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CONTENTS

CARDIOLOGY

Nonfatal myocardial infarction in women
E. Böszörményi, J. Szigeti, L. Mikes, J. Kiss, M. Kármán, J. Fehér, Margit Kovács ... 3

Incidence of rhythm disorders in hyperthyrosis with special respect of old age form
A. Mohácsi, F. Wórum, I. Lőrincz, E. Nagy, A. Leövey 21

Pattern recognition in evaluation of haemorheological and haemodynamical measurements in the cardiological diagnostics
K. Tóth, B. Mezey, I. Juricska, T. Jávor 31

IMMUNOLOGY

Cellular and humoral autoimmune responses against human eye muscle membrane antigen in Graves' disease
Ildikó Molnár, Katalin Németi, Ágota Bokk, Valéria Stenszky, Cs. Balázs, Nadir R. Farid 43

Correlations between serum alpha$_2$-HS-glycoprotein concentration and conventional laboratory parameters in systemic lupus erythematosus
L. Kalabay, L. Jakab, K. Cseh, Teréz Pozsonyi, L.A. Jakab 53

T-lymphocyte subgroups and the activity of human natural killer (HNK) cells in low-grade and high-grade malignant cases of non-Hodgkin lymphoma
T. Burger, Matild Schmelczer, Lenke Molnár, Gy. Fábián, Adél Szabó, Eszter Tóvári ... 65

Endogenous interferon (IFN) in patients with acute hepatitis B
A. Boron-Kaczmarska, R. Zachoval, V. Zachoval, F. Deinhardt 77

ENDOCRINOLOGY

The measurement of the serum sex-hormone binding globulin in various thyroid diseases
J. Földes, S. Vana, Cs. Bános, P. Lakatos, G. Tarján 81

GASTROENTEROLOGY

Gastric and colorectal cancer in the tropical part of Africa
G.A. Bálint 91

METABOLISM

Porphyrins and nucleic acid in some photosensitive skin diseases
M.M.H. El-Sharabasy, A.A. Hegazy, M.S. El-Soubky, M. Abd El-Maksoud 99

Activities of serum cathepsin (B, H and L) and metalloproteinase (MMP7-ase) in patients with gastrointestinal and bronchial malignant tumours
Aranka László, I. Sohár, S. Karácsonyi, A. Petri, I. Troján 107

BOOK REVIEWS 111

Acta Medica Hungarica, Vol. 47/1-2, pp. 3-20 (1990)

NONFATAL MYOCARDIAL INFARCTION OF WOMEN

E. BÖSZÖRMÉNYI, J. SZIGETI, L. MIKES, J. KISS, M. KÁRMÁN, J. FEHÉR, MARGIT KOVÁCS

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(Received: May 28, 1989)

Five-hundred women admitted for rehabilitation to the State Hospital for Cardiology 1 to 10 months after myocardial infarction were divided into two groups, viz. group I containing patients less than 40 years of age and group II, in which the patients were older than 41 years. Forty-nine per cent of the patients were blue-collar, whereas 22% of them were white-collar workers; 16.5% had a high qualification, 28% were housewives or retired. The leading symptom at admittance, that is in the post-infarction period, was angina pectoris (32% in group I and 73% in group II). Heart failure, rhythm disturbance and hypertension occurred less frequently. The groups considerably differed from each other in the frequency of risk factors. In group I, smoking (81%), use of anticoncipients (41%) and hyperlipoproteinaemia (32%), while in group II hypertension (49%), smoking (45%), obesity (43%) and hyperlipoproteinaemia (41%) were the main risk factors.

Keywords: Myocardial infarction, risk factors, rehabilitation

Introduction

Coronary heart disease (CHD) is the major cause of disability and the leading cause of death of women, although women lag men in incidence of CHD by 10 years /14/. The advantage of women over men wanes with advancing age and is practically lost after the menopause /14/. On the other hand, myocardial infarction (MI) in young menstruating women occurs rarely /11/.

Abbreviations: CHD = coronary heart disease; Gr = group; pts = patients

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Akadémiai Kiadó, Budapest

The Framingham study has been in continuous operation since 1948. Over 30 years of surveillance of 2873 women, 574 developed initial manifestations of CHD. There were 196 coronary fatalities /14/.

It is a surprise that a large proportion of myocardial infarctions (MI) remained unrecognized. Among women and men sustaining a first MI 35% and 28%,respectively, went unrecognized /13/.

The weight of evidence indicated that the risk of both nonfatal and fatal MI increases three to fourfold in women who use high estrogen oral contraceptives /14/. Oral contraceptives alone, or especially combined with other risk factors, significantly increase the incidence of MI /9, 10, 12, 18, 19, 24, 28, 30, 32, 33/.

Hypercholesterolaemia as a primary risk factor for CHD and myocardial infarction is generally accepted in the literature /6/.

Elevated serum cholesterol level is considered by some investigators a further risk factor for recurrent myocardial infarction /7, 31/, others disagree with this, at least in the male sex /20, 27/.

Wilhelmsen et al. /35/ found that women with infarction had higher blood pressure values and higher triglyceride levels, and smoked considerably more than women in the general population. All the same, the cholesterol values were not significantly higher in the women with infarction /35/.

In this hospital 3 to 4000 patients admitted for rehabilitation after MI are treated every year. In the last years, the number of myocardial infarction has increased among our female patients.

This is put in evidence by the modification of the men/women ratio, which was 5.3:1 in 1977 and 3.9:1 in 1987. Considering the increasing number of women among our infarct patients, we started a prospective study, trying to find out the characteristics, if there are any, of myocardial infarction in women.

Patients and Methods

We performed the complex investigation of 500 women, admitted consecutively between 1 January 1985 and 31 December 1987, for rehabilitation 1-10 months (mean: 3.5 months) after MI.

In the acute phase of illness the patients had been treated in various hospitals and clinics in different towns of Hungary. The diagnosis was done according to the WHO criteria, that is, the patients showed at least two of the three cardinal symptoms of MI: 1) Long-lasting (more than 20 min) angina; 2) ECG modifications typical of MI; 3) elevated plasma-enzyme levels.

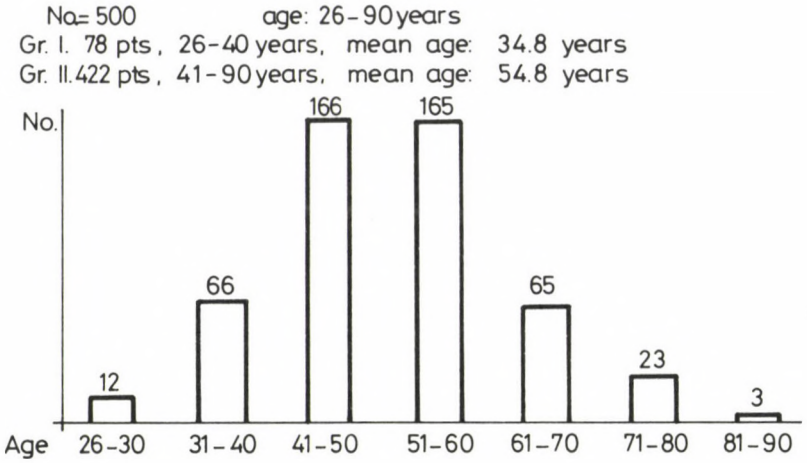

Fig. 1. Age distribution of the patients

Since we had no possibility to study this period of MI, we have no data concerning acute-phase mortality and symptoms.

The patients were divided into two age groups: group I including patients younger than 40 years, and group II including patients older than 41 years (Fig. 1).

We tried to evaluate the following items:

I. History
- profession
- heart complaints before MI
- stress situations

II. Clinical symptoms at the admittance in our hospital
- angina
- congestive heart failure
- hypertension
- rhythm disturbances
- site of infarction

III. Non-invasive investigations

- Bicycle ergometry

All patients performed gradual bicycle exercise tests. Workload was increased in a graded fashion from 30 W with 30 W increase every 3 min. The exercise was continued until 85% of the maximal predicted heart rate was reached, or ischaemic changes occurred or anginal pain developed. ECG leads were arranged according to the CM_5 system, with the reference electrode over the manubrium sterni, an exploring electrode in the V_5 R, and another in the V_5 position.

- Phonomechanography

We calculated the PEP/LVET ratio. Normal values in our laboratory: 0.34 $\pm$ 0.03 Ratio above 0.37 was considered a sign of impaired left ventricular function.

- Radioisotope examinations with ^{113}Indium

We determined the:

- stroke volume index (stroke volume/m^2 body surface): normal values $\geq$ 45 ml/m^2 b.s.;
- estimated ejection fraction (Van Dyke): normal values $\geq$ 50%.

IV. Biochemical laboratory investigations

- cholesterol, normal values: $\leq$ 6.4 mmol/l
- slightly elevated 6.5 - 6.6 mmol/l; elevated $\geq$ 6.7 mmol/l
- HDL cholesterol, normal values: $\geq$ 1.0 mmol/l
- LDL cholesterol, normal values: $\leq$ 4.0 mmol/l
- Triglycerides, normal values: $\leq$ 2.0 mmol/l
- Type of hyperlipoproteinaemia (HLP)
- Uric acid, normal values: $\leq$ 350 mmol/l

V. Risk factors:

- Positive family history
- Smoking
- Anticoncipients
- Hypertension
- Hyperlipoproteinaemia
- Diabetes
- Impaired glucose tolerance
- Elevated uric acid
- Psychical stress
- Obesity (Broca index $>$1.1)

We evaluated the data of Group I and Group II patients separately.

Results

I. History

- Profession: The majority of the patients (pts) in both groups were blue-collar workers. In group II, the number of retired persons was considerable. We included in this group also a small number of housewives (Fig. 2).

- Heart complaints before infarction

The percentage of symptomfree patients before infarction was much higher in group I than in group II.

The younger patients had angina less frequently and arrhythmias more frequently than the older. None of them had heart failure (Fig. 3).

- Predisposing factors immediately before infarction: 89, that is 17.8%, of the 500 patients had stress situations several months or weeks before the infarction (group I: 22 = 28.2%; group II: 67 pts = 15.8%).

Hard, long-lasting or unusual physical activity was a predisposing factor only in 68 cases (group I: 40 = 51.2%; group II. 28 = 6.6%).

	White-collar workers	Blue-collar workers	House-wives	Retired	
Gr. I. 26 - 40yrs	25	53	-	-	78
Gr. II. 41 - 90yrs	86	194	42	100	422
	111	247	42	100	500

Fig. 2. Profession

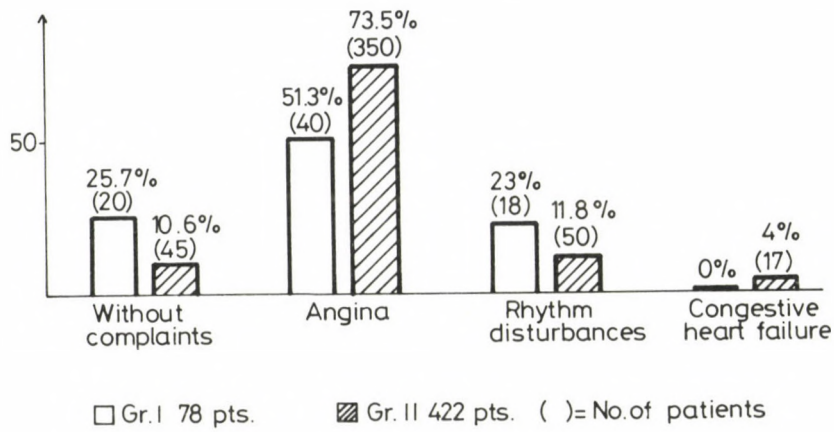

Fig. 3. Cardiac complaints 1-5 years before the infarction

II. Clinical symptoms at admittance

In group I, only one-third of the patients (25 = 32%) had angina and a few had heart failure (7 pts = 8.9%) or arrhythmia (6 pts = 7.6%); and none had hypertension.

In group II the majority of the patients had angina (363 = 73%) or heart failure (122 pts = 28.9%); only few had arrhythmia and hypertension before infarction (Fig. 4).

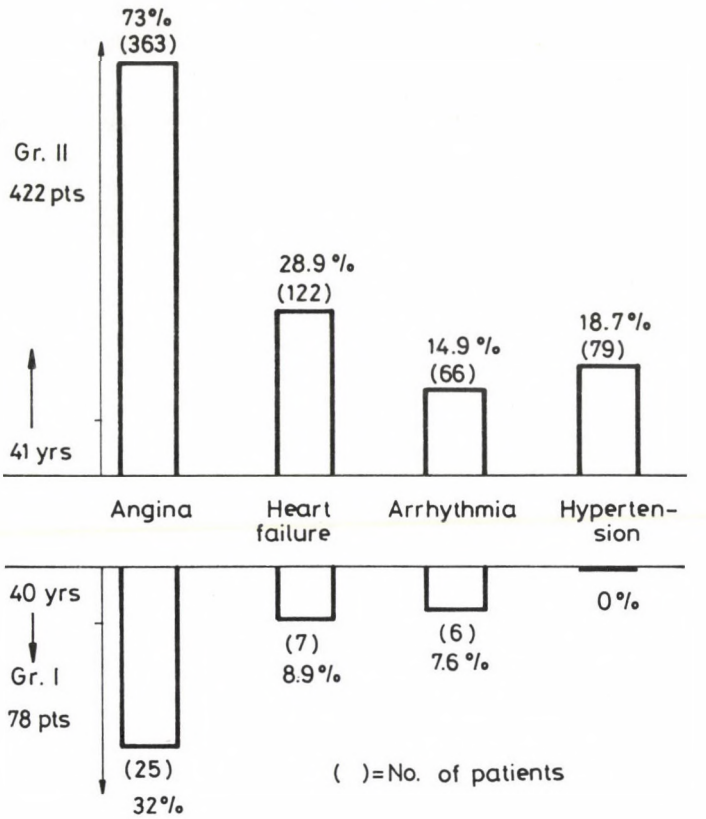

Fig. 4. Clinical symptoms at admittance

Site of the infarction

Only older patients had non-Q infarction (69 = 16.2%) or double infarction (20 pts = 4.6%). The majority of the young patients (33 = 42%) had anterior, the majority of the older patients had inferior (138 = 32.6%) infarction (Fig. 5).

Non-invasive investigations

- Bicycle exercise test. In both groups most patients had a good exercise capacity (120 W) (group I: 36 pts = 50.7%; group II: 105 pts = 42.7%); a small percentage could perform only 30 W (group I: 4 pts = 5.6%; group II: 11 pts = 4.5%) (Fig. 6).

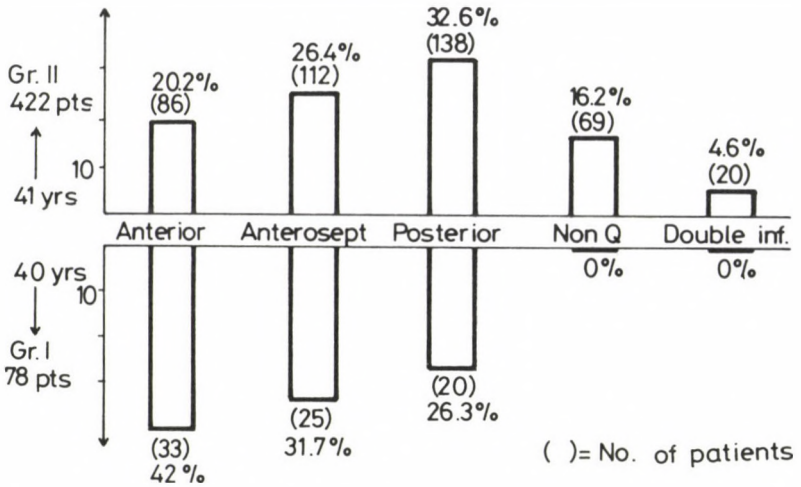

Fig. 5. Site of the infarction

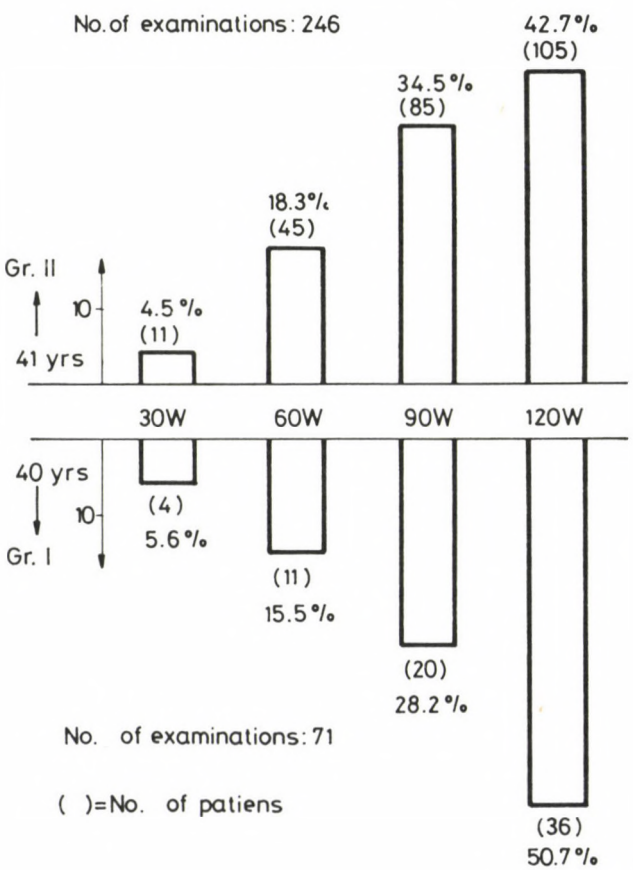

Fig. 6. Exercise performance

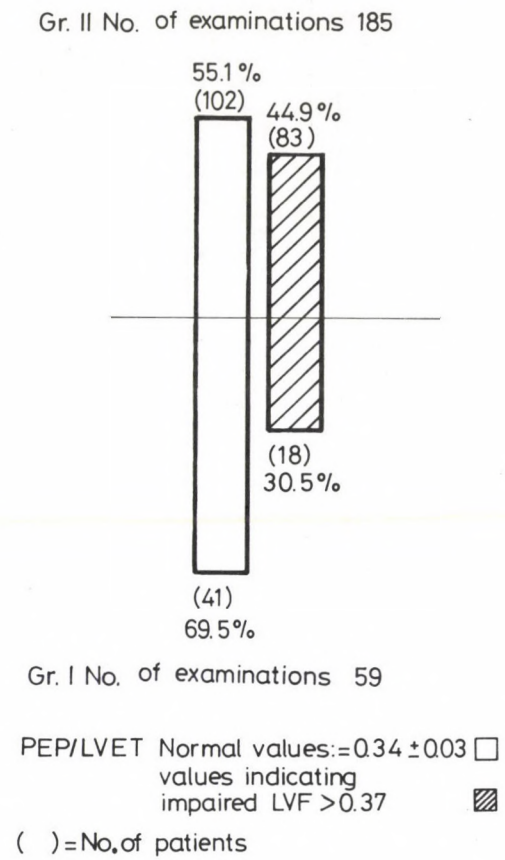

Fig. 7. Phonomechanography

- Phonomechanography

A PEP/LVET value over 0.37, indicating an impaired left ventricular function, was put in evidence more frequently among patients in group II (44.9%) than those in group I (30.5%) (Fig. 7).

- Radioisotope examinations

The stroke volume index was diminished in both groups in about 40% of the patients (group I: 39.2%; group II: 41.8%). The estimated ejection fraction was moderately decreased (35 to 50%) in half of the patients of both groups (group I: 50.0%; group II: 51.2%). The two parameters indicate a moderately impaired left ventricular function in 40-50% of the patients. A severely impaired LVF (EF $\leq$ 35%) was shown out only in a few cases (Fig. 8).

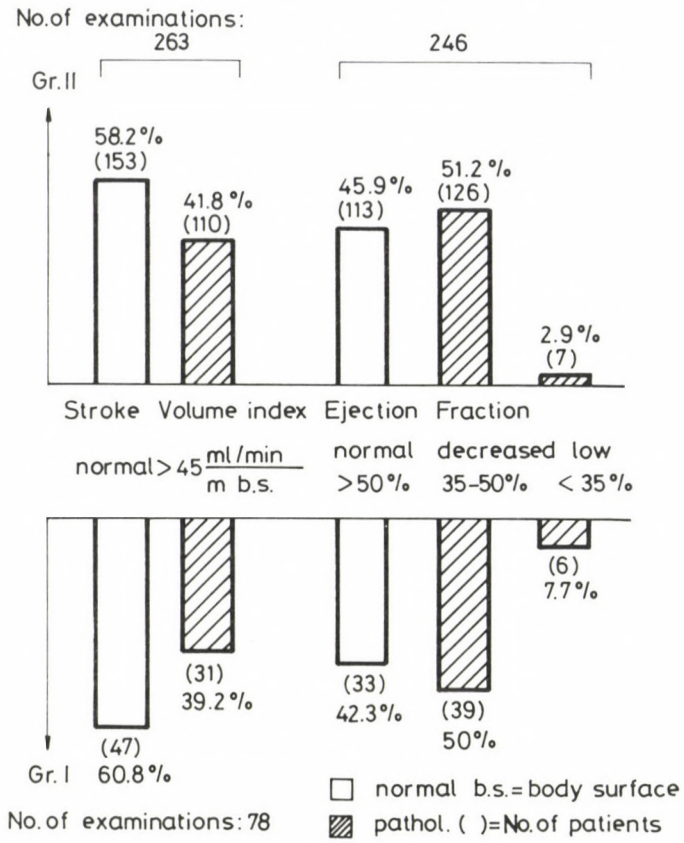

Fig. 8. Radioisotope examinations ^{113}Indium

- Biochemical laboratory examinations

In group I, we found pathologic values of cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and uric acid only in a small percentage (32.2; 29.3; 25; 16.7 and 15.4%), respectively. In group II, elevated cholesterol (42.0%) and triglyceride (46.5%) levels were found more common. Pathologic HDL (19.7%) and LDL cholesterol (37.3%) values were not uncommon. The plasma uric acid was elevated in 22% of the patients (Fig. 9).

- Risk factors

We analysed the frequency of each risk factor and of the combinations of risk factors in both groups. There was a considerable difference between the two groups.

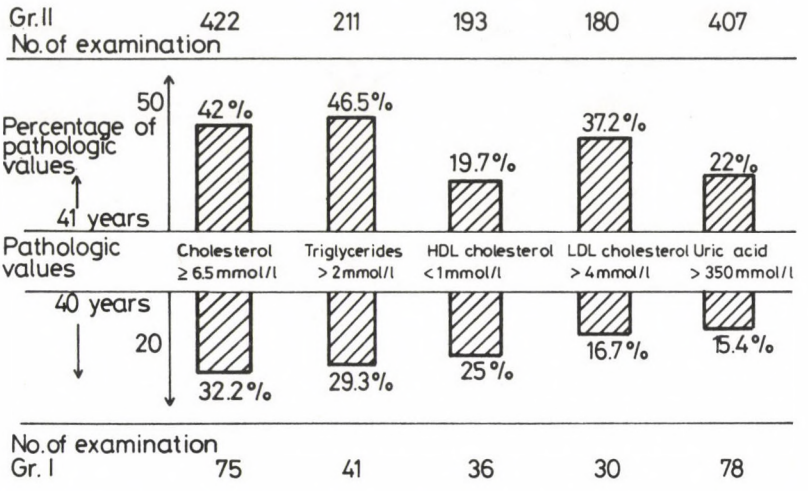

Fig. 9. Results of biochemical laboratory examinations

In group I the very important risk factor was smoking (80.8% of the patients). A quarter of all pts (25.6%) was heavy smoker (30 to 40 cigarettes a day).

The other important risk factor was use of anticoncipients (41%). Hyperlipoproteinaemia (32%) and psychical stress (28.2%) occurred in about one-third of the patients. In most patients with hyperlipoproteinaemia we observed type II/a (16.6%) and type III (11.6% of the 78 patients). Type II/b occurred only in three cases (3.8%). The other risk factors, such as hypertension (26.9%), positive family history (16.6%), diabetes (6.4%) and elevated uric acid level (5.4%) were less important; 29 patients (37%) were obese. Obesity occurred only in combination with other risk factors, such as smoking and hypertension, smoking and anticoncipients or smoking and hyperlipoproteinaemia (Fig. 10).

Only 4 pts (5.1%) were without any evident risk factor, 15 (19.3%) had a single risk factor (smoking: 13 = 16.7%) and use of anticoncipients: 2 = 2.6%. The others had 2 or 3, some had even 4 risk factors (Fig. 11).

In group II, the most important risk factor was hypertension (48.8%). The proportion of smokers was also considerable (45.2%), 5.9% of all pts were heavy smokers. Hyperlipoproteinaemia and diabetes were put in evidence more frequently than in group I (41.2 and 10.9%, respectively) (Fig. 10).

In this group we observed mostly the types II/a and II/b (20.6 and 13.7%, respectively, of the patients). Type III occurred only in a few cases

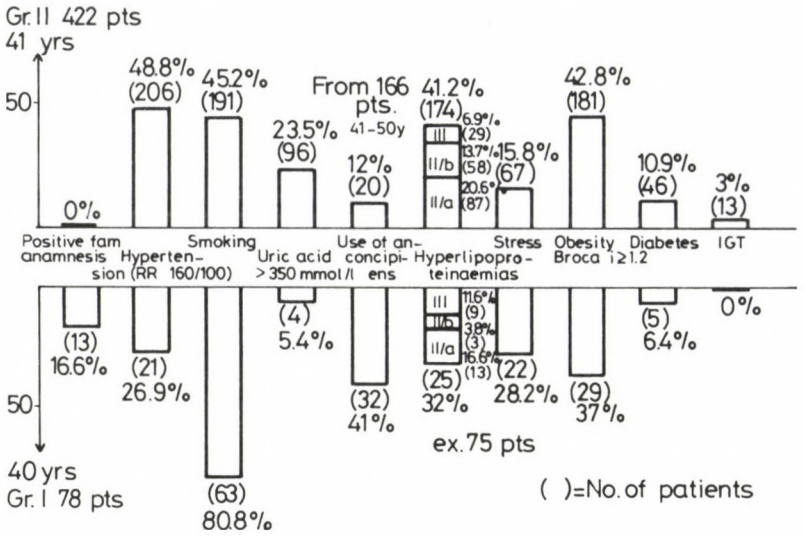

Fig. 10. Risk factors

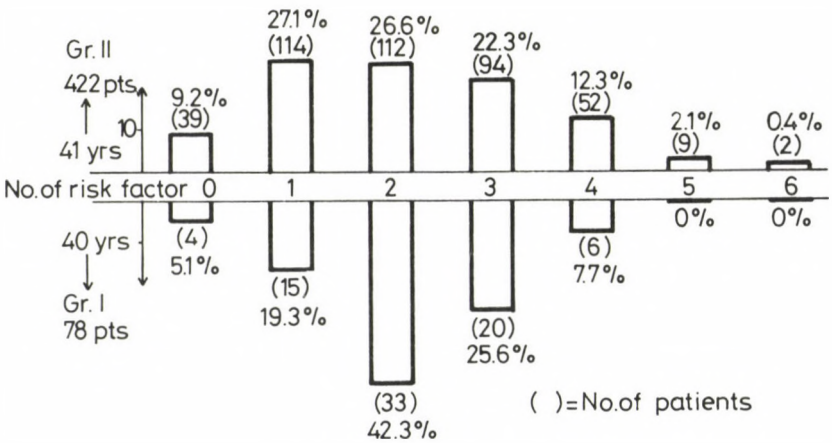

Fig. 11. Number of risk factors

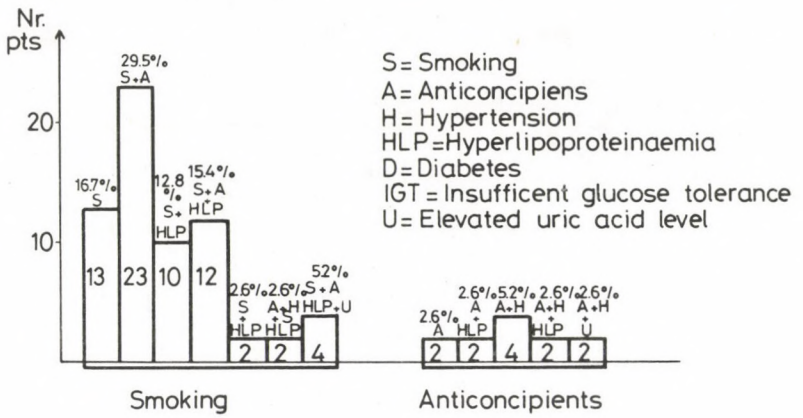

Fig. 12/a. The most frequent risk factor combinations
Gr. I

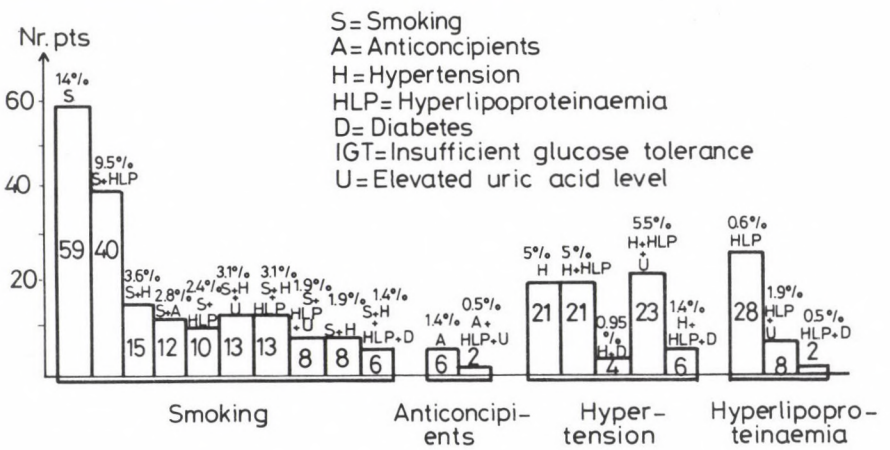

Fig. 12/b. The most frequent risk factor combinations
Gr. II

(6.9%). Obesity was observed more frequently than in the young group (181 pts = 42.8%); 32 pts (10.2%) were considerably obese (Br.i. $\geq$ 1.3).

In a few cases (11 pts = 2.6%) obesity was the single risk factor. Generally it was present in combination with another 2 or 3 risk factors, such as smoking, hypertension or hyperlipoproteinaemia. IGT (3.1%), stress (15.8%) and elevated uric acid level (23.5%) were not frequent.

From the 166 women aged 41-50 years 20 (12%) used anticoncipients. Some patients (39 = 9.2%) had no evident risk factor. Most patients had 1, 2 or 3 risk factors (27.1 and 26.6 and 22.33%,respectively, and only a few had more (Fig. 11).

Combination of risk factors

In group I, smoking (13 pts = 16.7%) and in 2 (2.6%) patients use of anticoncipients was considered single risk factors. The majority of the patients had 2 or 3 risk factors. The most frequent combinations were smoking + anticoncipients (23 pts = 29.5%), smoking + anticoncipients + hyperlipoproteinaemia (12 pts = 15.4%) and smoking + hyperlipoproteinaemia (10 pts = 12.8%). Other combinations occurred rarely (Fig. 12/a).

In group II, single risk factors were smoking (59 pts = 14.0%), hyperlipoproteinaemias (28 pts = 6.6%) hypertension (21 pts = 5.0%) and, in 6 cases (1.4%), anticoncipients. The most important risk factor combinations were smoking + hyperlipoproteinaemia (40 pts = 9.5%), smoking + hypertension (15 pts = 3.6%), smoking + anticoncipients (12 pts = 2.8%), and hypertension + HLP (21 pts = 5.0) (Fig. 12/b).

Discussion

Our observations indicating an increasing number of infarctions among women are similar to those reported by other research groups. Considering the fact that our patients were living in different parts of Hungary we can suppose that these observations reflect the situation in this country.

The majority of our patients with MI were 41 to 60 years (331 = 66.2%) old. It seems that this is the most dangerous age for women to get a MI.

The number of younger patients ($\leq$ 40) was also considerable. Some of them even younger than 30 years.

In both age groups the majority of the patients were blue-collar workers.

A quarter of the young, and only 10.6% of the older patients was without any cardiac complaint before the infarction. Some of them had angina or arrhythmia, and only a few older patients had heart failure.

Hard or unusual physical activity was a predisposing factor before the infarction in both groups, much more frequently in young than in old patients. The non-invasive investigations indicated an impaired left ventricular function in both groups, in 40-50% of the patients but severe LV dysfunction was observed only in a few cases.

Despite this phenomenon, the majority of patients from both groups had a good exercise capacity.

The most important risk factor of patients under 40 was smoking, which was in 13 cases (16.7%) the only risk factor, and was put in evidence in the majority of patients with other 1, 2 or 3 risk factors. The most frequent risk combination were smoking + hyperlipoproteinaemia and smoking + anticoncipients + hyperlipoproteinaemia. Use of anticoncipients was also a very important risk factor but with the exception of 2 cases (2.6%) occurred only in combination with 1, 2 or 3 others. This observations suggests that in this age group the anticoncipients represent generally a cardiovascular risk only in combination with other factors. Obesity, hypertension and hyperlipoproteinaemia were not uncommon, but only in combination. Positive family history and diabetes showed no considerable importance. Stress situations were present in about one-third of the patients.

In the older age group the important risk factors as hypertension, it was followed by smoking and hyperlipoproteinaemia. Each of these occurred also as a single risk factor, but mostly in combination with 1-4 others. Obesity occurred more frequently. In a few cases this was even the single risk factor, but generally it occurred together with others. Diabetes was observed in 10.9% of the patients.

Coronary spasm probably plays an important role in the pathogenesis of myocardial infarction in young menstruating women taking oral contraceptive pills and smoking cigarettes. A number of investigations /12, 19, 23, 24, 26/ suggest synergism between oral contraceptives and cigarette smoking in the pathogenesis of MI in young women /5, 17/. Cigarette smoking alone was suggested to be atherogenic in autopsy studies /1/ and to cause sudden death in women /29/.

Cigarette smoking can be associated with nicotine-induced release of norepinephrine /6/, which might theoretically cause vasoconstriction, probably by altering the prostacyclin thromboxane A_2 ratio /34/.

The question arises whether women who had suffered from a myocardial infarction, despite the overall low incidence of this disease in middle-aged women, were more heavily burdened by the risk factors hypercholesterolaemia, hypertension, and smoking than are men with MI. Wilhelmsen et al. /35/ found, however, similar distributions of these risk factors in MI patients in both sexes and in general, among pre- and postmenopausal women, a fact that does not support this hypothesis.

Interestingly enough, Olsson and Rehnqvist /25/ did not notice any difference in the 3 year prognosis between patients having low ($\leq$ 6.7 mol/l) and higher (6.7 mmol/l) serum cholesterol levels. Treatment with metoprolol for 3 years did not influence the total serum cholesterol level (one quarter of patients were women).

On the other hand, Leren et al. /15/ found elevated cholesterol levels after several years treatment with propranolol. The HDL cholesterol level decreased.

Hyperuricaemia seems to have an independent influence on the overall mortality in women, although there is no relationship between serum uric acid concentration and incidence of cardiovascular disease, such as MI or angina pectoris /4/.

Treatment of hypertension is a major objective in the prevention of cardiovascular disease. However, the effects of antihypertensive drugs on the CHD mortality have been contradictory and in many respects disappointing /2, 21, 22/.

Løchen /16/ in a large population study (Tromsø Heart Study) suggested that the increased level of total serum cholesterol in hypertensives probably was present originally, and was not aggravated by the antihypertensive treatment.

The lower HDL cholesterol and elevated tirglyceride levels in treated, compared to untreated hypertensives might have been evoked by drug therapy. The unfavourable effects of these drugs may in this way counteract the benefits of blood pressure reduction. Probably control of blood pressure alone is not enough to reduce the incidence of and mortality from CHD.

Despite the lipid changes, the Beta-Blocker Heart Attack Trial /15/ showed beneficial effects both on morbidity and mortality by long-term propranolol treatment.

A possible explanation for these conflicting results might be that these drugs inhibit not only lecithin-cholesterol acyltransferase, resulting in decreased HDL cholesterol, but also acyl CoA-cholesterol acyltransferase,

an effect that might result in a decreased deposition of cholesterol esters in the artery walls /3/.

The investigations indicate a remarkable difference between the myocardial infarction of men and women: high serum cholesterol is not such a strong risk factor for (non-fatal) myocardial infarction in women as in men. High triglycerides are apparently more common in infarct women /16, 35/.

It has also been demonstrated that the CHD risk in hypertensives is greatly magnified by daily smoking. It has been shown that it may be more important for subjects with mild hypertension to stop smoking than to put them on antihypertensive drugs /22/.

To prevent the MI of women or to stop the increase of casualties we could advise the following:

1. Cessation or moderation of smoking.

2. Women who have been using anticoncipients for a long period should be checked for other risk factors. They must know that the combination of smoking with use of anticoncipients may be dangerous at every age. The risk is much more increased for women older than 35 years.

3. Treatment of hypertension and prevention of obesity is also very important.

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INCIDENCE OF RHYTHM DISORDERS IN HYPERTHYROSIS WITH SPECIAL RESPECT OF OLD AGE FORM

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Sinus tachycardia and atrial fibrillation are frequent features in hyperthyrosis while sinus node dysfunction is regarded as a rare complication. Bradycardia may cause diagnostic problems mainly in atypic hyperthyrosis of the old age. The authors analysed distribution and age related association of the rhythm disoreders in hyperthyrosis. In case of the appearance of Sick Sinus Syndrome (SSS), parameters representing the function of sinus node were studied by electrophysiological investigations. Above the age of 50 years incidences of atrial fibrillation and SSS were significantly increased. The abnormal sinus node function proved to be reversible in a portion of the cases. In old age, in case of occurrence of the symptoms of SSS, possibility of hyperthyrosis also should be considered, especially when indication of permanent pacemaker is established.

Keywords: Hyperthyrosis, sick sinus node syndrome, implantation of pacemaker

Introduction

It was almost 170 years ago that Parry attributed great importance to the abnormality of the cardiovascular system in generating symptoms of hyperthyrosis (HT) /25/. HT may exacerbate existing ischaemic or myocardial impairment of other origin of the heart or may cause heart disease in itself /11, 21/. The nonspecific cardiovascular symptoms (palpitation, effort dyspnoe, weakness, peripheral vasodilatation) are reversible by attainment of the euthyroid state and they may be reproduced in animal experiment by

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Abbreviations: SSS = Sick Sinus Syndrome; HT = hyperthyrosis

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administration of thyroxine /15/. Besides circulatory hyperkinesis, arrhythmias form the most important group of the cardiac symptoms of HT. Apart from the persisting tachycardia during sleeping the most common type of arrhythmia in HT is atrial fibrillation; less frequently, other arrhythmias, like paroxysmal supraventricular tachycardia, atrial flutter, atrial tachycardia, ventricular and atrial extrasystolia, SSS-linked arrhythmias and transient conductivity disturbances may occur /5/. The frequency of this last type arrhythmia is poorly known. In case of occurrence of the typical clinical symptoms recognition of the existing endocrine disturbance, simultaneously existing together with rhythm disorders, does not cause any problem, but in old age patients the clinical symptoms may be absent or misleading (apathic form). Leading or single symptom can be just the rhythm disorder and/or the insufficiency of the pump-function appearing resistant to medical treatment. Regarding the fact that the beginning of the HT occurs in old age in almost the third of the cases (30-35 per cent), presence of rhythm disorders accompanied by bradycardia may cause diagnostic and therapeutic problems /5, 7/. The above-mentioned situation prompted us to investigate the incidence of rhythm disorders and their distribution in old-age HT, with special attention to the rhythm disturbances causing bradycardia. We analysed the sinus-node function of HT patients who were subjected to electrophysiological investigations because of SSS clinical symptoms.

Patients and Methods

The data of 219 cases, clinically verified and/or identified by laboratory methods were analysed. Diagnoses were based on clinical appearance, T_3 uptake (T_3U), total serum T_3 and T_4 values, ^{131}I storage curves and, occasionally, TRH-TSH test (200 μg TSH), anti-human thyroglobulin and anti-microsome antibody determinations, or thin needle-biopsy. The patients were subdivided into two groups: to a group of age below 50 years belonged 121 patients, 12 male and 109 female, with average age of 29.1 years (17-48 years), while the group of the age above 50 years contained 98 patients, 96 female and 2 male ones, with average age of 60.0 years (50-84 years). The distribution of diagnoses was as follows: Graves-Basedow's disease, 182 cases; autonomous adenoma, 35 cases; Graves' disease associated with Hashimoto's thyroiditis, 2 cases. The type of arrhythmia was established at admission and during disease process by electrocardiograph registration. During the investigations only those patients were included whose anamneses did not show hypertension, ischaemic myocardial disease and/or congestive heart failure. In five cases invasive electrophysiological investigations also took place. The following parameters were determined: sinus node recovery time (SNRT), sino-atrial conductivity time (SACT). His bundle electrogram, ventricular and atrial effective refractory periods (AERP, VERP) and atrio-ventricular (AV) impulse conductivity characteristic curve (Wenkebach point).

Results

Table I

Rhythm disturbances in 219 hyperthyrotic cases

	Number of cases	
	17–49 years	50–84 years
I. Normal	12	14
II. Disturbances of impulse formation		
A. Disturbances of sinus impulse formation		
sinus tachycardia	93	39
sinus bradycardia	-	7
B. Disturbances of heterotopic impulse formation		
atrial fibrillation	2	34
paroxysmal atrial tachy	2	1
premature atrial beat	4	3
III. Disturbances of impulse conductivity		
sinoauricular block	-	5
first degree AV block	5	4
Aberrant ventricular conductivity	5	4

Table I shows the distribution of arrhythmias observed during treating of 219 hyperthyrotic patients. In the group of patients below 50 years there were only 12 cases in which impulse formation and conductivity disturbances were not observed. An overwhelming majority of the arrhythmias were of sinus tachycardia, 93 cases (76.8 per cent). Atrial and paroxysmal tachycardia occurred exceptionally. The number of AV conductivity disturbances as well as ventricular and atrial heterotopies did not change with age. In the group of patients with age above 50 years occurrence of arrhythmias, raising the suspect of SSS (sino-atrial block and sinus bradycardia) and atrial fibrillation, was increased. Atrial fibrillation, sinus bradycardia, sino-auricular block were observed in 34, 7 and 5 cases, respectively. Besides the persistent atrial fibrillation the arrhythmias appeared paroxysmally in 12 cases. Figure 1 shows the age-dependent distribution of atrial fibrillation and deviations referring to sinus node dysfunctions. In the subgroup of age above 70 years, the frequency of atrial

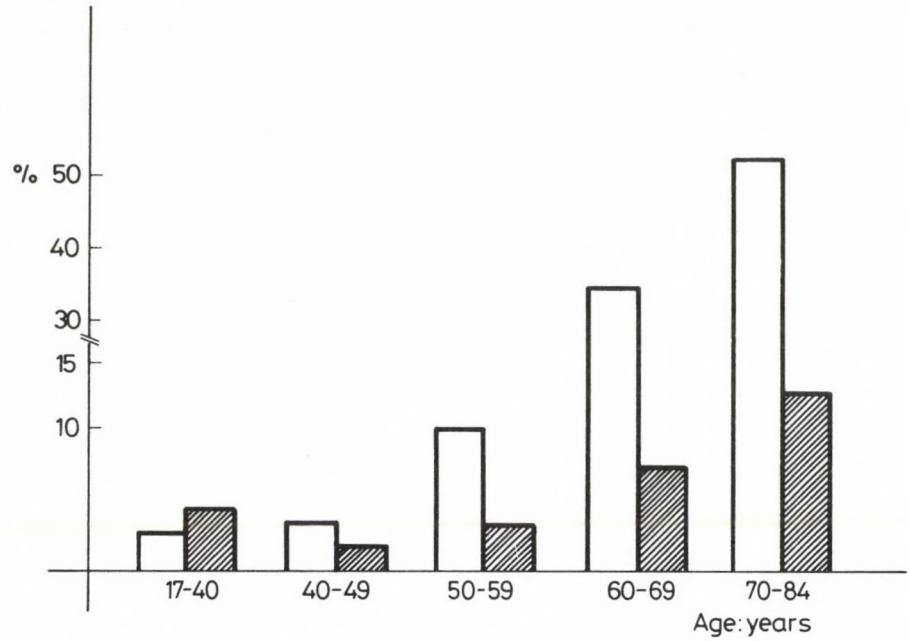

Fig. 1. Age dependence of the rhythm disturbances appearing in the forms of atrial tachycardia and sinus-node disease. (Open columns: atrial tachycardia, shaded columns: referring sinus-node disease.) When constructing the figure "normal" frequency heart function in HT was also taken into account.

fibrillation surpassed 50 per cent of the cases; the number of SSS cases also increased, although at a reduced rate. Table II depicts the data of patients who were admitted to electrophysiological investigation because of the suspect of sinus-node disease. A definite, but moderate, prolongation of sino-atrial conductivity was observed in 4 cases with an atrial effective refractory period inhomogenity. In two cases, because of persisting pathological sinus-node functions and significant symptoms, permanent pacemaker implantation was performed; in one case, symptoms were manifesting upon administration of beta-receptor blocker. These symptoms disappeared as dosage had been reduced. In two cases the euthyroid state was reached and the anomalies referring to sinus-node dysfunction ceased. Prolonged H-V interval referring to conductivity disturbance, was not observed.

Table II

Electrophysiological data of hyperthyrotic patients showing symptoms referring to sick sinus syndrome

Patient No.	Sex	Age (years)	ECG	SACT (120-240) ms	SNRT (800-1200) ms	AV node Wenkebach	AH (50-120) ms	HV (35-55) ms
1	female	61	SA-block	400	5000	180	70	35
2	female	55	SA-block	450	2000	220	75	45
3	female	74	Sinus brady-cardia (S.standstill)	260	1800	180	70	50
4	female		SA-block	240	1250	200	90	40
5	male		SA-block	260	1600	185 atrial fibr.	90	50

Discussion

The occurrence of atrial fibrillation in HT is estimated at 20 per cent in general; AV-conductivity disturbances and SSS are kept in evidence as rare complications; their prevalence is changing by publications /5, 6, 20, 23, 24/. The occurrence of AV-conductivity disturbances ranges from 5 to 20 per cent in the publications, though, Ferrer does not mention HT among the possible causes of SSS /10/. In our investigations, sinus bradycardia and sino-auricular block did not occur below 50 years; above 50 years we observed these arrhythmias in 12 cases. In old age HT we saw the sinus node syndrome, frequently. In the cases "normal" frequency heart work and an atrial fibrillation appearing paroxysmatically could be a partial phenomenon belonging to a pathological sinus-node function. Talwar et al. observed definite prolongation of the sinus-node recovery time and sino-atrial conductivity time at the investigation of HT patients sent to electrophysiology study with bradycardia, but these phenomena regressed when the euthyroid state was attained /31/. In two of our cases the deviations persisted even in the euthyroid state, only in three cases they proved to be reversible. The uneven character of the refractory period of the atrium referred to its vulnerability in one case. We found no deviations characteristic or AV-conductivity damages. In our cases age-dependence of the arrhythmias was obvious: in younger ages, together with frequent sinus tachycardia, a tachycardia of paroxysmal character occurred in ex-

ceptional cases (in serious thyrotoxicosis), while above 50 years the number of both the permanent and the paroxysmal type atrial fibrillation was increased. Arrhythmia together with bradycardia occurred only above 50 years of age.

