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N E U R O L O G Y

# TREATMENT OF CHRONIC PAIN SYNDROMES WITH TRANSCUTANEOUS IONTOPHORESIS OF VINCA ALKALOIDS, WITH SPECIAL REGARD TO POST-HERPETIC NEURALGIA

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(Received: August 1, 1988)

Successful treatment of 35 post-herpetic neuralgia (PHN) patients by means of transcutaneous iontophoresis of Vincristine is reported. This technique, based on transganglionic regulation — a novel neurobiological principle discovered by Csillik and Knyihár-Csillik —, alleviated pain in both fresh and inveterated PHN cases. Statistical analysis of the results excludes a placebo-like action.

Keywords: pain, post-herpetic neuralgia, Vincristine, iontophoresis

## INTRODUCTION

Nociception (i.e. reception for acute pain), an efficient mechanism preventing imminent tissue damage, serves to protect the organism, while neuralgic and neuralgiform pain syndromes are either residual or resultant products of various specific diseases and serve no purpose in the continued existence of the individual. Despite numerous therapeutic trials, intense emotional involvement associates with chronic pain and thus the neuralgic and neuralgiform pain syndromes often culminate in suicidal attempts /26/. This is especially true in the case of post-herpetic neuralgia /23, 25, 30, 33/.

The discovery of transganglionic regulation of primary sensory neurons /5/ and the role of the nerve growth factor (NGF) in this regulato-

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ry system /2, 4, 7, 10, 14, 19, 29/ revealed new perspectives in the treatment of chronic pain syndromes. By means of blockade of retrograde axoplasmic transport in peripheral sensory nerves, brought about by iontophoretic application of Vinca alkaloids to the dermatomal representation of the impaired spinal segment /6, 17, 31/, hitherto intractable chronic pain syndromes have become accessible for therapeutic efforts. The aim of the present publication is to summarize and evaluate the case histories of 35 PHN patients treated with transcutaneous Vincristine iontophoresis in our Pain Control Unit, in the years 1986 and 1987.

## PATIENTS AND METHODS

Patients: 14 male and 21 female PHN patients were subjected to transcutaneous Vincristine iontophoresis.

Iontophoresis fluid: 1% Vincristine (obtained from Gedeon Richter Pharmaceutical Co., Budapest) dissolved in distilled water containing 1% hyaluronidase ("Hyase", obtained from Institute for Serobacterial Production and Research HUMAN, Gödöllő–Budapest). This fluid is applied to hydrophilic fabric, in the amount of 30 µg vincristine $cm^2$ of the dermatomal area to be treated.

Equipment: Nervostat (Standard Physiotherapeutic Apparatus, produced by Orvosi Műszeripari Szövetkezet, Budapest) DC: 1–5 mA for head and neck; 10–30 mA for limbs and trunk.

Electrodes: 0.5 mm ... 2.0 mm thick lead plates

Duration of treatment: 60 min

Number of sessions: 10 ... 30 (see Table I)

The pain state was determined each day before the iontophoretic treatment, using a multicoloured analogue scale /8/, first proposed for mentally retarded children by Dr. Alaine Polcz (personal communication). This scale yields surprisingly constant and reproducible results even in elderly (cerebral sclerotic) patients, ranging from "no pain" (white = 0–10%) throught various colours of increasing psychic or rather emotional meaning (shades of yellow, orange, red, lilac) up to black (100% = intolerable pain; Fig. 1).

## RESULTS

A positive change was observed in the pain state by employing the therapy described above, and by attempting to select patients with genuine PHN (note difficulties later). Results of the treatment are summarized in Table I.

It is tacitly accepted on the basis of common consensus that in any type of therapeutical procedure, placebo effects may play a role. It is also generally approved that placebo effects are responsible, in the

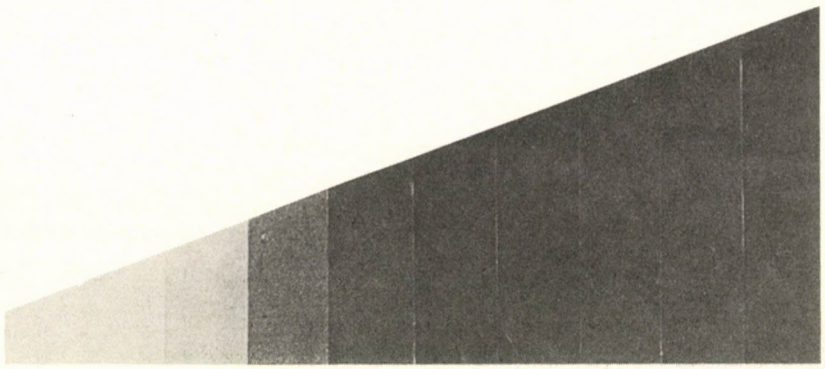


Fig. 1. The two-dimensional multicoloured analogue scale (TDMA) developed by Polcz and Szücs, used for the estimation of pain state of the patients. Colours of increasing psycho-emotional values range from white to black, indicating a continuous percentage scale from 0–10% (no pain) to 100% (intolerable pain)

statistical sense, for 1 ... to ... 40% of the results. Therefore, following the lines laid down by Wilcoxon et al. /34/ we calculated the probability of placebo effects in our trial. Simple arithmetical calculations, which in this case indicate a $P < 0.01$ unequivocally exclude any role of placebo effects in the improvements in the pain state.

As mentioned above, a basic prerequisite for the analysis of the efficiency of the iontophoretic treatment is that patients with obscure or mixed causes of chronic pain be excluded from the trial. In clinical terms, it is often impossible to abstain from treatment in subjectively desperate, yet not objectively