The exact mechanism through which HT leads to the above types of arrhythmias has not been clarified. Although the role of age is obvious, age in itself cannot serve as an explanation as it has been shown by the results of 24 h Holter monitoring on healthy subjects /4/. Symptoms referring to the increase of the sympathetic tonus as well as the advantageous effect of the beta receptor blockers it seemed to be obvious to conclude to the change of reaction of the sympathico-adrenal system /3, 12/. The clinical observation, however, that before and after beta-receptor blocker the frequency and blood pressure response of HT patients to work load were identical with those of the similarly-treated healthy subjects served as evidence against the hyperactivity of the sympathetic system /12/. In spite of the presence of the normal catecholamine level, the increase of the beta-receptor density with the simultaneous decrease in number of the alpha receptors, as well as the high cAMP level increment upon isoproterenol administration, investigated in vitro in isolated myocardial cells, makes very probable the increase of the beta-receptor hypersensitivity towards catecholamines /16, 30/. There is no evidence of the presence of cholinergic hypersensitivity of SSS in its pathogenesis either /13, 28/. Among the symptoms of the old-age HT the increase of the number of angina pectoris is striking. On this ground vasospasm, local ischaemia cannot be excluded in background /2/. In HT, for the high number of the arrhythmias probably the intracellular changes of the ion milieu can be regarded as responsible. Duration of the action potential (AP) decreased in the ventricular heart muscle cells as a result of the shortening of the plateau phase at first place; the steepness of the diastolic repolarization slope rose but the resting threshold potential of the membrane, the steepness and velocity of the ascending branch of AP remained unchanged /1, 17, 30/. For all these changes during development of the plateau phase the potential-dependent Ca^{2+} channels and the conductance changes of the K^{+} channels, playing role in the diastolic repolarization, could be regarded as responsible. However, opposing this view, Jaeger et al. described prolongation of the AP in Purkinje fibres as a result of thyroxine effect /17/. The above changes can be explained only in that case if the factors responsible for opening and closing of the Ca^{2+} channels (phosphorylation and dephosphorylation mechanism) as well as for the increase and decrease of the

K^+ conductance are changing simultaneously upon thyroxine effect. The actual change (decrease and increase of the duration of AP and/or excitability) will be defined as resultant of the above changes. In spite of the accelerated Na^+– K^+ ATP-ase function in HT, fast increase of the extracellular K^+ takes place because of the lack of ATP, all this resulting in an increase of the excitability of the cells /9, 26/. The increase of the intracellular Ca^{2+} concentration causes changes of the same direction /14, 19, 32/. The Ca^{2+} antagonist diltiazem favourably can be used in the treatment of arrhythmias occurring in HT /22/. This experimental fact is also related to the changes role of the Ca^{2+} channel's.

As to the occurrence of SSS in old age, it is important to take into consideration the possibility of HT because in the lack of adequate clinical symptoms too much time may elapse until the correct diagnosis is established while myocardial damage may develop. The correct interpretation of rhythm disturbances and their adequate treatment may essentially improve the life prospect of the patient /8/. The reversible nature of a rhythm disturbance should be taken into account in such a case during establishing the necessity of permanent pacemaker implantation.

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PATTERN RECOGNITION IN EVALUATION OF HAEMORHEOLOGICAL AND HAEMODYNAMICAL MEASUREMENTS IN THE CARDIOLOGICAL DIAGNOSTICS

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The non-invasive differential diagnosis of ischaemic heart disease (IHD) and acute myocarditis or secondary cardiomyopathy following myocarditis can be difficult on the basis of the complaints, resting and exercise ECG and nuclear cardiological tests. 92 patients (mean age: 46 years) in the first step and 100 patients (mean age: 44 years) in the second step all with heart troubles, were examined. Besides determination of the routine parameters, nuclear haemodynamical and haemorheological measurements were carried out. Then each group of the patients was classified into 4 subgroups: 1) myocardial infarction /n:9/, 2) IHD /52/, 3) myocarditis /28/, 4) chronic cor pulmonale (CCP) /3/ subgroups in the first group and 1) normal /n:20/, 2) IHD /50/, 3) myocarditis /16/, 4) chronic cor pulmonale /14/ subgroups in the second group. The patients were reclassified by our multivariate pattern recognition algorithm (PRIMA). The average effectiveness of our method was over 80%, the recognition abilities for the subgroups (classes) ranged between 71 and 100%. An analysis of the discrimination power of the properties has made it evident that the haemorheological features were more characteristic than the haemodynamic ones in distinguishing the two differential-diagnostically critical groups. Our results show that our multivariate statistical method can be useful for the computer-aided decision in cardiological diagnostics.

Keywords: PRIMA, haemorheological parameters, haemodynamical measurements, differential diagnostics.

Abbreviations: AMI: acute myocaridal infarction; CCP: chronic cor pulmonale; IHD: ishaemic heart disease

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Introduction

The nonivasive, and sometimes even the invasive differential diagnostics of ischaemic heart disease (IHD), acute myocardial infarction (AMI) and acute viral myocarditis can pose a hard problem in the clinical practice /1, 2, 6, 8-11/. In these diseases, both the acute and chronic phase, the complaints often are similar, the resting ECG does not always make distinction and the stress tests performed in the chronic phase often provide similar results. The virus serology tests performed in the case of the suspicion of myocarditis may be positive in all of the mentioned diseases. Even the value of the invasive endomyocardial biopsy is criticized by some investigators /1, 2, 6, 9/.

For a better approach to this differential diagnostic problem, radioisotope haemodynamical and haemorheological investigations were routinely carried out in our cardiopathic patients /14/.

To evaluate the collected results, our multivariable pattern recognition method /7/ was used to classify the patients into different diagnostic categories.

Patients and Methods

92 patients (mean age: 46 years) in the first and 100 patients (mean age: 44 years) in the second step, all admitted with cardiac complaints, were examined. On the basis of the case histories, the physical examination and of the performed tests each group could be divided into 4 subgroups. Tables I and II show the age and sex distribution of the patients within the diagnostic groups.

Table I

Sex distribution of the patients in the subgroups of the first group

Sex of patients	Subgroup 1 (AMI)		Subgroup 2 (IHD)		Subgroup 3 (carditis)		Subgroup 4 (CCP)	
	n=	mean age (yrs)	n=	mean age (yrs)	n=	mean age (yrs)	n=	mean age (yrs)
Male	7	55	37	48	9	38	3	49
Female	2	61	15	53	19	40	0	
Total	9	57	52	50	28	39	3	49

Table II

Sex distribution of the patients in the subgroups of the second group

Sex of patients	Subgroup 1 (normal)		Subgroup 2 (IHD)		Subgroup 3 (carditis)		Subgroup 4 (CCP)	
	n=	mean age (yrs)	n=	mean age (yrs)	n=	mean age (yrs)	n=	mean age (yrs)
Male	10	32	45	57	2	31	12	56
Female	10	30	5	52	14	32	2	50
Total	20	31	50	56	16	32	14	55

Resting ECG recording with 12 leads, routine laboratory tests and virus-serology tests were performed. Furthermore, haemodynamical parameters, such as cardiac output, cardiac index, stroke volume and stroke volume index were determined in the first group by first pass isotope method using a portable NK-362 gamma detector (Gamma Factories, Budapest, Hungary). Before the intravenous isotope injection (^{99}Technecium) blood samples were taken for the haemorheological assays. In the first group of patients whole blood and plasma viscosity was determined at low shear rates (0.53, 1.35, 2.48, 4.95 1/s) while in the second group at medium and high shear rates (10, 90, 200 1/s). Fibrinogen level was quantified by the Reiner-Cheung method. The rheological measurements were performed at low shear rates in Contraves Low Shear 100 viscosimeter, at medium and high shear rates in Hevimet capillary viscosimeter /3, 14/.

Table III shows the upper limits of the normal ranges as estimated by our method.

Table III

The upper levels of the normal range of whole blood and plasma viscosities and plasma fibrinogen level

Shear rates (1/s)	Whole blood viscosity (mPa s)	Plasma viscosity (mPa s)
0.53	30	
1.35	20	
2.48	15	
4.59	10	
90.0	4.5	1.35

Plasma fibrinogen level: 4.0 g/l.

To evaluate our collected haemodynamical and haemorheological parameters by computer analysis, the most relevant parameters were selected.

These data served the training set for the PRIMA (Pattern Recognition by Independent Multicategory Analysis) supervised pattern recognition techniques /4, 7/. This class modelling method derives in learning phase for each class independent decision rules that subsequently can be used for classification of samples (in our case patients) of unknown origin. The decision rules are based on class distances. Classification can be done by assigning the patients to that class (subgroup) for which the class distance is minimal or smaller than a suitably selected limit value, the so-called class distance threshold. After the learning phase, we could calculate the discriminating power of the different properties which can be

used to characterize the importance of the given property and to select the relevant data from the point of view of the given classification.

The efficiency of classification is characterized by the "recognition ability", which corresponds to the fraction of patients from the training set that are classified correctly.

The algorithm of the PRIMA method was programmed in BASIC for ROSY 80-B (Rolitron) microcomputer.

Results

Figure 1 shows the values of the cardiac output and of whole blood viscosity at low shear rate in the first group of patients.

The cardiac output was the lowest in chronic cor pulmonale. It should be noted that all the 3 patients were already in the decompensated phase of CCP as confirmed by right-heart catheterization. One of them even died in the hospital. In cases of AMI the nuclear test was carried out immediately after the mobilization from bed, mostly on the 5th day of hospitalization. The cardiac output in these patients was lower than the normal level. In IHD and myocarditis, the cardiac output was in the normal range, but in myocarditis it was higher and often showed a hyperkinetic state, though the difference from the IHD patients was statistically not significant.

The most pathological whole blood viscosity was found in chronic cor pulmonale. This parameter was pathologically high in AMI and IHD as well. In myocarditis the whole blood viscosity was significantly lower than in AMI and IHD ($P < 0.01$) and it was in the normal range.

The values of whole blood viscosity and haematocrit for the second group can be seen in Fig. 2. The highest values were measured in CCP due to the polyglobulia in this disease. In IHD, also increased whole blood viscosity was found. In the normal controls and in myocarditis both parameters were significantly lower than in the other two groups ($P < 0.01$) and were in the normal range.

Figure 3 shows the plasma viscosity and fibrinogen levels of all the examined patients. In AMI, IHD and chronic cor pulmonale pathologically elevated fibrinogen levels were measured. Due to the high fibrinogen level, plasma viscosity was increased in these patients, too.

In Table IV the ordered discriminating powers of the most relevant measured parameters are listed in the point of view of discriminating between classes 2 and 3 in the first group of patients. Haemorheological parameters have the highest values. The results of haemodynamic test are less reliable in the differentiation of these diagnostic categories.

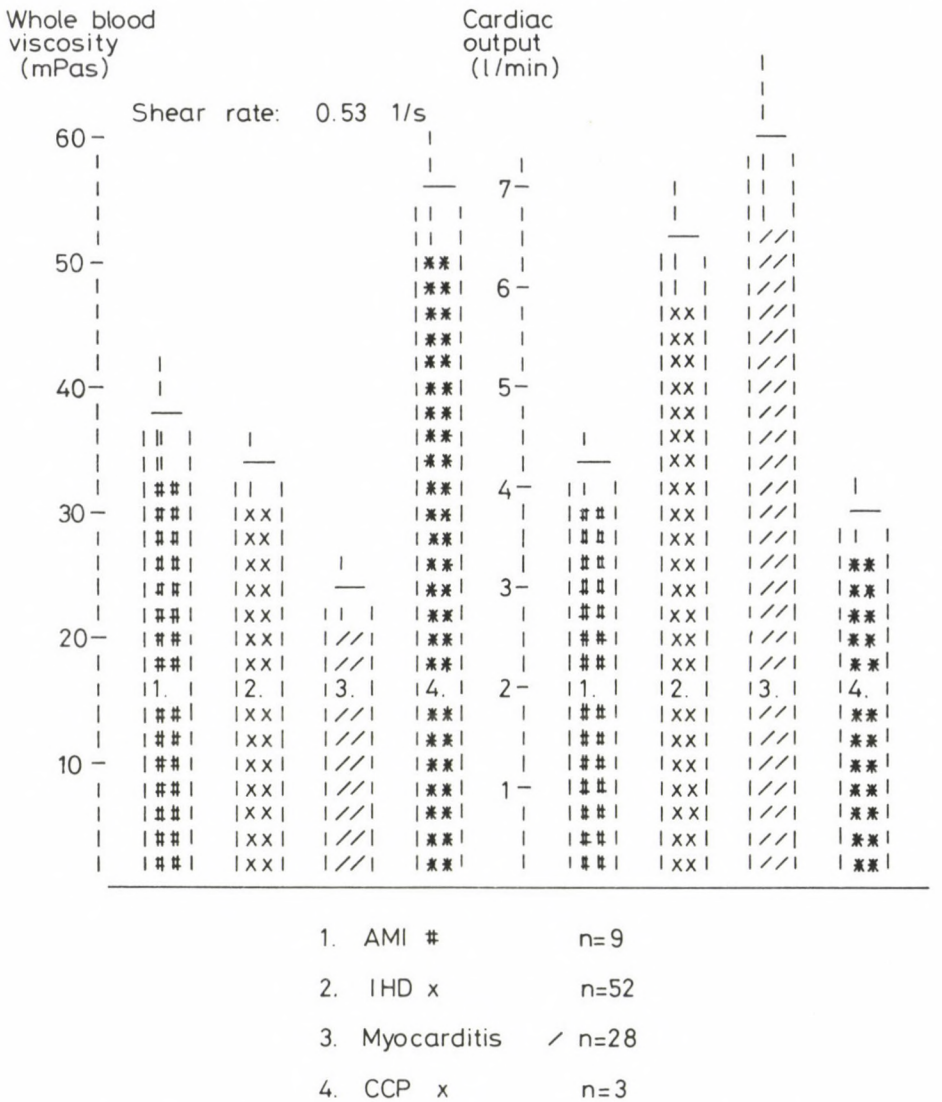


Fig. 1. The values of whole blood viscosity and cardiac output in the first group of patients

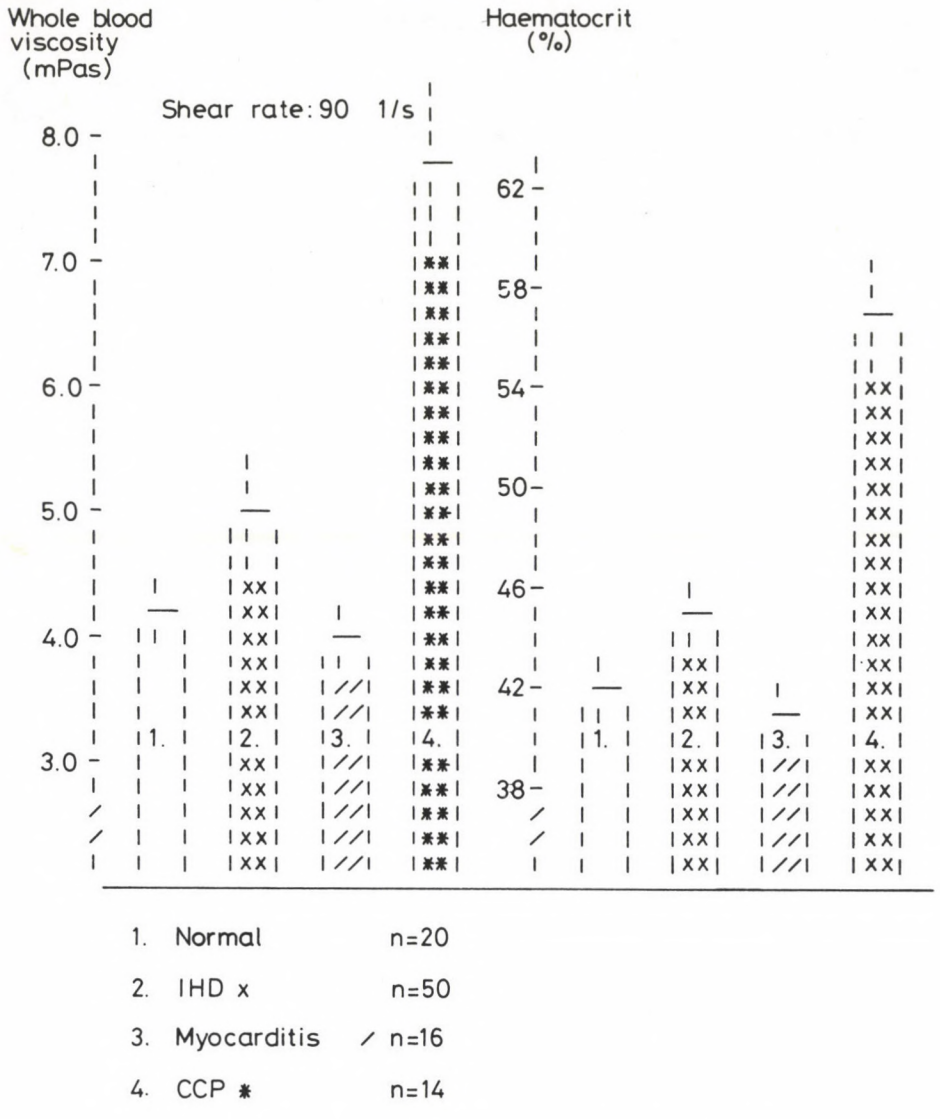
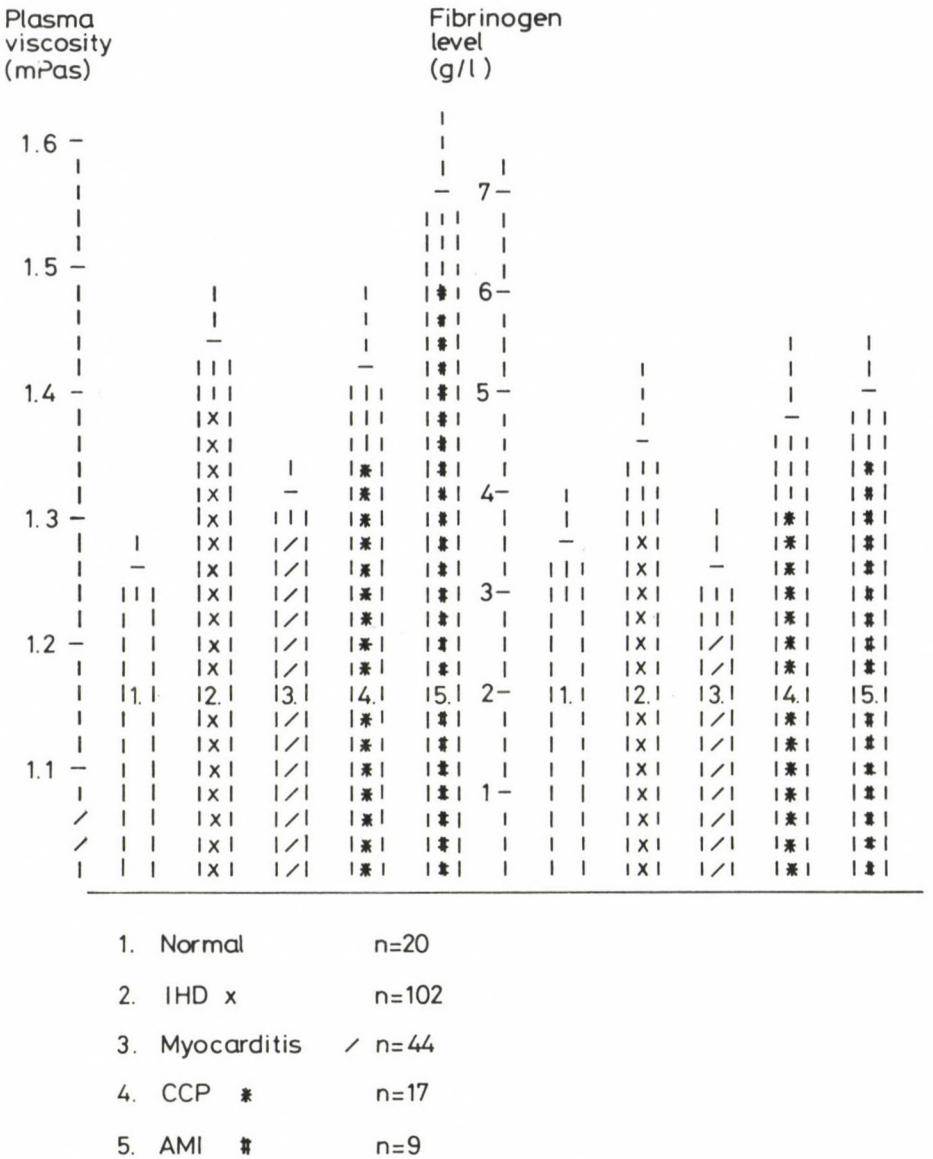


Fig. 2. The values of whole blood viscosity and haematocrit in the second group of patients



1. Normal n=20
2. IHD x n=102
3. Myocarditis / n=44
4. CCP * n=17
5. AMI # n=9

Fig. 3. The values of plasma viscosity and fibrinogen in the studied groups

Table IV

The ordered list of discriminating powers between classes 2 and 3 in the first group of patients

	Variables	Discriminating power
1.	Whole blood visc. at 1.35 1/s	1.568
2.	Whole blood visc. at 2.48 1/s	1.445
3.	Whole blood visc. at 4.59 1/s	1.443
4.	Whole blood visc. at 0.53 1/s	1.427
5.	Haematocrit	1.423
6.	Red blood cell count	1.085
7.	Plasma proteins	0.736
8.	Cardiac index	0.694
9.	Cardiac output	0.472
10.	Cholesterol	0.430

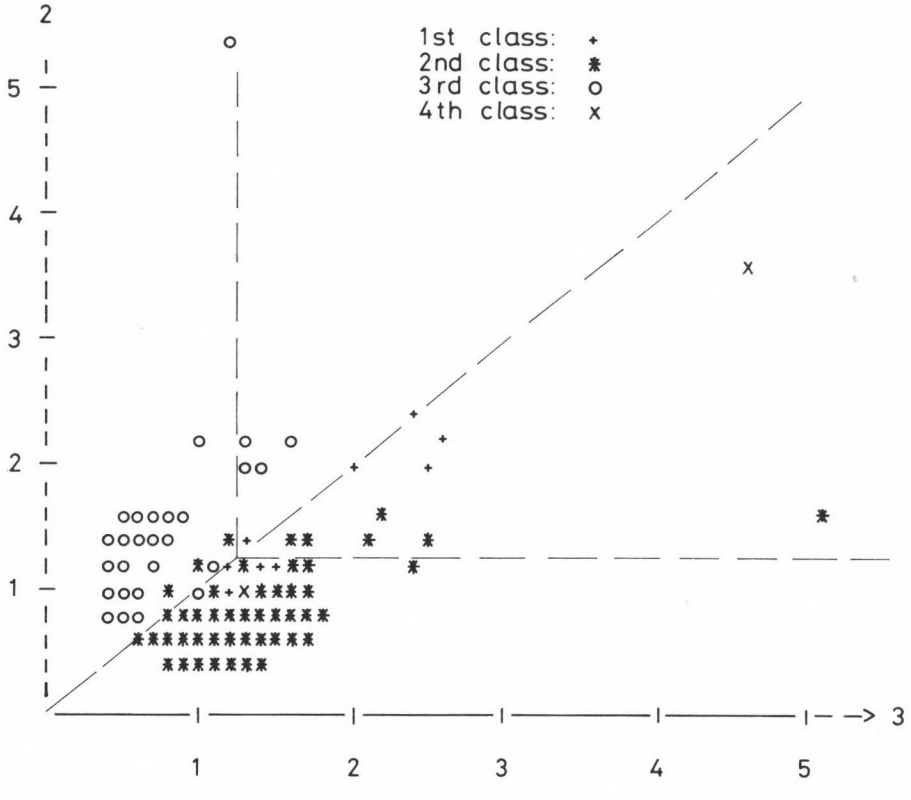


Fig. 4. Class distances of patients of the first group

The class distances from the 2nd and 3rd classes after the reclassification of patients of the first group is graphically presented in Fig. 4. It shows that the 2nd and 3rd classes, which give the most difficult differential diagnostic problem, are situated in well-separated regions.

Table V shows the average effectiveness and the recognition abilities for individual classes of the first group. The average effectiveness was 80%, which is a good result from the point of the clinical practice.

Table V

The average and individual recognition abilities in the first group of patients

Classes	No. of patients	Correctly classified	Incorrectly classified	Recognition ability (per cent)
1.	9	9	0	100
2.	52	37	15	71
3.	28	25	3	89
4.	3	3	0	100
Total	92	74	18	80

Figure 5 shows the class distances between the 2nd and 3rd classes after the reclassification of patients of the second group on the basis of their haemorheological parameters. A good separation of the classes is clear.

Table VI shows the average effectiveness and the recognition abilities for individual classes of the second group. The average effectiveness did not differ significantly from the firstly examined group, although in the second group only haemorheological parameters were taken into consideration.

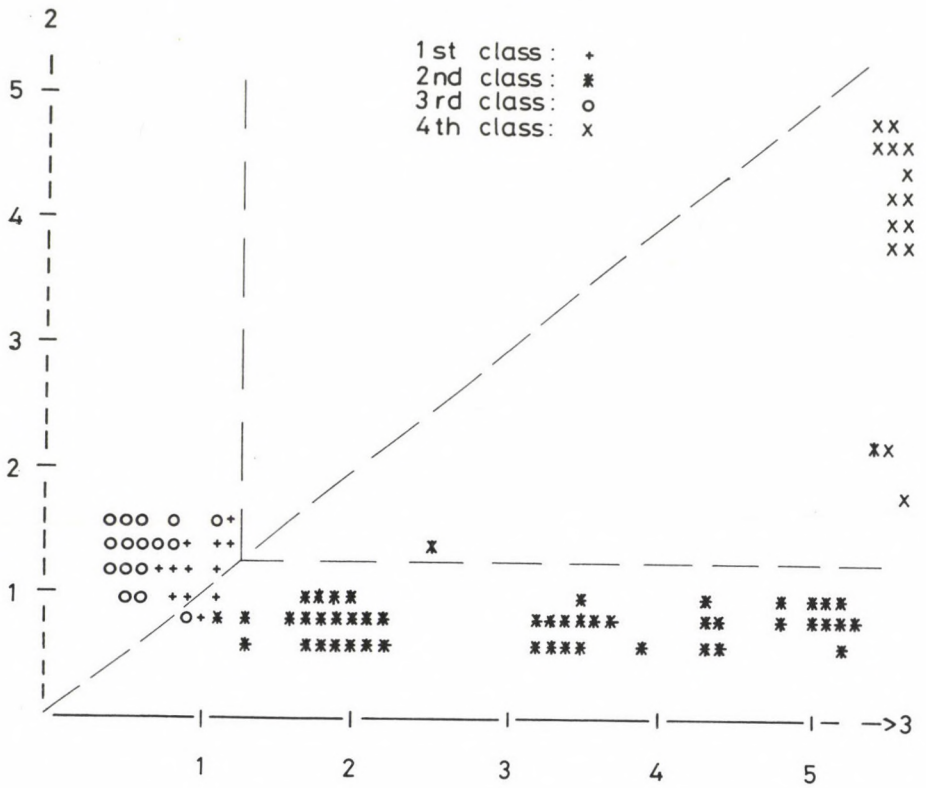


Fig. 5. Class distances of patients of the second group

Table VI

The average and individual recognition abilities in the second group of patients

Classes	No. of patients	Correctly classified	Incorrectly classified	Recognition ability (per cent)
1.	20	17	3	85
2.	50	45	5	90
3.	16	13	3	81
4.	14	13	1	92
Total	100	88	12	88

Discussion

The differential diagnosis of IHD, of its most serious manifestation form, the AMI, and of the acute and chronic viral myocarditis often pose a hard problem even nowadays /6, 8, 10, 13/. Even the previously widely used endomyocardial biopsy has been in widely criticized. Being an invasive method, it cannot be routinely used and the histological results may be ambiguous /1, 2, 6, 9/.

Therefore, in our ward a routinely used new noninvasive laboratory method was initiated to help to solve the mentioned problem. The role of haemorheological factors are well-known in different cardiological and other vascular diseases /5, 12, 13, 15/. The rheological measurements can play an important role in epidemiological, differential diagnostic and clinico-pharmacological studies. Our results suggest that the use of this measurement can be helpful in this differential diagnostic problem, too.

The complex evaluation of different tests often poses a serious problem because sometimes there are contradictions between them. Our results with the application of PRIMA in differential diagnostics verify that this method is useful for computer-aided decision in the clinical practice and is suitable for developing expert systems on the basis of new conceptions.

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CELLULAR AND HUMORAL AUTOIMMUNE RESPONSES AGAINST HUMAN EYE MUSCLE MEMBRANE ANTIGEN IN GRAVES' DISEASE

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75 patients with Graves' disease (54 with ophthalmopathy) were investigated using the tests of leucocyte adherence inhibition and immune adsorption with ^{125}I-labelled Staphylococcus Protein A, against human eye muscle "crude" membrane antigen. The results of positive leucocyte adherence inhibition (10 out of 26 vs. 1 out of 28, $P < 0.05$) and anti-human eye muscle membrane antibody index (mean $\pm$ S.D.) (1.89 ± 1.20 vs. 0.84 ± 0.38, $P < 0.001$) showed a correlation with the patients with clinically active eye disease and the HLA-B8 antigen in Graves' ophthalmopathy ($P < 0.01$). Positive leucocyte adherence inhibition was observed in 9 out of 21 cases of Graves' disease without ophthalmopathy, but its prognostic relevance has to be confirmed in the development of ophthalmopathy.

Keywords: Leucocyte adherence inhibition, antihuman eye muscle antibody, Graves' ophthalmopathy, HLA haplotypes

Introduction

Graves' ophthalmopathy is an autoimmune disease characterized by cell-mediated and humoral immune responses against orbital tissue /11, 19, 28/. A high frequency of minimal changes in the eye muscle can be found by ultrasonography and computerized tomographic scanning in Graves' disease without ophthalmopathy /21/. Early observations showed in retroorbital tissues receptors capable of binding TSH or its derivatives /33, 34/. Thyroglobulin-antithyroglobulin complexes causing a damage in the human eye muscle were also reported /15, 18, 23/. Autoimmune responses against eye muscle antigens have been described but it is not known whether the damage results from

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cell-mediated immunity, circulating immune complexes or pathogenic antibodies /5, 29, 30/.

Local release of lymphokines by infiltrating cells may also be important, since these lymphokines may produce many of the well-known pathological features of the disease /25/.

Graves' disease was one of the first autoimmune disorders where an association with certain HLA haplotypes was reported. The susceptibility to Graves' ophthalmopathy was strongly associated with HLA-B8 haplotypes rather than with DR3 /7/.

The aim of our investigation was to study the role of leucocyte adherence inhibition (LAI) against human eye muscle membrane antigen and to detect the circulating anti-human eye muscle membrane antibody (AEMA index) in Graves' ophthalmopathy. We investigated whether the activity of the eye disease in Graves' ophthalmopathy influences the cell-mediated and humoral immune responses against human eye muscle membrane antigen. Finally, we wanted to study the association between the LAI and AEMA index, on the one hand, and the HLA haplotypes in Graves' ophthalmopathy on the other.

Patients and Methods

Fifty-five patients of the IIIrd Dept. of Medicine of Kenezy's Hospital, Debrecen (14 males, 61 femalse, from 17 to 77 years (mean age 46 $\pm$ S.D. 13 years), with Graves' disease were investigated. Fifty-four had ophthalmopathy with an average duration of 6 $\pm$ 2 years. The clinical diagnosis of Graves' disease was on the elevated thyroid hormone levels, elevated thyrotrophin receptor antibodies and diffuse hyperplasia of the gland on thyroid scan. Classification of ophthalmopathy was performed according to the criteria of the American Thyroid Association /31, 32/. The patients with Graves' disease, who were age- and sex-matched were divided into two groups based on the presence (group B) or absence (group A) of ophthalmopathy. Group A consisted of 21 patients: 2 males, 19 females, from 17 to 77 years, mean age 47 $\pm$ (S.D.) 16 years. Group B consisted of 54 patients: 12 males, 42 females, from 23 to 68 years, of mean age 46 $\pm$ 12 years.

The cell-mediated immune response against human eye muscle "crude" membrane antigen was examined by Urist's leucocyte adherence inhibition test (LAI test) /27/. There are several tests to detect cell-mediated immune responses. Among them the LAI test seemed to be a sensitive, simple method for using as a routine diagnostic technique /4, 17/. A positive LAI test was defined as a value exceeding the upper limit of mean $\pm$ 2 S.D. for normal subjects tested concurrently (-12.64 $\pm$ 26.21).

Circulating antibodies against human eye muscle "crude" membrane antigen was tested by Faryna's method using immune adsorption with ^{125}I-labelled Staphylococcus Protein A (^{125}I-SPA) /6/. The antihuman eye muscle membrane antibody index (AEMA index) was calculated from data obtained in the presence of serum from 25 controls. Positive test was defined as a value exceeding th upper limit of mean $\pm$ 2 S.D. for normal subjects tested concurrently (0.997 $\pm$ 0.085).

HLA antigens were studied with lymphocyte cytotoxicity micro-method /24/.

The human eye muscle "crude" membrane antigen was obtained from patients who had not suffered from tumour, endocrine or infectious diseases within 4-6 h after death. After removal

of fat and the tendinous parts, the tissue was finely minced and homogenized. The homogenate was centrifuged at 500 x g to remove debris, and the supernatant was centrifuged at 3000 x g to obtain the "crude" membrane pellet. The protein concentration was measured by Lowry's method and the concentration 1 mg/ml was used in the test /16/.

All data were analysed by Student's paired t test or Welch's d test, and the proportions of the positive results by the Chi-square test /13/.

Results

We found a higher frequency of LAI in group A than in group B. Nine out of the 21 in group A and 11 out of the 54 in group B were positive for LAI. A borderline significance was found by the χ^2 test ($\chi^2 = 3.91$, $P > 0.05$) (Fig. 1).

Elevated AEMA indices were found in both groups A and B (1.19 ± 0.48 and 1.39 ± 1.05, respectively) (Fig. 2). The AEMA index was positive in 6

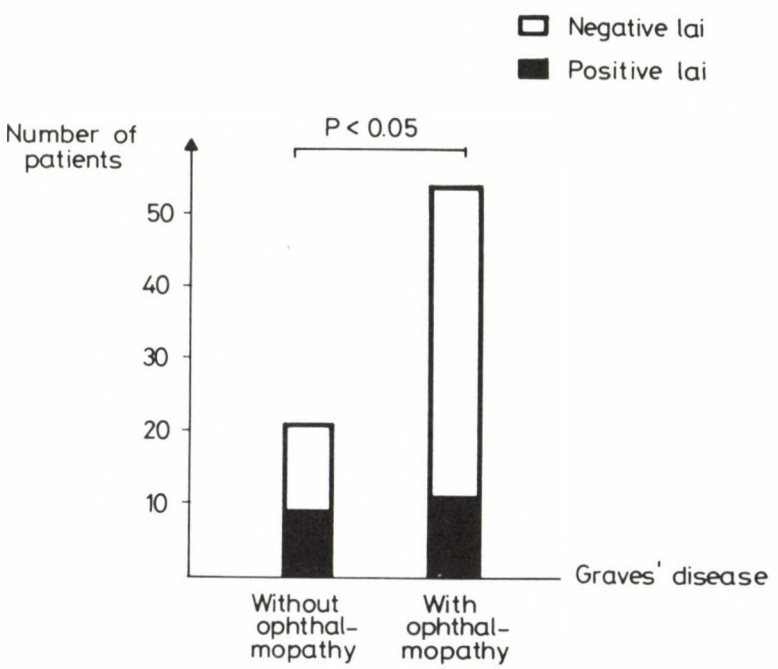


Fig. 1. Leucocyte adherence inhibition (LAI) against human eye muscle membrane antigen in Graves' disease. Positive LAI was defined as value >39.78. $P < 0.05$ by Chi-square test

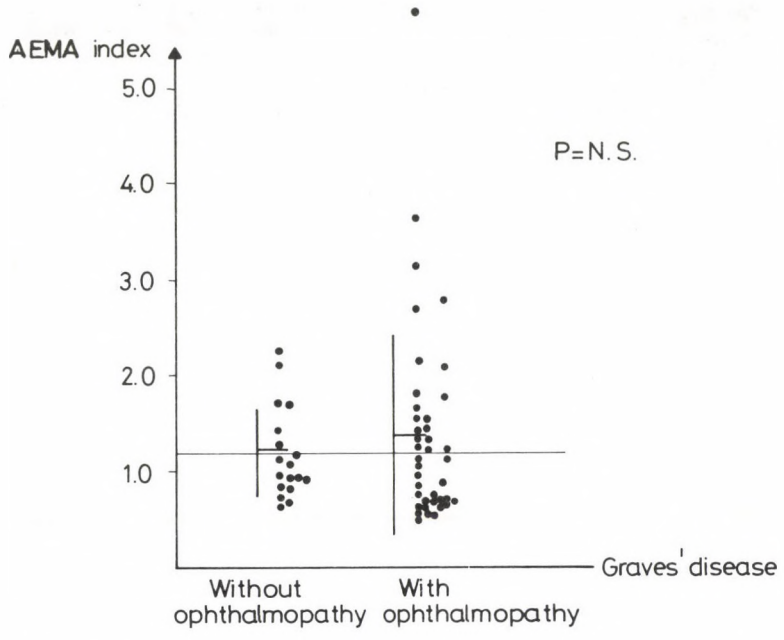
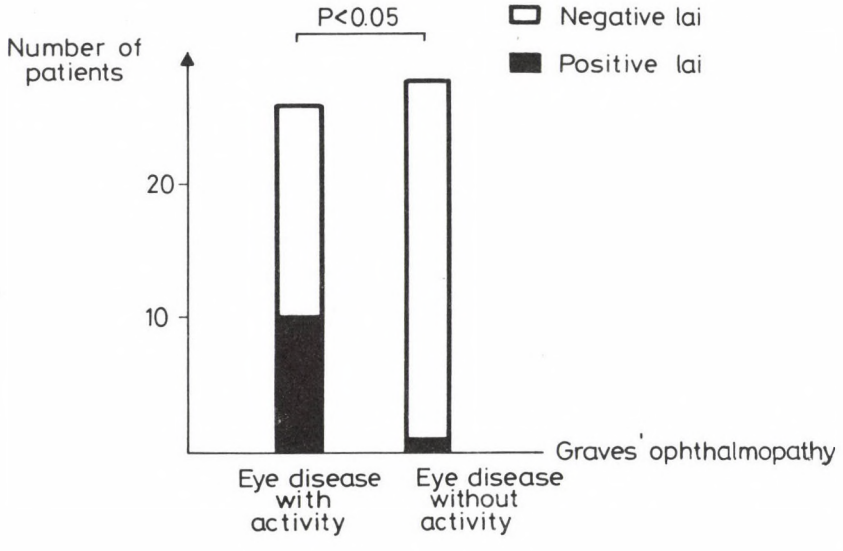


Fig. 2. The anti-human eye muscle membrane antibody (AEMA) index in Graves' disease. Positive AEMA index was defined as value >1.17. P = N.S. (non-significant)



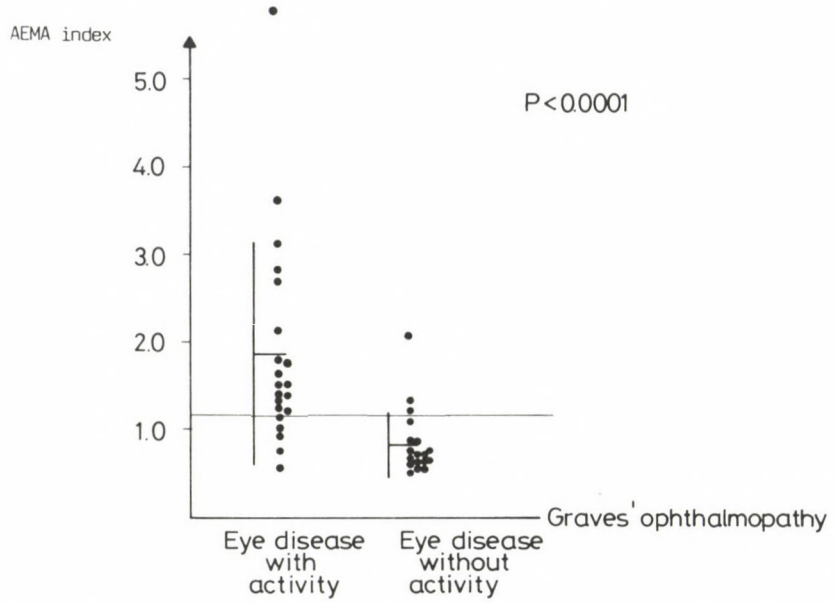


Fig. 4. The anti-human eye muscle membrane antibody (AEMA) index in Graves' ophthalmopathy with the presence and absence of eye disease activity. Positive AEMA index was defined as value >1.17. $P < 0.001$ by Welch's test

cases out of 18 in group A and 19 out of the 40 in group B. No significant difference was found between the two groups.

LAI (Fig. 3) showed a borderline significance, but the AEMA index (Fig. 4) was found to be significantly higher in the group with clinically active eye disease as compared to the group with clinically inactive disease(10 out of 26 vs. 1 out of 28, $\chi^2 = 5.1$, $P < 0.05$ and 1.89 ± 1.20 vs. 0.84 ± 0.38, $P < 0.001$ by χ^2 test with Yates' correction and Welch's d test).

Significant difference of LAI was observed between HLA-B8+ patients compared to those who were lacking the antigen ($\chi^2 = 8.59$, $P < 0.01$ by χ^2 test with Yates' correction) (Table I). A borderline significance of AEMA index was found between the DR3+ and DR3- patients ($P < 0.05$ by Welch's d test).

Fig. 3. Leucocyte adherence inhibition (LAI) against human eye muscle membrane antigen in Graves' ophthalmopathy with the presence and absence of eye disease activity. Positive LAI was defined as value >39.78. $P < 0.05$ by Chi-square test

Table I

Relationship between the frequences of two HLA antigens and the positive leucocyte adherence inhibition or anti-human eye muscle membrane antibody index in Graves' ophthalmopathy

Studied HLA antigens in patients with Graves' ophthalmopathy		Positive LAI[a]	AEMA index mean ± S.D.
HLA-B8+	N = 14	$^{x}6$	1.80 ± 0.95
HLA-B8-	N = 24	$^{x}1$	1.26 ± 1.37
DR3+	N = 12	4	^{xx}2.15 ± 1.68
DR3-	N = 9	0	^{xx}0.91 ± 0.37

[a] a value ≥ 39.78

$^{x}P < 0.01$, by Chi-square test with Yates' correction

$^{xx}P < 0.05$, by Welch's d test

Discussion

Although previous studies showed cell-mediated responses against the orbital tissue by the MIF test, LAI and test for release of leucocyte procoagulant activity in Graves' disease, but the role of these changes in the pathogenesis of ophthalmopathy is still questionable /9, 10/. The inconsistencies between the results obtained with the above tests may reflect different T cell functions in the development of ophthalmopathy /5/. In ophthalmopathy circulating antibodies against human eye muscle were detected by several studies /1, 14/. The pathogenic roles of these functions were attributed to antibody-dependent cell-mediated cytotoxicity. The antigens involved cross-reacted with TSH receptor on fibroblasts and globulin in eye muscle /2, 8, 20/.

It was shown that the MHC class I antigens on human eye muscle membrane were demonstrated in Graves' disease, suggesting their role in the development of ophthalmopathy. Positive LAI was more frequently observed in patients with HLA-B8 antigen than in those with DR3 in our cases with Graves' ophthalmopathy /3, 22/. The presence of these immunologic stigma associated with minimal changes in eye muscle, without clinical evidence ophthalmopathy can be presented by positive LAI. This presumption is supported by the fact that LAI-reactive cells are apparently antigen-specific

while MIF is species-specific. The suppression is correlated with the suppression of adaptive transfer of DTH (delayed T-cell hypersensitivity) /26/. The prognostic value of AEMA and LAI has yet to be confirmed by long-term studies. The positive results of LAI and AEMA index strongly correlated with the activity of the eye disease, therefore, this knowledge may be utilized in the therapy.

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CORRELATIONS BETWEEN SERUM ALPHA$_2$-HS-GLYCOPROTEIN CONCENTRATION AND CONVENTIONAL LABORATORY PARAMETERS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Serum alpha$_2$HS-glycoprotein (A2HSG)[1] concentrations of 63 patients with systemic lupus erythematosus (SLE) were determined, and found to be significantly low compared to those of 59 healthy blood donors. The diminution of serum A2HSG concentration was proportional to the degree of activity of SLE, and was not influenced by secondary infections. There was a statistically significant positive correlation between serum A2HSG and the C3 complement component levels. A negative correlation between serum A2HSG and IgG, IgA concentration and anti-DNA activity was observed. Serum A2HSG was significantly low in cases of positive for the following laboratory parameters: antinuclear antibodies, circulating immune complexes and LE cell phenomenon. We found no correlation between serum IgM concentration, cryoglobulins, latex agglutination and serum A2HSG levels. The unusually good negative correlation between A2HSG pathogenetical role of this glycoprotein in SLE. The determination of A2HSG concentration may be of clinical importance in SLE.

Keywords: Alpha$_2$-HS-glycoprotein, systemic lupus erythematosus

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[1] Abbreviations: ALAT: leucine aminotransferase; ANA: antinuclear antibody; ASAT: aspartate aminotransferase; A2HSG: alpha$_2$-HS-glycoprotein; CIC: circulating immune complexes; ENA: extractable nuclear antigen; SLE: systemic lupus erythematosus; VDRL: venereal disease rapid laboratory

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Introduction

Alpha$_2$-HS-glycoprotein (A2HSG) is a glycoprotein of 49 kd. It was first described by Heremans /11/ and by Schmid and Bürgi /27/. The terminal amino acid and the antigenic identity of the proteins isolated by these two groups of authors was demonstrated by Schultze /28/, who proposed the current name of this glycoprotein according to the discoverers' initials (H.S.).

Although many properties of A2HSG have been described, its biological role is still unclear. It accumulates in highly mineralized tissues, such as bone and dentin. Besides albumin, A2HSG is one of the major constituents of the non-collagenous bone matrix /9, 30/. It may play a role both in bone mineralization and resorption /5, 9, 20, 21/. Its serum concentration decreases in patients with Paget's disease of bone /2/, in those of protein-energy malnutrition /1, 26/ and in the hypercalcaemia of multiple myeloma /6/. A2HSG is produced by the liver /30/. Its serum level is decreased in alcoholic cirrhosis of the liver /12/. A2HSG is a negative acute-phase reactant, its plasma concentration decreases in severe bacterial infection, trauma, some solid tumours and myocardial infarction /3, 4, 14, 25, 29/.

The role of A2HSG in the defensive mechanism of the organism has also been suggested. Besides opsonic properties A2HSG can increase the phagocytic function of human neutrophil granulocytes and monocytes /17, 24/. It binds to Epstein-Barr virus transformed lymphocytes, thus it may play a role in the elimination of these cells /18/. A2HSG can affect the lymphocyte proliferation response in healthy individuals /19/ and in patients with lung and neck tumours /3, 4, 7/.

Apart from one report debating the biological role of A2HSG in rheumatoid arthritis /23/, the behaviour of its serum concentration in autoimmune disorders has not been studied. Therefore, having purified A2HSG from human serum, we determined the serum concentration of this glycoprotein in patients with systemic lupus erythematosus (SLE). We correlated these values to the activity, and clinical course of the disease, and some laboratory parameters conventionally measured in patients with this disease.

Patients and Methods

Patients. We determined the A2HSG concentration in 265 serum samples from 63 patients with SLE (59 females, 4 males, aged 43.2 ± 10.0 years, x ± S.D.) and that of 108 serum samples from healthy controls (32 females, 27 males, aged 40.4 ± 10.5 years, x ± S.D.). The diagnosis of SLE was based on the ARA criteria. The SLE was considered active when antinuclear antibodies (ANA) and circulating immune complexes (CIC) could be detected, the serum concentration of the complement component C3 was less than 0.9 g/l and the anti-DNA activity exceeded 25 IU/ml. Patients were given prednisolone (Prednisolone, 0-120 mg daily, orally) and/or azathioprine (Imuran, 0-50 mg/d, orally), depending on the activity of the disease.

Preparation of A2HSG. A2HSG was isolated from pooled human serum by the method of Matsushima et al. /22/, with some modifications. In brief, human serum was precipitated by ammonium sulphate (37%), and the precipitate was purified in the following steps: DEAE-Sephadex A 50 (Pharmacia, Uppsala) and DEAE-cellulose (Reanal, Budapest) ion-exchange chromatography, gel filtration on Sephadex G 200 (Pharmacia) column, hydroxylapatite adsorption chromatography (Bio-Gel HTP, Bio-Rad, Richmond) and Blue Sepharose CL-6B (Pharmacia) affinity chromatography.

The purity of the isolated A2HSG was determined by SDS-PAGE /8/ and crossed immunoelectrophoresis. The former was done in acrylamide gel (total gel: 7.5%, running gel: 5% acrylamide) staining with Amidoschwarz. The latter was made in 1% Litex agarose gel, on 5x5 cm slide glasses. Two µl of A2HSG preparation was used as antigen and 60 µl of polyvalent horse anti-human antiserum (Human, Budapest) and 60 µl of monospecific rabbit anti-A2HSG antiserum (Behringwerke) were used as antibody. The electrophoresis was done in Veronal-Na-Veronal buffer (pH 8.6) at 10 V/cm for 1 h in the first direction and at 1 V/cm, for 16 h in the second direction. The preparations were stained with Amidoschwarz. The crossed electrophoresis of our A2HSG preparation gave only one precipitation line against anti-human polyvalent horse serum, which was identified by monospecific rabbit anti-human A2HSG antiserum. Our A2HSG preparation proved to be homogeneous on SDS-PAGE, too.

The determination of serum A2HSG concentration. The serum level of A2HSG was determined by electroimmunodiffusion /15/ in 1% Litex agarose gel on 5x5 cm slide glasses. Two µl of serum was used as antigen and 60 µl of monospecific rabbit anti-human A2HSG antiserum (0.7 g/l; Behringwerke) incorporated in the gel were used as antibody. We used our purified A2HSG in different concentrations (0.2, 0.4, 0.6 and 0.8 g/l) as standard. The electrophoresis was made in Veronal-Na-Veronal buffer (pH 8.6) at 1 V/cm for 16 h. The preparations were stained with Amidoschwarz.

Other laboratory determinations. CIC was determined by precipitation (4% PEG 6000), cryoglobulins were detected by the conventional method (incubation at 4 ^{o}C for 24 h). In the ANA and latex agglutination tests the appropriate kits were used (Sevac and Human, respectively). The LE cell phenomenon was detected after destroying granulocytes mechanically in heparinized samples. The determination of serum concentrations of C3, IgA, IgG and IgM was made by radial immunodiffusion (Hyland monospecific antisera to C3 and Human antisera to immunoglobulins). Serum anti-DNA activity was measured by radioimmunoassay kits (Amersham). The serum bilirubin concentration was measured by the diazo reaction. ASAT and ALAT activities were determined kinetically (Boehringer). Alkaline phosphatase and prothrombin activities were measured by the method of King and Armstrong and the Sinplastin test, respectively.

The statistical analysis was done with Student's and paired Student's test.

Results

The serum A2HSG concentration of the healthy controls is shown in Table I. We found neither sex differences nor age variations among the serum A2HSG values of controls. The serum A2HSG concentration of patients with SLE was significantly lower than that of the controls (Fig. 1). The serum A2HSG level of patients with active SLE was further reduced, compared both to controls and to that of the inactive SLE group. During the 1-25 year clinical follow-up we observed remission and exacerbation of SLE in 15 and 16 cases, respectively. Compared to the previous values, we found a significant increment of serum A2HSG concentration during remission and a decrement during exacerbation of the disease (Table II). The serum A2HSG concentrations of patients with permanently active disease hardly differed from each other, and were substantially low during serial determinations. On the other hand, the serum levels of patients with permanently inactive disease were much higher, showing no significant change during serial determinations.

Table I

Serum A2HSG concentration of healthy individuals of different age (g/l)

Age (years)	n	A2HSG $\bar{x} \pm$ S.D.
11 - 20	6	0.671 ± 0.07
21 - 30	12	0.745 ± 0.06
31 - 40	20	0.694 ± 0.09
41 - 50	10	0.701 ± 0.08
51 - 60	7	0.675 ± 0.09
61 - 70	2	0.692 ± 0.06
totals	59	

n = number of healthy controls

Secondary infections (usually of pulmonary and urogenital origin) were observed in 16 patients. The serum A2HSG concentrations of these patients did not differ from the values of the patients without secondary infection (Fig. 2). The difference between the groups of active and inactive disease dominated over the changes observed during infection in both the active and the inactive groups of patients.

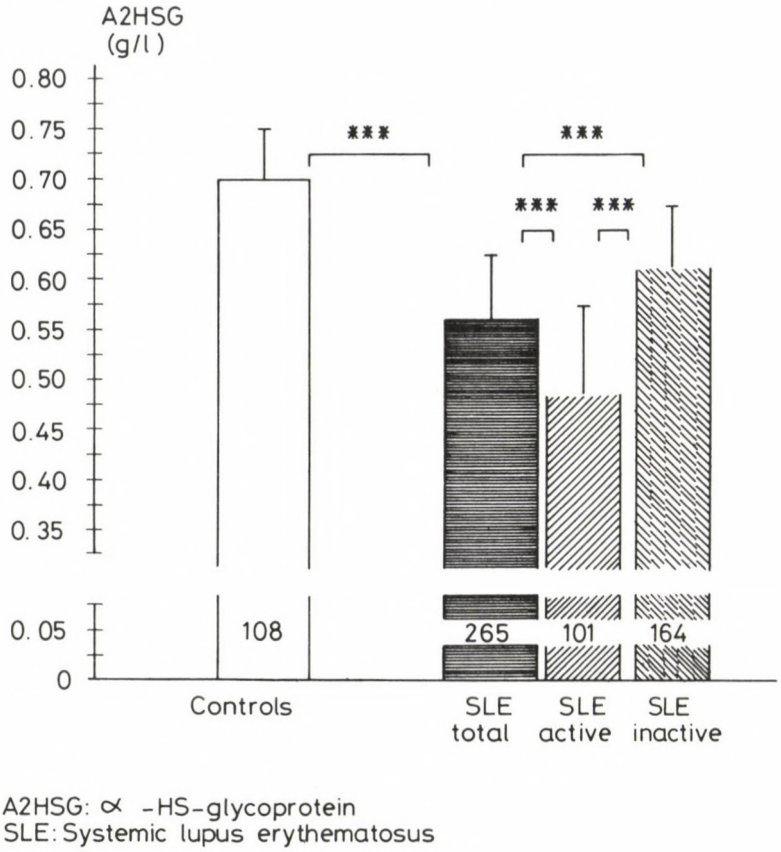
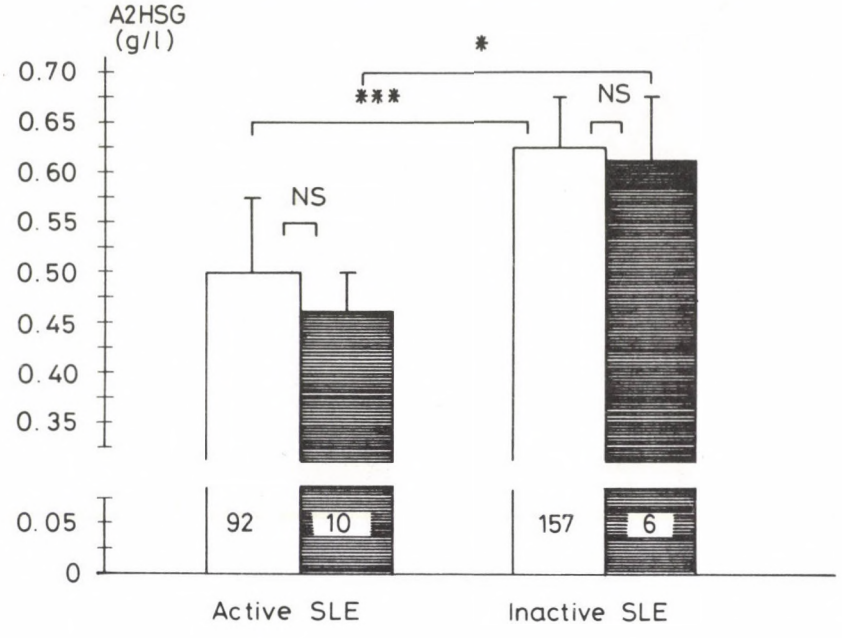


Fig. 1. Serum A2HSG concentrations ($\bar{x} \pm$ S.D.) of healthy controls (empty bars) and of patients with SLE (active SLE: ////, inactive SLE: \\\\, SLE totals: filled bars). The number of observations are represented by the figures within each bar. *** = $P < 0.001$

Correlating some conventional laboratory parameters measured in SLE to serum A2HSG levels, the serum A2HSG concentration of ANA-positive and that of CIC-positive patients were significantly lower than those of the ANA- or CIC-negative ones (Fig. 3). This could have been expected from the activity criteria of SLE mentioned above. However, the serum A2HSG concentration was significantly reduced in LE cell positive patients compared to negative ones. The serum A2HSG concentration did not decrease either in cryoglobulin-positive or in latex-positive patients significantly.

On the other hand, we found a statistically significant positive correlation between serum A2HSG concentration and serum C3 (Table III). We ob-



A2HSG: α_2-HS-glycoprotein
SLE: Systemic lupus erythematosus
NS: Not significant difference

Fig. 2. Serum A2HSG concentrations ($\bar{x} \pm$ S.D.) of SLE patients with (filled bars) and without (empty bars) infectious complication. The numbers of observations are shown within each bar. * = $P < 0.05$; *** = $P < 0.001$

Table II

Serum A2HSG concentration as a function of the clinical course of SLE (g/l)

		A2HSG $\bar{x} \pm$ S.D.	P
no change in activity:			
permanently active	9/34*	0.479 ± 0.10	***
permanently inactive	23/91	0.620 ± 0.10	
changes in activity:			
exacerbation	15/30	0.476 ± 0.06	***
remission	16/32	0.605 ± 0.08	***

Number of patients per number observations. *** = $P < 0.001$, compared to inactive status in permanently active and exacerbation group, and to active status in the remission group.

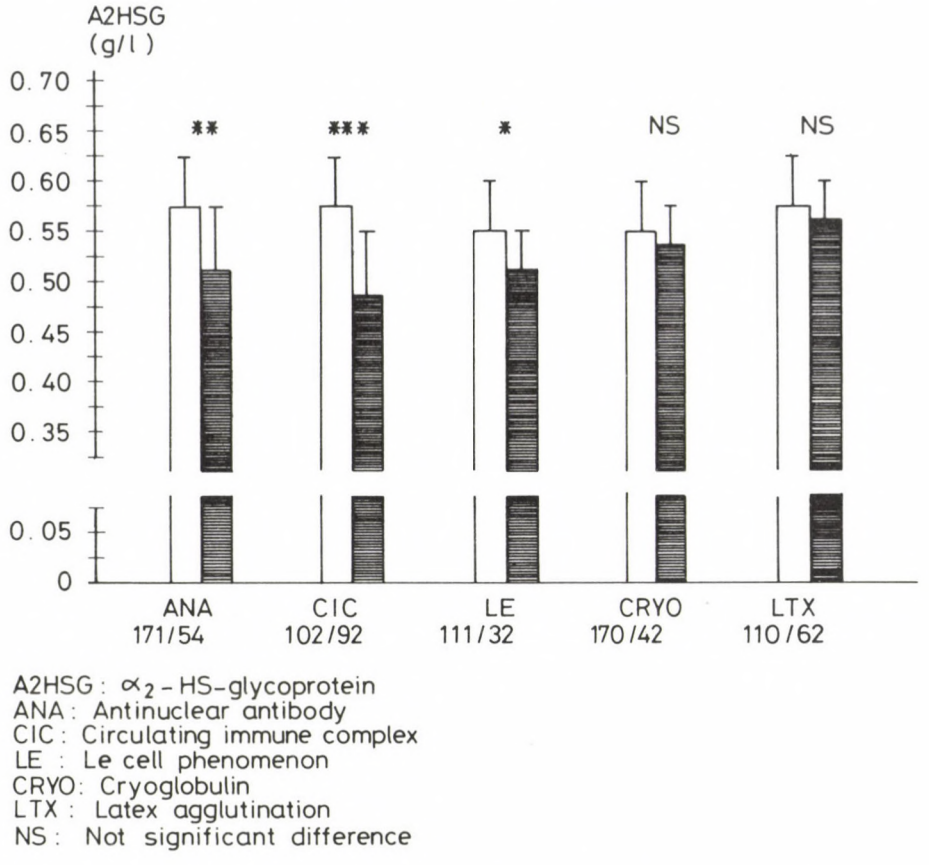


Fig. 3. Correlations between serum A2HSG concentration ($\bar{x} \pm$ S.D.) and the presence of antinuclear antibody (ANA), circulating immune complexes (CIC), LE cell phenomenon (LE), cryoglobulins (CRYO) and the latex agglutination test (LTX). Positive and negative values are represented by filled and empty bars, respectively. The numbers of observations of negative and positive values are shown before and after slashes under each bar, respectively.
* = $P < 0.05$; ** = $P < 0.025$; *** = $P < 0.001$; NS = not significant difference

Table III

Correlation between serum A2HSG levels (g/l; $\bar{x} \pm$ S.D.) serum C3, IgA, IgG, IgM concentrations (g/l; $\bar{x} \pm$ S.D.) and anti-DNA activity (IU/ml) in SLE

	n	regression curve parameters	r	P
C3	254	y = 0.096x + 444.7	0.274	***
IgA	178	y = -0.168x + 606.2	-0.255	***
IgG	178	y = -0.060x + 648.6	-0.364	***
IgM	178	y = -0.106x + 576.0	-0.118	NS
anti-DNA	95	y = -1.067x + 617.0	-0.547	***

n = number of observations; r = regression coefficient;
***P < 0.001; NS = not significant

served a statistically negative correlation between serum A2HSG levels and serum IgA and IgG. Similar correlation was not found with IgM. We detected an especially good negative correlation between serum A2HSG level and serum anti-DNA activity.

Retrospectively, we tried to find correlation between some types of autoantibodies and serum A2HSG concentration. The VDRL test was negative in each of our patients studied. Circulating lupus anticoagulant activity was demonstrated in 3 cases. The serum A2HSG levels of patients showing this activity did not differ from those of without it. Immunofluorescence methods for antibodies against mitochondria, smooth muscle and extractable nuclear antigen (ENA) were done only for differential diagnostic purposes (altogether 5 patients). All proved negative. Nine patients proved Coombs-positive. However, the serum A2HSG concentration of Coombs-positive SLE patients was reduced only when clinical and serological activity could also be demonstrated (data not shown).

Discussion

We separated A2HSG from human serum and used our preparation for the determination of serum A2HSG concentration successfully in the present work. In contrast to Dickson et al., who reported a progressive age-related decrease of serum A2HSG (from 0.74 to 0.64 g/l) in 167 healthy women /10/, we found neither age- nor sex-related differences between the 32 female and 27 male controls we studied.

The serum A2HSG concentrations of patients with SLE were reduced significantly compared to healthy controls. Low serum A2HSG levels can be due to decreased production, increased consumption or elimination. A2HSG is produced by the liver /30/. We failed to observe any correlation between serum A2HSG concentration and the enlargement of the liver or hepatic function tests (serum bilirubin concentration, ASAT, ALAT, alkaline phosphatase and prothrombin activity) in our patients. Increased urinary loss also seems unlikely, even in patients with lupus nephritis /13/.

The effect of prednisolone and azathioprine treatment on serum A2HSG levels cannot be excluded in this study. We could not find any reliable difference among the serum A2HSG concentrations of our patients because of the small number of untreated cases expressing activity. However, the serum A2HSG concentration of these few patients did not differ from that of the treated cases. Corticosteroid treatment resulted in an increase of serum A2HSG levels in hypercalcaemic patients with multiple myeloma /6/.

Thus, we think that diminished serum A2HSG levels are due rather to increased consumption than to any other cause in SLE. This is supported by the negative correlation between serum A2HSG level and anti-DNA activity, which seems very strong compared to the other laboratory parameters used in this work. A2HSG has been suggested to bind to DNA /16/. It is well-known that a large amount of native DNA is released into the circulation as a consequence of nuclear damage caused by autoantibodies; this process can induce a further increase in the anti-DNA activity of the serum. The binding of serum A2HSG to native DNA could counteract this process. Moreover, A2HSG could play a role in the elimination of native DNA or DNA-containing immune complexes from circulation by enhancing phagocytosis.

Although the presence of autoantibodies against glycoproteins is unusual in SLE, the occurrence of anti-A2HSG antibodies could provide another basis for the reduction of serum levels of this glycoprotein. Unfortunately, we could not investigate the presence of such antibodies because of the small amount of isolated A2HSG. We think this question needs further evaluation. We could not establish any relationship between serum A2HSG concentration and other nonspecific autoantibodies (those against cardiolipin, blood coagulation factors, ENA, mitochondria, smooth muscle cells and erythrocyte membrane).

Lebreton et al. reported a fall of serum A2HSG levels in patients with infection /14/. However, we could not find significant reduction of serum A2HSG in patients with secondary infections. This difference can be

attributed to that the secondary infections observed in our patients were less severe than those they reported on (e.g. Staphylococcus or Pseudomonas-speticaemia after burns or osteomyelities). Furthermore, the blood samples we investigated were taken from patients who had already been treated with broad-spectrum antibiotics.

Our data show that there is a considerable correlation between serum A2HSG level and the activity of SLE. This is supported by the difference between the serum levels of patients in the active phase and those in the inactive phase of SLE and the alteration of serum A2HSG concentration during the long-term follow-up. The variation of serum A2HSG concentration indicated well the change of disease activity both in relapse and remission. Since the behaviour of serum A2HSG has not been studied extensively in autoimmune diseases, the specificity of this parameter cannot be determined yet. In our practice ANA, and, especially, anti-DNA activity seem to be the most predictive among the conventional laboratory parameters in SLE. Since these parameters show the strongest correlation with serum A2HSG levels, we assume that the latter parameter may also be highly predictive. This is supported by the fact that serum A2HSG concentration was consequently less than 0.69 g/l (average of healthy controls) in samples showing anti-DNA activity higher than 25 IU/ml (criterion of SLE activity). On the basis of our observations, the determination of serum A2HSG may have clinical importance.

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T-LYMPHOCYTE SUBGROUPS AND THE ACTIVITY OF HUMAN NATURAL KILLER (HNK) CELLS IN LOW-GRADE AND HIGH-GRADE MALIGNANT CASES OF NON-HODGKIN LYMPHOMA

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T-lymphocyte subgroups and the percentage and activity of Human Natural Killer (HNK) cells were investigated in 24 patients suffering from low-grade and 24 patients with high-grade malignancies of non-Hodgkin lymphoma (NHL). The ratio of CD3, CD4, Leu-7, and HNK cells as well as the release by HNK cells of the ^{51}Cr bound to target cells were found decreased, depending on the pathological stage. The tests were performed with OKT-monoclonal sera. A significant change was observed in the reactivity of bone marrow cells to monoclonal sera; the change was identical in character with that observed when lymphocytes isolated from the peripheral blood were used in the same tests. No significant changes could be observed in the quantitative relations of immunoglobulins. Such changes could by no means be expected, on the basis of the unchanged number of T-suppressor lymphocytes (CD8). As to the supposed immunological relation in detail, the role of a plasma factor that reduces T-lymphocyte formation is assumed to have primary importance in this phenomenon.

Keywords: Non-Hodgkin lymphoma, T-lymphocyte subgroups, killer-Cells, monoclonal antibodies.

Abbreviations: CC: centrocytic; CB: centroblastic; HNK: human natural killer; MABs: monoclonal antibodies; NHL: non-Hodgkin lymphoma

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Introduction

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of lymphoid neoplasms showing wide histological and cytomorphological variation. The differences in immunological phenotypes are significant, and disorders of the immune system favour the development of NHL /17, 21, 28/. The damaged condition of the immune system shows different degrees, the accompanying immune status and its development are poorly known, and the pathogenesis is far from being clear. Studies on this problem have given an account of an increased B lymphocyte level associated with a simultaneous reduction of T-cell count, and of a disturbed cellular activity related to the function of the immune system. There are literary data on quantitative changes in the ratios of T-lymphocyte subgroups, and all this shows correspondence to the histological, type and the pathological stage /15, 16, 18/. The activity of human natural killer (HNK) cells in NHL and in different histological types deserves special attention /13/.

The classification in terms of histological type according to the so-called "Kiels" system, a system, introduced nearly 20 years ago /21, 29/ has revealed that the centroblastic and centrocytic pathological pictures originate from B cells, whereas those with typically convoluted and multilobulated T-cell lymphomas are located in the cortical substance of lymph nodes. An immunological characterization of these cells may fail. The introduction of monoclonal antibodies (MABs) in determining the phenotypes of NHL has been very helpful /1, 14, 20, 24/.

The aim of this report was to investigate those changes in patients with different pathological stages of NHL, which can be detected in the peripheral blood and the bone marrow with MABs. The staging system that has been suitable in chronic lymphoid leukaemias as improved by Binet et al. /2/ cannot be applied in NHL because of significant differences in the pathogenesis of this disease. Generally the Ann Arbor staging /7/ in Hodgkin disease is applied in NHL, though, even this can be fallible. The activity of peripheral blood and bone marrow cells, which differed from normal when tested with MAB, can adequately supplement the judging of the pathological stage.

Patients and Methods

Twenty-four low grade (CC-CB) and 24 high grade (CB) patients were included in this study. The patients were tested partly prior to the beginning of treatment, partly before its continuation. In addition to the usual haematological testing, the immunoglobulin levels as well as the lymphocyte subgroups were determined in the blood and in a part of the patients, in the bone-marrow aspirate. The following monoclonal sera were used: ORTHO-Pharmaceutical Corp., New Jersey: OKT3, OKT4, OKT8 and Becton-Dickinson, U.S.A.: Leu-7. Details of the tests were reported elsewhere /5/. In a part of the patients the activity of natural killer cells was determined in both pathological groups as practised by Stephenson et al. /15/.

For pathological tests bone-marrow biopsy (spina iliaca post. sup.) was performed with Jamshidi needle. Evaluation was made partially from aspirate smears using May-Grünwald's stain. Similarly to tests of peripheral blood, lymphocytes were isolated from the aspirate, and the percentages of lymphocyte subgroups were determined with MAB-sera.

The pathological tests were based on examination of the removed lymph node, spleen, stomach, or bowel. These investigations were performed in the Institute of Pathology, University Medical School, Pécs. For this valuable help, we express our thanks here. The Kiel classification was used.

All of our NHL patients had B-cell tumours.The group of low-grade malignancies as well as that of high-grade malignancies contained 24 patients. The ratio of males to females war 14/10 and 13/11, respectively. The distribution by stage of disease was as follows: (i) low-grade malignancy (CC-CB): I-II: 12, III-IV: 13; (ii) high-grade malignancy (CB): I-II: 10, III-IV: 14 patients. In the group of low-grade malignancy: lymphoplasmacytic: 1, centroblastic-centrocytic, follicular: 11, diffuse: 11, centrocytic: 1. In the group of high-grade malignancy; immunoblastic: 3, centroblastic: 1, Burkitt's lymphoma: 2, lymphoblastic: 4, not classifiable: 4. (In the following, the designations CC-CB and CB will be used.) The treatment of patients in the group of low-grade malignancies was carried out according to the MOP pattern, whereas in that of high-grade malignancies it was performed according to the "PROMACE" and "OVER 50" patterns, alternating with MOP. It is not the purpose of the present paper to give an account of the results of these studies /6, 8/. The results were compared with data of control patients 40-60 years of age who had undergone venipuncture for other reasons. These did not suffer from haemotopoietic or oncologic diseases. Most of them had hypertension. Literary data and, in part, data of individuals who were subsequently excluded from the groups of patients and proved to be healthy served as controls of the data of bone-marrow tests. The results were then evaluated in terms of mean, distribution and Student's tests.

Results

The CD3- and CD4-positive T-lymphocytes were reduced in number in the pathological stages II, III and IV of the CC-CB group. On the other hand, the CD8-positive cells were either identical or higher in number as compared to the normal controls. The CD4/CD8 ratio showed a definite reduction in all the four pathological stages. A modest reduction of the percentage of Leu-7 positive cells and a significantly lowered activity of HNK cells were observed. In the advanced pathological stages the E-rosette count was decreased in accordance with the foregoing, whereas no significant changes could be observed in the EAC rosette counts and absolute lymphocyte counts.

In the group of patients with high-grade malignancies (CB) a significant reduction of CD3-positive cells was revealed in stages III and IV; the CD8-positive cells were increased in number in groups I and II. In stages III and IV, on the other hand, only a slight change was observed. The CD4/CD8 ratio was definitely low. The activity of Leu-7 and HNK cells had lowered values in all groups, especially in pathological stages III and IV: E-rosette formation was reduced in the third and fourth groups only, whereas the production of EAC rosettes was left unchanged. No significant differences could be observed in the absolute lymphocyte counts. Significant differences were found in CD3, CD4. CD8 and HNK cell activities in both of groups CC-CB and CB. The difference was also significant in both pathological groups if the two III and IV stage groups are viewed. Significant changes of CD3, CD4, CD4/CD8, Leu-7 and HNK activities and in E-rosette formation were observed in groups CC-CB and CB, as related to the controls (Table I). MAB reactivities are expressed in column diagrams in Fig. 1.

Tests for bone-marrow lymphocyte subgroups were carried out in 15 cases of group CC-CB, and in 13 cases of the CB group. After a mechanical homogenization of the aspirates, the isolation of lymphocytes and from these the tests for subgroups were performed as referred to above. CD3-, CD4- and CD8-positive cells showed a marked and significant reduction, whereas there were no significant difference in the Leu-7, E-rosette and EAC-rosette and mouse rosette counts. This referred to every stage of the CC-CB group of patients, and similar results were gained in the CB group. However, the difference was marked and significant in both pathological forms between stages I and II,on the one hand, and IV on the other (Table II).

Table III shows the immunoglobulin levels. Significant differences between classes CC-CB, CB and controls were observed in class IgA only. The value of C4 was lower than that of controls in stages III and IV of the CB group only.

Table IV contains data on the clinical manifestation of the disease. The most general symptom was involvement of the lymphnode, sample (see, section 1). Mediastinal and generalized phenomena occurred in stages III and IV. In section 2: an involvement of Waldeyer's ring was observed in the advanced stages only, and even there, in less than a half of the cases. Splenomegaly (section 3) (examined by palpation or with ultrasonography), had a lower incidence in the initial stages, whereas it had a major incidence in stages III and IV. Extralymphatic process of a high incidence was, primarily found in the group of high-grade malignancies and in advanced

Table I

Reactivity with monoclonal sera and rosette formation.
Lymphocytes from the peripheral blood of CC-CB and CB patients
(mean ± S.E.)

Stage	CD3	CD4	CD8	CD4/CD8	Leu-7	HNK	R-Ros	EAC Ros.	Absolute Ly-count 10^9/L
CC-CB	45	40.2	30.3	1.3	9	8.5	60.0	19.0	2.0
I n=5	±4.2	±3.8	±2.8	±0.2	±1.4	±2.7	±3.1	±4.2	±0.4
II n=7	55.8	42.2	31.8	1.5	12.0	15.0	50.0	18.0	2.8
	6.1	4.1	2.2	0.2	2.1	3.0	7.1	3.4	0.5
III n=5	45.8	26.6	27.7	1.0	13.5	10.1	40.0	21.2	1.3
	±4.5	±2.9	±2.8	±0.3	±1.9	±2.0	±4.5	±2.9	±0.2
IV n=7	36.5	30.5	19.6	1.4	7.3	15.5	33.7	23.5	6.2
	±4.5	±2.9	±2.0	±0.2	±1.5	±2.9	±5.5	±7.5	±4.5
CB	43.5	38.6	23.5	1.6	11.9	34.6	64.2	24.5	2.7
I n=5	±2.5	±4.6	±3.9	±0.3	±2.9	±6.1	±6.7	±3.9	±0.2
II n=5	42.9	35.7	31.2	1.3	12.4	35.0	57.3	20.0	2.4
	±3.1	±3.2	±4.1	±0.2	±2.9	±3.9	±8.1	±4.9	±0.2
IIIn=7	31.8	24.9	19.3	1.3	7.5	11.1	38.6	27.2	1.5
	±4.5	±4.1	±3.9	±0.3	±1.5	±2.1	±8.0	±4.1	±0.3
IV n=7	35.7	18.6	16.8	1.1	7.7	4.0	34.0	21.0	2.7
	±6.0	±2.9	±4.1	±0.1	±2.1	±1.1	±6.0	±4.1	±0.3
CC-CB Total	45.7	34.8	27.3	1.3	10.4	12.2	45.9	20.4	3.0
n=24	±2.1	±1.9	±2.3	±0.3	±1.7	±3.1	±4.7	±3.0	±0.4
CB Total	38.4	29.4	22.6	1.3	9.9	21.2	48.5	23.1	2.3
n=24	±3.9	±2.9	±3.0	±0.3	±2.9	±4.1	±4.5	±2.7	±0.5
Significance CC-CB vs.CB	P<0.05	P<0.05	P<0.05	ns	ns	P<0.01	ns	ns	ns
CC-CB III-IV	41.1	28.5	23.6	1.2	10.4	12.7	36.8	22.3	3.7
n=12	±2.9	±2.9	±1.8	±0.2	±2.9	±2.0	±4.1	±4.1	±0.3
CB III-IV	33.8	21.7	18.0	1.2	7.6	7.5	36.3	24.1	2.1
n=14	±4.1	±3.1	±1.9	±0.2	±3.1	±1.9	±3.9	±2.9	±0.2
Significance CC-CB vs CB	P<0.05	P<0.05	P<0.05	ns	ns	P<0.05	ns	ns	P 0.05

Table I (cont.)

Stage	CD3	CD4	CD8	CD4/CD8	Leu-7	HNK	R-Ros	EAC Ros.	Absolute Ly-count 10^9/L
Control n=25	61.2 ±4.3	40.5 ±1.4	20.3 ±1.1	2.0 ±0.1	23.6 ±2.4	51.6 ±16.7	64.4 ±7.8	27.1 ±6.6	2.1 ±0.3
Sign.contr. vs. CC-CB-CB	P<0.01	P<0.01	ns	P<0.01	P<0.01	P<0.01	P<0.01	ns	P<0.01

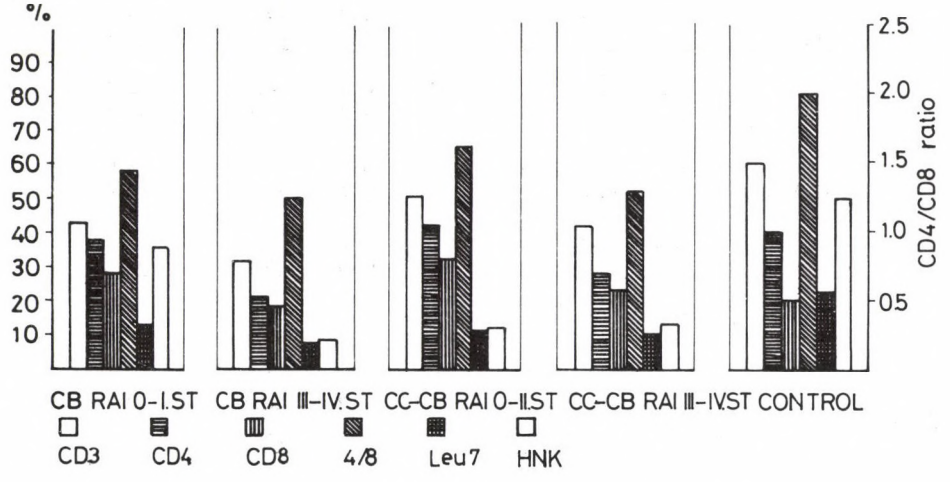

Fig. 1. Monoclonal antibody reactivity and HNK-cell ^{51}CR-delivery in %

Table II

Reactivity with monoclonal sera and rosette formation.

Bone-marrow lymphocytes from CC-CB and CB patients
(mean ± S.E.)

Bone-marrow	CD3 %	CD4 %	CD8 %	CD4/CD8	Leu-7 %	HNK ^{51}Cr fsz %	E-Ros. %	EAC-Ros %	Mouse Ros. %
CC-CB I-II n=7	22.4 ±4.5	14.8 ±6.1	11.4 ±4.5	1.3 ±0.4	6.7 ±2.1	N.G.	10.12	9.38	Ø
III-IV n=8	16.9 ±4.5	13.8 ±2.9	17.3 ±6.4	0.6 ±0.1	3.6 ±1.5	N.G.	6.0 ±2.5	34.5 ±12	Ø
CB I-II n=5	35.1 30.0	15.4 13.5	12.7 11.0	1.2	5.8 ±6.2	N.G.	12.5- 8.0	33.5- 30.0	Ø
III-IV n=8	11.6 ±6.2	9.5 ±6.1	9.6 ±7.5	0.9 ±0.4	7.0 ±2.1	3.0 ±1.0	26.0 ±1.5	33.0 ±2.0	Ø
Control n=10	44.2 ±3.5	21.6 ±4.1	20.2 ±2.2	1.1 0.1	4.5 ±1.8	6.3 7.1	0.1	7.3 12.8	0.05
Control vs. CC-CB CB	P<0.01	P<0.01	P<0.01	ns	ns	-	ns	ns	-

N.G. = not given
ns = not significant

pathological stages. The bone-marrow sample (section 5) showed a blastic incidence in more than 25 percents, of the pathological pictures with high-grade malignancy and in advanced cases. Anaemia appeared similarly in advanced clinical stages. A pathological, cellular infiltration of the liver was observed in a histological testing of liver biopsy, where similarly, the advanced stage of the disease appeared to be decisive.

The digestive tract (section 8) was affected rarely, mostly as primary manifestation. It could be traced in 6 of the 14 patients in the CB group in stages III and IV. The involvement of the central nervous system may primarily be characteristic of the CB group: we observed it in 3 patients, two of whom suffered from Burkitt's lymphoma. The lungs and the pleura were affected rarely showing generalized pathological pictures; we observed it in 4 cases. Infiltration of the skin was observed in 4 patients.

Table III

Mean immunoglobulin level in stages I-II and III-IV CC-CB and CB patients (mean ± S.E.)

Immuno-globulins	IgG g/l	IgA g/l	IgM g/l	CH-50 mg/dl	C_3 mg/dl	C_4 mg/dl
CC-CB I-II n:10	7.1 ±2.1	2.3 ±1.1	2.0 ±0.3	1.5 ±0.3	73.5 ±8.1	32.5 ±5.3
III-IV n:8	8.2 ±1.5	2.5 ±1.1	2.3 ±0.6	0.9 ±0.6	80.5 ±12.1	27.7 ±5.6
CB I-II n:10	10.6 ±2.1	3.3 ±0.9	1.0 ±0.3	0.9 ±0.2	60.4 ±10.9	30.6 ±4.8
III-IV n:12	9.6 ±2.1	2.2 ±0.4	1.1 ±0.2	1.5 ±0.3	81.0 ±11.2	31.6 ±3.1
Control n:50	11.0 ±1.8	1.1 ±0.7	1.1 ±0.5	1.5 ±1.1	100.0 ±22.0	40.0 ±9.0
Significance: CB-CBCC vs.control	ns	P<0.05	ns	ns	ns	ns

ns = not significant

Table IV

Organic changes of patients with CC-CB and CB lymphomas in different pathological stages

Organ affected Stage	CB-CC	No.24	CB	No.24
	I-II	III-IV	I-II	III-IV
	(n=10)	(n=14)	(n=10)	(n=14)
1. Lymph nodes region 1-2	10		10	
mediastinal:		5		14
generalised		5		14
2. Waldeyer's ring	0	6	2	6
3. Spleen (infiltrated)	2	10	4	9
4. Extralymphatic process:	2	4	2	9
5. Bone-marrow: <25% blast A	8	0	1	0
≥25% blast B	2	14	9	14
6. Blood >10 HB	8	1	2	2
<10 HB	2	13	8	12
7. Liver (infiltrated):	0	7	1	12
8. Gastric and intestinal tract:	0	1	1	6
9. Central nervous system:	0	0	1	3
10. Lung and pleura	0	1	0	3
11. Skin	0	1	0	3

Discussion

It has generally been accepted that NHL can be divided into two groups: the one with low-grade, the other with high-grade malignancy of the disease. This classification is based on the duration of the pathological process and the developing complications, i.e., on recognition of the severity of organic functional disturbances and the time of their appearance. Chronic lymphoid leukaemia, which is a malignant lymphoma, should definitely be distinguished from these; its classification can be considered to be solved by Rai's grouping, in terms of the modifications suggested by Binet et al. /2/. The staging of other malignant NHLs is far from being complete, as it disregards several pathological phenomena, those qualifying the duration of the pathological process and the severity of the disease /7/.

We made efforts to find quantitative changes from normal conditions by investigating lymphocyte phenotypes, primarily in T-lymphocyte subgroups. A marked reduction of CD3 and CD4 was observed in stages II-IV, while there were no significant changes in stages II-IV in the CD8-positive lymphocytes. The constancy of the suppressor/cytotoxic cell count can result from tumour-genetic disorders of the occurring cell markers /1/ on the one hand, and it can explain the disturbed immune balance and the enhanced inclination to infection on the other. The decrease of the ^{51}Cr-releasing capacity of HNK cells, and or the reduction of their absolute numbers, is marked /20/. The decrease of E rosettes in number as well as the increased number of B-phenotype cells may be in accordance with B-cell malignoma.

In the group of lymphomas of high-grade malignancy (CB) a similarly significant decrease of CD3, CD4, Leu-7 and HNK cells can be observed, especially in the advanced pathological stages. On the other hand, no significant quantitative changes were found in CD8-positive cells. Upsetting of the immune balance is indicated by a significantly reduced CD4/CD8 ratio.

The behaviour of the bone-marrow lymphocytes - in full accordance with literary data - differs from the quantitative relations of the lymphocyte subgroups in the peripheral blood /6, 8/. Although the technical difficulties of the tests in aspirates are evident, the changes related to the normal controls showed a direct relation. The reduction of CD3, CD4 and CD8-positive cells was significant, but no marked differences could be observed in the reduction of the Leu-7, E-rosette and EAC-rosette counts. This may be related to a malignant origin of bone-marrow lymphocytes, though, alternatively, the appearance of Leu-7 cells may be a consequence of recirculation

neither can it be excluded that bone-marrow lymphocytes may carry double markers.

Unfortunately, we had no opportunity to examine HNK cell activity. The numerical differences were markedly significant in the advanced pathological stages.

The immunoglobulin levels differed from the normal only in the IgA class, and the CD4 value differed only in stages III and IV of CB lymphomas. The lack of an increased antibody production in spite of recurring infections can be attributed to the activity of suppressing cells (CD8) and the reduction of the count and/or activity of Leu-7 and HNK cells that modulate these /4/. Further, it should be taken into account that by the reduction of CD4 lymphocytes interleukin-2 (IL-2) production, too, will decrease, and that this phenomenon may have a role in increasing HNK activity /19/. Presumably, IL-2 has - via interferon gamma - a direct and an indirect influence on the activity of HNK cells /4, 19/. One should reckon with a mutual reinforcing activity with interferon alpha, too /4/. The fact that the immunoglobulin levels are not far from being normal can be attributed to the ineffectivity of the quantitatively normal character of the CD8 T-lymphocyte group adjoined to the reduction of cellular activity of CD4, Leu-7 and HNK. Otherwise, significantly reduced immunoglobulin levels could not have been observed.

The significant reduction of the T-lymphocyte group observed in B-cell-type HNL can, perhaps, be explained by a phenomenon observed experimentally /11/. The cause of this pehnomenon is a significant decrease of T-cell colony formations. As observed by Hutchinson et al., this phenomenon is related to a plasma factor circulating in patients with B-cell-type NHL. The same does not occur in patients with T-cell-type NHL, though, it can be found in other lymphoproliferative clinical pictures /3, 23/. Malignant B cells might produce inhibitory lymphokin and/or there might be a competition between fluid and cell-bound IL-2s /9, 10, 12, 25, 26/.

The value of data about the organic localization of the disease (Table IV) needs confirmation by examination of further patients. The extension of the organic localization is in full accordance with the judgement of malignity on the clinical picture. More severe and vitally dangerous complications, such as an infiltration of the central nervous system and severe anaemia, can primarily be observed in cases of malignant CB. The latter was

accompanied by a ratio of blastic cells exceeding 25% in the bone marrow of our patients.

The problems concerning the aetiology and pathogenesis of NHL are to be solved. In spite of recent successes, we cannot yet be satisfied, not even with the therapeutic results.

At present, we cannot yet refer to clear agreements between the expression of cell markers and the morphological appearance of cells. All this fits to the reactivity of monoclonal antibodies with an "epistope" primarily on the cellular surface than with pathological molecules defining the tumorous character of cells.*

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ENDOGENOUS INTERFERON (IFN) IN PATIENTS WITH ACUTE HEPATITIS B

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The occurrence of serum interferon was studied in 39 patients with acute viral hepatitis B. Antiviral activity of interferon in serum was determined by measuring the inhibition of the CPE of vesicular stomatitis virus on bovine kidney cells (MDBK). Among these patients only 5 (12.8%) had detectable serum interferon level during the first week of hospitalization. The antiviral activity of the interferon-positive sera was low (5-10 IU/ml).

Keywords: Interferon, acute viral hepatitis B

Introduction

Recovery from acute viral infection involves a complexity of host-defense mechanisms, among them interferon (IFN), which affects mechanisms such as non-specific inflammation, antigen expression, phagocytosis, antibody formation and cell-mediated resistance /7/.

The role of IFN in human viral hepatitis has not been well-definited up today. Previous reports indicated that patients with different forms of viral hepatitis were unable to produce IFN /2, 6, 8/. With newly developed methods, however, certain authors /1, 3, 5/ found endogenous interferon in patients with acute hepatitis. These findings have been questioned by others /4/.

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The aim of the study presented here was to assess the frequency of occurrence of serum IFN in acute hepatitis B in a series of patients admitted to the Clinic of Infectious Diseases in Bialystok, Poland (head: Professor P. Boron).

Patients and Methods

Thirty-nine patients suffering from acute hepatitis B for positive (HBsAg and anti-HBc-IgM) were included in the study. None of the patients suffered from the severe (fulminant hepatitis) clinical from of the disease.

After hospital admission sera of the patients in the acute phase of disease were sampled and immediately frozen at -20 ^{o}C. From these patients a total of 92 blood samples were available for analysis. Blood samples were drawn at the time of hospital admission and up to 6 times during hospitalization.

Refrigerated sera were sent in dry ice to the Max von Pettenkofer Institute, Munich, FRG (head: Prof. dr. med. F. Deinhardt), in order to be tested.

Hundred and twenty healthy individuals served as a control group (none of them showed clinical signs of any viral infection.)

Antiviral activity of sera was determined by measuring the inhibition of the cytopathic effect (CPE) of vesicular stomatitis virus (VSV) on bovine kidney cells (MDBK).

Serialtwofold dilutions of sera were made in volumes of 0.05 ml in the wells of flat-bottomed 96-well microtiter trays (Nunc, Denmark). MDBK cells at concentration 0.4 x 10^{6}/ml in 0.05 ml minimal essential medium (MEM) with 10% FCS were added and incubated at 37 ^{o}C for 24 h in a humidified 5% CO_2 atmosphere. The resulting monolayers were then drained by inversion of the microtiter tray over a sterile pad and 0.05 ml of a suspension of VSV was added to each well. The final evaluation of CPE was done after reincubation of the microtiter trays for 48 h at 37 ^{o}C.

Antiviral units were calculated as the reciprocal of the serum dilution inhibiting 50% of the CPE. One unit is equivalent to approximately one reference unit of the World Health Organization human leukocyte reference IFN B 69/19. The lower limit of detection of this test was 5 IU/ml. As some of the sera were toxic to the MDBK cells, the sera were preabsorbed with the MDBK targets.

Every serum IFN-positive in the bioassay was reexamined using an immunoradiometric assay for IFN-alpha (SUCROSEP IFN - alpha, IRMA, Röhm - Pharma, West Germany).

Results

Healthy individuals had virtually no IFN in blood. Among the 39 patients with acute hepatitis B, 5 (12.8%) had detectable IFN level in serum during the first week of hospitalization. One patient had IFN up to the sixth week of hospitalization.

The activity of IFN and anti-HBc IgM in blood serum in 5 patients is illustrated in Table I.

No correlation between IFN level and clinical course of the disease was observed, and all IFN-positive patients with acute viral hepatitis B recovered.

Table I

The interferon level and anti-HBc-IgM in blood serum of patients with confirmed IFN activity

Patients	Week of hospitalization											
	I		II		III		IV		V		VI	
	IFN U/ml	anti--HBc IgM	IFN U/ml	anti--HBc IgM	IFN U/ml	anti--HBc IgM	IFN U/ml	anti--HBc IgM	IFN U/ml	anti--HBc IgM	IFM U/ml	anti--HBc IgM
1	10	+	(-)	+	(-)	+	(-)	(+)	(-)	(+)	(-)	+
2	5	+	(-)	+	(-)	+	(-)	(-)	(-)	(-)	(-)	(-)
3	5	+	(-)	+	(-)	+	(-)	(-)	(-)	(-)	(-)	(-)
4	5	+	(-)	+	(-)	+	(-)	+	(-)	+	5	+
5	10	+	(-)	+	(-)	+	(-)	+	(-)	(-)	(-)	(-)

The antiviral activity of the IFN-positive sera was low (5-10 IU/ml). Eighty-six sampes were negative for IFN.

The antiviral activity was characterized as IFN-alpha by neutralization with antibody to IFN-alpha and by SUCROSEP IFN-alpha IRMA. Incubation with antibody to IFN-beta and IFM-gamma had no effect.

Discussion

IFN is an early defense mechanism against viral infection, inducing an antiviral state in mononuclear cells.

Our study shown that only few of patients with acute hepatitis B responded to the infection by activation of their IFN system. The IFN levels were low in these patients during the early phase of disease and were not longer measurable thereafter but in one patient with a more severe from of hepatitis.

The failure of detecting circulating IFN in most patients with acute hepatitis B does not exclude a possible involvement of the IFN system in viral hepatitis B, for the tests might not be sensitive enough. IFN might preferably act in situ, at the tissue injury or IFN metabolism could be altered in acute liver disease.

Our finding that antiviral activity is present in at least some patients with acute hepatitis B be should encourage researchers to apply more sensitive test system to elucidate the role of IFN in acute hepatitis B.

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ENDOCRINOLOGY

THE MEASUREMENT OF THE SERUM SEX-HORMONE BINDING GLOBULIN IN VARIOUS THYROID DISEASES

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Synthesis of "sex-hormone binding globulin" (SHBG) is influenced by thyroid hormones and its concentration in the serum of femals subjects may be a marker of thyroid hormone effect at the peripheral tissue (liver) level. Compared to the levels found in euthyroid females (n=46), the mean (± S.D.) serum SHBG concentration was found elevated in overt hyperthyroidism (Graves' disease: n=56; 141.6 ± 37.6 vs. 48.3 ± 16.2; toxic nodular goiter: n=16; 119.9 ± 50.7 vs. 48.3 ± 16.2 nmol/l; $P < 0.001$). In contrast, it was decreased in manifest hypothyroidism (n=25; 24.9 ± 14.8 vs. 48.3 ± 16.2; $P < 0.001$). In the group of preclinical hyperthyroidism (n=43), despite suppressed TSH secretion, the serum value of SHBG was normal (47.4 ± 16.8), while its serum level approached the lower border of the normal range in subclinical hypothyroidism (n=10; 33.6 ± 6.1 vs 48.3 ± 16.2 nmol/l; $P < 0.01$). Data indicate that the pituitary responds more sensitively than the liver to a slight change of the serum thyroid hormone level. During thyroid hormone replacement for hypothyroidism, measurement of serum SHBG may provide help to assess the response of the target organ to the given therapy. In patients with generalized resistance to thyroid hormone, the serum SHBG level is within the normal range (51.3 ± 9.8 nmol/l), thus, its determination supports the diagnosis of this disease.

Keywords: Serum, sex-hormone binding globulin; thyroid diseases

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Abbreviations: FT_4: free-thyroxine; FT_3: free-triiodothyronine; TSH: thyroid-stimulating hormone; TRH: thyrotrophin-releasing hormone; SHBG: sex-hormone binding globulin; GRTH: generalized resistance to thyroid hormone

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Introduction

"Sex-hormone binding globulin" (SHBG) is a glycoprotein synthesized in the liver; it serves as a transport protein for testosterone and oestradiol. Its production is stimulated by oestradiol and reduced by testosterone /8, 9/. Previous data indicate that thyroid hormones enhance the synthesis of SHBG directly or via indirect mechanisms /13/ and that the serum concentration of this glycoprotein is a marker of thyroid hormone effect at peripheral tissue (liver) level. Recently, a sensitive radioimmunoassay has been developed for the measurement of serum SHBG. By means of this method in most of the cases with overt hyperthyroidism an elevated, while in the majority of patients with manifest hypothyroidism a decreased, serum SHBG level was detected /1, 10, 15/.

A study has been undertaken to clear the following questions: 1) To what extent is the serum SHBG level abnormal in cases with overt hyperthyroidism or hypothyroidism and when does the serum level return into the normal range after an adequate treatment; 2) To what degree is serum SHBG level influenced by subclinical hyperthyroidism or hypothyroidism and does its determination give any further information about the peripheral (liver) effect of thyroid hormone in these latent thyroid diseases? 3) Does the measurement of the serum SHBG level provide any help in the diagnosis of generalized resistance to thyroid hormone?

Patients and Methods

Untreated female patients aged 20 to 60 years, with overt hyperthyroidism (Graves' disease: n = 56; toxic nodular goiter: n = 16) or manifest hypothyroidism (n =25) were studied. The results were compared with data obtained in age- and sex-matched euthyroid subjects. In all hyperthyroid cases the serum free-thyroxine (FT_4) and free-triiodothyronine (FT_3) levels exceeded the upper limit of the normal range, while the serum TSH concentration was less than 0.1 mU/l. On the other hand, in hypothyroid subjects the serum FT_4 level was low (9.3 pmol/l) and the TSH concentration was elevated (5.0 mU/l).

The studies were extended to patients with latent hyperthyroidism and hypothyroidism. The clinical state of preclinical (subclinical) hyperthyroidism can be best observed in patients with functional autonomous thyroid adenoma. The subjects in this group were clinically euthyroid, a "hot" nodule was demonstrated in their thyroid gland, the basal serum TSH level was subnormal and the TSH response to TRH remained absent despite serum FT_4 and FT_3 levels still within the normal range. These studies were performed in 43 female patients with preclinical hyperthyroidism.

Ten female subjects were enrolled into the group of subclinical hypothyroidism. In these cases the subclinical hypothyroid state developed after subtotal thyroidectomy and radioiodine therapy, or it was the consequence of Hashimoto's thyroiditis. The patients were clinically euthyroid, their serum TSH concentrations exceeded the upper limit of the normal range in spite of normal FT_4 and FT_3 values.

We investigated the effect of an appropriate therapy on the serum SHBG level in patients with hyperthyroidism and hypothyroidism. In 8 hyperthyroid subjects the serum SHBG and TSH levels were measured before the onset of treatment and, again 4, 8, 12 and 20 weeks after methimazol (Metothyrin) therapy was begun. Three months following the start of the treatment all patients became clinically euthyroid, with serum FT_4 and FT_3 levels within the normal range.

We determined also the serum SHBG levels of patients receiving thyroid hormone replacement (Thyranon, Organon or Eltroxin, Glaxo) for hypothyroidism and referred to our thyroid out-patient clinic. Within this group all subjects seemed to be clinically euthyroid and their FT_4 - FT_3 levels were within the wide normal range. These patients were classified into three subgroups: a) normal serum TSH (n = 22); b) elevated serum TSH ($>$3.0 mU/l; n = 9); c) suppressed serum TSH concentration ($<$ 0.1 mU/l; n = 14).

At last, we measured the serum SHBG level of 3 female patients with generalized resistance to thyroid hormone. These subjects were clinically euthyroid despite elevated total-T_4, FT_4, total-T_3 and FT_3 values; in one of these cases the serum TSH level was normal, while in the remaining two it was slightly elevated; an exaggerated TSH response to TRH load was observed in each.

Serum SHBG level was determined by a sex-hormone binding globulin immunoradiometric assay kit (Farmos, Finland). The intraassay coefficients of variation (cv) for value in the low range (25 nmol/l, n = 10) was 5.1%, while in the high range (120 nmol/l, n = 10) it was 3.8%. The interassay cv was 8.5% for a value of 25 nmol/l and 6.7% for a value of 120 nmol/l. Blood for SHBG assay was taken at 8 o'clock in the morning after an overnight fast.

To measure serum hormone levels the following commercially available kits were used: total-T_4 (TT_4): RK-12 (Isotope Institute of the Hungarian Academy of Sciences; normal range: 70-155 nmol/l); total-T_3 (TT_3): RK-11 (Isotope Institute of the Hungarian Academy of Sciences; normal range: 1.2-3.0 nmol/l); free-T_4 (FT_4): Amerlex M FT_4 (Amersham; normal range: 9.3 - 24.5 pmol/l); free-T_3 (FT_3): Amerlex M FT_3 (Amersham; normal range: 3.5 - 8.6 pmol/l); TSH: IRMA-mat TSH (Mallinckrodt; normal range: 0.3 - 3.0 mU/l). For TRH loading test 200 ug TRH was administered intravenously and the TSH response was considered normal if ΔTSH (maximal-basal TSH level) was is the range from 2.0 to 25 mU/l after the TRH load. Thyroid scans were performed after administration of ^{99m}Tc-pertechnetate (50 MBq).

Student's two-sample t test was used in the statistical analysis. Mean $\pm$ S.D. are given in the text and the tables.

Results

Table I

Serum SHBG level (mean $\pm$ S.D.) in euthyroid female subjects and in age- and sex-matched patients with overt and subclinical hyperthyroidism and hypothyroidism. Significance of deviations from the control group: ** $P < 0.01$; *** $P < 0.001$

Diagnosis	Serum SHBG (normal range: 30-90 nmol/l)
Control (n=46)	48.3 $\pm$ 1.63
Overt hyperthyroidism	
a) Graves' disease (n=56)	141.6 $\pm$ 37.6***
b) Toxic nodular goiter (n=16)	119.9 $\pm$ 50.7***
Preclinical hyperthyroidism (n=43)	47.4 $\pm$ 16.2
Manifest hypothyroidism (n=25)	24.9 $\pm$ 14.8***
Subclinical hypothyroidism (n=10)	33.6 $\pm$ 6.1**

Data in Table I demonstrate that in manifest hyperthyroidism (Graves' disease, toxic nodular goiter) the mean value of serum SHBG was significantly higher, conversely, in overt hypothyroidism it was remarkably lower than in the euthyroid group ($P < 0.001$). The mean serum SHBG levels were identical in the preclinical hyperthyroid and control groups, while in subclinical hypothyrodism they were lower than in euthyroid females (P 0.01), but higher than in overt hypothyroidism.

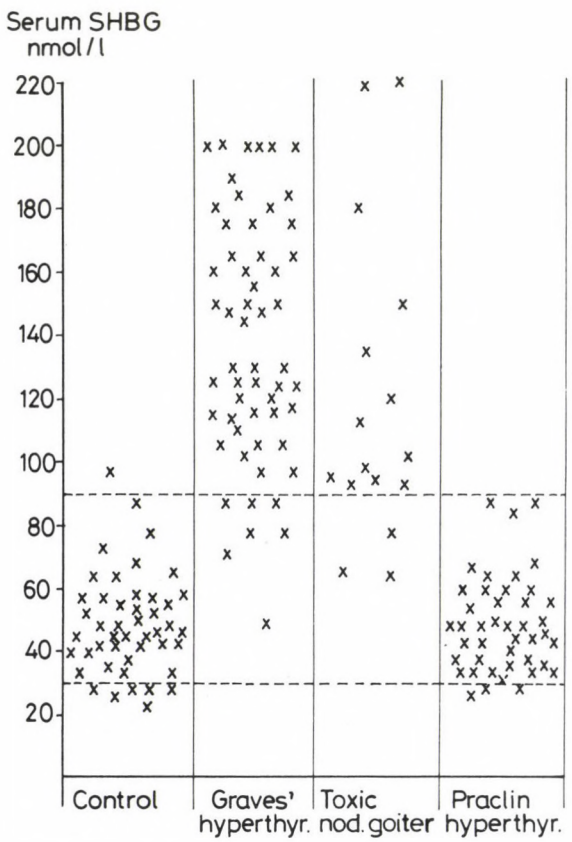

Fig. 1. Individual serum SHBG levels of euthyroid (control) females and of age- and sex-matched patients with manifest hyperthyroidism (Graves' disease, toxic nodular goiter) and with preclinical hyperthyroidism. Area between the dotted lines indicates the normal range of serum SHBG level

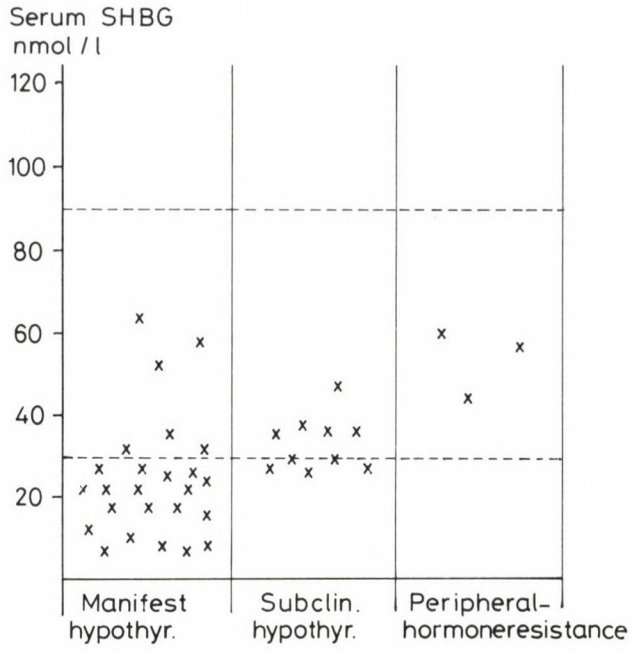

Fig. 2. Individual serum SHBG levels of female patients with manifest and subclinical hypothyroidism. Data for 3 female subjects with the diagnosis of generalized resistance to thyroid hormone, as well

Figure 1 and Fig. 2 illustrate the individual serum SHBG values in the examined groups. In 49 out of 56 patients with Graves' hyperthyroidism and in 13 out of 16 subjects with toxic nodular goiter elevated, while in 19 of 25 patients with overt hypothyroidism decreased, serum SHBG levels were found. In most of the subjects with subclinical hypothyroidism the serum SHBG levels were around the lower border of the normal range. At last, in all 3 patients with generalized resistance to thyroid hormone the serum SHBG concentrations fell within the normal range.

Table II shows the serum SHBG and TSH levels of 8 originally hyperthyroid female patients prior to initiation of treatment and 4, 8, 12 and 20 weeks following the beginning of methimazol therapy. It is demonstrated that the serum SHBG level returned into the normal range sooner than the TSH concentration to Metotyhrin treatment.

Figure 3 demonstrates the individual serum SHBG levels of those of our out-patients who were receiving thyroid replacement for hypothyroidism at least 6 months and were clinically euthyroid. On the basis of the serum

Table II

Changes of serum SHBG and TSH levels after initiation of methimazol (Metothyrin) therapy in 8 female hyperthyroid patients. Normal and abnormal cases at the given time

	SHBG		TSH	
	normal	high	low	normal
Before treatment	-	8	8	-
4 weeks after therapy	3	5	7	1
8 weeks after therapy	4	4	6	2
12 weeks after therapy	7	1	4	4
20 weeks after therapy	8	-	3	5

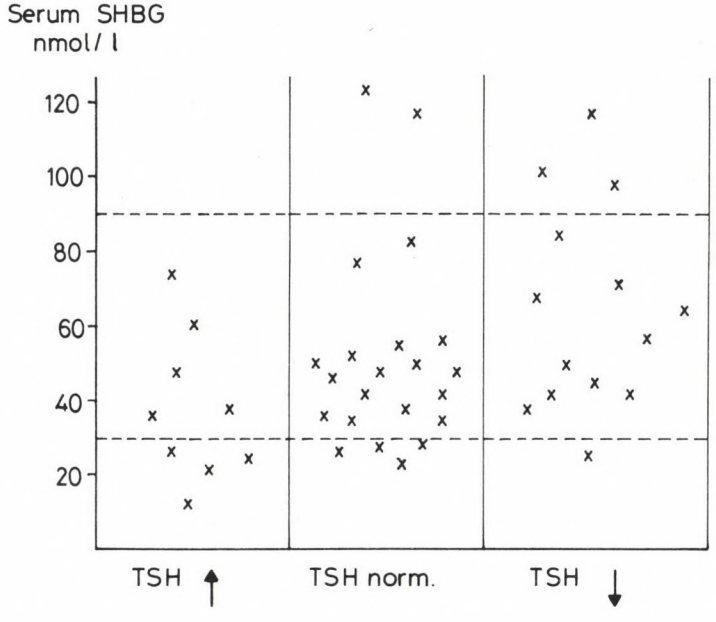

Fig. 3. Individual serum SHBG levels of female patients receiving thyroid hormone replacement for primary hypothyroidism and classified on the basis of serum TSH concentration. ↓ = TSH<0.1 m/l; ↑ = TSH >3 mU/l

TSH concentrations, these subjects were classified into 3 subgroups. In 9 out of 45 patients the serum TSH levels still exceeded the upper limit of the normal range (4.0 - 20.0 mU/l) and among them serum SHBG levels remained decreased (< 30.0 nmol/l) in 4 cases. On the other hand, in response to the therapy, serum TSH disappeared in 14 subjects. Out of them serum SHBG values exceeded the upper limit of the normal range in 3 cases.

Discussion

Sex- and age-related differences have an explicit influence on serum SHBG level. Previous data have demonstrated that the serum concentration of this binding protein is higher in females than in males /1, 15/. For sake of a better comparison and evaluation of the results in the present study only 20 to 60 years old untreated female patients were included. The outcome of this investigation refers only to these subjects.

In accordance with previous findings /1, 10, 15/ our present results demonstrate that in the majority of the patients with overt hyperthyroidism the serum SHBG level is elevated and after the administration of methimazol treatment it shows a decline. Our observation discloses that after the onset of methimazol therapy the synthesis of SHBG in the liver returns to normal sooner than the TSH secretion in the pituitary.

In cases with functional thyroid adenoma toxic nodular goiter usually evolves in a gradual way through the clinical state of preclinical (subclinical) hyperthyroidism. The latter condition is regarded as an intermediate state. It is still questionable whether in preclinical hyperthyroidism - beside the hypophysis - further target organs are affected by changes of serum thyroid hormone levels still within the normal range or by transient increases of serum T_4 or T_3 levels. Former studies based on serum SHBG assay had not yielded an unequivocal answer to this question /5, 16/. According to our findings the mean level of serum SHBG was normal in preclinical hyperthyroidism indicating that the pituitary is more sensitive than the liver to a slight change of serum thyroid hormone concentration.

As shown by previous data, the serum SHBG level is decreased in most of the cases with manifest hypothyroidism. This is corroborated by our findings. In the group of subclinical hypothyroidism the mean value of serum SHBG approaches the lower limit of the normal range. This indicates that the liver as a peripheral organ might be affected by a decrease of

serum thyroid hormone level within the normal range, however, in this respect the pituitary is a much more sensitive organ.

It is still uncertain whether the measurement of serum SHBG supplies any help in evaluating the appropriate thyroid hormone replacement dose for hypothyroidism. Our data demonstrate that in subjects whose serum TSH concentration normalizes in response to substitution therapy, disregarding some exceptional cases serum SHBG level returns into the normal range. However, in some clinically euthyroid patients the TSH content remained elevated in spite of serum FT_4 and FT_3 values within the wide normal range. In these subject, a decreased serum SHBG level was found quite often, indicating that "tissue"-hypothyroidism still existed. Thus, the necessity to increase the replacement dose was raised. Conversely, in some patients TSH secretion became already suppressed in response to the hormone therapy, though the serum FT_4 and FT_3 levels remained still within the wide normal range. In the majority of these cases a normal serum SHBG level could be disclosed; in some subjects, however, the serum SHBG values became elevated, giving additional evidence to the development of "tissue"-thyrotoxicosis. It is supposed that in these patients subclinical hyperthyroidism had developed, which may be detrimental in the long-term and it needs further therapeutical implications.

At last, our study was extended to subjects suffering from generalised resistance to thyroid hormones (GRTH). The diagnosis of this disease is difficult, because sensitive methods for demonstration of peripheral tissue responses to thyroid hormones are lacking; furthermore, the sensitivity of the different organs to T_4 and T_3 may be divergent /4/. Our results are consistent with previous data showing normal SHBG levels despite elevated T_4 and T_3 values in GRTH /11, 14/. This varies due to the change of serum SHBG level found in hyperthyroidism, thus, the measurement of SHBG provides help in the diagnosis of GRTH. However, the value of this test seems to be limited, as in some cases of hyperthyroidism the serum SHBG level remains within the normal range. For this reason, in suspected cases of GRTH Serne et al. /14/ combine the determination of the basal SHBG level with its response to short-term T_3 administration while Ceccarelli et al. /3/ apply a complementary in vitro method by which it is demonstrated that the normal suppressive effect of T_3 on fibronectin synthesis is blunted in fibroblasts from patients with GRTH. Anyhow, it can be stated, and this has been corroborated by our studies, that SHBG determination serves as a test for the assessment of end-organ (liver) senstivitity to thyroid hormones and it may provide help in the diagnosis of GRTH.

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GASTRIC AND COLORECTAL CANCER IN THE TROPICAL PART OF AFRICA

(A REVIEW)

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Until recently the practitioners in Africa, and also those in the temperate climates, had to rely on literature written in an environment that is very different from the tropical circumstances. In the rapidly changing world the physician must be well-informed about the differences which exist between the medical practice of the tropical and of the temperate climates. In this article the characteristics of gastric and colorectal cancer in the tropical (sub-Saharan) part of Africa are briefly reviewed.

Keywords: Gastric cancer; colorectal cancer; tropics; Africa

Introduction

Gastric cancer is one of the most common lethal malignancies all over the world. Men are affected about twice as often as women.

The mean age usually reported is 55 years but patients are even older in some North American series. Only 5 per cent of the patients are less than 40 years of age.

Cancer of the large bowel accounts for about 20 per cent of all deaths due to malignant disease in the United States. Colon is the second most common site for carcinoma in both males and females. Unfortunately, the death rate for this disease has not changed in the past 40 years.

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There is no doubt that some diseases have different patterns - symptoms, incidence rates, etc. - in the tropics than in the temperate climates.

Social change is relentless and rapid all over Africa. It has already changed the character of some diseases and it will expectedly continue to change it as the traditional and rural society is replaced by a new urban one.

The aim of this article is to briefly review the differences regarding to gastric and colorectal cancer in the tropical part of Africa.

Gastric cancer

Gastric carcinoma is less common throughout the African continent than it is Europe, though it is widespread all over the tropics.

The main problem (similarly to colorectal cancer) is that the available figures are scarce and do not reflect the true incidence of the disease.

Reporting the exact morbidity and mortality rates of a disease depends on a satisfactory case history (which is very difficult to obtain in most parts of the developing world, where the physician is so dependent on interpreters), on good diagnostic facilities (endoscopy is a costly method, therefore the local budget can rarely afford it; mass screening is impracticable in African - rural - circumstances); and finally on autopsy reports. (To perform post-mortem autopsies in all cases seems to be practically impossible because of the lack of family's consent and/or due to religious principles.)

There are, however, considerable variations in incidence rates. The disease is, e.g. more common in Nigeria (where its frequency is about five per cent of all malignancies and the male/female ratio is 2.6:1.0), than in Uganda and Mozambique /12, 13, 17, 22, 23, 28, 42, 53, 55, 56/.

In Rwanda and Burundi, and eastern Zaire gastric cancer seems to be the most common tumour in men, and its frequency is also high around Mt. Kilimanjaro, in (some parts of) Western Kenya and in the West Lake province of Tanzania /32, 33/ but, interestingly, in Dar-es-Salaam (Tanzania) Dolmans et al. /21/ found only 5 cases of gastric cancer in 446 endoscopically investigated patients, between September 1978 and March 1980. Dent /20/ gives data for Rhodesian (Zimbabwean) Africans.

Apart from a significant relationship to blood-group A, there seems

to be no suggestion of a genetic or ethnic factor in the aetiology; - the cause most probably lies in environmental factors /31/.

Relation to different dietary staple-foods has been suggested but not proved /7/.

Alcohol, smoking, carcinogens in grilled or smoked meat or fish, vitamin A deficiency, pernicious anaemia, etc. have all been suggested as significant factors but none have been proved yet to be important /12/.

The clinical course of the disease is very similar to that in the western world. The male/female ratio is about 2:1 in most part of Africa.

Most of the tumours originate in the pyloric or prepyloric region /28, 44/. Besides the ulcerative form, which is the most common, polypoid and infiltrative types also occur. Gastric cancer is an adenocarcinoma with a spectrum from well-differentiated to anaplastic /50/.

Two main groups are distinguishable /41/: the intestinal type, which is characterized by cells with a tendency to cohesion and formation of glandular structures and "islands"; and the diffuse type, characterized by separate cells that infiltrate individually. A higher rate of the former variety had been reported in areas with high-incidence rate of gastric carcinoma /56/. It was therefore unexpected that in northern Nigeria, which is a low-incidence area /44/, of 65 specimens classified, by using the criteria of Lauren /41/, 40 were intestinal, 16 diffuse, 2 mixed and 7 unclassifiable; these patients were mostly young, i.e. at an age which has been thought to favour the diffuse lesion.

Because early symptoms are often minimal or nonexistent in the potentially curable phase, patients usually seek medical advice too late. Thus, despite improved diagnostic and surgical techniques and facilities, less than 10 per cent of the patients survive 5 years.

Colorectal cancer

This tumour has for long been known to be infrequent in Africans in Africa, an observation that applies to most of the third-world countries /26, 29, 47, 49, 54, 61/.

The infrequency of colonic carcinoma in Uganda, as compared to European countries, was noted by Trowell /57/, who remarked that this applies to most parts of Africa. Turner /58/ in Mombasa (Kenya) reported only two (adult) cases per year in a retrospective study; Sande found 49 cases in a

five-year period in Nairobi, Kenya /54/, and White in Bulawayo (Zimbabwe) reported three adult cases in a period of three years /60/.

The Kampala Cancer Registry (Uganda) recorded in a period of 9 years (1952-1960) 39 cases, i.e. 1.3 per cent of all cancers /18/.

Umerah and Obadike reported 5 cases during one year from Zimbabwe /59/, while Johnson failed to find a single case in Botswana in a period of 6 years /37/. Bodoe published data for Ghana /5/.

The real incidence of the disease in Nigeria is unknown; a ratio frequency of 2.6 per cent was reported from Ibadan /24/.

Clinical series from Enugu, Ibadan, Lagos and Zaria indicate that the teaching hospitals each manage from 5 to 20 cases annually /1, 3, 46, 48/.

So rare is the condition that single case reports have been considered worthwhile in Africa /38/.

However, during the last decade evidence of a rising tendency of colonic carcinoma has appeared (in Africans) in Africa /27, 43, 51, 62/.

There is no doubt that environmental factors are of great importance in colorectal cancer /31, 36/.

The very low incidence of colonic carcinoma in the tropics is perhaps surprising in the view of the very high incidence of parasitic infections and other inflammatory conditions of the colon. Since amoebiasis and schistosomiasis are very common throughout Africa, they are obviously not involved in the aetiology of colorectal cancer.

Regions of the world with a high risk for carcinoma of the colon and rectum are characterized by high socio-economic conditions with diets rich in beef and saturated fats but poor in fibre.

It seems very probable that the diet of common people living in tropical (sub-Saharan) Africa is very poor in carcinogens. In the more developed countries saturated fats are frequently used for frying; in Africa, on the other hand, the animal fat intake is usually very low, and due to the very high carbohydrate and fibre content of the diet carcinogens may be diluted considerably by the large faecal mass. The high fibre content of the usual diet also may offer some protection /10, 40/. Moreover, the different (cosiderably lower) intestinal bacterial counts might also account for the great variance of the rate of carcinogen production /35/.

A shorter intestinal transit-time is also typical in the third-world; consequently the contact-time between the carcinogens and the colorectal mucosa is shorter.

Whether or not "high-fibre" diet protects against colonic cancer is still under debate /8, 9, 14, 15, 30, 39/.

Cleave /11/ has sought a correlation between the consumption of refined sugar and colonic cancer without convincing results.

In the high-risk regions there is also a high prevalence of premalignant conditions, such as adenomatous polyps, multiple polyposis, villous adenoma and chronic ulcerative colitis /34/. These conditions are uncommon in the tropical Africa.

The tumour and the clinical picture, when it occurs, is similar to that observed in the developed countries.

Two-third (or more) of the tumours develop in the left half (rectosigmoid) of the colon; the caecum is also involved, whereas the intermediate areas have a much lower incidence rate. (Short transit-time?)

In Nigeria, right-sided colon carcinomas and obstructing tumours are more frequent, but treatable pre-malignant conditions are less common than in the western world /2/.

The tumour is an adenocarcinoma; ulcerative, protuberant and infiltrative growths occur, the last being more common in Africa than in temperate countries /25/.

Mucoid and anaplastic forms are apparently also more common in Africa. Intenstinal obstruction is a very rare complication in Africans /16/.

The mean age at presentation is lower than in western countries /2/, and the history of the disease is, interestingly, longer /4/.

According to Owor /52/, on the whole, patients with carcinoma of colon and rectum, are about a decade younger than their counterparts in Europe and North America. This statement has been supported by Adenkule /2/, and by Opiyo and Din /51/, who have reported four cases of colorectal cancer from Zambia under the age of 20 years.

Rectal amoebiasis is the most important diagnostic problem in the tropics.

A mass in the right iliac fossa in a tropical country is likely to be caused by pyomyositis (in the ileopsoas muscle), an appendix mass or abscess or, possibly, by a helminthoma; carcinoma of the caecum is very low on the list of possibilities. In areas where schistosomiasis is common, peritoneal granulomas (in association with an irregular, hard liver) can be mistaken for abdominal and hepatic secondary deposits from a colonic carcinoma; those granulomas, however, tend to be uniform in size and are especially dense on the pelvic floor.

A course of metronidazole should be given initially on the supposition that the lesion is caused by amoeba; if amoebiasis is the correct diagnosis, some reduction in the size of the mass should occur in 72 hours. Colonic carcinoma and appendix abscess do not respond to that regime /12/.

The current approach to treatment of colon cancer is primarily surgical. The chemotherapy of colorectal cancer has been reviewed by Moertel /45/.

At last but not at least, one should be borne in mind that in tropical Africa, a comparatively large number of the patients are infected by HIV-virus. According to Bernstein, AIDS (in some cases) may cause a differential diagnostic problem /6/.

Another very important statement is that the unanimously accpeted principle, namely mass-screening by endoscopy, detection of occult bleeding, etc. as a preventive step against colorectal and gastric malignomas /19/, is out of question in most of the third-world countries, for the local budget cannot afford the necessary expenses.

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PORPHYRINS AND NUCLEIC ACID IN SOME PHOTOSENSITIVE SKIN DISEASES

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This study was suggested to evaluate the possible role of porphyrins and DNA, and their interaction, in some photosensitive premalignant and malignant dermatoses. Twenty-five patients with photosensitive skin diseases viz. xeroderma pigmentosum and basal cell carcinoma, were randomly selected at the outpatient clinic of Dermatology in Mansoura University Hospital. Twenty-five matched normal individuals were used as a control group. In basal cell carcinoma patients, a high increase in skin DNA and decrease in skin total porphyrin, haemoglobin and haem concentrations were observed. In xeroderma pigmentosum, a significant decrease in both skin DNA and skin total porphyrin were found, at the same time, there were elevations in urinary total porphyrin, PBG and ALA concentrations, and a high decrease in haemoglobin and haem levels.

Keywords: Porphyrins, nucleic acid, photosensitive skin disease, Xeroderma pigmentosum

Introduction

Reactions of the skin to sun exposure are of particular importance in Egypt where sunshine is intensive almost all over the year and its damaging effects are primarily due to its ultraviolet (UV) component /1/. Chronic exposure to solar rays over decades often results in production of carcinoma of the exposed areas of the skin /25/.

Sun exposure plays an important role in the development of basal cell carcinoma, the most common carcinoma of the skin, and the tumour has its

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Abbreviations: ALA = δ-aminolaevulinic acid; ALA-D = δ-aminolaevulinic acid dehydrase; PBG = Prophobilinogen; DNA = Deoxyribonucleic acid

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highest incidence over areas most exposed to sun. Some 3/4 of the initially presenting lesions occur on the head and neck /14/.

Xeroderma pigmentosum is a rare autosomal recessive disease, in which patients develop the solar damage, pigmentation abnormalities and malignancies in the area of skin exposed to sunlight. The disease has UV hypersensitivity and abnormal DNA repair /19/.

The accumulation of porphyrins in the skin with aging leads to photodynamic carcinogenesis, which results from biologically significant damage to DNA in the form of single strand breaks and sister chromatid exchange /16/. Porphyrins, to evoke cutaneous photosensitivity, must be present close enough to the skin surface to absorb the radiant energy transmitted through the skin /2/.

Pathak and Burnett /23/ showed that porphyrins are normally present in trace amounts in the human skin and that in porphyria these levels are increased. By using fluorescence microscopy, it was demonstrated that porphyrins are present in the stratum corneum, in the Malpighian cells and in the dermis.

The present study was designed to evaluate the possible role of porphyrins and DNA and their interaction in some patients suffering from xeroderma pigmentosum or basal cell carcinoma.

Materials and Methods

Materials

Twenty-five cases of photosensitive premalignant or malignant skin diseases of different ages (15-50 years) and both sexes were examined. The patients were randomly selected from outpatients presenting at the Clinic of Dermatology, Mansoura University. They were classified into two groups:

- Xeroderma pigmentosum, 10 patients and
- Basal cell carcinoma, 15 patients.

The diagnosis was based on strict history, clinical examination and histopathological examination. A group of 25 apparently healthy persons were taken as a control group. Skin biopsy from the involved skin, especially the sun-exposed parts, hands, head and neck. Venous blood samples and urine samples were collected.

Methods

The following components were assayed:

Total porphyrin in the blood /24/ and skin biopsies /3/. Erythrocyte protoporphyrin and haem /20/. ALA-D activity /32/. Haemoglobin per cent /12/. DNA in skin and blood /28/. Total protein in leucocytes-a modified method of lowry /21/. Urinary ALA /30/. Urinary PGB /26/. Urinary coproporphyrin and uroporphyrin /29/. Creatinine in the urine /31/. The statistical analyses of the results were carried out according to the method described by Burn et al. /5/.

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highest incidence over areas most exposed to sun. Some 3/4 of the initially presenting lesions occur on the head and neck /14/.

Xeroderma pigmentosum is a rare autosomal recessive disease, in which patients develop the solar damage, pigmentation abnormalities and malignancies in the area of skin exposed to sunlight. The disease has UV hypersensitivity and abnormal DNA repair /19/.

The accumulation of porphyrins in the skin with aging leads to photodynamic carcinogenesis, which results from biologically significant damage to DNA in the form of single strand breaks and sister chromatid exchange /16/. Porphyrins, to evoke cutaneous photosensitivity, must be present close enough to the skin surface to absorb the radiant energy transmitted through the skin /2/.

Pathak and Burnett /23/ showed that porphyrins are normally present in trace amounts in the human skin and that in porphyria these levels are increased. By using fluorescence microscopy, it was demonstrated that porphyrins are present in the stratum corneum, in the Malpighian cells and in the dermis.

The present study was designed to evaluate the possible role of porphyrins and DNA and their interaction in some patients suffering from xeroderma pigmentosum or basal cell carcinoma.

Materials and Methods

Materials

Twenty-five cases of photosensitive premalignant or malignant skin diseases of different ages (15-50 years) and both sexes were examined. The patients were randomly selected from outpatients presenting at the Clinic of Dermatology, Mansoura University. They were classified into two groups:

- Xeroderma pigmentosum, 10 patients and
- Basal cell carcinoma, 15 patients.

The diagnosis was based on strict history, clinical examination and histopathological examination. A group of 25 apparently healthy persons were taken as a control group. Skin biopsy from the involved skin, especially the sun-exposed parts, hands, head and neck. Venous blood samples and urine samples were collected.

Methods

The following components were assayed:

Total porphyrin in the blood /24/ and skin biopsies /3/. Erythrocyte protoporphyrin and haem /20/. ALA-D activity /32/. Haemoglobin per cent /12/. DNA in skin and blood /28/. Total protein in leucocytes-a modified method of lowry /21/. Urinary ALA /30/. Urinary PGB /26/. Urinary coproporphyrin and uroporphyrin /29/. Creatinine in the urine /31/. The statistical analyses of the results were carried out according to the method described by Burn et al. /5/.

Results

Some biochemical abnormalities were observed in the blood, skin and urine of xeroderma pigmentosum and basal cell carcinoma patients as compared to the control values.

It is clear from Table I taht the blood haemoglobin per cent was highly significantly decreased in the xeroderma pigmentosum group while it was significantly decreased in basal cell carcinoma. These decreases were generally accompanied by decreased haem levels. In the same patients, there were insignificant changes in total porphyrin, protoporphyrin and ALA-D activity.

Table I

Blood haemoglobin (g%), total porphyrins (ug%), δ-aminolaevulinic acid dehydratase (ALA-D, units), protoporphyrin IX (umol%) and haem (mol%) in xeroderma pigmentosum (XP) and basal cell carcinoma (BCC) in comparison to the control group

Item	Control	XP	BCC
Haemoglobin			
Mean $\pm$ S.D.	12.70 ± 1.21	10.30 ± 1.55	10.95 ± 1.69
Number	22	10	12
P		<0.001	<0.01
Total porphyrin			
Mean $\pm$ S.D.	27.40 ± 6.03	23.90 ± 6.58	30.77 ± 8.05
Number	20	10	12
P		>0.05	>0.05
ALA-D			
Mean $\pm$ S.D.	17.07 ± 5.95	13.85 ± 4.25	16.10 ± 8.17
Number	22	10	12
P		>0.05	>0.05
Protoporphyrin			
Mean $\pm$ S.D.	$(8.73 \pm 3.58)10^{-4}$	$(9.78 \pm 4.90)10^{-4}$	$(7.76 \pm 2.01)10^{-4}$
Number	15	10	9
P		>0.05	>0.05
Haem			
Mean $\pm$ S.D.	$(14.65 \pm 2.47)10^{-4}$	$(12.21 \pm 2.04)10^{-4}$	$(12.30 \pm 2.39)10^{-4}$
Number	22	10	12
P		<0.05	<0.01

Table II

Blood DNA (ug/gm protein), skin DNA (ug/gm tissue) and skin total porphyrin (ug/gm tissue) in xeroderma pigmentosum (XP) and basal cell carcinoma (BCC) in comparison to the control group

Item	Control	XP	BCC
Blood DNA			
Mean ± S.D.	72.52 ± 7.45	69.20 ± 5.21	72.60 ± 5.78
Number	22	10	11
P		>0.05	>0.05
Skin DNA			
Mean ± S.D.	311 ± 17.53	274 ± 33.46	427 ± 94.90
Number	25	8	11
P		<0.01	<0.001
Skin total porphyrin			
Mean ± S.D.	0.966 ± 0.421	0.695 ± 0.170	0.583 ± 0.352
Number	25	7	10
P		<0.01	<0.01

Table III

Urinary total porphyrin, Porphobilinogen (PBG), and δ-aminolaevulinic acid (ALA) in mg/gm creatinine in xeroderma pigmentosum (XP) and basal cell carcinoma (BCC) in comparison to the control group

Item	Control	XP	BCC
Total porphyrin			
Mean ± S.D.	$(17.75 \pm 3.90)10^{-3}$	$(31.8 \pm 13.27)10^{-3}$	$(18.90 \pm 12.91)10^{-3}$
Number	16	7	10
P		<0.001	>0.05
PBG			
Mean ± S.D.	6.11 ± 2.83	8.64 ± 3.28	6.67 ± 4.23
Number	22	7	9
P		<0.05	>0.05
ALA			
Mean ± S.D.	2.51 ± 0.92	4.09 ± 1.70	2.59 ± 1.62
Number	22	7	9
P		<0.01	>0.05

Table II reveals that blood DNA did not change in either the xeroderma pigmentosum and basal cell carcinoma group. However, skin DNA was significantly decreased in xeroderma pigmentosum and highly significantly increased in basal cell carcinoma group. Skin total porphyrin was significantly decreased in both groups of patients.

In xeroderma pigmentosum, the mean concentrations of urinary total porphyrin, ALA and PBG were increased, however, there were no considerable changes in these parameters in basal cell carcinoma (Table III).

Discussion

Oxidized porphyrins are fluorescent and their importance in clinical dermatology is related to the fact that they may accumulate in the skin and their exposure to certain wave length is followed by cutaneous photosensitization /27/. Cutaneous photosensitivity may be associated with increased porphyrin levels in the skin, erythrocytes and/or blood plasma /9/.

In 1935, Korbler /19/ suggested that the accumulation of porphyrins in the skin with aging leads to photocarcinogenesis. Since then, a number of reports have supported the interplay between porphyrins and DNA damage in inducing tumours.

As shown by the present results, haemoglobin per cent was decreased in both xeroderma pigmentosum and basal cell carcinoma. This decrease may be attributed to the fact that the disease is reported mainly in patients from rural regions; our patients were mainly farmers living at a low standard of life, they must have been predisposed in the form of nutritional deficiency brought about by shortage of intake or by gastrointestinal upsets, which may have been due to intestinal worm infestation or schistosomiasis /13/. On the other hand, their anaemia may be attributed to multiple neoplasia in xeroderma pigmentosum cases /22/.

In xeroderma pigmentosum, urinary total porphyrin and the porphyrin precursors, ALA and PBG were elevated. These elevations could be understood on the light of abnormalities in the activities of most enzymes of the haem biosynthestic pathway /11/.

The present study indicates also that both the DNA and total porphyrin levels in the skin samples from xeroderma pigmentosum patients where decreased. There is no satisfactory explanation for the decrease of skin DNA level in these cases. The decrease of the total porphyrin might be understood as a consequence of the inhibition of the enzyme copro-oxidase, as previously suggested by Hegazy et al. /17/. These authors stated that the decrease of total porphyrin in xeroderma pigmentosum may be due to blocking of copro-oxidase and accompanying parallel changes in the corresponding protoporphyrin/haem ratio. The inhibition of copro-oxidase will

result in an accumulation of coproporphyrin and uroporphyrin, which are both highly hydrophilic and almost completely excreted in the urine. Accordingly, the urine level of total porphyrin will be elevated as in the present study.

Our results indicate that the photosensitivity in xeroderma pigmentosum cases is associated with a decrease of both DNA and total porphyrin in the skin. The degree of photosensitivity may depend on the type and quantity of porphyrins in skin and plasma /10/. So, the estimation of skin total porphyrin concentration seems not to be a reliable diagnostic factor helping in the clinical management of the patients. Rather, it is probable that the estimation of some specific porphyrin isomer(s) (type and quantity of that isomer) may be of diagnostic value in these patients.

Epstein et al. /15/ reported that the inhibition of DNA, RNA and protein synthesis and mitosis formation occurred within the first hour after irridation and persisted for several hours thereafter. In 1968, Cleaver /6/ reported that skin fibroblasts from xeroderma pigmentosum patients are unable to repair normally after exposure to a certain type UV light because this light induced damage in their DNA. This DNA repair defect is most likely due to a lack or insufficient amount of DNA endonuclease, which is an essential enzyme that inhibits the repair of UV - damaged DNA /7/.

In basal cell carcinoma patients, there are no considerable changes in the urinary total porphyrin, ALA and PBG while a significant increase in cutaneous DNA is observed in addition to a decrease in the total porphyrin concentration in the skin. This finding is in accordance with the findings of Bohm and Sandrilter /4/ and Collste et al. /8/ as regards the increase in DNA in solid tumours and its value in serving diagnostic purposes. However, the decrease in the skin total porphyrin may be understood on the basis of the possibility that porphyrins participate in the induction of actinic damage.

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ACTIVITIES OF SERUM CATHEPSIN (B, H AND L) AND METALLOPROTEINASE (MMP7-ASE) IN PATIENTS WITH GASTROINTESTINAL AND BRONCHIAL MALIGNANT TUMOURS

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Serum lysosomal cysteine proteinases cathepsin B, H, L and metalloproteinase (MMP7-ase) activities of 14 patients suffering from gastrointestinal and bronchial carcinomas were investigated. The serum cathepsin B and H activities were significantly diminished in the carcinoma group as compared to the controls, while the activity of cathepsin L and of MMP7-ase was normal.

Keywords: Serum, cathepsin B, H, L, metalloproteinase, patients, gastrointestinal, bronchial carcinomas

Introduction

A lysosomal cysteine proteinase, cathepsin B has been reported to be involved is proliferation and metastatic potential of malignant experimental tumours /3/ and human mammary and cervical carcinomas /2/.

Elevated proteinase-like peptidase activities (collagenase, cathepsin B, cathepsin B + L, cathepsin H and D) were observed in homogenates of gastric cancer tissue, suggesting an important role of these enzymes in tumour invasion /5/.

Activities of serum cysteine proteinases and metalloproteinase were investigated in the present study in gastrointestinal and brocnhical cancerous patients of the Department of Surgery (Szeged).

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Material and Methods

Serum cathepsin B, H, L and metalloproteinase (MMP7-ase) activities of 14 patients having gastrointestinal (hepatic, gastric, pancreatic, rectal) and bronchial cancer confirmed by histological examinations were analysed.

Serum cathepsin B, H, L and MMP7-ase were determined fluorimetrically using synthetic peptide substrates (4-methyl-7-coumaryl-amide = MCA) /1/. MMP7-ase hydrolyses succinyl-alanyl-alanyl-prolyl-phenylalanyl-(Suc-Ala-Ala-Pro-Phe-MCA) substrate /4/.

Results

Serum cathepsin B and H activities were significantly diminished in the tumorous group as compared to controls (Table I), while the cathepsin L and MMP7-ase activities were approximately equal in both groups.

Table I

Activities of serum cysteine proteinase (cathepsin B, H, L) and metalloproteinase (MMP7-ase)

Enzyme	Tumours group			Control group	
	mean mU/ml	± S.D.	t probe	mean mU/ml	± S.D.
Cathepsin B	3.66	4.1	$P < 0.001$	20.65	11.84
Cathepsin H	79.64	29.99	$P < 0.001$	104.8	6.5
Cathepsin L	1.6	1.03	$P > 0.05$	1.89	0.98
Metalloproteinase	7.26	4.33	$P > 0.05$	7.55	3.29

U = micromol x min^{-1}

Discussion

Watanabe et al. /6/ published their results after we had finished our investigations; they found higher cathepsin B and L activities in the tumorous tissue than in the surrounding tumourfree one, indicating that the elevated cathepsin activites are related to proliferation and envolvement of gastric cancer.

Independently of Watanabe's group we measured the activity of cystine proteinases and MMP7-ase in serum samples from tumorous patients not directly from the cancer tissue.

The lower enzyme activities in the tumorous patients' sera may reflect increased utilization for the cellular activity and for tissue destruction.

Further cysteine proteinase and metalloproteinase investigations should be made after resection of different tumours to evaluate the role of the above-mentioned enzymes in the proliferation and metastatic capacity of gastrointestinal and bronchial carcinomas.

Furthermore, it is known that alfa-2-macroglobulin can inhibit lysosomal cysteine proteinases. The lower proteinase activities in serum can also be produced by higher concentration of proteinase inhibitors found in serum.

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BOOK REVIEWS

Hexachlorobenzene

Proceedings of an International Symposium. (IARC, Scientific Publications No. 77.)

Eds: C.R. MORRIS and J.R.P. CABRAL 1987

Price: 50.00 £

This volume contains the proceedings of an international symposium on hexachlorobenzene (HCB), held in Lyon, France 24-28 June 1985. HCB is used as a fungicide in various regions of the world. Being present in some pesticides and industrial wastes it is also important as a source of impurities. HCB is highly resistant to degradation and easily dispersed in the environment, as is illustrated by the epidemic poisoning that occurred in Turkey during the 1950s. The book contains a collection of interpretative overviews of the occurrence, chemical properties and manufacturing, as well as the distribution of HCB the environment. It summarizes the persistence and pathways of HCB from the technosphere into the athmosphere. Several health effects of HCB are described in the chapter "Human Observations". HCB is one member of a small group of polyhalogenated aromatic hydrocarbons that are capable of causing cutaneous porphyria in humans.

In Turkey the porphyria was accompanied by neurotoxic symptoms, viz. sensory motor neuropathy and myotonia. There are some evidences based on epidemiological data suggesting that HCB exposure or poisoning may associate with an elevated incidence of primary liver cancer. This volume of the IARC Monographs concludes that there is still a considerable burden of HCB in human population as well as in our environment. Even with the obvious technological advances, several questions remain unsolved. Their solution would require highly innovative research, new critical approaches to develop international cooperations. A register would be useful to assess world research in this field. This volume of the WHO IARC Monographs seems to be the first step in this direction.

ANNA TOMPA

Some Halogenated Hydrocarbons and Pesticide Exposures

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 41. WHO, IARC, Lyon, France 1986

Price: Sw.Fr. 65.-

This volume of IARC Monographs deals with 11 of halogenated aliphatic hydrocarbons, bis(2-chloro-1-methyl)ether, polybrominated biophenyls and amitrol. Some of these compounds have already been evaluated by the IARC and their list is published in this volume again. The importance of halogenated alkanes is due to the large quantity of the annual production in the developed countries. Therefore, IARC analysed this group of chemicals with special interest and reevaluated their carcinogenecity, based on the very recent data base.

Because of the extreme toxicity of some polychlorinated dibenzo-dioxins and polychlorinated dibenzofurans, highly selective and specific ana-

lysis is required to detect even low levels of these compounds in order to minimize occupational exposure. These compounds have a considerably high odour threshold and this may cause problem in their everyday use and explains the relatively high number of intoxications.

Evaluation of these chemicals was not easy for the Working Group. All short-term test systems were taken into consideration, although, some of them were not reproducible between laboratories.

Case control studies offered an alternative approach to cohort studies for assessing the correlation between certain exposures and the sporadic appearance of rare diseases. In these studies the increased risk has been associated mainly with chlorophenoxy herbicides and with chlorophenols. Four compounds were found to have sufficient evidence of the carcinogenicity in experimental animals, but none of them showed any evidence of human carcinogenicity. Most of these chemicals were suspect of increasing other environmental contaminants' toxicity and of exhibiting synergistic effects. Looking at their inhalation and skin toxicity, it is concluded that chlorophenoxy herbicides have only limited evidence of the carcinogenicity of occupational exposure to humans.

ANNA TOMPA

Some Naturally Occurring and Synthetic Food Components,

Furocoumarins and Ultraviolet Radiation

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 40. WHO, IARC, Lyon, France 1986

Price: Sw.Fr. 65.-

This volume of IARC Monographs contains 20 items from naturally occurring and man-made food and food additives or other compounds which may have been used in therapy with UV radiation.

Diets have an increasing role as factors causing cancer in humans and some compounds taken together with food may influence the endogenous formation of different carcinogens (nitroso-compounds). Therefore, in the future the composition of the human meals will have a prominent importance in cancer research and cancer prevention. This fact underlines the importance of this volume, which gives a detailed list of the current data related to this topic.

The importance of analysing the carcinogenicity of the natural and synthethic food is not new in the IARC Monographs. Since 1969, the Agency has evaluated more than 30 food additives and food contaminants and more than 20 naturally occurring edible materials, and gave evidence of carcinogenicity for both animals and men for more than 30 food additives.

In this volume the Agency considers naturally occurring toxins: bracken fern and some of its constituents viz. citrinin, patulin and rugulosin. Among the food additives benyzl acetate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and potassium bromate were evaluated. In these two groups three compounds were found carcinogenic to experimental animals: bracken fern, BHA and potassium bromate. However, there is no sufficient evidence for the carcinogenicity of these substances to humans.

The third group of compounds dealt with in this volume comprises seven heterocyclic amines in food: these appear during pyrolysis of different heterocyclic amino acids. They are formed from heated proteins consisting two classes of N-heterocyclic primary amines of either carbolines

or imidazoquinolines. A final conclusion of these compounds' carcinogenic potential has not been made yet because satisfactory human data fail. The final group of the compounds dealt with includes a number of furocoumarins which are used clinically together with UV-treatment. Citrus oils, such as bergamot oil, contain psoralen, which is used not only in food but also in different suntanning products. In the absence of UV-light, furocoumarins have no significant therapeutic or genotoxic effect. This is important not only for the therapeutist but also for users of bergamot-oil-scented cosmetics, or for any other practical use of thes products.

ANNA TOMPA

The Relevance of N-nitroso Compounds to Human Cancer. Exposures and Mechanisms

IARC Scientific Publications No. 84. WHO, IARC, Lyon France 1987

Eds: H. BARTSCH, I. O'NEILL and R. SCHULTE-HERMANN

Price: 50.00 £

IARC Scientific Publications serve the programme conducted by the WHO to study potential carcinogens in human environment. They intended also to contribute to the dissemination of authoritative information on different aspects of cancer research.

This volume contains the Proceedings of the Ninth International Meeting on N-nitroso Compounds held in Baden (Austria) in 1986. It is dealing with the carcinogenic and mutagenic effects of different N-nitroso compounds, considering their mechanism of action at the molecular and biochemical level. There is a detailed description of their metabolism in vivo as well as their reaction with macromolecules in vitro. The volume also contains methods for detection of N-nitroso compounds in gastric juice, and in food and drinks.

The biological effects of N-nitroso compounds are described in Chapter 5 in different in vivo and in vitro experimental models. Chapter 6 deals with endogenous formation of nitrosamines from the nitrite-reactive compounds in vivo; this process was analysed also after alcohol intake and after high cysteine intake, which modulates the process.

The measurement of volatile nitroso compounds in the air, in food or in different drugs give some evidence about the level of environmental exposure.

The role of the nitroso compounds in the tobacco and in betel-quid, which can cause cancer, is discussed in Chapter 9. Methods for analysis of specific markers which have been detected in some tobacco smokers is also included. ^{32}P-postlabeling assay is used for determining the different adduct formations. By adding proline to the betel-quid mixture and then analysing the saliva of chewers it has been demonstrated that some of these nitrosamines are formed during chewing.

In the last Chapter of the volume papers are presented on the clinical and epidemiological studies, convincing the reader of the relevance of the experimental data to humans.

Some of the conclusions of human studies are really important. For example they emphasize the importance of maternal and paternal exposure to N-nitroso compounds in the increase of childhood brain cancer in their offsprings. Although it was already known that food intake and the quality of the food can influence the incidence of gastric cancer, the beneficial ef-

fect of citrus fruit consumption and that fibres can reduce gastric cancer incidence is a new discovery.

Besides these examples, the Proceedings present a number of recently developed and sensitive methods for measuring biologically active doses of N-nitroso compounds and give strategies to their application in human studies in cancer prevention.

ANNA TOMPA

THE NINTH ANNUAL MEETING OF
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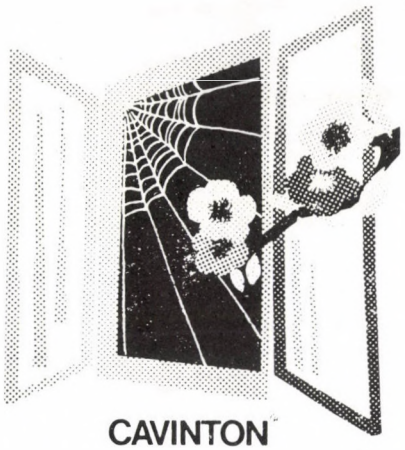

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CONTENTS

NEUROLOGY

Somatosensory evoked potentials elicited by stimulation of the posterior tibial nerve in patients with multiple sclerosis
J.L. Czopf, L. Kellényi, J. Czopf 115

Non-specific peroxidase (Donor: H_2O_2-oxidoreductase, Ec '.11.1.7) activity in multiple sclerosis
J. Najbauer, L. Vécsei, G. Pálffy 129

GYNAECOLOGY

Tissue characterization by transvaginal colour Doppler for the evaluation of gynaecological tumours
First of two parts: review of the literature
B. Juhász, A. Kurjak, L. Lampé, Z. Hernádi 135

Tissue characterisation by transvaginal colour Doppler for the evaluation of gynaecological tumours
Second of two parts: clinical experiences
B. Juhász, A. Kurjak, L. Lampé, Ivica Zalud, G. Crvenkovic, Z. Hernádi 149

CIRCULATION

The effect of vasodilator therapy on the limb circulation in hypertension
A. Váradi, F. Solti, M. Iskum 157

GASTROENTEROLOGY

Ultrasonography and water content of the liver in chronic diffuse liver disease
Agnes Szebeni, Elizabeth Pintér, Gy. Stotz, Gy. Tolvaj, M. Juhász, K. Dávid 163

Successful treatment of intractable gastric ulcers with acetazolamide
A. Erdei, I. Győri, A. Gedeon, Ildikó Szabó 171

PATHOPHYSIOLOGY

Effects of hypothyroidism and hyperthyroidism on thermogenic responses to selective and nonselective beta-adrenergis agonists in rats
I. Ilyés, J. Stock 179

IMMUNOLOGY

Killer-cell activity in alcohol-originated diseases of the liver, and the effect of alcohol on the K-cell functions under in vitro conditions
R. Schnabel, M. Bokor, Gy. Malatinszky, T. Garam 189

LABORATORY

Effects of the medium on the filterability of human red blood cells
P. Tamás, P. Gresele, D. Polidori, G.G. Nenci 201

Physiological values of glucose-6-phosphate-dehydrogenase (G-6-PD) in chorionic villi
Aranka László, Mária Simon, J. Szabó 209

BOOK REVIEWS 213

"INTO EUROPE" 219

CONGRESSES, FELLOWSHIPS, COURSES 223

Acta Medica Hungarica, Vol. 47/3-4, pp. 115-127 (1990)

NEUROLOGY

SOMATOSENSORY EVOKED POTENTIALS ELICITED BY STIMULATION OF THE POSTERIOR TIBIAL NERVE IN PATIENTS WITH MULTIPLE SCLEROSIS

J.L. CZOPF, L. KELLÉNYI and J. CZOPF

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(Received: June 5, 1990)

Normal control somatosensory evoked potential data (elicited by stimulation of the posterior tibial nerve), of 20 healthy subjects were compared to the electrophysiological data of 20 patients with the diagnosis of definite or probable multiple sclerosis. The demyelinating process affects all segments of the response, causing increase in latency and decrease in amplitudes as well as lack of the potentials during the disease. Based on the literature, the neurological localization values of TPSEP are summarized. Due to its outstanding sensitivity in these cases, the procedure as advised by the authors should be used in demyelinating processes and in other spinal cord disease of different aetiology.

Keywords: multiple sclerosis, clinical electrophysiology, evoked potential, posterior tibial nerve.

Introduction

Multiple sclerosis (MS) is a common organic neurological disease, affecting mostly young adults and resulting in stepwise progression to early disability. In the clinical diagnosis of multiplicity (i.e. foci in white matter) the evoked potential (EP) technique has gained an important role in the last two decades. EPs are electric changes of brain activity of low intensity elicited by repetitive external stimuli. In 1947, Dawson /12/ succeeded for the first time in registering somatosensory EPs by electric stimulation of the lowerlimb nerve which he recorded as "cortical response".

Abbreviations: MS = Multiple sclerosis; EP = evoked potential; TPSEP = somatosensory evoked potentials elicited by stimulation of the posterior tibial nerve; SEP = somatosensory evoked potential

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Akadémiai Kiadó, Budapest

In 1963, Liberson et al. /31/ recorded potentials over the spinal cord, evoked by electric stimulation. - Following the investigations of Cracco et al. /9/ - the procedure has become a routine procedure in the clinical electrophysiological diagnosis. This method, by monitoring the propagation of the stimulus along the somatosensory system, enables the clinical neurologist to localize pathologic functions and detect clinically silent foci.

PATIENTS AND METHODS

Data from 20 healthy adult persons were compared to those from 20 MS patients. All the patients had a definite or probable form of the disease; three of them were of completely normal sensory function, the others showed moderate signs of motor or deep sensory disturbance of spinal cord origin. Degrees of clinical certainty have been established according to Bauer's criteria /4/.

Stimulation. The posterior tibial nerve was stimulated behind the medial ankle with superficial electrodes. A constant current generator produced square pulses of 0.5 ms duration and 1/s frequency. After determining the sensory threshold, an intensity - somewhat higher than the motor threshold - was induced, which elicited a twitch in the short flexors of the hallux. (This value was 2-5 times higher than the sensory threshold.) Both lower extremities were examined in each case.

Registration. The potentials were registered at points C3 and C4 - being the active sites and at point CO - being the reference point -, according to the 10-20 electrodes arrangement system. Frequency transmission of the amplifier was 50-2000 Hz. The sampling frequency of the averager (Kell-512-D-81-2), was 2 kHz. 512 individual responses were averaged, and every registration was repeated at least twice. Taking into account the differences in the length of the extremities, corrections were performed in latency measurement.

Evaluation of the response. The absolute latency values of waves in the cortical potential measured within the first 120 ms (P1-N1-P2-N2-P3-N3-P4), the amplitudes from peak to peak of the individual segments were evaluated. The mean value, standard deviations of the normal material and the so-called confidence ellipses (i.e. the distribution field of normal with 2 SD probability) were calculated, limit values (low and high extremes) were also determined and used to differentiate the normal from pathological response.

RESULTS

Figure 1 and Table I show statistical data for the normal responses. The abnormal TPSEP values in all cases of MS reflect lesion in the afferent sensory system. In cases with spinal cord lesions TPSEP may be absent (Fig. 2). As shown in Fig. 3, an increase in latency, a decrease in amplitude, and lack of certain components were observed. Pathological alterations were also frequent in patients devoid of detectable clinical sensory symptoms (documentation of subclinical lesion). In three patients the neurological sensory findings were normal in every respect - and in two out

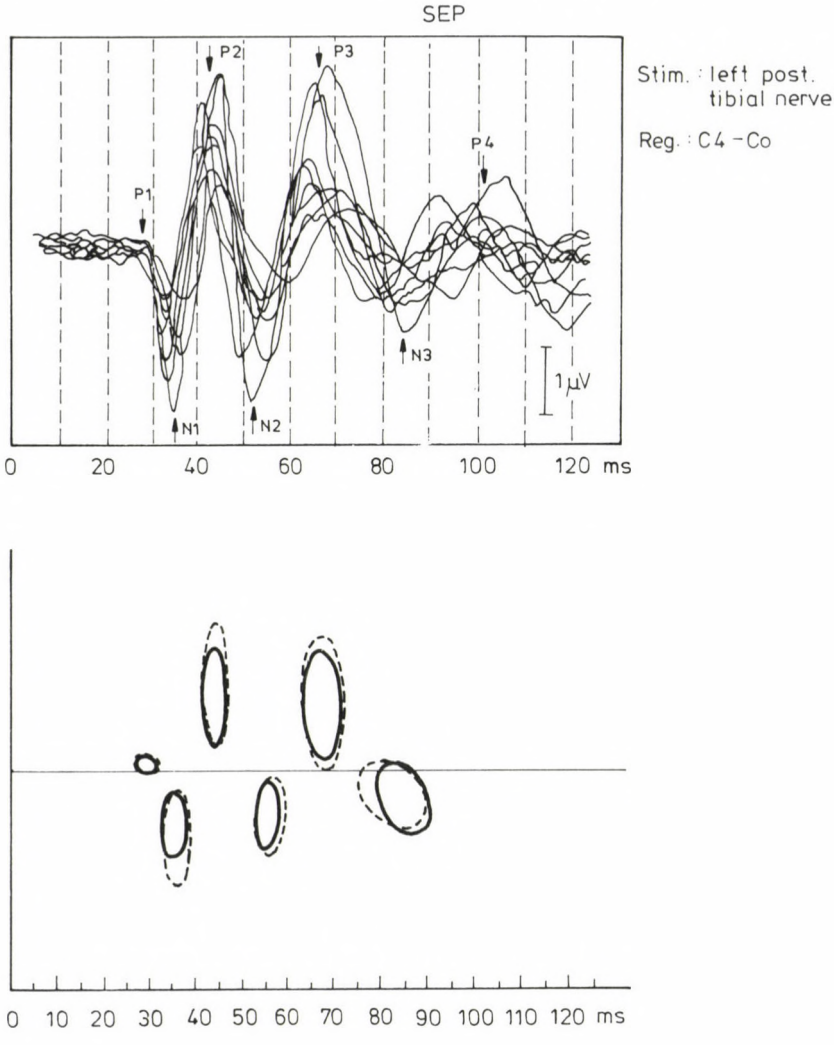

Fig. 1. Upper part: The normal TPSEP. (The single EPs of 9 normal subjects were superimposed for demonstrating the interindividual stability of the response). Lower part: The "confidence ellipses" of the peaks (i.e. the field which contains the normal value at 95.4% probability)

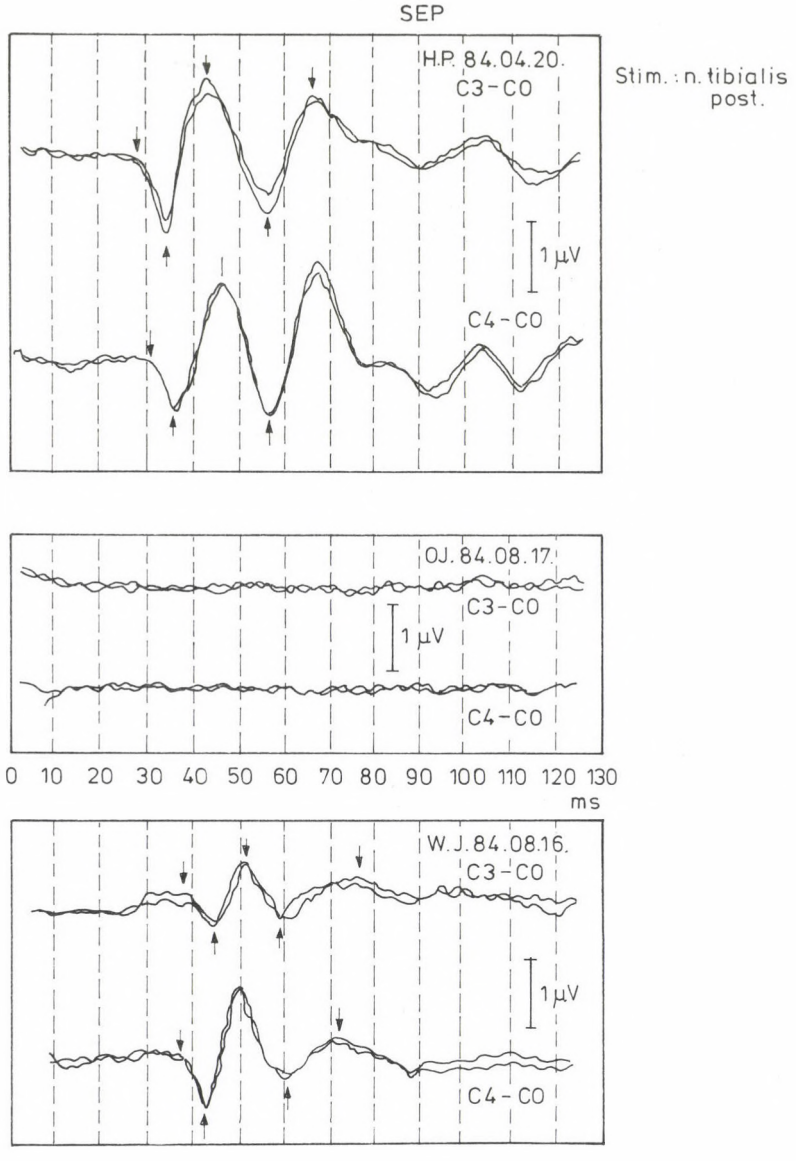

Fig. 2. Upper part: normal TPSEP. Middle: TPSEP of a 46 years old man. (Clinically definite MS with generalized symptomatology. No cortical response could be recorded.) Lower part: The response of a 56 years old man. (Clinically definite MS with optospinal symptomatology, and mild sensory deficit. Note the prolonged latency of all components.)

Table I

SEP (Stim.: n. tibialis post.) Latency (ms)

		P1	N1	P2	N2	P3	N3	P4
C3-Co	Mean	29.7	35.75	44.3	55.73	67.2	84.86	101.4
	SD	2.08	2.06	1.76	2.11	2.70	4.41	4.51
	Lim.v.	27--33	33.5--40	42--47	53--61	63.5--73	77--90	96--105
C4-Co	Mean	30.0	36.0	44.63	56.03	67.88	82.68	100.08
	SD	1.93	2.01	1.81	2.57	3.49	5.65	5.59
	Lim.v.	27--34	33.5--41	42--48	51--62	63.5--75	74--94	92--106.5

SEP

Amplitudes (μV)

		P1-N1	N1-P2	P2-N2	N2-P3	P3-N3	N3-P4
Left C3-Co	Mean	6.3	12.3	11.2	10.4	10.0	9.4
	SD	2.9	5.4	5.3	5.7	5.6	3.2
	Lim.v.	2.6-12	4.6-22.4	4.0-23.2	4.6-22.8	3.6-22	4.0-12
Right C4-Co	Mean	6.8	14.2	12.6	10.9	10.1	9.5
	SD	3.2	7.2	6.8	7.0	6.6	4.0
	Lim.v.	2.2-14.1	5-30.4	4.4-28.8	2.0-27.2	2.8-24.4	4.0-12.4

Lim.v. = limit values (low and high extremes)

of the three cases severe alterations were observed in the EPs (subclinical lesions). With repeated examinations the responses may well serve in long-term monitoring of steroid treatment (Fig. 4).

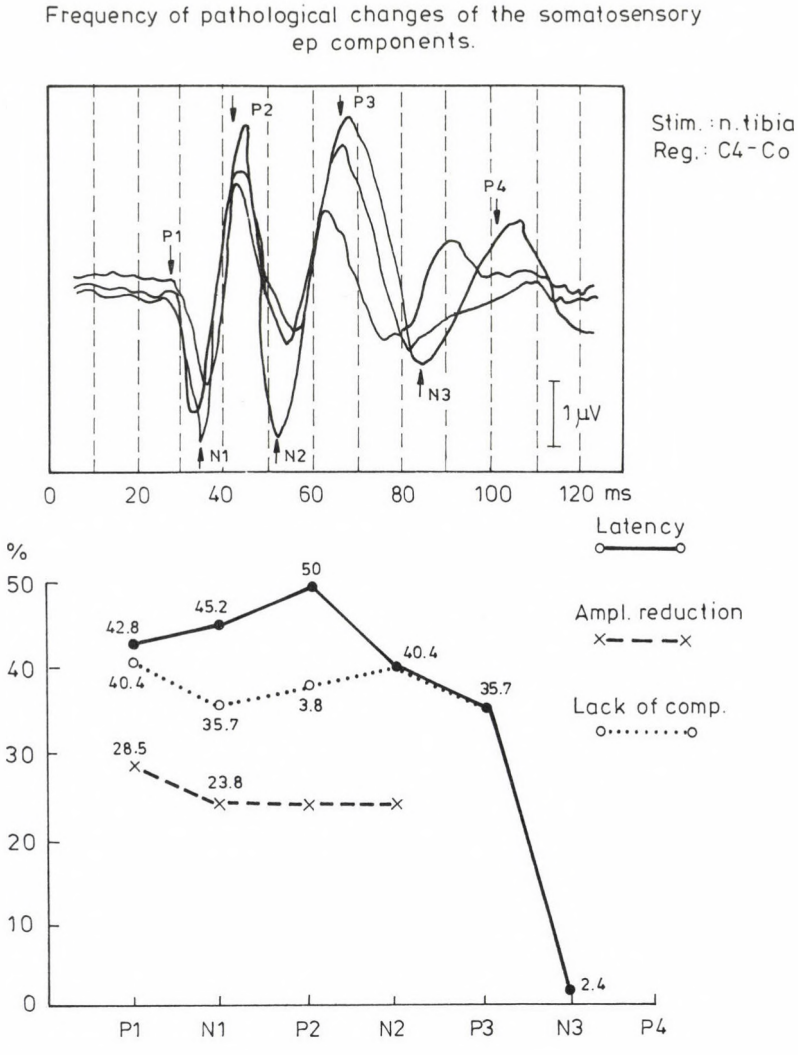

Fig. 3. Upper part: the normal TPSEP. Lower part: the frequency (in%) of the different alterations of the evoked potentials

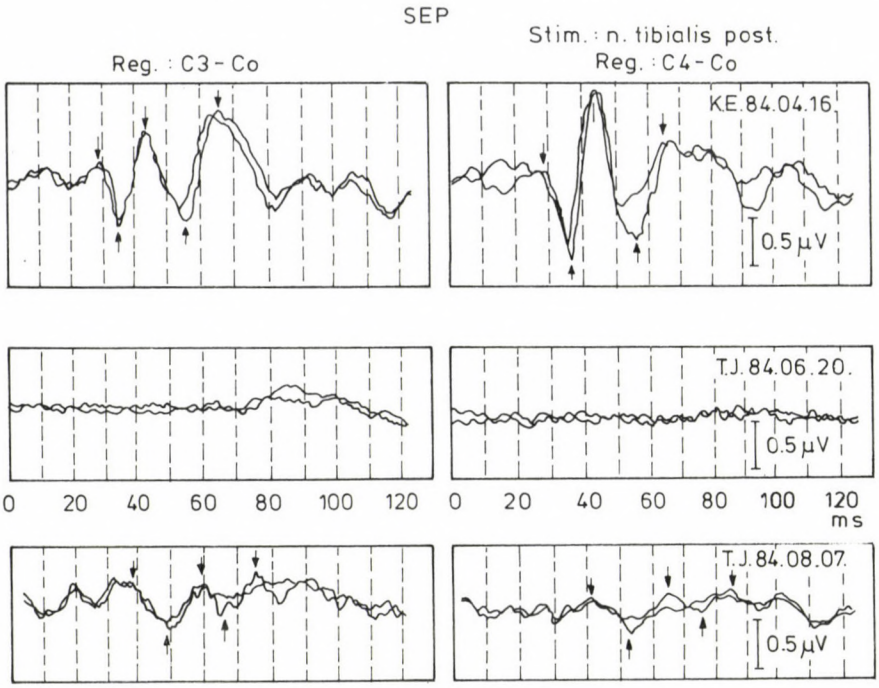

Fig. 4. Upper part: Normal TPSEP. Middle: The record of a 24 years old women. (Clinically definite MS with generalized symptomatology.) No response is detectable. Lower part: The response of the same patient after 6 weeks steroid therapy. The components can be identified, their latencies are increased

Discussion

The proposed value of the components in localization - based on the literature - are summarized in Table II. The potential of the plexus ischiadicus appears to be within 11-21 ms; transmission of the sensory impulse in the spinal cord lasts 21-27 ms. Potentials of the nucleus gracilis and the lemniscus medialis are reflected by the waves between 24 and 31 ms, those of the thalamus about 30 ms, the cortical response appears between 30 and 75 ms /1, 15, 21, 25, 26, 28, 29, 32, 33, 34, 38, 42, 43, 44, 46, 48, 52, 54, 55/. Because of the optimal recording possibility of the vector of the cortical generator of the lower limb, the EPs elicited by the stimulation of the posterior tibial nerve is of higher voltage on the side of the stimulation /10/. It seems possible that the stimulation of the

Table II

The probable origin of the SEP components

	Plexus and roots	Posterior column: Lumbal	Thoraic	Cervical	N.gracilis	Lemn.med.	Thalamus	Gy.centr.ant.	Gy.centr.post.	Pariet.gyr.
PHILLIPS et al. 1980	N1	N2								
LUEDERS et al. 1980		N 24			N 26 - P 27		N 30		P 35	
KAKIGI et al. 1981		P 29			N 27 - P 28 - N 31				P 36	
LEANDRI et al. 1982	P11-N17-P21	N24(conus)			P 27		N 31			
ABBRUZZESE 1982	N17 - 18	P21 - N23								
LASTIMOSA 1982					P 24 - 27			N 33 - P 37 - N 45 - P 55		
KAKIGI 1983						P 28-N 31		N 32(c)	P 36	
SEYAL et al. 1983						P 28	P 31	N 34	P 38 N 38	
LUEDERS 1983				N24 P27		N 30				
MAUGIERE et al. 1983							N 16		N 20 P 27-45	
YAMADA et al. 1983								N 29	N 32	N 34-60
SMALL et al. 1984		N21			N 27 - 29 P 33					
TSUJI et al. 1984		N18-N20		N18-N20	N 24	P 27 N 30			P 38 N 46	
DESMEDT et al. 1985		N20-F21-P26				P 30		N33(c) N37(c) P58(b) N75(i)	N33(c) P58(b) N75(i)	N33(c) P38(i)P58(b) N75(i)
GILMORE et al. 1985	(N5)	N14		N20						
SEYAL et al. 1985		P22(-N22)		$^{O}22$						

posterior tibial nerve is mainly transmitted through the posterior fascicle, wherease that of the sural nerve is through the spinothalamic system /42/. An increase in the speed of conduction at the central areas was observed when the proximal part of the tibial nerves was stimulated /37/. The conduction velocity at different segments can be calculated on the basis of latency differences corresponding to different levels of the afferent system or by using the results of stimulations at various heights /6, 19/ viz. cauda equina: 54 m/s (38), "whole" spinal core: 65.8 m/s /23/, 45-65 m/s /6/, 64 m/s /22/, lower thoracic part: 49 m/s upper thoracic part: 100 m/s /37/.

SEP analysis may also be helpful in localization of vascular diseases /5, 8, 13, 14, 20, 51/, it can be properly used for intraoperative monitoring, and has been widely used in the analysis of root and peripheral nerve lesions and myelopathies and degenerative diseases /2, 17, 47, 53, 56/, but the most fruitful field of its application is MS.

It was Namerov /36/ who succeeded for the first time in eliciting somatosensory EPs and observed altered recovery function of the sensory system. Baker et al. /3/ found pathological EP alterations in more than two-thirds of their MS patients. Since the early seventies this procedure has become a routine method /7, 45/. According to Walsh et al. /50/ the SEP was altered in a higher proportion than the visual evoked potentials; only one patient was found (out of 56) with normal response.

Jörg /24/ summarized the alterations observed in his MS patients (following stimulations of the median nerve, the posterior tibial nerve and segmental stimulation): (1) an increase in the latencies of the cortical SEP components, (2) an increase in the difference between the potentials registered at the left and right sides, (3) an increase in the central conduction time; (4) and increase in the inter-peak latency of the subcortical potential; (5) an increase in the refractory period; (6) disseminated alterations in the segmental SEP. According to our data the reduction of amplitude (or lack) or components is frequent as well.

Responses elicited by stimulating the lower limb nerves were more often pathological than the potentials evoked by stimulating the upper ones /7, 18, 39, 40, 45, 54/. The prolonged latencies of the potentials usually do not disappear in the course of the pathological process, however, in rare cases, they might return to normal /16, 17, 22/. Davis et al. /11/ concluded - on the basis of analysing mainly spinal cases - that subclinical lesions were detected in high proportions.

Among EPs the response elicited by the stimulation of the tibial nerve is of outstanding significance. When patients with MS are examined, it is the most sensitive electrophysiological parameter, which can mainly be used for detecting spinal cord lesions.

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NON-SPECIFIC PEROXIDASE (Donor: H_2O_2-oxidoreductase, EC 1.11.1.7) ACTIVITY IN MULTIPLE SCLEROSIS

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The non-specific peroxidase (donor: H_2O_2-oxidoreductase, EC 1.11.1.7) activity of red blood cells in patients with multiple sclerosis, patients with other neurological diseases, and healthy control individuals was investigated. To this end, a simple method was developed. No significant difference was found in the non-specific peroxidase activity of red blood cells from patients with multiple sclerosis and controls.

Keywords: multiple sclerosis, red blood cells, non-specific peroxidase activity.

Introduction

Red blood cells (RBC) of patients with multiple sclerosis (MS) show several abnormalities. It has been reported that there is an increase in the osmotic and mechanical fragility of MS RBC /3, 5, 13/. Also, these cells show decreased electrophoretic mobility /17/, and an abnormal correlation between the levels of linoleate and arachidonate has been found /4/. A decreased adherence of MS RBC to myelin basic protein has also been reported /10/.

Glutathione peroxidase is believed to be a key enzyme in the maintenance of the integrity of RBC membranes /9/ and plays a major role in the

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Abbreviations: HC = healthy controls; HRPO = horseradisch peroxidase; MS = multiple sclerosis; OND = other neurological diseases

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disposal of peroxides from living cells /11/. It has previously been proposed that abnormal lipid peroxidation may play a role in the pathogenesis of MS /8, 12/. Several authors have found a mild, but significant, erythrocyte glutathione peroxidase deficiency in MS /6, 14-16/, while others failed to detect any significant change in the activity of this enzyme /1, 7/.

These observations prompted us to study the non-specific peroxidase activity of RBC in patients with MS, patients with other neurological diseases (OND) and in healthy control (HC) individuals. A simple method has been developed to measure the non-specific peroxidase activity of red blood cells, using hydrogen peroxide (H_2O_2) and o-phenylenediamine (OPD) as substrates in a colorimetric reaction.

MATERIALS AND METHODS

Thirty eight MS patients (22 F, 16 M, mean age 43 years, range 25-64; mean duration of disease 12 years, range 1-26), 31 patients with OND (18 F, 13 M, mean age 45 years, range 18-73), and 21 HC individuals (13 F, 8 M, mean age 35 years, range 24-81) were included in the study. Two of the cases in the MS group were later autopsy-proven, 20 fulfilled the criteria of definite, 6 of probable and 10 of possible MS /2/. Two of the MS patients received steroid treatment, while the others were on vitamin B treatment, or were given no drugs at the time their blood samples were collected. The OND group included patients with epilepsy (four), Guillain-Barré syndrome (one), cerebrovascular syndromes (eighteen), Parkinson's disease (one), amyotrophic lateral sclerosis (one), and others (six).

Fifty µl of blood was drawn by fingertip puncture during the morning hours after a light breakfast, and was immediately diluted, and washed 3 times in 10 ml of phosphate-buffered saline (PBS; 10 mM phosphate, 150 mM sodium chloride, pH 7.2). The cells were collected by centrifugation at 200 x g for 10 min. The cell density was adjusted to 1×10^6 RBC per ml of PBS, the cells were centrifuged, the supernatant was aspirated and replaced with the same volume of distilled water. After haemolysis, the samples were stored in 1 ml volumes at $-20^{\circ}C$.

The defrosted samples were diluted 1:5 in distilled water, giving a haemolysate equivalent to 2×10^5 RBC per ml. One hundred µl of haemolysate was applied, in triplicates, into the wells of a 96-well microtiter tray. Also, 100 µl of 0.5×10^{-3}, 1.0×10^{-3}, 1.5×10^{-3}, 2.0×10^{-3}, 2.5×10^{-3}, 3.0×10^{-3} or $t.0 \times 10^{-3}$ µ g of horseradish peroxidase (HRPO, type I, EC 1.11.1.7, specific activity 95 U/mg, Sigma) per ml of distilled water, was used in triplicates as a standard in separate wells. The reaction was started by adding 100 µl of freshly-prepared colorimetric reagent (0.2 M citric acid-phosphate buffer, pH 5.0, containing 1 mg/ml of OPD, and 0.022% H_2O_2) to each well with samples prewarmed to $37^{\circ}C$. After 7 min of incubation at $37^{\circ}C$, the reaction was terminated by adding 50 µl of 1 M sulphuric acid. The optical densities were measured at 492 nm in a Specord (GDR) spectrophotometer equipped with a microcuvette. The activity detected in 100 µl of cell lysate (equivalent to 2×10^4 RBC) was expressed as the amount of HRPO producing colorimetric reaction of the same intensity as that detected in the cell lysate. In order to reduce the error of the method, samples of 15 subjects (5 from each group) were assayed simultaneously on the same tray.

The amount of H_2O_2 sufficient to ensure saturation of the enzyme, and the time during which the relationship between the amount of oxydized OPD and the reaction time is linear, was determined in preliminary experiments. In intra- and interassay reproducibility experiments the average standard error of the mean was 1.6% and 10.7% of the mean value, respectively.

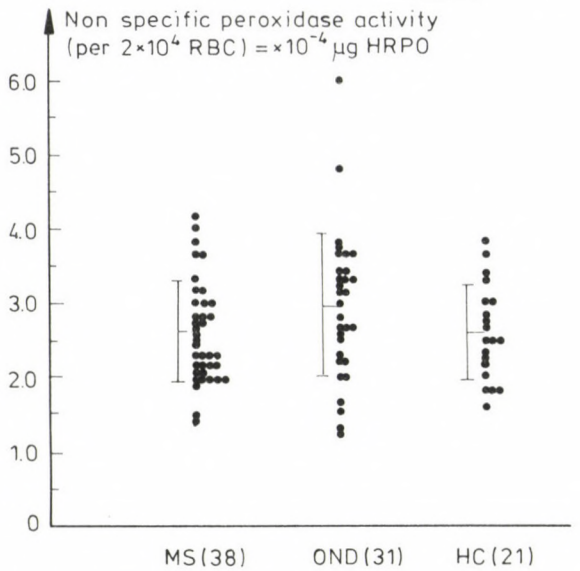

Fig. 1. Distribution of non-specific peroxidase activity in red blood cells (RBC) of multiple sclerosis (MS) patients, patients with other neurological diseases (OND), and healthy control (HC) individuals. Results are expressed as the amount of horseradish peroxidase (HRPO) producing colorimetric reaction of the intensity detected in $2x10^4$ RBC.4
Bars represent the mean ± S.D.

RESULTS AND DISCUSSION

The distribution of non-specific peroxidase activity in RBC of patients and controls is shown in Fig. 1. The mean peroxidase activity measured (± standard deviation) in 38 patients with MS, 31 with OND and 21 HC individuals was equivalent to the activity of: $2.6\pm0.7 \times 10^{-4}$; $2.9\pm1 \times 10^{-4}$; and $2.6\pm0.6 \times 10^{-4}$ µg of HRPO in $2x10^4$ RBC, respectively.

The method used in this study is very similar to that in the enzyme-linked immunosorbent assay (ELISA). The hydrogen peroxide is degraded by H_2O_2-oxidoreductase and the OPD is converted into its oxidized form, resulting in a colorimetric reaction. Experiments with purified catalase showed that although H_2O_2 is readily degraded by this enzyme, no colorimetric reaction develops in the presence of OPD (data not shown). Furthermore, when increasing amounts of catalase were added to the reaction mixture 7 min prior to the addition of HRPO, a competitive inhibition of colorimetric reaction was observed. Since for a 50% inhibition, a catalase/HRPO ratio of 2000 was necessary, it is unlikely that the RBC catalase and

peroxidase interfere with each other at a significant level in our assay system.

Earlier studies have reported decreased glutathione peroxidase activity in RBC of multiple sclerosis patients /6, 14-16/, however a causative role has not been established. Other studies failed to detect a glutathione peroxidase deficiency in MS /1, 7/. Our work does not reveal a deficiency in the non-specific peroxidase activity of RBC in MS.

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GYNAECOLOGY

TISSUE CHARACTERIZATION BY TRANSVAGINAL COLOUR DOPPLER FOR THE EVALUATION OF GYNAECOLOGICAL TUMOURS

First of two parts: review of the literature

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This is the first review published in Hungary about the usefulness of transvaginal colour Doppler (TVCD) in gynaecology. The history of "ultrasound tissue characterization", the conventional and colour Doppler methods are discussed as well as the pathomorphological basis of abnormal Doppler signals. The summary of tumourangiogenesis, of neovascularization and its manifestation in Doppler spectrum are given. The differences between normal and pathological Doppler signals in female small pelvis are shown. It is concluded that the routine application of TVCD will reduce the rate of false positive results and "luxury" operations from screening procedures.

Keywords: colour Doppler ultrasound, colour flow mapping, transvaginal sonography, gynaecological malignancy, tissue characterization.

Introduction

The term "tissue characterization" covers a range of meanings from qualitative assessment to scientific measurement /10/. Many attempts have been made in the past 20 years to derive more quantitative information relating to tissue structure from the returned ultrasound beam. Various acoustical properties of tissues - absorption, speed, dispersion, density, compressibility, bulk modulus, scattering characteristics, attenuation - might be probed by "ultrasonic telehistology" (a term coined by Chivers and Hill /9/).

Abbreviations: TAF = tumour-angiogenesis factor; TVCD = transvaginal colour Doppler

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Of particular appeal was the possibility that information relating to specific histology was already available in the returned echoes and, at least potentially, specific predictors of tissue type might be defined merely by more elaborate signal analysis. An index of quantitative tissue echogenicity was developed for the diagnosis of liver disease /71/.

Others used a simple electronic system adaptable to a standard echograph with the aim of obtaining an index of echogenicity /14/. A further team /31/ undertook to develop a system for obtaining information from B-mode ultrasonography, which converts information into digital values and processes them in a microcomputer to produce an acoustic intensity histogram. Display information on the histogram pattern includes echo intensity and echo frequency in the region of interest. The curve of attenuation rate derived from the histogram was used to distinguish between liver cirrhosis and the tissue of normal or fatty liver.

Other conceptual developments that have taken place since then have been noninvasive detection of blood flow by Doppler /72/, tissue motion /68/ and image texture /43/ as possible indicators of histology and pathology.

There are at least seven characteristics that can be measured by ultrasound:

1) the attenuation of ultrasound /45, 73/
2) the speed of ultrasound /25/
3) the acoustic impedance /29/
4) the scattering characteristics /11/
5) the nonlinearity of signal propagation /42/
6) the motion of tissue /68/
7) blood flow can be detected and quantified by techniques based on the Doppler effect /4, 5, 72, 75/.

Despite all this effort, only image inspection and Doppler techniques have shown progress toward the concept of telehistology, and it is really only through them that tissue characterization has achieved routine clinical application.

Tissue characterization by conventional Doppler equipments

The presence of abnormal flow spectra around the periphery of malignant tumours was documented in cases of breast cancers /4, 72/. The results were confirmed by several groups /32, 34, 44, 46, 53, 54, 61-64/.

The abnormal flow signals consisted of an increase in signal amplitude when compared with the contralateral normal side (corresponding to a greater number of moving cells within the beam), an increase in peak systolic flow; a characteristic distribution of the Doppler spectrum, showing a predominance of high-power, low-frequency element; high diastolic shift (in some tumours the systolic/diastolic variation is absent).

The major difficulty with both continuous-wave Doppler (CWD) and pulsed-wave Doppler (PWD) is the need for operator expertise and time to search the periphery of the tumour and detect one of the limited number of localized abnormal vessels or shunts. Colour Doppler, by showing a two-dimensional display of vascularity, can expedite the examination by displaying simultaneously the presence of the vessels, which can then be interrogated for quantitative analysis by pulsed Doppler.

Tissue characterization by colour Doppler

Most colour Doppler machines have been developed for cardiac ultrasound; they are insensitive for detecting neovascular flow. It is not enough to merely demonstrate a vessel in the vicinity of a tumour, the diagnostic features of tumour vascularity are the high velocities and low impedance. Considerable modification in signal processing was necessary to optimize the colour Doppler machines for detecting neovascular flow characteristics. Nevertheless, some of the more sensitive machines already show this possibility (Aloka SSD-350, Aloka SSD-860, Sonotron Vingmed CFM 700).

Using an ultrasonic dynamic flow imager it was possible to display neovascularization in a rabbit VX2 carcinoma and also to visualize areas of multidirectional flow presumably due to complex arterial patterns and arteriovenous shunts /50/.

Blood flow in several types of tumours was assessed with colour Doppler echography, and invasive moles were considered to be the best indication of response to chemotherapy /51/.

Taylor et al. /62/, then Hata et al. /26, 27/ were the first to publish Doppler ultrasound studies in pelvic diseases of women, moreover the latter group used transabdominal colour Doppler equipment, (previously only the Doppler ultrasound identification and assessment of deep-lying vessels of the normal /58-60/ and pregnant /6, 69/ female pelvis were made. In all cases of endometrial carcinoma, ovarian carcinoma and trophoblastic disease typically abnormal flows were observed /26, 27/, but all cases of cervical carcinoma with abnormal flows were of stage II/b or over. It was concluded that Doppler ultrasound is a pertinent diagnostic tool that can be used to observe changes in tumour vascularity in gynaecologic malignancies before and after treatment.

Colour Doppler sonography has some advantages in comparison with other methods.

i) The visualization of blood flow in the region of interest makes the orientation much easier and makes the search for disturbed flow in small, non-visible vessels possible. This feature is of utmost importance in the examination of tumours where the distribution of blood vessels is never known in advance. It is this search ability which makes it potentially so powerful in solving some of the diagnositc problems unsolved so far.

ii) The most important advantage of this diagnositc tool is the display of blood flow over the whole female pelvis as compared with the one line of sight available with conventional pulsed Doppler. By this means the technique is simple to perform, easy to interprete and quick.

iii) The more accurate placement of pulsed Doppler beam has made the reproducibility of Doppler measurements increased.

Transvaginal colour Doppler

Kurjak et al. /25, 36-40/, and Zalud and Kurjak /77/ were the first to report that transvaginal colour flow imaging can be used in the assessment of pelvic circulation, and to differentiate between benign and malignant pelvic tumour.

With transvaginal colour Doppler it was shown /3/ that the absence of intratumoural neovascularization and a normal (high) pulsatility index can be used to exclude the presence of invasive primary ovarian cancer, thus, potentially reducing the rate of false positive results from the screening procedure using conventional ultrasonography while maintaining the detection rate of the disease. The early recognition of ovarian cancer is the only approach to achieve a reduction in the specific mortality. Transvaginal colour flow mapping may be used to identify potentially malignant ovarian masses and help in elucidating the early stages of tumourigenesis. The routine application of this new technique will enable us to develop a screening programme based on ultrasonography.

In a preliminary report /28/ it has been confirmed that transvaginal colour Doppler is expected to be an important diagnostic method for assessing blood flow in physiologic and pathologic conditions of the pelvis. A transoesophageal probe was used in these studies.

What is the feature that provides the abnormal Doppler signals? This pathomorphological entity is the tumour-neoangiogenesis = neovascularization.

Neovascularization

Many influences involving the neoplasm and host modify the growth rate of cancers. One of the most important conditions is an adequate vascular supply of the tumour.

During experiments with heterologous tumour transplantation in guinea pig eyes, it was observed that a few implants, which apparently did not vascularize failed to grow for almost 2 years. However, when the same tumours were reimplanted into their original host, they vascularized and grew progressively /23/.

Others suggested that an attribute of tumour cells is their capacity to elicit continuosly the growth of new capillary endothelium in vivo /2/.

Folkamn et al. /16/ demonstrated that the growth of tumours which have been implanted in any one of several different organs and maintained by a long-term perfusion stops when the tumour reaches a diameter of 3-4 mm.

Further growth of tumour tissue in in vitro organ cultures cannot be sustained without neovascularization of the tumour /22/.

Neovascularisation does not require direct contact with tumour cells since vessels have been elicited from the hamster cheek pouch by tumours contained in a millipore filter /12, 24/.

Folkman et al. /17/ have shown that a diffusible factor, mitogenic for capillary endothelium, can be isolated from animal and human tumour cells. This factor called "tumour-angiogenesis factor" (TAF) and the concept of "anti-angiogenesis" was proposed to indicate that blockade of TAF might prevent solid tumours from growing beyond a diameter of 2-3 mm /18/.

When small fragments of anaplastic Brown-Pearce carcinoma were implanted directly on the iris in susceptible rabbits, the fragments always vascularized. A characteristic growth pattern, consisting of prevascular, vascular, and late phase was observed, which terminated with destruction of the eye within 2 weeks. The beginning of exponential volume increase was shown to coincide with vascularization of the implant.

In contrast, implants placed in the anterior chamber, at a distance from the iris, did not become vascularized. After initial growth into spheroids, they remained arrested at a small size, comparable to prevascular iris implants, for periods as long as 6 weeks. Although dormant in terms of expansion, these avascular tumours contained a population of viable and mitotically active tumour cells. When reimplanted on the iris, vascularization was followed by a rapid, invasive growth. These observations suggest that neovascularization is a necessary condition for malignant growth of solid tumour /22/.

Recent studies /19/ with transgenic mice have shown that for at least one type of cancer angiogenesis occurs during the transition from hyperplasia to neoplasia, and the induction of angiogenesis is an important step in carcinogenesis.

Both animal and human tumour cells have been shown to produce angiogenesis factors /8, 13, 15, 49, 55, 57, 70, 76/.

Other workers have recently identified and cloned a new platelet derived substance that stimulates endothelial cell growth and chemotaxis in vitro, and angiogenesis in vivo /30/.

The identification of TAFs raised an interesting possibility cancer growth was blocked by inhibiting the angiogenesis. Indeed, an "anti-angiogenesis" factor has been extracted from cartilage, a tissue that normally lacks vascularization. Infusion of this component of cartilage reduces the growth rate of experimental neoplasms in animals /41/. It was also demonstrated /66/ that heparin released by mast cells (accumulated at a tumour site before the ingrowth of new capillary sprouts but that mast cells alone could not initiate angiogenesis) increased the migration of capillary endothelial cells in vitro and enhanced the intensity of angiogenesis induced by tumour extract in vivo (but heparin alone could not initiate angiogenesis).

In contrast, protamine (a heparin antagonist) blocked the ability of mast cells and heparin to stimulate migration of capillary endothelial cells in vitro and inhibited tumour angiogenesis and subsequent tumour growth when it was applied locally but it had no effect on established capillaries that were not proliferating.

Another inhibitor of angiogenesis, which is produced by cells when they are capable of expressing an active cancer-suppressing gene, has also been discovered /48/. The loss of this inhibitor activity occurs concomitantly with expression of both angiogenesis and tumourigenesis.

Summary of the connection between tumour growth and neovascularization

Rapid cellular proliferation of and increased metabolic demand by, cancer cells call for an increased supply of nutrients and oxygen. This increased demand is met with by simple diffusion of tissue fluid in the initial part of tumour growth. However, with continued growth this proves inadequate as the central part of the tumour becomes distant from the source of supply, therefore, the need for a well-developed tumour vasculature of its own arises. This is initiated by production of tumour angiogenesis factor(s) - by the tumour cells. TAF appears to induce mitosis and migration of endothelial cells from the surrounding small vessels. These vessels soon canalize and grow towards the tumour, forming a complex network of tumour vasculature. Vascularization of tumours is shown to be associated with an increase in growth rate and acquisition of metastatic potential. Tumour volume doubling time and tumour cell loss factor decrease as the tumour vascularity increases. The fraction of cells in the S phase and growth fraction are higher, having higher vascularity. Thus, high vascularity is associated with rapid growth. The new vessels must be formed very early on in the development of the tumour and therefore are early markers of a tumour.

Characteristics of neovascularization

The original description of arteriographic neovascularity or "tumour vascularity" is credited to Strickland /56/. He described a tumour vessel as one that was "deployed seemingly without purpose, keeps to no set course and shown no progressive diminution in caliber". Several benign and malignant tumours were examined /20/ to evaluate the histologic picture as shown on angiograms as neovascularity. Newly-formed vessels are large capillaries (rather large endothelial lined capillary-like channels), or sinusoids, and neither contain smooth muscle in their walls, only some fibrous connective

tissue. "Puddling", "laking" and "staining" represent the collection of contrast medium in small capillaries or sinusoids. Smooth muscle neither regenerates nor proliferates to any significant degree in adults.

If normal smooth muscle can be found in the walls of arteries with a normal architecture, then one may assume that the artery is not new and it does not represent neovascularity.

Another vascular alteration in malignancy is the presence of arteriovenous shunting represented angiographically by early venous opacification. Arteriovenous shunting may result from a large, focal, direct communication /47/, or may reflect multiple microscopic communications in the abnormal tumour microcirculation /33, 67/. "Pooling" or "laking" result from tumour vessels terminating in amorphous spaces within the tumour parenchyma with or without associated necrosis. A tumour "stain" or "blush" reflects the accumulation of contrast material in microscopic spaces. Since the vessels are collapsed in a histologic section, it is difficult to know how large these spaces are in vivo. Shubik /52/ contrasted the appearance of a living tumour - in which the vessels may occupy more than 50% of the tumour volume - with the collapse of the vascular bed in the dying host and the dramatic decrease in tumour vessel size in the fixed specimen.

The manifestation of neovascularization in Doppler spectrum

Since most of the resistance to flow resides at the level of the muscular arterioles, vessels deficient in these muscular elements present diminished peripheral resistance to flow and thereby receive a larger volume flow than vessels with a high impedance. The abnormal, thin-walled vessels would exhibit low-impedance signals with little systolic-diastolic variation. Because of their lack of normal muscle components, the amount of vasoconstriction by sympathetic stimulation cannot be increase - this is the basis for differentiation between benign and malignant tumour vascularity by their response to epinephrine /1/.

The Doppler detection of the haemodynamics of flow through these spaces is thus a more specific tumour marker than the mere vascular morphology shown by imaging studies alone /63/.

Differences between normal and pathological Doppler signals in female small pelvis

Normal waveforms

The use of Doppler ultrasound in the clinical assessment various diseases suggested that each artery appears to have its own characteristic Doppler waveform, which may be modified by disease. Taylor et al. /58/ and Taylor and Burns /60/ have demonstrated characteristic waveforms for iliac, uterine and ovarian vessels and also the changes in these waveforms associated with hormonal changes during menstrual cycle and/or early pregnancy.

It should be noted that AORTA, COMMON ILIAC and EXTERNAL ILIAC arteries all show similar waveforms of plug flow, which is virtually a square wavefront. The waveforms outline the envelope of the spectrum with "no filling in", that is, not "spectral broadening". In early diastole, there is a reversed component due to reversed flow from the high impedance vasculature of the legs during diastole.

In contrast, INTERNAL ILIAC waveform shows complete spectral broadening. In other words, there is parabolic flow with all velocities present from zero at the wall to a maximum in the mid-stream. A characteristic of the internal iliac waveform is a diastolic hump. It is important to recognize this characteristic waveform since the internal iliac artery is seen most frequently just inferior to the ovary and is easily detected in the vicinity of the ovary. If the edge of the vessel is sampled with pulsed Doppler, a waveform is seen which looks very similar to the resting ovarian signal.

The UTERINE waveform varies by the state of pregnancy. In the non-gravid the waveform is of high impedance with little or no diastolic flow. Both ascending and descending branches of the uterine artery can be located even in the non-pregnant patient.

The OVARIAN vessels can be sampled by the Doppler beam utilizing a small sample volume and locating the cursor in the infundibulopelvic ligament just lateral to the ovary. In normal individuals it was noted that the ovarian signals appeared to change with time over the menstrual period. It means, enhanced flow was documented with an increased diastolic component during the formation and activity of the corpus luteum. At the first week both ovaries show a high impedance flow with virtual absence of diastolic flow. In the artery of the inactive ovary these high impedance signals

persist during the cycle. At ovulation in the artery of the active ovary there is a marked diastolic component as well as increased peak velocities. Compared with those of the inactive ovary, these signals are very easy to elicit. These changes persist and become more marked by middle luteal phase but regress to a high impedance signal by the day of next menstruation.

Pathological waveforms

Features of neovascularity determine types of Doppler signals.

ARTERIOVENOUS SHUNTS give rise to high-velocity flow because of the pressure gradients. These shunts are remarkable on account of the extreme velocities that may occur in them. Pressure energy is converted to kinetic energy to give the high velocities which can be detected by a Doppler device. These small vessels around the periphery of a tumour have blood velocities (70-700 cm/s) many times exceeding those in the normal aorta /63, 64/. It is unlikely that these high velocities could be supported in vessels smaller than 1.5 mm in diameter. Although vessels of this magnitude can be seen angiographically on the periphery of many tumours, such large vessels are seldom seen histologically even when specifically sought, because of collapse and shrinkage of tumour vessels during fixation.

ENDOTHELIAL LINED LARGE CAPILLARY-LIKE CHANNELS or SINUSOIDAL SPACES make a flow accross a moderate pressure gradient into a very low impedance vascular place possible. The type of signal originated from this sinusoidal channels exhibits little systolic/diastolic variation with relatively high velocity in addition to their very low impedance - this makes these signals highly abnormal.

For this reason, the several pulsatility and resistance indexes alone may be insufficient to characterize such tumour signals since similarly low pulsatility and resistance is found in normal parenchymal flow but without high velocity. Moderately high Doppler shifts indicate that some cells are moving rapidly, but the complete broadening and even the concentration of energies at the lower velocities (indicating by the whiteness of the tracing) suggests that many red blood cells are moving slowly, so that the peripheral flow in these sinusoidal spaces may be quite sluggish, accounting for the prolonged opacification interpreted as "dense parenchymal staining" on angiograms.

Remarks

Angiogenesis, the growth of new capillary blood vessels, does not occur normally in the postnatal life of males, but in women new vessels are produced in the vascularization of the corpus luteum each month, and, of course, during pregnancy. Neovascularization is also a component in pathological processes, such as wound healing, inflammatory and certain immune responses and continuous growth of solid neoplasms.

For the above-mentioned corpus luteum formation we screen premenopausal women for early ovarian cancer only during the first week of the menstrual cycle and take care to avoid the time of ovulation and the presence of corpus luteum and pregnancy.

Campbell et al. /7/ reported the results of a conventional transabdominal ultrasound screening for ovarial carcinoma, and they concluded that it was not possible to differentiate between ultrasonic appearance of early malignant and benign tumours. The odds against a positive screen result indicating the presence of primary ovarian cancer were only 1:67.

The routine application of transvaginal colour Doppler will enable us to determine the extent to which women with hydrosalpinx, tumour-like conditions or benign tumours can be identified and save unnecessary operations, but a low resistance index can be used as a mark of malignity even if the ovary is normal in size and there are no other suspicious signs.

In this way one can reduce the rate of false positive results and "luxury" operations from screening procedure using conventional ultrasonography while maintain the detection rate of ovarian cancer.

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TISSUE CHARACTERIZATION BY TRANSVAGINAL COLOUR DOPPLER FOR THE EVALUATION OF GYNAECOLOGICAL TUMOURS

Second of two parts: clinical experiences

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Transvaginal colour Doppler was used to evaluate the blood flow patterns in pelvic vessels in a group of 315 patients including 168 with uterine tumours and 147 with adnexal masses. Neovascularization of malignant tumour tissue was successfully displayed by colour Doppler in the vases of endometrial and ovarian cancers but no abnormal blood supply was observed in the cases of early cervical cancers. A comparison between the characteristics of blood flow within benign and malignant lesions showed lower resistance index in cases of malignancy. The sensitivity, specifity, positive predictive value, negative predictive value and the diagnostic accuracy of this new method in the recognition of endometrial and ovarian cancers are higher than 95%. By the help of transvaginal colour Doppler (together with the classical methods as colposcopy, cytology etc.) it will be possible to establish of complex screening programmes for all types of gynaecological cancers.

Keywords: colour Doppler ultrasound, colour flow mapping, transvaginal sonography, gynaecological malignancy, tissue characterization.

Introduction

We have shown /5/ that image inspection and Doppler techniques are in progress toward the concept of tissue characterization by ultrasound. Colour Doppler and transvaginal sonography are the most exciting recent developments in the field of obstetrical and gynaecological diagnostic ultrasonic techniques /6-11/.

Abbreviations: NPV = negative praedictive value; PPV = positive praedictive value; RI = resistance index

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In the present work we investigated the vascularity of female pelvic tumours by obtained colour Doppler signals, using a transvaginal probe.

Subjects and Methods

PATIENTS

In a group of 168 patients with proven uterine tumours transvaginal colour Doppler studies were performed before operation at the Ultrasonic Institute University of Zagreb. The ultrasonographer was informed about the result of transabdominal ultrasound and date of the last menstrual period, but he was not about clinical findings and indications for treatment.

Table I

Types of operations because of uterine tumours (n=168)

HISTOLOGY	OPERATIONS		
	Conisatio	TAH	Wertheim op.
Adenoca. endom.		7	2
Leiomyosarcoma ut.		1	
Ca.cerv.ut.st."0"	4		(16 malignant)
Ca.cerv.ut.st.I/b			2
Adenomyosis ut.		21	
			(152 benign)
Myoma ut.		131	

The type of operations in relation to the pathological diagnosis is listed in Tabel I.

Laparotomies were performed on 147 other patients with adnexal masses, among them 22 cases with primary cancer, 2 metastatic ovarian cancer and 123 with benign lesions or tumour-like conditions.

TRANSVAGINAL COLOUR DOPPLER (TV-CD)

Patients with empty bladder were examined in lithothomy position with a 5 MHz transvaginal colour and pulsed-wave Doppler (TV-CD + PWD) probe (Aloka colour Doppler SSD-860, Aloka Co., Japan). The probe was covered with a coupling gel and inserted in a condom, which was coated with gel and inserted into the vagina. Premenopausal women were scanned during the first week of the menstrual cycle to exclude changes in intraovarian blood flow that are known to occur during corpus luteum formation. The spatial peak temporal average intensity was 80 mW/cm^2, which is well within the highest limit recommended by the Food and Drug Administration of the U.S.A. In the 2-D Doppler mode, the flow directed toward the transducer was displayed in shades of red, the flow directed away from the transducer was in shades of blue, and the velocity of flow was coded by the brightness of colour. Frequency dispersion was determined as the variance of the signal by the autocorrelator. It was added to the velocity signal as an alternate hue such as green, which made the turbulent blood flow appear not as a pure red or blue, but as a distinctive speckled yellow or cyan (mosaic appearance). The maximum velocity of the flow which exceeded the Nyquist limit was presented as colour alias-

ing. Pulse repetition frequencies of this apparatus ranged from 2 to 32 kHz. Blood flow velocities were displayed within a 90^{o} sector at depths ranging from 2 to 20 cm. In the 2-D and PWD modes, velocities from 10 to 150 cm/s can be measured. Wall filters (100 Hz) were used to eliminate low frequency signals originating from noise. In addition to colour Doppler, the machine is equipped with a conventional PWD system, which can be used simultaneously with B-mode imaging superimposed with colours as in "duplex"-systems.

Colour Doppler was used first to visualize circulation in major vessels, then a systematic evaluation of the uterine, ovarian and tumour tissue was done. The ovaries were often easier to identify in premenopausal women, owing to the presence of follicles. After menopausa, the ovaries tended to decrease in size and became more uniformly echogenic, but their position was invariably found or confirmed by reference to the uterus and the iliac vasculature.

Blood flow characteristics were further analysed by PWD. A sample volume on the line of the PWD beam was placed at the region of interest, where the colour flow was clearly noted. Prominent areas of vascularization (probably reflecting neovascularization) usually appeared as continuosuly fluctuating colour rather than the colour seen with normal arteries. The angle of the transducer was moved to obtain the maximum waveform amplitude and clarity, then the picture was frozen and the resistance index (RI) was calculated electronically from a smooth line fitted to the average waveform over 3-5 cardiac cycles, according to the formula: RI = (A-B)/A, where A equals the peak systolic Doppler shift frequency, B the maximum end diastolic Doppler shift frequency. The lowest RI was used for flow velocity waveform characterization. Sonographic findings and RI-s were compared with operative and histopathological diagnoses. Student's t-test was used for statistical analysis of the obtained RI-s for distinction between benign and malignant lesions. Data were then analysed, based on the principles of test interpretation (see Table II) as published by Griner et al. /3/. Sensitivity addresses the probability of a test being positive if the disease is present. Specificity indicates the likelihood of the result being normal in the absence of the disease. Positive predictive value (PPV) expresses the probability that the disease is present when the test is positive. Negative predictive value (NPV) indicates the probability that disease is not present in a case giving a negative test. The diagnostic accuracy (DgAcc) was calculated to determine the percentage of correct test results whether positive or negative.

Table II

Indices for a test interpretation

Sensitivity	= true positive / (true positive + false negative)
Specificity	= true negative / (true negative + false positive)
PPV	= true positive / (true positive + false positive)
NPV	= true negative / (true negative + false negative)
DgAcc	= correct results/total patients tested

HISTOPATHOLOGY AND CLASSIFICATION

The extent of malignant disease was estimated by the surgeon and the final diagnosis was based on the histology report. All abnormal tissues were classified according to the criteria recommended by the World Health Organisation /12/.

The stage of each primary cancer was determined from the operation records according to the revised recommendation of the International Federation of Gynaecologists and Obstetricians /13/.

Results

There were 16 cases of malignant and 152 cases of benign uterine tumour. Colour flow inside the endometrium and/or myometrium was detected in 8 out of the 9 endometrial carcinomas. The peripheral impedance was always low (RI < 0.5), mean RI $\pm$ S.E. = 0.38 ± 0.06.

Table III

Blood flow patterns of benign and malignant uterine tumours

HISTOLOGY	COLOR FLOW present	not present
Adenoca. endom.	8	1
Leiomyosarcoma ut.		1
Ca.cerv.ut.		6
Adenomyosis ut.		21
Myoma ut.	28	103

Table III shows that in 1 case out of 9 endometrial cancer, in the only 1 case of leiomyosarcoma uteri and in all of 6 early cervical cancers (4 with stage "0" and 2 with stage I/b) we failed to detect any pathological blood flow.

In all of 21 adenomyosis and in 103 cases of myoma uteri, we failed to detect abnormality of blood flow. In 28 out of 131 fibroids we saw flow inside the myometrium (mostly around the order of the fibroid) but RI was always higher than 0.5 (mean RI $\pm$ S.E. = 0.73 ± 0.08). When the RIs were compared in cases of myoma uteri and carcinoma endometrii, significantly lower RIs were obtained in cases of endometrial cancer ($t = 6.9$; $P < 0.01$).

If we leave the cervical cancers out of consideration (because our attempts to visualize blood flow within them were completely unsuccessful but we have an excellent possibility for screening of cervical cancer by a combination of Papnicolau smear with colposcopy) (see Table IV) the sensitivity, specificity, PPV, NPV and DgAcc show good results in the prediction of the dignity of the uterine tumours (Table V).

Table VI shows that colour flow was detected inside the malignant adnexal masses, except in 1 case (stage III, endometrial cancer with metastases in both ovaries), and no flow or flow with high RI was detected in all benign lesions, again except 1 case (hydrosalpinx covered by well-vascularized omentum).

Table IV

Resistance indices (RI) detected in benign and malignant uterine tumours (except cervical cancers)

	Malignant	Benign
RI < 0.5	8	0
RI > 0.5	0	28

(Remark: those cases from Table III when colour flow was present)

Table V

The capability of TV-CD in differentiating of benign and malignant uterine tumour (except cervical cancer) (n=162)

Sensitivity	80%
Specificity	100%
PPV	100%
NPV	98.7%
DgAcc	98.8%

Table VI

Blood flow patterns and resistance indices (RI) of malignant and benign adnexal masses (n = 147)

HISTOLOGY	Number of patients	COLOR FLOW present	not present	RI (±S.D.)
Cystadenoca ov.	20	20	0	0.34 (± 0.09)
Metast.ov.ca.	2	1	1*	0.275(± 0.035)
Granulosacell tu.	2	2	0	0.35 (± 0.10)
Dermoid cyst	8	0	8	
Cysta ov.simpl.	57	11	46	0.68 (± 0.12)
Hydrosalpinx	16	1	15	0.71
Cystadenoma ov.	15	4	11	0.65 (± 0.13)
Endometriosis ov.	19	0	19	
Abscessus	5	0	5	
Pseudocyst	1	1**	0	0.41**
Adhaesions	2	0	2	

(Remarks: * the only one false negative case

** the only one false positive case

explanations see in text)

Table VII

The capability of TV-CD in differentiating benign and malignant adnexal masses

Sensitivity	95.8%
Specificity	99.2%
PPV	95.8%
NPV	99.2%
DgAcc	98.6%

The efficacy of colour Doppler is excellent (Table VII).

Undoubtedly, the most important fact is that 4 out of the 23 true positive cases were recognized with early stages (3 with stage I/a, 1 with stage II) without any symptom, clinical sign or specific ultrasound finding on the transabdominal ultrasonography (only 2 of them had slightly enlarged ovaries). Only the positive transvaginal colour Doppler test (Tabel VIII) served as indication of the operation.

Table VIII

Distribution of patients with ovarian cancer by stages

Stage	Number of patients
I/ai	3
II/a	1
III+IV	20

Discussion

Many clinical problems remain in differentiating benign and malignant tumours. The increasing number of imaging techniques have led to improved detection of space-occupying lesions but created many more problems requiring solution. The major clinical problem is now characterization of such masses.

Ultrasound imaging when carefully done and interpeted by an experienced operator is one of the better techniques for tissue characterization. One feature of malignancy that is well known to radiologists, however, is the bizarr vascular morphology that characterizes many malignant tumours. Flow in these abnormal channels gives rise to characteristics

that can be detected by Doppler techniques. Colour Doppler in the same vaginal probe produces a superb simultaneous visualization of structural and flow information about the female pelvis and offers new insights in dynamic studies of blood flow within tumour masses /6-11, 14/.

Our results show that the absence of intratumoural neovascularisation or flow with a high RI can be used to exclude the presence of invasive endometrial and ovarian cancer. Tumour vascularity can successfully be used for characterization of these tumours. Sensitivity, specificity, PPV, NPV and DgAcc are acceptably high.

Hata et al. /4/ failed to detect colour Doppler signals in early cervical cancers. The vascularity of the uterine cervix might not be so affected by the neoplasm at the early stage of cervical carcinoma and/or the newly-formed vessels are too small, thus the velocity and volume flow are below the resolution power of the equipment.

The goal of transvaginal colour Doppler should be identification of tumours in not enlarged ovaries /2/. This excellent method may be used to screen for ovarian and endometrial cancer; furthermore, the assessment of vascular changes and the peripheral resistance to blood flow may reduce the number of conventional ultrasound scans and other invasive procedures that are required nowadays to give a definite diagnosis /1/.

These developments, together with appropriate training and quality control programmes will facilitate the establishment of screening clinics for gynaecological cancers.

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THE EFFECT OF VASODILATOR THERAPY ON THE LIMB CIRCULATION IN HYPERTENSION

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The effect of vasodilator drugs on limb circulation was investigated in 59 hypertensive patients. Forty-six of them suffered from obliterative arterial disease, too. The drugs administered were: hydralazine, nitroglycerine and sodium nitroprusside. Isotope dilution method was employed to assess limb blood flow before and after acute treatment. Limb vascular resistance was calculated from the limb blood flow and the mean blood pressure.

A marked increase in limb blood flow and decrease in vascular resistance following vasodilator treatment were observed in hypertensive patients suffering from obliterative arterial disease. No difference was found between the various vasodilators investigated. Normotensive patients with arterial disease exhibited the same response to a lesser degree. In exceptional cases, where blood pressure dropped abruptly, limb blood flow decreased and limb vascular resistance increased.

The data show that vasodilator therapy in hypertension results in diminished limb vascular resistance and improved limb circulation in patients with obliterative arterial disease.

Keywords: vasodilators, obliterative arterial disease, hypertension, limb circulation.

Introduction

The strategy of antihypertensive therapy in cases of impaired peripheral - limb - circulation has been controversial for decades. Overestimation of the significance of perfusion pressure in limb arterial circulation discouraged clinicians from using effective antihypertensive

Abbreviations: LBP = limb blood flow; LVR = limb vascular resistance; MBP= mean arterial blood pressure

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agents in the therapy of such patients, because the decrease of blood pressure in patients with peripheral arterial stenosis was regarded as harmful for the local circulation. The depression of the pathologically elevated peripheral vascular resistance with antihypertensive agents, however, seemed to be beneficial for the limb circulation. We planned therefore to study the changes of the limb circulation in hypertensive patients under short-term antihypertensive therapy. On theoretical grounds, we chose drugs with peripheral vasodilating property to depress systemic arterial pressure.

Patients and methods

Fifty-nine hypertensive patients, 11 women and 48 men were examined after having obtained their consent. The mean age of the patients was 57.3 ys (17-82). Forty-six of the patients suffered from obliterative peripheral arterial disease, too. The patients had intermittent claudication or rest pain. The obliterative arterial disease was established by serial angiography and Doppler's sonographic technique. In 13 hypertensive patients the peripheral circulation was intact. Three types of vasodilators were chosen to achieve acute decrease of systemic blood pressure: 39 patients were treated with hydralazine as an i.v. bolus, nitroglycerine was administered to 10 and sodium nitroprusside to another 10 patients, in form of continuous i.v. infusion, 1-3 µg/kg/min.

Before drug administration and after the decrease of systemic blood pressure limb blood flow was assessed according to the venous isotope dilution principle /15/. Briefly, two needles were inserted into the femoral vein at a distance of 2 cm from each other. Labelled albumin iodine was infused through the distal needle with a constant flow rate and samples were taken through the proximal one. The dilution of radioactivity in this set-up is a function of blood flow in the femoral vein which represents the total arterial flow, being the sole outflow tract of the lower limb.

Limb blood flow (LBF) can be calculated as follows /14/:

$$LBF = I \left(\frac{\text{act. const.}}{\text{act.sample}-V/2} - 1 \right) \text{ml/min} \quad .$$

where I = the flow of isotope through the distal needle (ml/min)
act. const.= activity of the isotope infused (cpm)
act.sample = activity of the sample taken through the proximal needle while infusing isotope through the distal one (cpm)
V = blank, activity of a sample, taken 1 min after the isotope infusion has been stopped. On repeated measurements this factor to be subtracted is the mean of the blanks before and after the actual measurement: (V1+V2)/2.

On the basis of limb blood flow and mean systemic arterial pressure (MBP), limb vascular resistance (LVR) can be calculated as follows:

$$LVR = \frac{80000 \times MBP}{LBF} \quad \text{din sec cm}^{-5}$$

$$\text{MBP being } P_{diast} + \frac{P_{syst} - P_{diast}}{3} \text{ (Wiggers)}$$

Results

Table I

Effect of vasodilators on arterial circulation of the limb in hypertensive patients with obstructive arterial disease. $\bar{x} \pm$ S.D. Level of significance is marked with asterisks+

No. of pts	Drug	MBP(mmHg) before	MBP(mmHg) after	LBF(ml/min) before	LBF(ml/min) after	LVR(dinseccm^{-5}) before	LVR(dinseccm^{-5}) after
21	hydralazine	137 $\pm$18.8	108*** $\pm$13.3	149 $\pm$74.3	244*** $\pm$89.3	75560 $\pm$21703	35410*** $\pm$19031
10	nitroglycerine	131 $\pm$23.3	107*** $\pm$20.1	197 $\pm$46.6	301** $\pm$132	54748 $\pm$13732	31313*** $\pm$ 9790
10	nitroprusside sodium	128 $\pm$12.6	97*** $\pm$12.0	162 $\pm$51.2	272* $\pm$158.5	70265 $\pm$25175	38105*** $\pm$21592

MBP: mean blood pressure; LBF: limb blood flow; LVR: limb vascular resistance

+ $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Table I shows the data measured before and after drug administration in hypertensive patients, suffering from obstructive arterial disease. The decrease in systemic blood pressure was a predictable effect of drugs with vasodilator activity. Increase in limb blood flow and decrease in limb vascular resistance pointed to a favourable effect of vasodilator therapy on the diseased limb circulation, even with the fall of perfusion pressure. Table II represents the haemodynamic data of hypertensive patients without peripheral arterial disease. On administration of hydralazine a tendency of increase in LBF was observed in these patients, but it did not reach the level of significance. The marked decrease in LVR is another change that points to the vasodilating effect.

Exceptionally, some hypertensive patients exhibited a very marked, abrupt drop in blood pressure under hydralazine medication. Table III represents the data of this group showing a definite decrease in LBF and a slight upward tendency of LVR.

Table II

Effect of hydralazine on arterial circulation of the limb in hypertensive patients with intact vessels. $\bar{x} \pm$ S.D. Level of significance is marked with asterisks (see footnote to Table I)

No. of pts	MBP(mmHg)		LBF(ml/min)		LVR(dinseccm^{-5})	
	before	after	before	after	before	after
13	138	105**	468.5	511.9	25077	18782***
	±12.1	±11.7	±133.5	±198.0	±7845	±7912

Table III

Effect of abrupt fall in systemic hypertension due to administration of hydralazine on circulation of the limb. $\bar{x} \pm$ S.D. Level of significance is marked with asterisks (see footnote to Table I)

No. of pts	MBP(mmHg)		LBF(ml/min)		LVR(dinseccm^{-5})	
	before	after	before	after	before	after
5	118.0	83.4**	400.6	261.4**	26376	27620
	±12.8	±8.8	±183.0	±59.2	±12520	±9899

Discussion

Since, according to a generally accepted concept about the leading role of perfusion pressure in maintaining adequate limb circulation of patients with peripheral arterial disease, a decrease of blood pressure is harmful /7/, therapy should be directed to augment the blood pressure /5, 8/. Our present data showed, in agreement with some earlier studies /6, 9, 10, 11, 17/ that most of the hypertensive patients had elevated limb vascular resistance due to a continuous peripheral vasoconstriction caused by a continuous sympatho-adrenergic dominance /9, 10, 18/. Drugs with peripheral vasodilating property (relaxing the smooth muscle of vessel wall) will diminish systemic vascular resistance and, if so, produce a favourable change in peripheral circulation even in cases with low perfusion pressure. Another possible danger of vasodilator treatment of patients with extremital obstructive arterial disease is the danger of steal phenomenon /1, 3/. Some authors observed no increase in limb blood flow with hypertension, limb vascular resistance being higher in hypertensive patients than in normotensive subjects /4, 6, 11, 12, 17/. We failed to find experimental signs of steal phenomenon and did not observe any pathological change in limb circulation on administration of vasoactive drugs.

Our data show that vasodilator therapy in hypertension results in diminished vascular resistance and improved limb circulation in patients with obliterative arterial disease.

Three more questions have remained to be answered:

i) Can the beneficial short-term effect of vasodilators be extrapolated to a long-term benefit of vasodilator therapy? To answer this question, studies of at least 2-3 month duration should be completed.

ii) How should be avoided a further impairment of the limb circulation if the danger of abrupt fall in blood pressure could cause a decrease in limb blood flow, as shown on Table III? We must suggest that patients of such type of response should not be treated with this sort of antihypertensive agents.

iii) Which type of vasodilator agent should be chosen for antihypertensive therapy in cases of obstructive arterial disease?

The three types of vasodilators in our experiment showed no significant differences in respect of increase in LBF. We consider all the three suitable, sodium nitroprusside, however, cannot be used for long-term therapy. Nitrogylcerine and hydralazine are accepted for long term therapy, so on the basis of the data above their use as antihypertensive drugs is justified in patients with obstructive arterial disease.

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GASTROENTEROLOGY

ULTRASONOGRAPHY AND WATER CONTENT OF THE LIVER IN CHRONIC DIFFUSE LIVER DISEASE

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Water content in the liver in vivo was determined in 89 patients (33 with normal liver and 56 with chronic liver disease), simultaneously with ultrasonography and histopathological examination. A part of each biopsy specimen was used for this purpose. The difference between wet and dry weights was calculated from the pre- and post-lyophilization weights. According to the attenuation type of ultrasonic images, the patients were divided into two groups, viz., patients of low attenuation type (i.e. patients with type I bright liver) and those of high attenuation type (i.e. with type II brigh liver). As to water content, no significant difference was observed between the two groups. No correlation was found between liver water content and histopathology either. It is concluded that knowledge of correlation between numerous parameters is needed to clarify the reason of attenuation differences.

Keywords: liver, ultrasonography, water content, diffuse liver disease.

Introduction

The normal echopattern of the liver - as suggested by some authors /2, 5/ - is caused by a series of alternate collagen-water interfaces.

Chronic diffuse liver diseases cause well-recognizable changes in the liver texture, characterized by high-amplitude echos /16, 18/ and a dense echopattern known as "bright liver" /10, 11, 13/. These signs are not specific for a certain liver pathology; normal echopattern may occur in pathological cases as well /11, 13, 25/. Numerous authors have described also attenuation values lower or higher than normal in certain liver

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diseases /3, 14, 15, 17, 22, 26/. In previous studies /19, 20/ we differentiated two groups of patients with characteristic ultrasonic signs of chronic diffuse liver disease: one with low attenuation characterized by bright liver (throughout in the organ) (type-I bright liver as designated by us) and another with high attenuation that can be recognized on the basis of mainly superficial appearance of bright liver pattern (type-II bright liver). Some authors /8, 21/ have suggested that the increased liver attenuation is caused by collagen fibrosis; others /3, 17, 22/ attribute it to fat. Decrease of liver attenuation is interpreted as being due to the water content of the liver /15/.

In this study in vivo water content determination of the liver was made in patients with normal ultrasonography, as well as in patients with chronic diffuse liver disease displaying type-I or type-II bright liver pattern on the ultrasonic scan.

Materials and methods

Ultrasonography. A high-resolution real-time scanner (Siemens Sonoline-SL2) was used. We took into account the gallbladder and the right renal parenchyma as reference organs. Echogenicity and attenuation of the liver were evaluated only if the gallbladder was echofree and the right renal parenchyma showed a homogeneous echopattern.

89 patients were divided into three groups.

1. 33 patients with normal ultrasonic echopattern of the liver (Fig. 1).

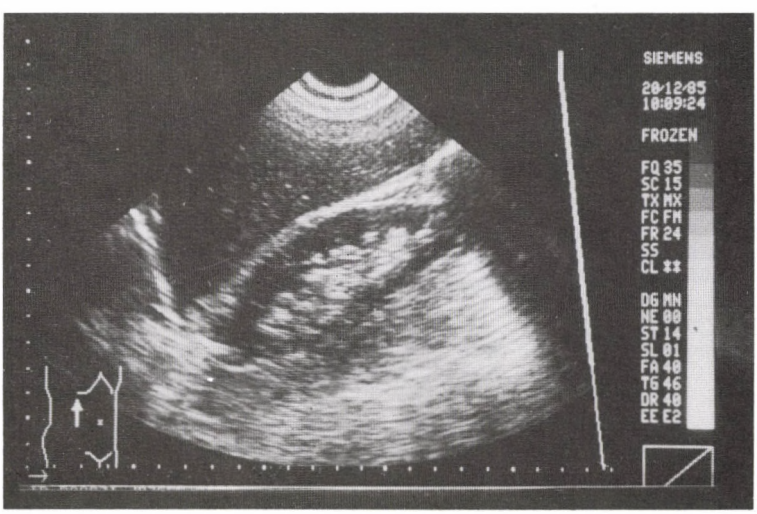

Fig. 1. Normal liver texture. Echo amplitude distribution and echodensity are similar those of the renal parenchyma

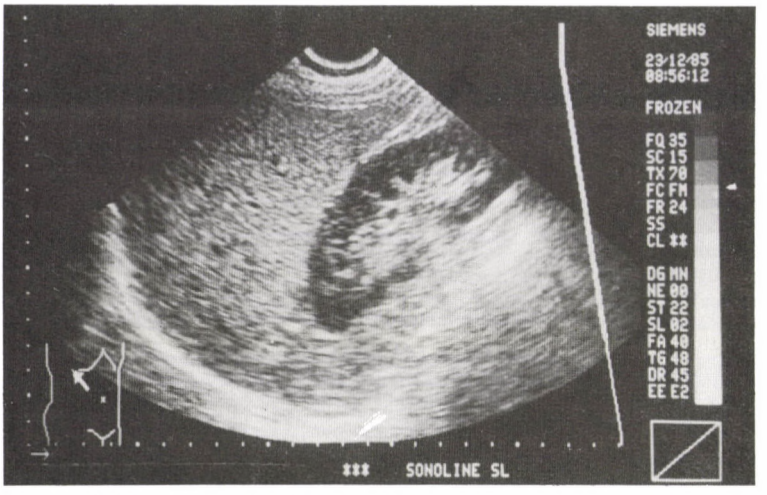

Fig. 2. Type-I bright liver. Note the high-level echos and dense echopattern throughout the whole thickness of the liver. Renal parenchyma exhibits normal echogenicity and echodensity. The difference between echopattern of the liver and renal parenchyma is obvious

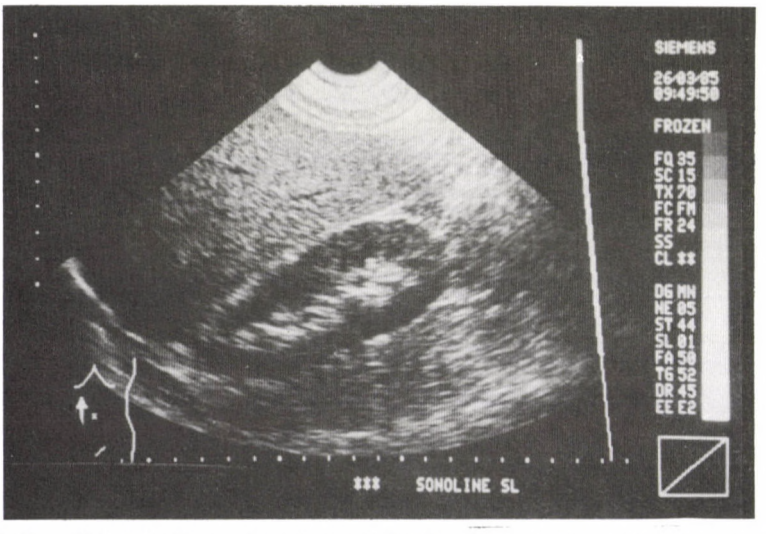

Fig. 3. Type-II bright liver. Note the high-level echos and dense echopattern near the liver surface and their absence in the deeper region. The renal parenchyma is normal. Behind the right kidney considerable amounts of echos are seen

2. 24 patients with type-I bright liver, i.e. low attenuation pattern (Fig. 2).
3. 32 patients with type-II bright liver, i.e. high attenuation pattern (Fig. 3).

Distribution of the two types was determined and compared with previously examined patient groups.

The maximum liver thickness was measured, and the averages for type-I and II bright livers were compared.

Water content. The water content of the liver of the 89 patients was determined in biopsy material obtained mostly by blind percutaneous liver biopsy performed with Menghini needles; in a minor part direct liver biopsy was made during surgery. The ultrasonography always preceded the biopsy. A portion of the biopsy material (appr. 10 mg wet weight) was immediately measured on a Sartorius balance 1207 MP2. This material was then lyophilized in a Janetzki LGR 05 apparatus at $-10^{\circ}C$ and 70 to 80 torr for 2x5 h, i.e. until constant weight. Repeated measurements showed steady values of the dry weight. Water content was calculated as follows:

$$\text{water content \%} = 100x(\text{wet wt.} - \text{dry wt.})/\text{wet wt.}$$

The remaining part of the biopsy material was utilized for histological examination and evaluated according to international criteria /1, 4, 6, 9, 12, 23/.

Final diagnosis was established by detailed clinical examination of the patients.

Results

Table I

Distribution of type I and type II bright livers in patients with chronic diffuse liver disease

Time interval of scannings	Ultrasonic scanner	No. of patients	Type-I birght livers %	Type-II
1976-1979	Kretztechnik bistable	144	41%	59%
1980-1981	Brüel-Kjaer compound gray-scale	55	45%	55%
1984-1986	Siemens H-R real-time	89	43%	57%

Table I shows the distribution in per cent of type I and II bright livers in three groups formed according to the date and technique of the examination. For evaluation of ultrasonic scans the above-mentioned criteria were considered in each group. The first group was studied between 1976 and 1979 with a Kretz bistable equipment, the second between 1980 and 1981 with a Bruel Kjaer compound gray scale scanner, and the third, the actual group between 1984 and 1986, with a high-resolution real-time scanner. As seen in Table I the distribution of the two bright liver types shows no significant difference in the studied patient groups. Neither the

date of examination, nor the type of equipment influenced the distribution considerably.

To eliminate the possibility that high attenuation pattern of bright liver is caused only by increased liver thickness, i.e. the longer propagation time of ultrasound, the maximum liver thickness values were measured. In the present study the average maximum thickness for the 24 type I bright livers was 11.5±1.5 cm (mean ± S.D.), while that for the 32 type II bright livers was 11.9±1.8 cm (mean ± S.D.). Accordingly, no significant difference exists between the maximal average thickness of type I and II bright livers.

The water contents of the 33 normal, 24 type I and 32 type II bright livers are shown in Table II. No significant difference was observed.

Table II

Water content of the liver in 89 patients with different ultrasonic patterns

Ultrasonic pattern of the liver	Number of patients	Water content of the liver ($\bar{x}$±S.D.%)
normal	33	40.9 ± 12.5 %
type I bright liver	24	47.0 ± 21.5 %
type II bright liver	32	48.9 ± 16.4 %

Table III

Correlation between histology, ultrasonography, and water content of the liver (89 patients)

Liver histology	Number of patients	Ultrasonic pattern normal	type I	type II	Water content in per cent ($\bar{x}$±S.D.)
normal	27	20	3	4	40.3 ± 13.4
Alcoholic hepatitis	6	-	5	1	49.3 ± 24.1
Fatty liver	39	9	11	19	46.6 ± 17.2
Cirrhosis	12	2	3	7	51.2 ± 17.2
Others (toxic, etc.)	5	2	2	1	45.5 ± 21.6

Table III shows the water content of the liver in relationship to ultrasonography and histomorphology. There was no correlation between these parameters.

Discussion

Water content of the liver and its correlation to acoustic parameters was determined in vitro by Bamber and Hill /3/ in a postmortem study. From the numerous biochemical and acoustic parameters water content and attenuation coefficient of normal and cancerous livers were investigated and correlated with each other. A negative correlation was demonstrated between the water content and the attenuation coefficient of the liver. On the contrary, fat content and attenuation coefficient of the liver showed a positive correlation /3/. Cloostermans failed to correlate attenuation and water content but found a relationship between water content and reflectivity in freshly excised livers originating from autopsy /8/.

No literary data are available on in vivo water content determination of the liver. In this study a simple method was developed using liver tissue obtained with biopsy. Indication of liver biopsy was always based on clinical decision. If histology of the liver was needed to establish the diagnosis, one part of the material was used for water content determination.

It has been published that attenuation values in patients with chronic diffuse liver disease might be lower or higher than normal /3, 14, 17, 22, 26/. These findings are in accordance with our experiences, namely, patients with chronic diffuse liver diseases exhibiting bright liver by ultrasound, can be classified into two groups according to attenuation. An expert examiner using standard criteria is able to differentiate bright livers of low-attenuation (type I) from those of high-attenuation (type II) /20/. Maklad et al., examining attenuation values in normal livers and in a group of patients with liver diseases, concluded that an increased water content of the liver is responsible for the lower attenuation in patients with tissue necrosis, and the higher attenuation values are due to decreased water content in patients with liver cirrhosis /15/. Bamber and Hill also found, in an in vitro study, a negative correlation between water content and attenuation coefficient /3/. Our results do not agree with these findings but are in accordance with those published by Cloostermans

et al. /7/. We found that in vivo water content determined in liver biopsy material of patients with bright liver patterns showed a large individual variability, but no significant difference could be observed between the low (I) and high (II) attenuation types.

In a few cases we attempted to differentiate between free and bound water in biopsy specimens obtained at surgery by the NMR measurement of spin-lattice relaxation time. No difference was observed between the groups. Nevertheless, the small number of measurements did not allow reliable evaluation.

Unfortunately, a specimen from liver biopsy does not represent the liver as a whole. In this study, in which parts of the same specimen were used for histology and for water content determination, presence or absence of fatty liver or cirrhosis, or some other chronic diffuse liver disease was diagnosed with high accuracy and correlation between water content and ultrasonic pattern could be analysed reliably. The in vitro findings of Bamber and Hill /3/ eventually provide some explanation of our results. These authors failed to observe any correlation between attenuation coefficients when plotted versus fat content after correction for variations in water content. Presumably, changes of numerous parameters must be studied and correlated in a more complex manner to explain low or high attenuation type of ultrasonic bright liver pattern /24/.

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SUCCESSFUL TREATMENT OF INTRACTABLE GASTRIC ULCERS WITH ACETAZOLAMIDE

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An open-controlled trial performed in gastric ulcer cases resistant to previous cimetidine, antacids, vitamin A and polyvinylbutylether therapy applied for at least 4 weeks. A group of 21 patients treated with acetazolamide was compared with 16 patients treated with cimetidine (controls). The period of management was 3 weeks. The number of healed patients ($P = 0.009$), the surfaces of ulcers after treatment ($P = 0.0166$) and the duration of complaints ($P = 0.0003$) differed favourably and significantly in the acetazolamide group as compared to the cimetidine group. In the acetazolamide group, however, several side effects (in 11 cases metabolic acidosis, in 9 cases tingling of extremities) were registered. Side effects were not seen in the control group. It is supposed that in the treatment of gastric ulcers a compound with less carbonic anhydrase inhibition but with the same or more cytoprotective effect would have wider clinical perspectives than acetazolamide alone.

Keywords: carbonic anhydrase, metabolic acidosis, ulcer, therapy, side effects.

Introduction

H_2 antagonits are effective drugs against duodenal ulcer disease, though, the results in the conservative treatment of gastric ulcers are controversial. That is why we examined the possibility of acetazolamide's (Ac) application.

Abbreviations: Ac = acetazolamide, CA = carbonic anhydrase, d = day

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The carbonic anhydrase enzyme (CA) catalyses the reaction: $H_2O+CO_2 \rightleftharpoons H_2CO_3$. The fundamental change is from a nonpolar gas with the linear structure $O = C = O$ to an acid or its conjugate bases with the coplanar configuration. CA concentration is high in mammals' stomach parietal cells, red blood cells, salivary glands, kidney and eye /14/. The early data about the role of CA in the gastric secretion were controversial /1, 4, 5, 8/. This enzyme appears to be important in controlling gastric acid synthesis in parietal cells and in regulating the bicarbonate secretion by surface epithelial cells. Some data suggest a protective function for CA in the gastric mucosa /2, 10/. Ac is a thiadiazole, a potent CA inhibitor. It is absorbed from the intestinal tract completely, and has about 100 min as plasma half-life. The decay of Ac from enzyme sites is slow; after one week Ac is still found in the organs, though, in a low concentration. A high dose of Ac blocks gastric acid secretion /8/. The oral or intravenous dose necessary to inhibit gastric acid secretion in human beings is about 50 mg/kg. When CA inhibitors are used, complete suppression of CO_2 hydratation cannot be expected, i.e. the rate of uncatalysed reaction is enough to provide a moderate reaction /13-15/. Gastric haemorrhagic ulcers were induced by hypovolaemic shock and indomethacin in rats; high doses of Ac enhanced gastric ulceration, assumed to inhibition both CA enzyme and mucus secretion /2, 10, 11/. Other studies have proved that Ac protects against ethanol-, aspirin- or histamine-induced gastric lesions and increases the degree of gastric cytoprotection /6, 19/.

Recent data have shown that the gastroprotective effect of Ac may be related to its sulfhydryl content and not to CA inhibition /12/. Ethoxzolamide causes a complete CA inhibition but does not prevent ethanol-induced lesions. Bismuthiol-I, a thiadiazol with sulfhydryls, is a weak inhibitor of CA. It reduced the lesion area induced by ethanol. The acute gastric mucosal protection by Ac and its derivatives might be related to their content of sulfhydryls in the oxidized or reduced state and not to their CA-inhibiting activity in the stomach /12, 21/.

Despite its side effects, Ac has already been applied for treating ulcer disease with good results. However, there are no well-controlled clinicopharmacological studies. Our purpose was to evaluate the effect of Ac on therapy-resistant benign gastric ulcers, and monitoring the side effects of the therapy.

Patients, methods

We treated endoscopically-controlled benign gastric ulcers resistant to an at least 4-week pretreatment indicating that the size of ulcer had diminished by less than 20%, or remained unchanged or even grew, despite continuous medication. Their pretreatment was carried out with cimetidine 1 g/day, antacids of an average of dose 50 mEq/d, Vitamin A 2x50 000 U weekly /9/, 1 tablespoonful of Shostakovsky's balsam (Medexport, Moscow) before bedtime (a polyvinylbutylether, which provides a protective barrier on mucosa). Our study was a prospective, open, controlled, randomized examination. Patients with renal or cardiac insufficiency, hepatic cirrhosis, respiratory diseases, chronic alcoholism, and/or diabetes mellitus were excluded. Gastroscopy was performed on three occasions, with an Olympus GIF D4 upper panendoscope 1) before the initial treatment, 2) at the beginning of the three-week programme, 3) and within 2 days after finishing it. We took biopsy samples at least four from the margin, one from the bottom, and some from the surrounding areas of the ulcer. All of the

samples were evaluated histologically. The size of the ulcer was measured with an open biopsy forceps, computing the surfaces of circle, ellipse or quadrangle. The therapy was known by the endoscopists. The period of management was 3 weeks, not more because of the expectable side effects of Ac.

The symptoms and the side effects also were registered. We used Ac monotherapy for three weeks, giving 25 mg/kg body mass daily doses in four parts (Fonurit tablet, manufactured by Chinoin, Budapest). In the first week of treatment we examined three times, and in the 2nd and 3rd weeks weekly twice, the acid-base relations, capillary pO_2, pCO_2, serum Na^+, K^+, Cl^-, BUN and creatinine values. We registered the serum bilirubin, AST, ALT, alcalic phosphatase, glucose, Ca^{++}, P levels, ECG, spirometry, creatinine clearence and the patients' complaints. Urine Na^+, K^+ and Cl^- were measured every day in 4 of the cases.

The control group members received 1 g of cimetidine (Histodil tablet, Richter, Budapest), antacids of an average of 45 mEq buffer capacity per day and vitamin A 50 000 U twice weekly. In the control group, laboratory examinations were performed at the beginning and at the end of the treatment. Spasmolytic (papaverinum) was allowed to take in time of pain in both groups. Statistical analysis was made with the 2-tail Fisher's exact test for evaluating the proportion of healed patients in both groups. Student's t-test was used to compare the duration of complaints and extent of ulcers.

Results

The trial was carried out in a period of 36 months. Twenty-one patients (male 16, female 5) average age 47 received Ac. With 2 patients the therapy was discontinued due to hyperkalaemia and urethro-prostatitis. One of the patients did not improve, the other was completely cured despite that the therapy lasted only one week. By the end of the third week, the ulcer had healed in 15 cases and improved in 4. In 16 members of the control group (average age, 52; male 12, female 4) in three weeks of treatment 5 healed, 8 improved, 3 showed no changes (Table I). The average extension of ulcers in the Ac group diminished from 143.5 mm^2 to 7.6 mm^2 while in the control group from 120.8 mm^2 to 32.1 mm^2. 2-tail Fisher's exact test proved significant as regards the number of recovered patients ($P = 0.009$, including the 2 withdrawn cases). The extent of the ulcers before treatment did not differ significantly ($P = 0.564$), after treatment significant differences were realized in the two groups ($P = 0.0166$). The patients' pain in the Ac group lasted an average of 4.4 days (S.D. 2.8), in the control group 11.4 days (S.D. 5.9). The value of Student's t-test was 0.0003 (Table II).

In the Ac group 9 patients complained of tingling of extremities, 2 of diarrhoea, 1 of temporary visual disturbances. We observed acidosis in 11 patients of the Ac group. Five of them were normal anion gap hyperchloraemic metabolic acidosis, 2 mixed hyperchloraemic-high anion gap metabolic acidosis. Four were mixed metabolic acidosis and respiratory acidosis with metabolic dominance. We measured in some cases other tran-

Table I

Results of the trial

Medication	Response of patients			Total
	Healed	Improved	Did not improve	
Acetazolamide	16	4	1	21
Control	5	8	3	16

Table II

Change of ulcers' extent and duration of pain

Acetazolamide group			Control group		
Ulcers' extent (mm^2)		Duration of pain (days)	Ulcers' extent		Duration of pain (days)
before treatment	after treatment		before treatment	after treatment	
50	0	2	78	13	15
51	0	2	106	29	12
191	0	10	81	0	7
313	0	4	153	31	11
315	0	7	60	0	5
48	0	3	83	32	12
54	0	6	80	80	21
80	0	3	28	0	8
155	0	3	51	13	15
79	0	3	314	80	8
131	0	5	106	106	21
314	30	3	190	31	9
190	0	2	81	0	3
154	29	7	28	49	21
52	0	1	52	0	4
79	0	2	441	53	10
438	80	5			
29	0	1			
51	0	3			
156*	no data	11			
87*	0*	3			

*Not included in the statistical analysis

sient mixed acid-base disorders during the treatment (e.g. metabolic acidosis with respiratory alkalosis) but the characteristics of the disturbances were as described above /16/. Below pH 7.25 (5 cases) 50-100 mEq/day $NaHCO_3$ was administered per os. In the course of the Ac therapy, excretion of potassium increased, though the diet was the same. In 4 cases before therapy the excretion of potassium was 33.1 mEq/day, on the average, during the first 3 days of the Ac therapy 38.2 mEq (115.4%) and an average of 36.0 mEq/day between the 4th and 21st days (108.8%). This equals to 67.5 mEq loss in 3 weeks compared to the pretreatment excretion level. However, hypokalaemia was not seen at all; for that very reason, we did not give K^+ salt. Excretion of Na^+ before therapy was 106.5 mEq/d, during Ac medication 131.4 mEq/day on the average (123.4%). We registered once a mild hypernatraemia (146 mEq/l). The Cl^- excretion before the Ac therapy was 80.3 mEq/day and during it 87.3 mEq/day (108.7%). Hyperchloraemia was found in 5 cases. Diuresis grew by 46% (mean: 805 ml/day before Ac and 1177 ml/day after Ac). Creatinine clearence showed temporary decrease in four cases (max.: 22%); after 3-4 days the values returned to the earlier level. The blood urea nitrogen rose with an average 37% but remained inside the normal range. The other parameters showed no change. The side effects and complaints disappeared by the end of the treatment.

The control (cimetidine) group showed no side effects. There were six smokers in each of the Ac and the control group.

Members of the Ac group and those of the control group spent an average of 10.8 days and 8.6 days, respectively, in hospital. In the Ac group 3 patients used temporarily spasmolytics and 2 patients minor tranquillants (diazepam and meprobamat). In the control group 8 persons used spasmolytics, and one of them used tranquillant (diazepam).

We examined 4 patients for Helicobacter pylori in the Ac group; bacteria were found 3 of them before and after the treatment.

Discussion

Under clinical conditions, good effects of Ac have been reported as regards the treatment of peptic ulcers. Gailitis et al. /7/ treated 125 peptic ulcer patients with 1 g/day Ac and found a decrease of acid secretion and a good clinical response, and tingling of extremities, metabolic acidosis, electrolyte loss as side effects. We obtained similar

results, but our patients did not complain of loss of appetite. Puscas /17/ reported on treatment of 148 patients (96 duodenal and 52 gastric ulcers); after 24 days 94% of duodenal and 91% of gastric ulcers were found healed. They found paraesthesia in the limbs in 35%, moderate asthenia and drowsiness in 17% of the cases; no other clinically important side effects were recorded. In another paper /18/ Puscas related their experiences of his group in the treatment with the Ac-containing drug Ulcosilvanil to more than 2500 gastroduodenal ulcer patients. They registered the inhibitory effect of their medicine both to basal and stimulated acid secretion. We failed to find in their studies detailed analysis of the effects on the acid-base and electrolite household. Our purpose was to gain some data on the territory mentioned above, and to study the efficacy of Ac alone, for the medicine "Ulcosilvanil" used by Puscas and his co-workers contained - in addition to Ac - potassium bicarbonicum, sodium bicarbonicum, sodium citricum, magnesium oxydatum and aluminium hydroxydatum. Probably, the latter components modified the effect of Ac.

Solt /20/, too, reported good effects of Ac in the management of peptic ulcers. We found good responses to the Ac treatment both in the clinical symptoms and the endoscopic appearance of gastric ulcers, although we had to supplement Ac monotherapy with sodium bicarbonicum in 5 cases. The effect of CA inhibition on the kidney is important. CA is present within the cells of both the proximal and distal tubules. When Ac is used, the absolute proximal bicarbonate reabsorption is found to vary with load, but the fraction reabsorbed is subnormal. When the filtered bicarbonate load is gradually reduced by progressive metabolic acidaemia, a decreasing amount of bicarbonate is escaping distally. When the distal tubule is able to handle the delivered bicarbonate, the acidosis will not worsen /3/. In the distal nephron the H^+ and NH_4^+ output is repressed under Ac medication. According to physiological experiments on rodents the toxicity of Ac proved to be similar to NaCl (lethal dose 50: 3-6 g per kg). When rodents and dogs were given Ac for several months the animals tolerated the drug well. During chronic administration of Ac normal anion gap metabolic acidosis occurs and some ten per cent loss in whole body Na^+, K^+ content may be observed, while these disturbances do not increase under continuous medication, and steady state sets in. These ionic patterns were observed for experiments lasting up to 16 months. Due to the blocking of CA of red blood cells, respiratory acidosis may develop. On humans, side effects (anxiety, tingling, breathlessness) appeared with a single dose over 114 mg/kg /8, 14/.

We observed in the Ac group several side effects, most frequently metabolic acidosis. The acid-base disturbance and electrolyte loss showed no progress. We had to discontinue the management only in one patient for that reason. Our patients found the subjective side effects, which were accompanied by simultaneous rapid pain relief, tolerable. However, we found side effects more frequently than Puscas. All side effects disappeared after the treatment was finished.

In conclusion, our view is that ac should not be the first-step management. We suppose that a compound with less CA inhibition but with the same or more cytoprotective effect would have wider clinical perspectives than Ac in the treatment of gastric ulcers.

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PATHOPHYSIOLOGY

EFFECTS OF HYPOTHYROIDISM AND HYPERTHYROIDISM ON THERMOGENIC RESPONSES TO SELECTIVE AND NONSELECTIVE BETA-ADRENERGIC AGONISTS IN RATS

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Oxygen consumption (VO_2) and mitochondrial guanosine diphosphate (GDP) binding of interscapular brown adipose tissue (BAT) were measured in hypothyroid, hyperthyroid and euthyroid rats after stimulations with selective and nonselective beta-adrenoceptor agonists: BRL 35135A (BRL) and Isoprenaline (ISO). Resting VO_2, VO_2 increment and mitochondrial GDP binding after beta-adrenergic stimulations were lower in hypothyroid rats than in the euthyroid group. The reduced responses were more marked for ISO than for BRL. Restion VO_2 and VO_2 increment after beta-adrenergic stimulations were higher in hyperthyroid rats than in the eurthyroid group; the increment was more marked for BRL than for ISO. In hyperthyroidism, mitochondrial GDP binding after BRL and after ISO was in the same magnitude; it was higher in the hyperthyroid than in the euthyroid group after BRL but not after ISO. The different thermogenic responses after ISO and BRL stimulations suggest that BRL is acting on a beta-adrenoceptor differing from the beta-1 and beta-2 adrenoceptors responsible for the effects of ISO. Activation of thermogenesis via the beta-3 adrenoceptor seems to be less dependent on the permissive levels of thyroid hormones than activation via beta-1 and/or beta-2 adrenoceptors. The beta-3 adrenoceptor may be more sensitive to increased levels of thyroid hormones.

Keywords: thermogenesis, oxygen consumption, mitochondrial guanosine diphosphate binding, beta-agonists, hypothyroidism, hyperthyroidism.

Abbreviations: BAT = brown adipose tissue; BRL = BRL 35135A; DIT = diet induced thermogenesis; GDP = guanosine diphosphate; ISO = Isoprenaline; NA = noradrenaline; NST = nonshivering thermogenesis; VO_2 = oxygen consumption

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Introduction

Brown adipose tissue (BAT) is the main effector of cold-induced non-shivering thermogenesis (NST) and diet-induced thermogenesis (DIT) in rodents /17/. BAT is controlled by the sympathetic release of noradrenaline (NA), which acts on beta-adrenoceptors on brown adipocytes to stimulate heat production via a unique mitochondrial proton conductance pathway that uncouples oxidative phosphorilation /12, 14/.

It is generally accepted that thyroid hormones play a permissive role in sympathetic-mediated thermogenesis /5/, acting via modulation of catecholamine sensitivity by regulating beta-adrenoceptor number and adenylate cyclase activity in sensitive tissues /2, 8, 19, 25/.

Radioligand and other studies indicated that BAT adrenoceptors consist of both the beta-1 and beta-2 subtypes /19, 20/. The use of a new and novel group of beta-adrenergic agonists, however, has shown that BAT beta-adrenoceptors might not conform to the beta-1/beta-2 classification /1/. These agonists stimulate brown adipocyte lipolysis much more selectively than heart rate and tracheal relaxation. Since the heart and the trachea mainly contain beta-1 adrenoceptors and beta-2 adrenoceptors, respectively, these results suggested that the novel agonists activated BAT via a beta-adrenoceptor that was different from the beta-1 and beta-2 subtypes. The presence of this "atypical" beta-adrenoceptor on BAT was confirmed in other studies /6, 10/ and it has been designated as beta-3 adrenoceptor.

However, it is not known how thyroid hormone status affects the beta-3 adrenoceptors and the thermogenic response to so-called beta-3 agonists. BRL 35135A (BRL) is a novel beta adrenergic (beta-3) agonist (Fig. 1) which selectively stimulates BAT and thermogenesis /6/. The aim of

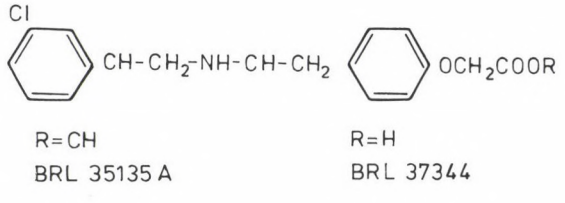

Fig. 1. BRL 35135A and its active metabolite

the present study was to investigate the effect of BRL on thermogenic responses in euthyroid, hypothyroid and hyperthyroid rats and to compare its thermogenic effects of BRL to those of Isoprenaline (ISO), which is a non-selective beta-agonist acting via beta-1 and beta-2 adrenoceptors.

Materials and Methods

Male Sprague-Dawley rats (Charles River, UK) were caged in pairs in a metabolic room maintained at 24°C with a 12 h light/dark cycle. They were maintained in a stock diet (PRD; Christopher Hill Group, UK). Hypothyroidism was induced by providing drinking-water containing 0.04% (w/v) methimazole (Sigma) /15/; hyperthyroidism was induced by addition of 0.03% thyroid powder (Sigma) to the diet /9/.

Oxygen consumption (VO_2) was measured in closed circuit respirometers /23/. Data of VO_2 were collected with an on-line microcomputer and computed to express the values as a ml O_2/min/kg$^{0.75}$ (STBD) in every five min. A 90 min period of measurement was done before any treatment was given to the rats, and VO_2 was measured for 2 h after treatments. To define responses to drugs VO_2 was measured in the 20-80 min period after treatment and increment was expressed as a difference between average VO_2 in this period and the basal resting VO_2. BRL and ISO were used in so-called effective and maximal thermogenic doses (BRL: 2 µg/kg and 200 µg/kg and 400 µg/kg, respectively) on the basis of our previous experiments.

Serum thyroid hormone levels were not measured, since the alteration in resting VO_2 of the treated groups was similar to that found by others in hypo- and hyperthyroidism /15, 16/.

On the day of GDP binding measurement after 80 min the maximal effective doses of beta-agonists the rats were killed by cervical dislocation, and interscapular BAT was dissected and weighed.

Mitochondria were prepared from separate BAT samples according to the method described by Slinde et al. /22/. The procedure was carried out at 0-4°C. The tissue was homogenized in 15 ml of 0.2 M sucrose buffer using a B Braun homogenizer. The homogenate was centrifuged (Beckman J32-21) at 3500 g to separate tissue fragments. The supernatant was then spun at 11000 g and the mitochondrial pellet was resuspended in 0.7-0.5 ml sucrose buffer, to give a final protein concentration of about 2 mg/preparation.

Mitochondrial GDP binding was carried out as described by Brooks et al. /3/. The binding of ^{3}H-GDP to isolated mitochondria was measured in the presence of unlabelled GDP at a concentration of 2 µM (approximately equivalent to the KD of the binding site and at 200 µM (maximum displacement concentration) to measure nonspecific binding. To measure the extramitochondrial space ^{14}C-sucrose was used. GDP binding was expressed as pmol/mg mitochondrial protein. The protein content of mitochondrial samples was determined by the Bio-Rad method.

The statistical significance of differences was computed with Student's t-test.

Results

1. Effect of methimazole and thyroid powder treatment on body and BAT weight

The growth rate of the methimazole-treated rats was significantly lower than that of the control ones. The interscapular BAT from hypothyroid rats increased in weight: the BAT weight corrected for body size (relative

Table I

Body mass and BAT mass during methimazole and thyroid powder treatment ($\bar{X} \pm$ S.E.M.)

Parameters	Methimazole	Control	t-test	Thyroid powder	Control	t-test
Initial body mass (g)	112.9 ± 2.0	113.2 ± 1.9	NS	128.4 ± 1.4	127.1 ± 1.2	NS
Final body mass (g)	193.3 ± 7.0	319.9 ± 6.4	+++	286.4 ± 7.5	297.1 ± 4.3	NS
BAT mass (mg)	215.8 ± 6.2	277.5 ±14.0	+++	354.2 ±25.0	295.8 ±18.0	NS
Relative BAT mass (mg/g body mass)	1.09± 0.18	0.85± 0.15	+++	1.24± 0.08	1.02± 0.07	+

n = 16; NS = non significant;

$^{+}P < 0.05$, $^{+++}P < 0.001$ } vs control

BAT weight) was significantly increased in the hypothyroid group. The body weight and BAT weight of the thyroid-treated group did not differ significantly from those in the control rats. However, the relative BAT weight in hyperthyroid rats significantly exceeded that in the control group (Table I).

2. Effect of methimazole and thyroid powder treatment on basal oxygen consumption

Resting VO_2 of hypothyroid rats was decreased gradually in the course of the methimazole treatment and it was approximately 75% of the VO_2 measured in euthyroid group after 2-3 weeks of the treatment. Thyroid treatment increased the resting VO_2 which was elevated approximately 70-75% after 2-3 weeks of the treatment in the hyperthyroid group, compared to VO_2 in the control group (Table II).

Table II

Resting O_2 consumption during methimazole and thyroid powder treatment (ml/min/$kg^{0.75}$; $\bar{X} \pm$ S.E.M.)

Treatment and time	Control groups	Treated groups	t-test
Methimazole			
2nd week	14.83 ± 0.40	10.35 ± 0.16	P 0.001
3rd week	14.63 ± 0.65	9.19 ± 0.30	P 0.001

Table II (cont.)

Treatment and time	Control groups	Treated groups	t-test
Thyroid powder			
2nd week	14.04 ± 0.28	20.55 ± 0.44	$P < 0.001$
3rd week	12.50 ± 0.22	18.12 ± 0.22	$P < 0.001$

n = 15 or 16

3. Changes in VO_2 following administration of BRL and ISO

VO_2 significantly increased after injections of either BRL or ISO in the euthyroid, hypothyroid and hyperthyroid groups. Increments after effective and maximal thermogenic doses of BRL and ISO are summarized in Fig. 2.

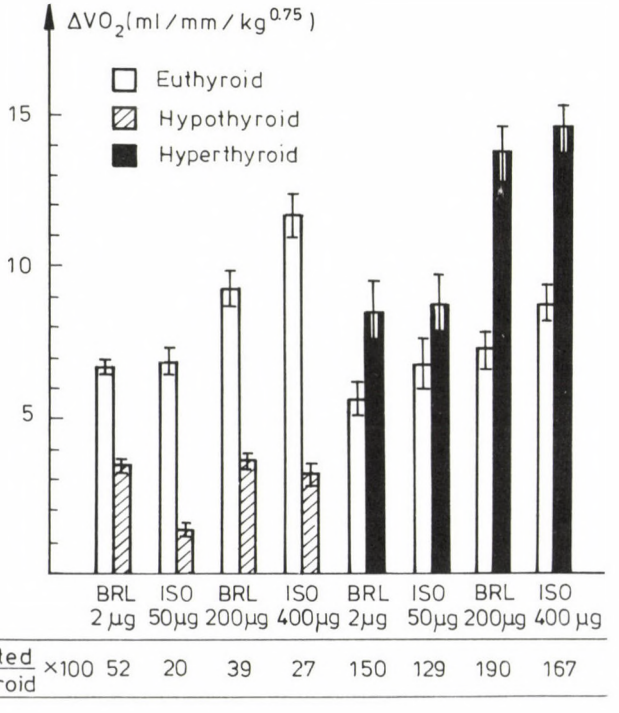

Fig. 2. Increase of VO_2 of euthyroid, hypothyroid and hyperthyroid rats after different thermogenic doses of BRL or ISO (n=8)

Increments in VO_2 were different according to the thyroid function of the animals and the administered drugs. The VO_2 responses to beta-agonists were in the hypothyroid rats lower than in the euthyroid ones. The reduction of response was more marked for ISO than BRL. The increments were 20% and 27% of the control response after ISO, and 52% and 35% after BRL, using effective and maximal thermogenic doses of the drugs.

In hyperthyroid group, despite the increased basal VO_2, after administration of beta-agonists, VO_2 elevated further and the increments were higher than those in control rats. This increased response was more marked for BRL than for ISO. The increments were 150% and 190% of the control response after BRL, and 129% and 167% after ISO, respectively, using effective and maximal thermogenic doses of the drugs.

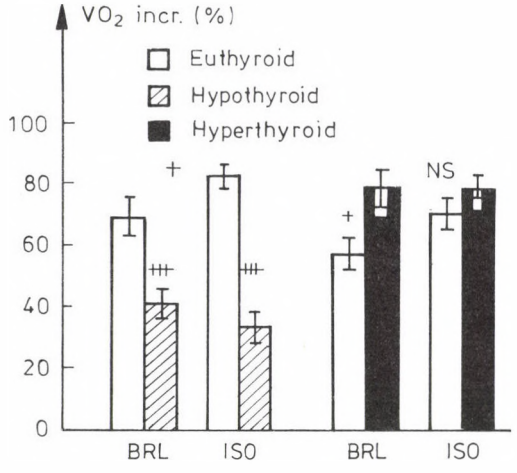

Fig. 3. Increment of VO_2 of euthyroid, hypothyroid and hyperthyroid rats after maximal thermogenic doses of BRL or ISO (n=8), * $P<0.001$; + $P<0.05$

Figure 3 contains the percentage increments in VO_2 after maximal thermogenic doses of BRL and ISO in euthyroid, hypothyroid and hyperthyroid rats. This parameter in the hypothyroid group was significantly decreased after administration of beta-agonists. However, the reduction of response was more marked for ISO than for BRL. Whereas, the percentage increments in hyperthyroidism were similar after BRL and ISO, the euthyroid-hyperthyroid difference in this parameter was more marked after BRL than after ISO.

4. Mitochondrial GDP binding after maximal thermogenic doses of BRL and ISO

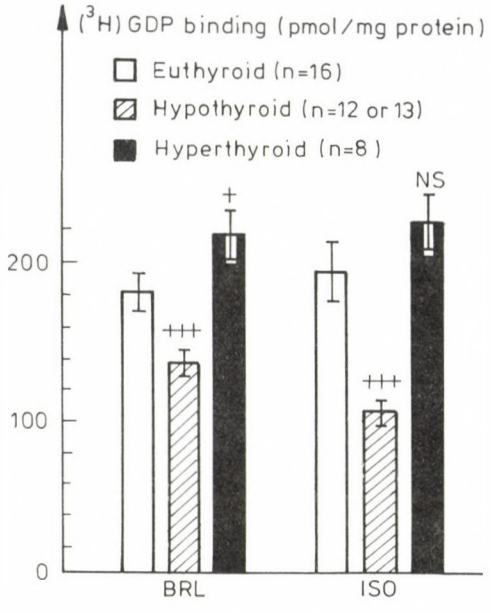

Fig. 4. BAT mitochondrial GDP binding of euthyroid, hypothyroid and hyperthyroid rats after maximal thermogenic doses of BRL or ISO, $^{+++}P < 0.001$; $+ P < 0.05$

The results of mitochondrial GDP binding are shown in Fig. 4. The specific GDP bindings induced by beta-agonists were lower in the hypothyroid rats than in the euthyroid group; they were higher after BRL than after ISO. The bindings of mitochondria from hyperthyroid rats were higher than those in euthyroid animals after BRL but not after ISO, although the response to BRL and ISO in hyperthyroidism were in the same magnitude.

Discussion

In the present study hypothyroidism resulted in a remarkable reduction in growth rate and BAT mass, but BAT mass corrected for body weight was larger in the hypothyroid group than in the control one. Enlargement of BAT in hypothyroidism was already observed by earlier workers /4, 11, 15, 26/, and it was also pointed out that the hypertrophic BAT of hypothyroid rats has a limited physiological role (incapacity to respond to NA nor-

mally). The relative BAT weight was also slightly increased in hyperthyroid rats was thought to be entirely due to deposition of lipid in the tissue /13/.

Despite the equally increased relative BAT weight after methimazole and thyroid powder treatment resting VO_2 was remarkably different, proving the existence of a hypothyroid and a hyperthyroid status. Alterations in resting VO_2 of the treated groups were similar to that found by others in hypo- and hyperthyroidism /15, 16/.

After administration of beta-agonists VO_2 in whole animals was increased in all groups. However, VO_2 response to beta-agonists was lower in hypothyroid than in the euthyroid group. Other workers /7, 15, 26/ showed that the thermogenic effect of NA on BAT decreased in hypothyroid rats and mice. On the other hand, VO_2 responses to beta-agonists were improved in hyperthyroid rats as compared to the euthyroid group. An increased NA-stimulated VO_2 response in hyperthyroidism has also been shown /15/.

The reduced VO_2 response in hypothyroid rats was more marked for ISO than for BRL. Although VO_2 responses in hyperthyroidism were similar after ISO and BRL, the increment to BRL was more marked than to ISO. The results of measurement of BAT mitochondrial GDP binding agree with the data of VO_2 measurements. The basal binding was not measured but the difference in GDP binding between hypothyroid and euthyroid groups was more marked after ISO than after BRL. These results also prove that a hypothyroid status can make more effective impression on the mechanism responsible for the thermogenic effect of ISO than for that of BRL. BAT mitochondrial GDP binding after the maximal thermogenic doses of BRL and ISO in euthyroid and hyperthyroid rats did not differ significantly, verifying that BAT thermogenic response to BRL in hyperthyroidism reaches the thermogenic capacity ot ISO.

How to explain the results of the present study?

The reduced VO_2 response in hypothyroidism and the marked increment in VO_2 in hyperthyroid rats to beta-agonists as well as results of the measurement of mitochondrial GDP binding can be explained with modulating catecholamine sensitivity due to alterations of the thyroid hormone status. It is well-known that hyperthyroidism is associated with an increase in a receptor density in heart, white adipose tissue and BAT /2, 19/, whereas a decreased receptor number in rat brown adipocytes has also been found by others /21, 24/.

BAT adrenoceptors have been classified not only as a mixed beta-1/beta-2 but as a beta-3 subtype, too /1, 18, 20/ and it is not known how

thyroid hormone status affects the beta-3 adrenoceptor or the thermogenic responses to a so-called beta-3 agonist, such as BRL. The present results suggest that hypothyroidism can exert a more expressed influence on beta-1 and beta-2 adrenoceptors than on beta-3 adrenoceptors responsible for the thermogenic effect of BRL. It seems that activation of thermogenesis via the beta-3 adrenoceptor is less dependent on permissive levels of thyroid hormones than activation via beta-1 or beta-2 adrenoceptors. Furthermore, our results suggest that the beta-3 adrenoceptor may be more sensitive to increased levels of thyroid hormones, since hyperthyroidism produced a greater potentiation of BRL responses than of ISO responses.

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IMMUNOLOGY

KILLER-CELL ACTIVITY IN ALCOHOL-ORIGINATED DISEASES OF THE LIVER, AND THE EFFECT OF ALCOHOL ON THE K-CELL FUNCTIONS UNDER IN VITRO CONDITIONS

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Non-specific cell immunity and, within it, the change in K-cell activity, can be relevant in alcohol-induced diseases of the liver. It was examined for this reason how alcohol in its different concentrations influences the activity of K-cells under in vitro conditions. Furthermore, the cytotoxic capacity of K-cells was defined in 22 chronic alcoholics and 112 patients with alcohol induced hepatopathies. The latter were divided into subgroups. Cytotoxic capacity of lymphocytes in the peripheral blood was determined in a test against human red blood cells. 123 healthy volunteers made up the control group. A high concentration of alcohol was needed to impede K-cell capacity under in vitro conditions. It is supposed that the gradual growth in K-cell activity registered in cases of alcohol-induced hepatopathy may point - though only indirectly - to the development of an antibody-dependent cellular cytotoxic reaction.

Keywords: liver immunology, alcohol-induced liver disease, Killer-cell, ADCC.

Introduction

Both experts and lays have long been speculating on the reason for the variation in susceptibility to alcohol. The enormous individual variations in both the acute symptoms and chronic consequences are well known. In some individuals heavy drinking habits carried on systematically for decades induce no liver aliments, while in others shorter alcoholization period - lasting a few months - can cause serious hepatic lesions. The healthy organism can metabolize 160-180 g of alcohol/day. Alcohol addicts

Abbreviations: ADDC = antibody dependent cellular cytotoxicity; LSP = liver specific membrane lipoprotein

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will generally metabolize even more, since chronic alcohol abuse causes enzyme induction, increasing enzyme capacity. Today we consider that high alcohol-content beverages develop a disease faster than those of a lower alcohol-content. Under equal conditions, women are more prone to alcohol-induced diseases of the liver, and usually show faster progression than men, while advanced forms of the diseases occur more frequently in females than in males.

Besides many other factors, it is possible that in some specific cases the immune system and, within it, the non-specific cell immunity, can play a leading role in alcoholic liver diseases. This can be explained by the fact that in the course of alcohol-induced liver cell damage, liver cell plasma membrane fragments of antigen properties may come into contact with lymphocytes and, finally, through a series of complicated processes, may result in production of auto-antibodies. The lipoprotein related to the liver cell plasmamembrane (liver-specific membrane lipoprotein: LSP) was isolated by Meyer zum Büschenfelde/13/. The distribution of LSP on liver paranchyma cells is diffuse. LSP can appear parallel on the membranes of hepatocytes and the plasma membrane /8/. The LSP is basically an antigen complex, many factors of which e.g. the hepatic lectin, sometimes referred to as asialoglycoprotein /12, 24/ have been recognized. Anti-LSP autoantibodies were first traced by Hopf et al. who used an immunofluorescence method. The autoantibodies were visible on the membrane of liver cells in the form of linear IgG deposits /9/. The anti-LSP is connected to the antigen-determinant area of the liver cell membrane by means of its Fab part, while it binds to the killer (K)-cell with its Fc fragment, the actual cause of cytolysis. The appearance of the anti-LSP autoantibody subsequently means the development of the conditions for an antibody-dependent cellular cytotoxic (ADCC) reaction directed against the liver cell /21/, with the killer-cell as its effector cell. The activity of K cells is partially genetically determined, although their cytotoxic capacity - when activated - may increase during an ADCC reaction. With all the above in mind, the determination of K-cell activity could be a useful indirect parameter to indicate a hypothetical ADCC reaction against the liver cell.

After considering the above theoretical presuppositions and data from medical literature, we made efforts to find an answer to the following questions:

1) How does alcohol influence the Killer-cell activity under in vitro conditions?

2) Is there a characteristic change in K-cell activity in the case of alcohol-induced liver diseases?

Materials and Methods

We have studied antibody-mediated cytotoxic capacity in an antihuman red blood cell test. The cytotoxic capacity test was developed by Garam and Bakács following Urbaniak's basic methodology /5, 23/. The test is modelled on the enzymekinetic structure of Zeijlemaker et al., as well as Thorn and Hanney /22, 26/. According to this model, the cytotoxic capacity of lymphocytes also depends on the number of target cells, in consequence, the cytotoxic mechanism is similar to certain enzyme reactions.

The lymphocytes were separated from 20 ml heparinized blood. We separated mononuclear cells according to Böyum, following the Ficoll-Uromiro gradient centrifugation method /3/. We used as target cells human "0" (R1, R2) red blood cells. Target cells were washed, incubated at room temperature with 2% papain for 10 min, then were labelled for 120 min at 37 °C with 200 μCi ^{51}Cr isotope (Na-chromate, Amersham, England). Following the incubation period, the cells and the required concentrations were set in RPMI-FCS nutritive solution. Anti-D immunoglobulin (National Institute of Haematology, Budapest) was used as antibody in 1:1200 final dilution. From the target cells were prepared a dilution set of $1x10^6$ to $14x10^6$/ml concentration. From these dilutions 50 μl units were put into the V-shaped well of a microdisch. Also 50 μl units of the $2x10^6$/ml concentration lymphocytes were added to each culture, then 50 μl anti-D serum of the above-mentioned concentration, and finally the end volume was augmented to 200 μl by the addition of 50 μl medium. The test units were incubated for 18 h in a thermostator of 37 °C in an atmosphere containing 5% CO_2. Alcohol was added in 50 μl volumes, at 0.5-1.0-5.0-10.0% concentration to the in vitro testing units.

The calculation of cytotoxic capacity: First the specific release per cent was calculated by the following formula:

$$\frac{\text{Overfloating "cpm" - spontaneous release "cpm"}}{\text{Total built-in activity "cpm" - spontaneous release "cpm"}} \times 100$$

From here, the cytotoxic capacity, expressed by the maximal number of target cells destroyed by a unit of lymphocytes, can be calculated by this formula:

$$\frac{\text{No of saturating target cells x related specific release \%}}{100}$$

The number of saturating target cells refers to the smallest possible number of target cells by which we can still measure the maximum of lymphocyte activity. In all lymphocyte and target cell combinations, we used two parallel testing units and the average of the two was used in calculations. In each case, we observed the direct effect of alcohol on red blood cells, but in none of the concentrations could we register a lytic effect. We ran all our calculations on a programme obtained from the University of California Los Angeles Biomedical Department.

Student's two-sample t-test was used for calculation of significance between the two groups.

Patients and Controls

K-cell activity was determined in two groups of patients and a group of healthy volunteers.

1. Patients' group 1. Twenty two chronic alcoholics (21 male and 1 female), none of whom was suffering from liver disease, and all being on voluntary therapy to break the drinking habit. Average age 38.0 (from 17 to 51) years.

2. Patients' group 2. 112 (64 male and 48 female) patients suffering from alcohol-induced hepatic ailments. The patients first underwent a thorough medical check-up: anamnesis, determination of physical status; laboratory tests including SGOT, SGPT, alkaline phosphatase, protrhombin, cholesterol, cholinesterase, GammaGT, electrophoresis, immunoelectrophoresis, anti-nuclear factor, AFP, HBsAg, complete urine analysis, complete blood count, sedimentation rate; more recently, it has become possible to determine serum-conjugated bile acid concentration as well as procollagen-III-peptide level; abdominal ultrasound, and in more than half of the cases, histology. In some cases additional gastroscopy, isotope liver-spleen scintigraphy and computer tomography were performed. The fatty liver diagnosis was in all cases supported by histology. In acute alcoholic hepatitis, liver biopsy was conducted in 11 cases out of the 25. Where biopsy was impossible, alcohol abuse in the anamnesis and a syndrome consisting of high fever, jaundice, anorexia, vomiting and diarrhoea helped in setting up the diagnosis. Virus marker (HAV, HBV) tests were negative. Leukocytosis was always traceable in the blood count. Laboratory tests were characterized by increase in serum transaminase, GammaGT and alkaline phosphatase. Ultrasound found 1st-degree diffuse liver lesion. 40 out of the 70 liver cirrhosis cases were examined histologically. Due to absolute or relative contraindications, biopsies were not conducted in advanced, vascularly decompensated and parenchymatous cases. In these cases the diagnosis was based on typical clinical patterns, the presence of the above-mentioned symptoms of decompensation, the laboratory tests detailed above, as well as a 2nd-stage diffuse liver lesion found by ultrasound.

The 112 patients were divided into three groups, viz.:

acute alcoholic hepatitis:	25 cases
fatty liver:	17 cases
hepatic cirrhosis:	70 cases

Following the revised Child scheme /4/, 3 subgroups were distinguished within the hepatic cirrhosis group. Child developed a score system based on the 3-level variations of 5 parameters, which represents efficiently the graveness of the patient's clinical state. We have summed up the revised Child scheme in Table I. Child's group "A" is represented by the 35 mildest clinical-state patients, in Child's group "B" we have the 21 patients with a clinical state of medium severity, and in Child's group "C" 14 cases of the most serious clinical state. Important data concerning groups and subgroups are shown in Table II.

3. The killer-cell activity of 123 (55 male, 68 female) healthy volunteers was the control. Average age: 56.6 years. This group, too, was subjected to a thorough examination, including anamnesis, determination of physical status, and laboratory tests (complete blood count, urine analysis, blood sugar level, SGOT, SGPT, GammaGT, alkaline phosphatase, serum bilirubin, cholinesterase). Only individuals found healthy in the above testing were included in the control group.

Table I

The revised Child scheme

points	1	2	3
bilirubin µmol/l	<25	25-50	>50
albumin g/l	>35	30-35	<30
encephalopathy	none	subclinical EEG +	liver coma (I-IV)
ascites	none	responsive to medication or surgical therapy	resistant to therapy
state of nourishment	normal	muscle atrophy	cachexia

Evaluation: 5 to 7 points = stage "A"
8 to 10 points = stage "B"
above 10 points = stage "C"

Table II

Main data of alcohol-induced liver disease groups

	Liver diseases						
	Acute alcoholic hepatitis	Fatty liver	Liver cirrhosis			Chronic alcoholic non-liver disease	Healthy control
			Child				
			A	B	C		
No. of cases	25	17	35	21	14	22	123
Male	14	9	25	11	5	21	55
Female	11	8	10	10	9	1	68
Average age	37.3	52.2	65.0	60.3	47.3	38.0	56.6
Age brackets	29-54	31-76	38-71	44-71	37-49	17-54	16-94

Results

Killer-cell activity of the healthy control group

We determined the average K-cell activity within the control group also on the basis of breakdown by sex and age (18-40, 41-65, 66+ years). Since we wish to publish the details of our findings in another paper, we restrict ourselves to giving only the most relevant connections in the present article. The K-cell activity (both on the whole and in the age groups) was higher in the female group than in similar male groups, although

the differences were not significant. Highest K-cell activity values were found in the group between 41 and 65 years of age, but the differences from the values obtained in the other age brackets were not significant.

Effect of alcohol on killer-cell activity under in vitro conditions

We found that under in vitro conditions Killer-cell activity needed high concentrations of alcohol (5.0-10.0%) to be impeded. We did not find trace influence affecting K-cell activity in the case of lower concentrations (0.1-0.5-1.0%). Our results are shown in Fig. 1. It is discernible from there that the alcohol concentration found in real life situations (in vivo) did not alter the cytotoxic capacity of K-cells under in vitro conditions. Both the 5.0 and 10.1% alcohol concentrations, which caused considerable decrease in K-cell capacity, would by no means appear under normal (in vivo) circumstances, since even lower concentrations than those can prove lethal.

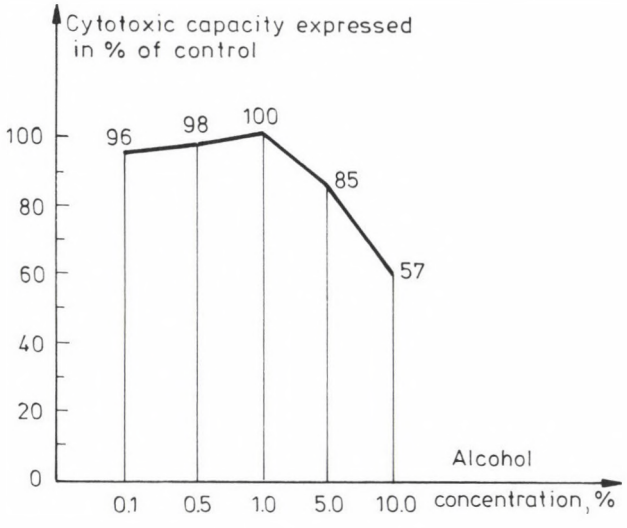

Fig. 1. K-cell activity alterating effect of alcohol measured in "0" Rh-positive human red blood cell cytotoxic capacity test under in vitro conditions. $2x10^6$/ml lymphocytes were used in the test

Killer-cell activity of chronic alcoholics and patients with alcohol-induced liver diseases

The average Killer-cell activity of chronic alcoholics (patients with no hepatic ailments was $1.97 \times 10^6 \pm 1.19 \times 10^6$. This result does not differ from the K-cell activity average measured in the control group: $1.95 \times 10^6 \pm \pm 1.43 \times 10^6$. In acute alcoholic hepatitis, K-cell activity decreased ($1.65 \times 10^6 \pm 0.93 \times 10^6$) in respect to both the control and the chronic group, although not significantly.

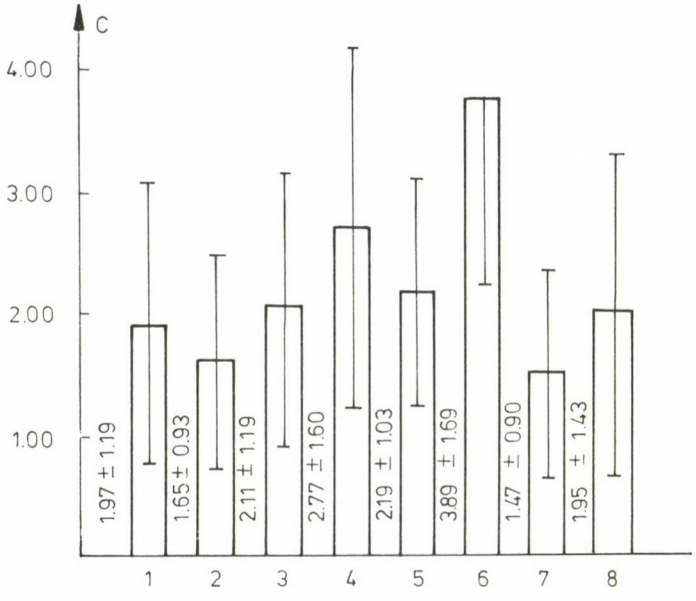

Fig. 2. Cytotoxic capacity of peripheral lymphocytes against human red blood cells (C=average of cytotoxic capacity x 10^6 ± S.D.) of chronic alcoholics (group No. 1; n=22) and patients of alcohol originated liver diseases. Group No. 2 is acute alcoholic hepatitis (n=25). Group No. 3 = fatty liver (n=17). Group No. 4 the total No. of hepatic cirrhosis (n=70). Group No. 5 hepatic cirrhosis - Child's "A" (n=35). Group No. 6 hepatitic cirrhosis - Child's "B" (n=21). Group No. 7 hepatic cirrhosis - Child's "C" (n=14). Group No. 8: healthy control (n=123)

Significance differences

group 1 vs 4	$P < 0.01$	group 2 vs 6	$P < 0.001$
group 1 vs 6	$P < 0.001$	group 3 vs 6	$P < 0.001$
group 2 vs 4	$P < 0.001$	group 4 vs 8	$P < 0.001$
group 2 vs 5	$P < 0.01$	group 5 vs 6	$P < 0.001$
group 5 vs 7	$P < 0.01$	group 6 vs 7	$P < 0.001$
group 6 vs 8	$P < 0.001$		

No significant difference between groups 1, 2, 3, 5, 7, and 8.

In further groups of liver-disease patients, we registered an increasing K-cell activity parallel to the seriousness of the illness. In this way, e.g., in the group of fatty liver $2.11x10^6 \pm 1.19x10^6$, and in hepatic cirrhosis $2.77x10^6 \pm 1.6x10^6$ were the registered average values of K-cell activity. The increase in K-cell activity in all hepatic cirrhosis cases is strongly significant ($P < 0.001$), compared to the control or acute alcoholic hepatitis groups. We also found interesting results within the subgroupings - established by following Child's score system - of the cirrhosis group. In the least severe stage, Child's subgroup "A", the K-cell activity ($2.19x10^6 \pm 1.03x10^6$) was equal to that of the clinically most similar characteristics, the fatty liver group. The K-cell activity of the medium-gravity clinical state Child "B" cirrhosis patients increased significantly ($3.89x10^6 \pm 1.69x10^6$). This value is significantly higher ($P < 0.001$) than in any other group examined. The K-cell capacity of Child's "C" stage patients falls sharply ($1.47x10^6 \pm 0.90x10^6$). For an easier survey of the findings we have summed them up in Fig. 2; in which the significance of differences between groups is also shwon.

Discussion

Alcohol causes a very significant change in the immune system, although many of its effects are still unclear.

The in vitro study of Glassman et al. registered alcohol-induced abnormal chemotaxis and functioning of granulocytes /6/. The function of macrophages decreased in the presence cf alcohol /6/. In alcoholic hepatitis and cirrhosis Con-A-induced lymphocyte proliferation decreased /14, 25/. Some in vivo studies show that the total number of T lymphocytes of the peripheral blood does not change in alcoholic liver diseases /25/, while according to others a certain decrease can be registered /10/. Considerably dissenting results between T lymphocyte subgroups have also been demonstrated. T-helper cells significantly increased in number in all alcoholic liver diseases, whereas T-supressing lymphocyte growth was only registered in alcoholic cirrhosis /25/. Actis et al. studied the cytotoxic activity of peripheral blood lymphocytes on autologous hepatocytes of patients with alcohol-induced liver disease. From the 10 hepatic cirrhosis and acute alcoholic hepatitis patients the cytotoxic activity increased in 9 cases. On the other hand, from the 8 cases of fatty liver and/or liver

disease accompanied by minor histological alterations, an increase in cytotoxic activity was observed in only one case /1/. Within the framework of the same study, the authors tested which were the ways leading to the lesion of liver cells: they found that in those six alcoholic hepatitis cases in which high cytotoxic activity was registered, lesion of cells recurred in the majority (five out of six) of the cases and it was not tied to T-cells. On the basis of these findings, the pathogenetic role of non-specific cellular cytotoxicity and auto-immunity in the development and progression of the disease might be questioned, consequently, attention turned towards natural cytotoxic cells and the cell lesion resulting from them. These cells are capable of killing target cells without prior immunization /22, 24/.

The non-specific cell-mediated immunity has two effecting mechanisms:

(a) lymphocytes may cause cytotoxicity directly, without specific antibody, this is why it is referred to as natural cell - mediated cytotoxicity (NCMC), where the executing cell is the natural killer (NK)-cell /7, 11, 16, 17, 19/. Müller et al. proved a diminishing of NK-cell activity in hepatic cirrhosis cases /15/. Since the most important function NK-cells is to destroy tumour cells, it seems obvious, although a bit far-fetched, to establish a relationship between the high incidence of malignant liver disease in these hepatic cirrhosis cases and low NK-cell activity registered in the same /15/.

(b) The condition for the other effecting mechanism of non-specific cell-mediated immunity is the presence of specific antibodies, which are needed for the destruction of target cells. This mechanism is termed antibody-dependent cellular cytotoxicity (ADCC). The effector cells of the ADCC reaction as mentioned above, are the killer (K)-cells.

The aim of our study was to gather data on the effect of alcohol on Killer-cells under in vitro conditions, and furthermore, to study the change of K-cell activity in groups of patients with liver diseases of alcoholic origin. We found that K-cell activity was impeded only under high in vitro alcohol concentration. It is an interesting result of the tests that K-cell activity shows a tendency to decrease in the acute alcoholic hepatitis group. It is possible that acute, toxic and large dosis of alcohol intake resulted in this group in diminishing activity. This hypothesis may also account for why no K-cell capacity change took place in case of a long-term, even alcohol intake (the chronic alcoholic, non-liver-disease patient group). From Fig. 2 it is also evident that parallel to

growing of seriousness of clinical diagnosis, the average of K-cell activity increases steadily until Child's stage "B" cirrhosis. There was barely any difference between Child's stage "A" cirrhosis and fatty liver groups. This is understandable, since there is a possibility if dynamic relationship between the mildest form of cirrhosis and the more serious form of fatty liver, which could occasionally run together with inflammation and fibrosis. The gravest Child's stage "C" group shows very low K-cell activity value, which could be explained, on the one hand, by the general deterioration process the body is undergoing when all biological functions, including cell functions, fail. On the other hand, it is also possible that at this stage the ADCC reaction, being completely over, the process burns out.

Our results - Killer-cell capacity growths registered in more serious cases of alcohol-induced liver diseases - seem to support the presumption that the possibility of an ADCC reaction developing in the course of the disease has to be taken into consideration. Although our findings are naturally only an indirect proof of the ADCC reaction taking place, they prompt us to verify with further testing the presence of theoretical anti-liver cell-specific ADCC reaction. The individual proof of an ADCC reaction can perspectively lay the foundation for an aimed immune therapy and could lead to the introduction of new therapeutical procedures.

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LABORATORY

EFFECTS OF THE MEDIUM ON THE FILTERABILITY OF HUMAN RED BLOOD CELLS

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Red blood cell filterability as an appropriate method for evaluating red blood cell deformability was studied with different suspending media of erythrocytes. Comparison of autologous plasma and buffer suspensions of healthy subjects' erythrocytes showed no significant difference in filterability. Albumin alone resulted in a dose-dependent increase, while fibrinogen caused a decrease in red cell filterability. In the presence of fibrinogen, albumin showed controversial effects. The results suggest that measurements of red blood cells in their original surroundings give more accurate information about the microcirculation because plasma components may have a crucial influence on erythrocyte deformability.

Keywords: erythrocyte deformability, absolute and relative filterability, healthy population, albumin, fibrinogen.

Introduction

At a given blood pressure the tissue blood supply is determined by the diameters of the involved vessels and the rheological properties of the blood. RCD is one of the factors of haemorheology. Damaging of this physiological parameter of RBCs shortens the life-span of the cells, increases whole-blood viscosity and worsens microcirculation /4, 21, 22/.

RCD is determined first of all by the cell's conditions (the geometrical state of the cell, the membrane viscoelastic properties and the intracellular viscosity) /4/, and influenced also by plasma parameters e.g.

Abbreviations: CP = number of clogging particles; PBS = phosphate-buffered saline; RBC = red blood cell; RCD = red cell deformability; Tc = red cell transit time

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albumin /14/, fibrinogen /7, 18/ and pH /1, 22/. Therefore, it seems to be justified to distinguish absolute and relative RBC deformability (RCD determined in buffer and autologous plasma, respectively). The application of this distinction is supported by discordant findings, e.g. in pre-eclampsia when the RCD depends on the suspension medium of RBCs /1, 9, 10, 16/.

The aim of our study was to evaluate the absolute and relative RCD in a healthy population and to measure the separate and joint effects of albumin and fibrinogen on RCD.

Materials and Methods

19 ml of venous blood anticoagulated by K_2-EDTA, obtained from 40 (19-40 years) healthy volunteers (17 male and 23 females) were centrifuged at 2000 g for 15 min. Erythrocytes were aspirated from the centre of the packed cell volume and washed twice with PBS (pH: 7.4; 290 mOsm/kg) containing 2 g/l of bovine albumin (SIGMA, fraction V) and 5 mMol/l glucose. The cells were suspended in 30 cases in PBS and autologous plasma and in five occasions in 1:1 and 1:4 mixtures of plasma and PBS as well. In 5 cases the RBCs were suspended in PBS containing 0, 10, 20, 40 and 70 g/l bovine albumin, in another 5 cases in PBS containing 40 g/l albumin, 1/g/l human fibrinogen (SIGMA) or both or none. Erythrocrits of aliquots were adjusted to 10% before incubation at 37°C for 30 minutes. Leukocyte contamination varied from 0.5×10^4 to 3×10^4/l in the samples under visual control.

RCD was assessed by measuring RBC filterability with a St. George's filtrometer (Mikron GMK, Budapest). The determination of the suspension's flow dynamics through the pores of a Nucleopore filter models the microcirculation regarding cell concentration, shear force and pore diameter. As in diameter the erythrocytes exceed the filter pores (7-8 μm vs 5 μm) only cells with appropriate deformability are capable of passing the pores easily. Red cells with decreased ability to change their shape in response to the external force will meet difficulties in traversing the pores or will be even entrapped. The data given by the filtrometer, the erythrocrit and the number of open pores at the beginning of the measurement gave the following characteristics of RCD when appropriate software and microcomputer were used.

Tc: red cell transit time (relative to the medium transit time of one cell through a pore 11.5 in length and 5 μm in diameter).

CP: number of clogging particles (number of blocked pores after filtration of 1 ml of suspension if one pore is blocked only by one cell; 10^6/ml) /8/.

Measurements were carried out in duplicates at room temperature within 3 h after blood sampling. Upon counting the filterability parameters, their means were taken for statistics. For the comparison of plasma and PBS suspensions Student's paired t-test, for the others Wilcoxon's test was used. Level of significance was taken at $P \quad 0.05$.

Results

When RBCs were suspended in their own plasma the Tc was 7.13 ± 0.69 (mean $\pm$ S.D.), in PBS it was 6.99 ± 0.65. The CP (10^6/ml) equalled in plasma 1.055 ± 0.210 and in PBS it was 1.103 ± 0.246. Similar results were obtained when the plasma was changed for buffer (Fig. 1). No significant dif-

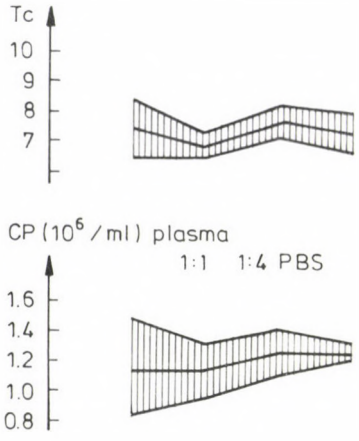

Fig. 1. Red cell transit time (Tc) and number of clogging particles (CP) values (mean ± S.D.) in autologous plasma, buffer and in their 1:1 and 1:4 mixtures (n=5)

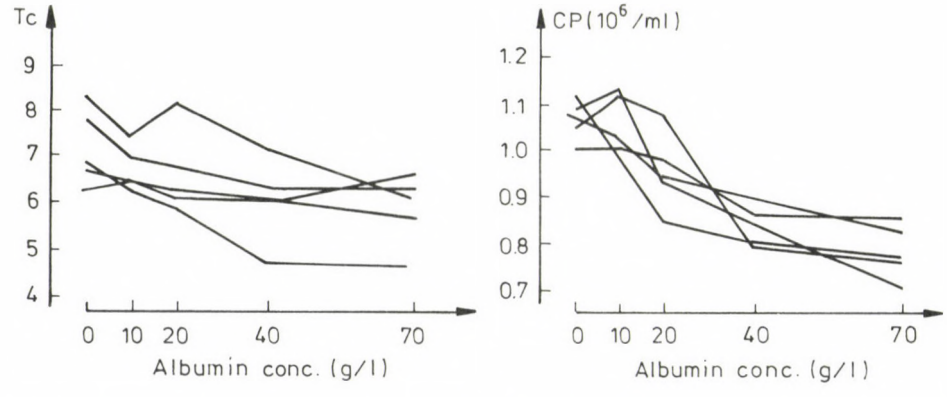

Fig. 2. Individual red cell transite time (Tc) and number of clogging particles (CP) values with increasing albumin concentration of the buffer medium

ference was found between plasma and PBS or mixed suspensions in any case and parameter.

Increasing albumin concentration caused a decrease in Tc. Except for the value with albumin 10 g/l (6.72 ± 0.48), the values with consecutive albumin concentrations (6.66 ± 0.90, 6.08 ± 0.90 and 5.90 ± 0.75) were significantly different from the control (7.21 ± 0.79) obtained without albumin. The CP found in albumin free PBS was 1.061 ± 0.051, with increasing albumin concentration it was 1.050 ± 0.072, 0.956 ± 0.081, 0.840 ± 0.042 and 0.781 ± 0.063. Samples with 40 and 70 g/l albumin concentrations were

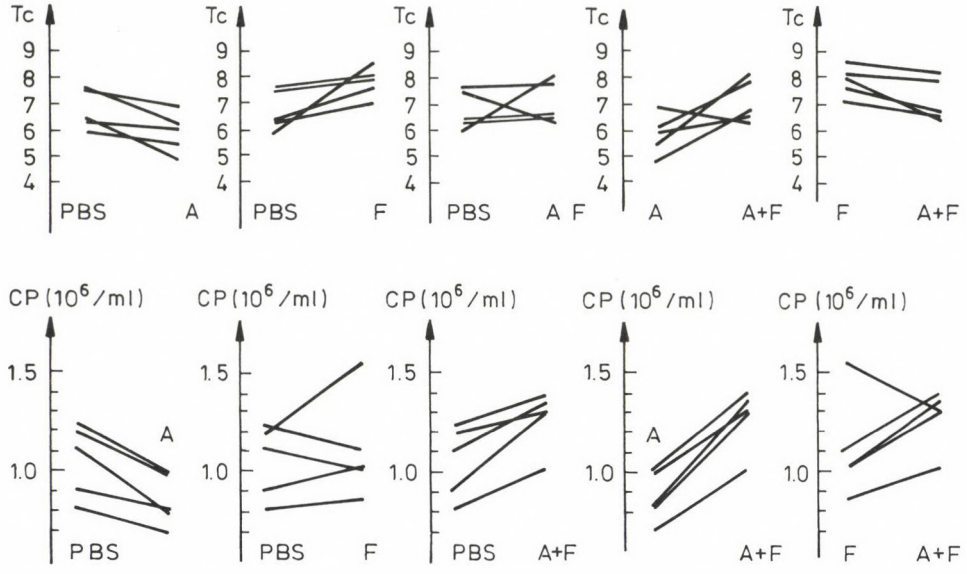

Fig. 3. Directions of changes of the individual red cell transit time (Tc) and number of clogging particles (CP) values obtained in protein free PBS, in the presence of 40 g/l albumin (A), 1 g/l fibrinogen (F), or both (A+F)

significantly different from the control. Individual values for Tc and CP are shown in Fig. 2, respectively. Further significant differences can be found between data regarding both parameters.

One g/l fibrinogen in the media (Fig. 3, F) resulted in a significant increase of Tc (from 6.73 ± 0.75 to 7.85 ± 0.54). 40 g/l albumin (A) caused again a significant decrease (5.90 ± 0.78) while the presence of both proteins in equal concentrations (A+F, 7.10 ± 0.77) the Tc did not differ significantly from the control value. As regards CP albumin alone caused a significant enhancement (from 1.049 ± 0.192 to 0.862 ± 0.122), fibrinogen alone (1.112 ± 0.248) did not, but in the presence of albumin (1.266 ± ± 0.149) did result in a significant decrease in filterability.

Discussion

RCD could be defined and measured as a relatively absolute value in a given system if cells were examined in buffer after a washing procedure. This value, however, may be remarkably affected by the cells' environment. Therefore, it might be considered that the measurement of RCD

in autologus plasma should give a more relevant information to the microcirculation than measurements performed in any other medium.

RCD measurements in the presence of fibrinogen, and especially in plasma, may raise a doubt whether changes in RCD indeed or other effects originate from the medium are responsible for the changes in filterability. One of such effects might be the changes in the medium's viscosity. In our system the count of relative (medium/suspension) flow values allow the exclusion of this effect. On the other hand, fibrinogen (and also globulins) may interfere with the suspension's flow because of RBC aggregation /3, 6, 17, 23/. However, this phenomenon occurs with a continuous slowing down of flow, at very low shear force (under 0.05-0.2 Pa), practically at the stop of the flow /2, 5, 13, 20/. Because in our instrument the applied driving force was 400 Pa the shear force was about 45 Pa in the pores /19/. Beyond that, the first few seconds of the flow, when RBC aggregates might have formed, were excluded from the measurements and initial flow rates were determined only in a mathematical way.

Also our data support that alterations in filterability were caused by RCD changes. Namely, we would have obtained a worse filterability in plasma than in buffer if effects of fibrinogen and globulins had caused RBC aggregations. We failed to find significant difference in filterability by the exchange of plasma medium to buffer.

Albumin enhances the disaggregation of rouleaux formations, but only in the presence of fibrinogen /15/. In our experiments albumin resulted in a dose-dependent enhancement in filterability of RBCs in the lack of fibrinogen. This effect existed in the same extent in hyperosmolar PBS (unpublished data). Our observations are inconsistent with results of Koyama and Kikuchi /12, 14/, who found a significant worsening in RCD by increasing concentration of plasma or albumin alone in the incubation medium; the worsening was especially high, when cells were suspended in hyperosmolar environment. The discordant observations can be explained partly by methodical differences.

Our data suggest that albumin alone increases, fibrinogen decreases the RCD on the whole, though, in the presence of fibrinogen albumin acts controversially: most of red cells become more flexible (lower Tc) but the remainder cells tend to be more rigid (higher CP). Regarding the Tc values they oppose each other's effects apparently, such as in thixotropy /11/. In the light of our findings with albumin and that both proteins adhere to the surface of erythrocytes /12, 18/, it may indicate a com-

petition when the adherence of albumin and fibrinogen molecules may have an opposite result on RCD. At the same time, the rate of pore clogging was significantly higher with both proteins than with fibrinogen alone. This observation could be explained by the presence of different cell populations in the examined samples.

Our data differ from that of Rampling and Sirs /18/, who experienced a decreased RCD by repeated washings of the RBCs which could have been protected by fibrinogen. It should be noted, however, that their method was basically different from ours. On the other hand, our findings are in accordance with the opinion of Dormandy /7/ that increasing fibrinogen concentration decreases the effective RCD.

Our results with PBS as medium were not significantly different from that with plasma medium but differend from the results with the PBS containing both proteins. These findings, even though the used fibrinogen concentration was lower than the physiological level, may indicate different RBC - environment interactions in buffer and plasma and/or the influence of other plasma factors accordingly. Investigation of details of RCD - environment connections needs further experiments.

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PHYSIOLOGICAL VALUES OF GLUCOSE-6-PHOSPHATE-DEHYDROGENASE (G-6-PD) IN CHORIONIC VILLI

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(Received: June 19, 1990)

The physiological activity values of glucose-6-phosphate dehydrogenase (G-6-PD) from chorionic villi homogenized in Triton X was evaluated. The frozen, non-cultivated chorionic samples were obtained from artificial abortion in an early period of gestation (6-11 weeks). The mean G-6-PD activity was 0.43 U/mg protein. The G-6-PD enzyme activity showed no correlation with the week of gestation.

Keywords: glucose-6-phosphate dehydrogenase, chorionic villi, early gestational period.

Introduction

Glucose-6-phosphate dehydrogenase (G-6-PD) catalyses the oxidation of D-glucose-6-phosphate to D-glucono-delta-lactone-6-phosphate by $NADP^+$ (or NAD^+). The enzyme was discovered by Warburg and Christian, initially in horse erythrocytes /1/ and subsequently in other mammalian erythrocytes /2/. The principal function of the hexose monophosphate shunt in human erythrocytes is the generation of NADPH and limited amounts of 5-phosphoribosyl pyrophosphate. NADPH is used for several functions, the most important being the coenzyme function for glutathione reductase, the enzyme that maintains glutathione in its reduced form. In addition, NADPH appears to be needed for the optimal functioning of catalase /3/. The pentose phosphate shunt provides the NADPH necessary for the fatty acid synthesis, which is related to the oxidative part of the shunt where the enzymes

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Akadémiai Kiadó, Budapest

G-6-PD and 6-phosphogluconate dehydrogenase produce the NADPH. G-6-PD was purified by Kanji et al. /4/ from pig liver. This enzyme exists as an active dimer of molecular weight of 133,000 dalton and an inactive monomer of 67,500. G-6-PD is highly specific for $NADP^+$ and glucose 6-phosphate, localizing mostly within the soluble portion of the cytoplasm.

Cultured fibroblasts may represent a mosaicism of two cell types. The product of the normal G-6-PD allele is designated the B form. A variant electrophoretic form G-6-PD type A,- is common mostly in blacks /5, 6/.

Kinetic characterization of the inhibition effect of nickel on G-6-PD and glutathione reductase (GR) was investigated by Cartana et al. /7/. The effect of nickel on G-6-PD activity was consistent with a mixed-type inhibition pattern.

Schuster et al. /8/ mathematically modellized the metabolic pathways of G-6-PD enzyme deficiency, the energy and redox metabolism of G-6-PD deficient erythrocytes the donors of which showed an increased sensitivity to energetic load.

Since data about the physiological values of G-6-PD activity from the chorionic villi have not been published so far, we have determined this enzyme activity from chorionic villi.

Materials and Methods

The activity of G-6-PD measured in Triton X (0.1%) homogenized chorionic tissue obtained from the first trimester (6-11 weeks) of gestations interrupted by artificial abortion. Purified chorionic villi were selected under dissection microscope by separation from maternal decidua. The tissue samples were obtained at legal termination of pregnancy performed for socioeconomic indications with regular consent of the mothers. The gestational age was calculated from the first day of the last menstrual period. The tissues were finally minced in 10 volumes of icecold buffer (50 ml TRIS-HCl 1% Triton X-100, pH 7.4) and homogenized at three 20 sec bursts (Ultra-Turrex homogenizer). The homogenate was centrifuged at 105,000 g for 60 min and the supernatant (cytosolic fraction) was used for enzyme assay.

Enzymatic activities were measured as described by Kanji et al. /4/. The change in absorbance at 340 nm (1 cm light path) was followed with a Varian DMS 70 spectrophotometer. The reaction mixture had the following composiiton: 2.5 ml of 0.1 M triethanolamine buffer (pH 7.6), 0.1 ml of 0.035 M glucose-6-phosphate, 0.1 ml 0.011 M NADP and 0.2 ml of 0.1 M magnesium chloride. The reaction was initiated by the addition of 0.1 ml of properly diluted enzyme.

One unit of G-6-PD is defined as that amount of enzyme which catalyses the formation of one micromole/min of NADPH under the conditions described above.

Results

After storage at $-70^{o}C$ the mean G-6-PD activity was 1.43 U/mg protein, SD ± 1.75; the mean of gestational weeks was 9.4 SD ± 1.86; the mean protein concentration of the chorionic villi homogenized in Triton was 2.99 SD ± 1.78 (Table I). The G-6-PD enzyme activities showed no correlation with the week of gestation ($r = -0.27$, $P > 0.05$).

Table I

Physiological activities of G-6-PD enzyme from homogenate of chorionic villi

week of gestations	X 9.4 SD ± 1.86
protein concentration	X 2.99 SD ± 1.78 mg/ml
G-6-PD n = 22	X 1.43 U/mg protein SD ± 1.75
Correlation coefficient = r	
G-6-PD and gestational weeks r = -0.27 P > 0.05	

Discussion

Salgó and Pál /9/ determined the reference range for 10 enzymes (alkaline phosphatase, ALAT, ASAT, GGT, CK, LDH, etc.) in amniotic fluid and maternal samples at 14-42 weeks of gestation. The determination of gamma-glutamyl-transferase, heat-stable alkaline phosphatase and creatine kinase was found to be of appreciable diagnostic significance in the clinical practice.

In this study, our team firstly evaluated the physiological activity values of G-6-PD from in Triton X-homogenized chorionic villi. The frozen, non-cultivated chroionic villi were obtained in an early gestational period.

Previously, the activity of the G-6-PD had been analysed from the amniotic fluid in the last trimester /10, 11, 12/; the mean activity of G-6-PD enzyme was 0.8 U/l (extreme range: 0-2.9). The activity of the lactate dehydrogenase was 82.4 U/l (56.7-132); that of the above-mentioned enzymes was higher than that of G-6-PD.

In the next future we will investigate the physiological activity of the G-6-PD during the second and the third trimester of gestation. The change of the activity of G-6-PD enzyme might be interesting among pathological circumstances like toxaemia, intrauterine fetal retardation and fetal distress.

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BOOK REVIEWS

"Cardiac lymph circulation and cardiac disorders"

Solti, F., Jellinek, H.

Akadémiai Kiadó, Budapest, 1989, pp. 1-187, with 160 figs

This is an excellent and up-to-date review on the correlation of cardiac lymph circulation and some cardiac disorders based mainly on experimental data and clinical observations of the authors obtained from several decades' study. The different forms and consequences of impeded cardiac lymph circulation are thoroughly discussed. Mechanical cardiac lymphatic insufficiency can be induced by obstructing cardiac lymph flow, e.g., by some inflammatory process in patients or by ligation of the cardiac vessels and regional lymph nodes in experimental conditions. The dynamic insufficiency can be produced by overloading the transport capacity of the cardiac lymph vessels, e.g., by increase of heart frequency, etc. or by increasing local lymph production provoked by elevated venous pressure in experiments. Due to the lymphatic blockade, the protein (and fluid) transport from the cardiac interstitium will be deteriorated with the consequences of protein accumulation in the myocardium and coronary vessels wall. These consequences are investigated by histological and electrophysiological (ECG) methods, and by measuring coronary blood flow.

The most important conclusion of the authors is that cardiac lymphatic blockade or insufficiency induces protein (plasma) accumulation in the wall of small coronary arteries, resulting in a decreased coronary blood flow and its early and late consequences. The early complications are focal necrosis, late complications coronary stenosis and cardiac slerosis.

The interrelations between acute myocardial infarction and arrhythmias with lymphostasis are also discussed.

The documentation is excellent. The aspect of the treatment of the book is critical. Unfortunately, repetitions occur frequently and a quantitative analysis of the late complications due to lymphostatis is missing.

The book is a good guide into the pathophysiology of the heart function and may be very useful for clinicians and physiologists dealing with some special problems of cardiology.

MIKLÓS PAPP

"Intrinsic Asthma"

Proceedings of the 4th International Symposium held at Davos from September 18-20, 1988

Edited by M. Schmitz-Schumann, G. Menz, U. Costabel and C.P. Page.

Birkhäuser Verlag, Basel-Boston-Berlin, 1989, 378 pages,

Price: SFr 96.0

For decades, asthma was considered to be an exchesively allergic disease. When IgE and specific antibodies were first detected and measured only patients with proven exogenic allergic asthma were considered real asthmatics. Later, it became apparent that status asthmaticus, serious

forms of asthma or deaths from asthma often the resulted from asthmatic illnesses showing no evidence of allergic cause. Thus, many clinicians returned to the traditional classification of tronchial asthma, i.e. into extrinsic (exogenic-allergic) asthma and intrinsic (postinfectious) asthma.

Clinical research interest is currently focused on bronchial hyperresponsiveness, which, as a common feature of all forms of asthma interferes with the old asthma categories. This becomes evident when genetic factors are being investigated as a possible cause of bronchial hyperresponsiveness.

In this book the pathogenesis and pathophysiology of intrinsic asthma are discussed by excellent lecturers in depth in comparison with other forms of asthma. The assumed viral origin, as well as the different cell populations of immune system involved in respiratory infection, and their mediators, are considered. Regulatory peptides, toxic radicals, the neuronal components of bronchial asthma and the specific biochemical lesions being related to intrinsic asthma are also dealt with. In the 33 series of lectures there are also included exhaustive discussions of the role of humoral immune factors, aspects of intrinsic asthma therapy, alternative methods of steroid treatment such as cytostatics, leukotrienes and lipoxygenase inhibitors, PAH-antagonists, phosphodiesterase inhibition, up-to-date anti-histamines and stabilizers for immune-competent cells.

The book contains the most important, latest theoretical and practical information in the field of intrinsic bronchial asthma.

ANDOR LEÖVEY

"Generalized epilepsy"

Neurobiological approaches

Avoli, M., Gloor, P., Kostopoulos, G., Naquet, R.

Birkhauser, Boston, 1990, pp. 1-481

Price: SFr 198.0

This book contains selected papers of the Symposium on Generalized Epilepsy held in Montreal, 1988. The prominent editors and authors are of the opinion that, in contrast to partial epilepsies, the neurobiological background of generalized epilepsy (GE) has not been satisfactorily cleared up as yet, although, there are significant and promising scientific results hopeful for use in the near future in clinical practice.

The book starts with a comprehensive thorough historical review of the theories and the models of GE, from Hippocrates to the up-to-date hypotheses.

In Chapter 1, that introducing the clinical features of GE, unfortunately, not the actually accepted classification of epilepsy is used. A very interesting paper of Andermann summarizes the unanswered questions of the GE, such as genetical determination, age dependence, benign nature, plasticity, myoclonus, simple and combined absences, connections between absences and tonic-clonic seizures, particular features of the West and the Lennox syndromes, etc. The common origin of sleep spindles and of the spike and wave complex is evidenced in this chapter by a relevant study of Kellaway. Chapter 2 deals with the basic cellular and neurotransmitter mechanisms of GE. The repetitive firing of the Betz cells is controlled by three slow potassium currents (two of them calcium-dependent, the third one

sodium-dependent). In the extracellular fluid, among others ionic changes measured with selective microelectrodes are demonstrated. Neurotransmitter mechanisms of GE are based on n-methyl-D-aspartate and GABA studies; some new excitant amino acids have been isolated which may play an important role in the pathomechanism. New epilepsy models based on the connection with the transmitter mechanism exist, e.g. the intracortical GABA infusion and the in vitro postsynaptic potential studies in slices. Chapter 3 demonstrates the thalamic and cortical mechanisms of GE. The role of the thalamic relay cells, the theory of the thalamic pacemaker and the relation of the thalamic "GE-specific" and "non-specific" parts are discussed. Considerable efforts have been made for a neurophysiological distinction between partial and generalized epilepsies in animal models and on the basis of the pharmacological experiences at cellular and synaptic level as well as chronic neuronal excitability. The study of Mirsky and Duncan on behaviour in GE is one of the most interesting papers in the volume; it is of great practical value. Chapter 4 is connected with the neurobiology of photosensitive GE. Exciting characteristics of this mechanism might be the focal features seen quite frequently, the participation of the frontal lobe and the decrease of dopamine release in the cortex. An excellent example for the utilization of the results of basic research is the paper of Wilkins et al. on "epileptic" and "non-epileptic" photosensitivity and their connection with headache and possible harmful effects of certain common visual environmental patterns. The topic of Chapter 5 is the GTCS in GE. Based on experimental models the significance of the calcium channels, the role of the substantia nigra and of the mesencephalon are demonstrated. Chapter 6 reviews a topic of outstanding practical importance: the results of metabolic and neurochemical studies of GE. The most modern method for them is positron-emission tomography (PET). Ictal hypermetabolism and postictal hypometabolism are characteristic of both partial and generalized mechanisms, so up now no specific differences have been found between the two main kinds of epilepsies. The application of PET revealed the function of the GABA-benzodiazepine complex during the epileptiform discharges. The last chapter's theme is the mechanism of action of the anticonvulsant agents in GE. The cellular effects develop through the calcium channels. A summarizing review deals with the importance and perspectives of valproate, another valuable paper interprets the cellular electrophysiological background of the epileptic symptoms occurring as a consequence of withdrawal of sedative durgs.

The editors and authors mode an excellent work. The volume contains all the important results of the neurobiological research of GE. It is a very useful survey not only for researchers but - in my opinion - it is even more valuable for epileptologists striving to treat their patients on the basis of the up-to-date knowledges achieved in this field.

Finally, a subjective comment: neurobiological approaches of particular physiological, psychological and environmental factors greatly involved in the clinical picture of epilepsies - especially of GE - still remain to be released up.

PÉTER RAJNA

"Obstetric genetics"

Zoltán Papp

Akadémiai Kiadó, Budapest, 1990

During the last two decades, two major forces in clinical studies on human reproduction have been the management of infertility and the early detection of fetal abnormalities. With this fascinating book, Obstetric Genetics, the well-known expert, Zoltán Papp is a pioneer in basic and clinical genetics in Hungary. Following the Hungarian edition of the book in 1986 the English version proves that the book is filling a gap in the border area of clinical genetics and obstetrics not only in Hungary, but in international means, too. Prevention and detection of fetal abnormalities may be feasible with the help of combined use of basic theoretical knowledge and clinical application of results of antenatal care and prenatal diagnosis, and in this way to apply fetal therapy among the most recent possibilities of antenatal care.

The book contains 80 chapters, each with several entries. The first eleven chapters deal with basic genetic knowledge that brings clinical genetics and obstetrics nearer to each other and is needed for all the experts interested in obstetric genetics. The next 9 chapters are devoted to the membranes and amniotic fluid. The emphasis is put on the information that can be obtained from examination of the amniotic fluid and from the exfoliated amniotic fluid cells. The following "obstetrical" chapters describe the methods of fetal examinations (amniofetography, ultrasound, fetoscopy, amniocentesis, CVS, fetal blood sampling).

Further on, besides the description of prenatal therapy and procedures for pregnancy termination, very important ethical and legal questions are discussed concerning the general aspects of decision making regarding the fate of pregnancy. These problems and the psychological aspects of prenatal diagnosis are not strictly involved in the topic of obstetric genetics, but are extremely important from the view of a couple with increased genetic risk. That is why we agree with the author when he involves these topics and tries to hand over his personal experience, too.

The second half of the book is devoted to the different genetic, metabolic, morphologic disorders, which also involve the questions of intersexuality and gonadal dysgenesis. There are some further chapters which do not belong closely to genetics (intrauterine infections, fetal haemolytic disease, diabetic embryopathy), but these disorders may, or rather should be involved because patients with such problems usually turn to genetic counselling.

In conclusion, this book can be highly recommended to obstetricians, paediatricians, medical students and to all the experts interested in the field of obstetric genetics. With the English edition this book may become accepted as an invaluable and accurate reference book.

IMRE CSABA

"Clinical observations and therapy of lung cancer"

Ungár, I., Böszörményi, M., Hanovszky, M., and Németh, Gy.

Akadémiai Kiadó, Budapest, 1989

Price: 140.- Ft

Carcinoma of the lung still remains a leading cause of death among the cancer patients in Hungary, especially among the smoker women. Although surgical therapy, chemotherapy and irradiation have considerably improved in the past decades, therapy remains unsatisfactory. The limited success of surgical therapy have forced the development of new approaches in treatment protocols. Combinations of these therapies seem to be promising for the majority of the patients with advanced disease.

The authors of this book present their own experiencies in the progress of lung cancer treatment, prevention and rehabilitation. The book contains some statistical analysis about the appearance and mortality of lung cancer and the present state or therapeutical trials in Hungary. The authors give short reviews of the atiology, histology and biology of this disease. The most interesting part of the book consists of case-reports which describe the clinical course and therapy of patients in detail. The descriptive style of the presentation helps the reader to think over the cases and to draw the necessary conclusions.

In addition, the book helps the clinician to build up the optimal strategies for lung cancer therapy.

ANNA TOMPA

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26 July 1990

The Editor
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Dear Sir

As 1992 approaches, this Society is keen to do all it can to promote closer liaison in medicine in Europe.

Our Journal of the Royal Society of Medicine carried in July an editorial from Mr Ian Burn, who is Senior Vice President and Chairman of the Society's recently established European Committee, and we wonder whether you would be prepared to publish this either in the form of a letter to your journal from Mr Burn or as an editorial or in any other way that you think appropriate. I enclose a copy of Mr Burn's editorial.

We are writing similarly to other journals, as we would like our colleagues in Europe to know that we are open to new ideas which will bring European and British medical men and women closer together. We would also like to receive news from European countries which we might publish in our Journal.

Yours faithfully

Robert N. Thomson

Robert N Thomson
Executive Director

Akadémiai Kiadó, Budapest

Journal of the Royal Society of Medicine Volume 83 July 1990 419

Editorials

Into Europe

Last Year's Annual Report of the Royal Society of Medicine referred to the Society's new educational television channel. The launch film on 30th May 1989 contained a message from Her Majesty The Queen, which went as follows:

'As your Patron, I send you every good wish for the inauguration of the Society's new televison channel on 30th May. I congratulate you on this exciting technological achievement, which will, I hope, benefit people throughout this country and in Europe, now and in years to come.'

The reference to Europe emphasizes the Society's commitment to involve itself much more actively on the European scene than hitherto. The Single European Act scheduled for 1992 is approaching fast and, as again stated in the Annual Report, the Society fully intends to prepare itself for this major event.

At present the Society has formal contacts with only three European societies, namely the Danish Medical Society, the Royal Academy of Medicine in Ireland and the Swedish Society of Medicine. This contrasts with the extensive and close links now forged with our colleagues in the United States of America, where the Royal Society of Medicine Foundation Inc was established 23 years ago and has office accommodation at the Metropolitan Club, New York. Its Anglo-American Visiting Professorship scheme and the regular Anglo-American conferences have become important elements in the Society's affairs. Evidence of the success of our relationships with the USA is the substantial Affiliate membership there, currently around the 3000 mark. As Fellows of the Society will know, in recent yeras there have been a number of developments in the context of medical practice which reflect the European trend. During the last decade a number of successful European medical societies and journals have been established, with participation by all countries within the Continent. Many British medical societies now hold joint meetings with colleagues in various parts of Europe. Occasional meetings of certain Sections of the Society have already taken place in venues on the Continent. There has also been an increasing number of invited speakers to Section meetings from European academic centres.

We have the basis and stimulus for making some major new moves now. The Officers of the Society have had preliminary discussions and certain initiatives are thought possible. These include encouraging more Sections to hold meetings in Europe or to meet jointly with European colleagues at the RSM, encouraging European participation in the activities of our Forums, and extending the Society's television activities to other European countries.

We would hope also to extend our formal links with other academic bodies within Europe.

There may also be scope for encouraging links between our library and similar libraries on the Continent. The Royal Society of Medicine also would hope to assist in exchange mechanisms for European colleagues, of all grades. Hopefully, these and like activities will encourage eligible colleagues from the mainland of Europe to apply for Fellowship and Affiliate membership of the Society.

To enable the matter to be pursued further, the Officers have proposed and Council have approved the setting up of a European Committee to consider how the Society's objectives may be promoted in Europe, and the establishment of a small named European Office to act as a focal point for European activity at the Society. The European Committee will meet frequently and would welcome any relevant suggestions and comments from member of the Society, many of whom travel frequently to the mainland of Europe where they have their own contacts and responsibilities. If you have such an interest, your help and ideas would be greatly appreciated.

Ian Burn

Chairman, European Committee

Royal Society of Medicine

1 Wimpole Street, London

Acta Medica Hungarica, Vol. 47/3-4, pp. 223-234 (1990)

University of London
Royal Postgraduate Medical School
Hammersmith Hospital

DIPLOMA
IN
ENDOCRINOLOGY (PATHOLOGY)
Regulations and Syllabus

Akadémiai Kiadó, Budapest

ROYAL POSTGRADUATE MEDICAL SCHOOL

DIPLOMA IN ENDOCRINOLOGY

REGULATIONS AND SYLLABUS

1. Qualifications for Admission

1.1 The Course for the diploma is open to graduates in Medicine or Science whose previous training and experience have, in the opinion of the School, qualified them for registration as students for the Diploma.

1.2 Candidates for the Diploma in Endocrinology (Pathology) must be experienced in light and/or electron microscopical morphology and have basic training in biochemistry and histology.

2. Course of Study

2.1 Students are required to attend an approved full-time course extending over a period of not less than one academic year.

2.2 The course for Pathology students will consist of

(a) a period of about 8-9 weeks of lectures seminars and practical classes based on current advances in laboratory techniques. The following topics will be included: immunohistochemistry; raising antibodies; monoclonal antibody technology; advanced histopathology; techniques for the localisation of regulatory peptides at light microscopical level; immunolabelling for electron microscopy; electron microscopical immunocytochemistry of regulatory peptides; recent advances in quantification.

(b) regular attendance at guest lectures and other activities in the School. These include Histopathology guest seminars (weekly during term-time), lectures on regulatory peptides presented at other teaching courses (averaging one per fortnight), and the weekly Medical Staff Round.

(c) regular Departmental meetings which include seminars on current research topics, discussion groups on and their characterisation, updates on technology, research reports from other research groups, national and international meetings and research in progress photographic sessions. Case report meetings with discussions on interesting pathology form an integral part of the teaching programme. Informal guest lectures are frequently held. Active participation in all of these activities will be encouraged.

(d) an extended period of practical work on approved projects in the field of endocrine pathology. This work will be carried out in co-operating laboratories either within the school or outside and will commence from the date of registration. Projects will be on defined aspects of regulatory peptide pathology/biology and are likely to be in one of the following broad areas: systematic survey of regulatory peptides, involvement of peptides in diseases

of the gastrointestinal tract, respiratory system, cardiovascular system, central and peripheral nervous systems; peptide biosyntheses - abnormalities in disease states, hybridisation histochemistry and pro-peptide translation; factors affecting grown of tumour cells; markers for use in neoplastic differentiation and diagnosis (e.g. neuron-specific enolase, chromogranin A); mixed endocrine tumours - morphology, biology and effects.

3. Scheme of Examination

3.1 Each student will be assessed by means of

(a) set coursework related to the course of lectures. Such coursework should normally be completed by 30 April of the year in which the lectures were presented.

plus (b) a written report on the approved project which must be submitted to the examiners by the 30th June of the year in which the candidate completes the course.

plus (c) an oral examination on the approved project.

plus (d) a written paper of not more than three hours duration on the subject as a whole.

(e) or a dissertation which must be written in English and must afford evidence of serious study by the candidates and of his ability to discuss critically a difficult problem in the filed of endocrine biochemistry or pathology.

3.2 The subject of the dissertation must be approved by the School by 30th March in the year in which the course of study is completed. Candidates must submit their dissertations within one year of completing the course of study.

3.3 Successful candidates will be awarded the "Diploma in Endocrinology (Pathology)". A "Distinction" grade will be given to candidates who show exceptional merit.

3.4 The Board of Examiners will be appointed by the Academic Board of Royal Postgraduate Medical School and will contain at least one examiner external to the School.

UNIVERSITY OF LONDON

ROYAL POSTGRADUATE MEDICAL SCHOOL

OUTLINE PROGRAMME OF COURSES SCHEDULED FOR 1991

in the series

CURRENT ADVANCES IN LABORATORY TECHNIQUES

Further details and application forms are available from:
Professor Julia M. Polak
Histochemistry Unit

or the appropriate course organiser at the
Wolfson Conference Centre
Royal Postgraduate Medical School
Hammersmith Hospital, Du Cane Road
London W12 ONN, U.K.

we regret we cannot accept telephone enquiries

MODERN IMMUNOCYTOCHEMISTRY
7-11 OCTOBER 1991

Course organisers: Julia M. Polak and Susan Van Noorden (Histochemistry)

IN SITU HYBRIDISATION
21-25 OCTOBER 1991

Course Organisers: Julia M. Polak, Giorgio Terenghi and Sally Gibson
(Histochemistry)

MONOCLONAL ANTIBODY TECHNIQUES
18-22 NOVEMBER 1991

Course organisers: Mary A. Ritter, Heather Ladyman and Russel Hargreaves
(Department of Immunology)
(Closing date: 31st March 1991)

IMAGE ANALYSIS AND MORPHOMETRY IN MEDICINE AND BIOLOGY
4-8 DECEMBER 1991

Course organisers: Julia M. Polak, David R. Springall (Histochemistry)
and Richard Wootton (Medical Physics)

MOLECULAR BIOLOGY

Course organiser: Steve Legon, Lecturer in Molecular Biology
(Dept. of Chemical Pathology)

6TH WORLD CONGRESS IN ULTRASOUND
1-6 SEPTEMBER 1991

The 6th World Congress in Ultrasound will be held in Copenhagen, from September the 1st to 6th 1991

The congress is sponsored by the World Federation for Ultrasound in Medicine & Biology

Jens Jørgen Kjer, M.D.
Herlev Hospital
Department of Ultrasound
DK-2730 Herlev
Denmark

Congress Secretariat: 6th World Congress in Ultrasound
Spadille Congress Service
Sommervej 3
DK-3100 Hornbaek
Denmark

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

World Health Organization

Lyon - France

FELLOWSHIPS FOR RESEARCH TRAINING IN CANCER

1991-1992

Applications for training fellowships in 1991-1992 are invited from junior scientists wishing to be trained in those aspects of cancer research related to the Agency's own programmes: epidemiology, biostatistics, environmental and viral carcinogenesis and mechanisms of carcinogenesis.

Applicants should be engaged in research in medical or allied sciences and intend to pursue a career in cancer research.

Fellowships are awarded for one year and are tenable at the Agency or in another suitable institution abroad. Fellows will, in general, be selected from applicants with some postdoctoral research experience related to cancer in medicine or the natural sciences. Applicants requiring basic training in cancer epidemiology will also be considered. They must have an adequate knowledge, both written and spoken, of the language of the country in which their fellowship is tenable.

Applications cannot be accepted from people already holding fellowships enabling them to study abroad.

Stipends will vary according to the cost of living in the country of study. The cost of travel for the applicant, and in certain circumstances, that of one dependent, will be met.

VISITING SCIENTIST AWARD

1991-1992

This award is intended for established cancer research workers with a minimum of five years postdoctoral experience who wish to spend up to one year at IARC working on the implementation of a collaborative research project related to the Agency's own programmes: epidemiology, biostatistics, environmental and viral carcinogenesis and mechanisms of carcinogenesis.

Applicants must belong to the staff of a university or a research institution. They must provide a written assurance that they will have a position to return to at the end of the period of award.

Candidates should submit their applications after consultation with an IARC scientific staff member. Applications will be reviewed by the Fellowships Selection Committee each year.

There will be an annual renumeration and the cost of travel will be met.

Fellowship application forms and more detailed information are available from:

Chairman of the Fellowships Selection Committee
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
150 cours Albert-Thomas, 69372 Lyon Cedex 08
France

THE TENTH ANNUAL SCIENTIFIC MEETING AND EXHIBITION

of the

SOCIETY OF MAGNETIC RESONANCE IN MEDICINE

will be held August 10-16, 1991,
in San Francisco, California, USA

For more information, contact SMRM,
1918 University Avenue, Suite 3C, Berkeley, CA
94704 USA.

Telephone: (415) 841-1899.
Fax: (415) 841-2340.

PROSTAGLANDINS, LEUKOTRIENES AND LIPOXINS '91

Washington, DC
May 13-17, 1991

XIth Washington
International Spring Symposium

c/o Dr. J.M. Bailey
Department of Biochemistry & Molecular Biology
The George Washington University School of Medicine
and Health Sciences
2300 Eye Street, N.W.
Washington, DC 20037
U.S.A.

The Society of Magnetic Resonance in Medicine

TENTH ANNUAL

SCIENTIFIC MEETING AND EXHIBITION

AUGUST 10-16, 1991

The San Francisco Hilton and Towers

San Francisco,
California, USA

Young Investigator's Award Competition

The Society of Magnetic Resonance in Medicine announces the competition for the Young Investigator's Award in Magnetic Resonance in Biology and Medicine. The competition is open to young scientists at the undergraduate, graduate, and postgraduate levels in academia, industry, or research institutions. Applicants must be less than 38 years of age on January 1, 1991, and have had no more than the equivalent of five years' full-time postdoctoral research experience, as documented by a curriculum vitae and letter of confirmation from their supervisor, mentor, or department head.

To enter the competition, the applicant must submit a single manuscript describing original work in the field of MR in biology and medicine. The applicant must be the sole or primary author of this work. The manuscript may not have been published prior to the submission deadline. The Young Investigator's Award Committee will select up to five finalists, who will make oral presentations at the Annual Meeting. The winner will be selected on the basis of the scientific quality and originality of his or her written and oral presentations and will be named before the close of the Annual Meeting. The winner is awarded $1,500, and all other finalists are awarded $500.

MAIL TO:
SMRM Business Office, 1918 University Avenue, Suite 3C
Berkeley, CA 94704, U.S.A.

CADMIUM IN THE HUMAN ENVIRONMENT
TOXICITY AND CARCINOGENECITY

An International Symposium

Gargnano, Italy

25-27 September 1991

International Agency for Research on Cancer

International Union of Pure and Applied Chemistry

Institute of Occupational Health, University of Brescia

International Agency for Research on Cancer
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F-69372 Lyon Cedex 08

XIIIth International Congress of Lymphology

September 29th--October 5th, 1991

at the MERIDIEN MONTPARNASSE Hotel, PARIS - FRANCE

MAIN TOPICS

- Anatomy of lymphatic system
- Surgery and lymphatic system (oncology, reconstructive and microsurgery)
- Physiology and rheology of lymphatic system
- Microcirculation and lymphatic system
- Dermatology and lymphatic system
- Immunology and lymphatic system
- Transplantation and lymphatic system
- Lymphatic system imaging (conventional radiology, CAT, NMR, radionuclides...)
- Oncology and lymphatic system
- Pharmacology, pharmacokinetics and lymphatic system
- Lymphedema
- Venous, arteries and lymphatic system
- AIDS and lymphatic system
- Others

The Congress Secretariat:

DIXIT International: Tel (1)47 88 53 47 Fax: (1)47 88 56 63
Telex: 611 989

THE PFIZER AWARD FELLOWSHIP

Dear Sir/Madam,

As part of our continuing efforts to support innovative research in the field of cardiology, Pfizer is offering the:

Pfizer Award Fellowship: to be given to an investigator presenting a high quality research project on calcium and membrane transport mechanisms.

Applications will be considered by the award committee of the **International Society of Hypertension.**

The award fellowship will be conferred at the 14th. Scientific Meeting of the International Society of Hypertension, Madrid, Spain, June 14th-19th 1992.

The award is US$ 20,000 per year for 2 years. A research proposal together with curriculum vitae and 2 letters of recommendation should be mailed to the

Secretary of The International Society of Hypertension
Dr. Hans Ibsen
Medical Department C
Glostrup Hospital
DK-2600 Glostrup
Denmark

One of the letters of recommendation should be from the chief of the laboratory in which the planned research is to be carried out.

Deadline of submission: March, 31st, 1992.

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INFORMATION FOR AUTHORS

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

Form of manuscripts

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

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

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

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

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

References

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

Examples:

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

Tables

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

Illustrations

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

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The first authors will receive (1) comments and suggestions of the Editorial Board for improving their paper; (2) a set of proofs for correction; corrected proofs should be returned without delay to the editorial office; (3) 100 reprints free of charge.

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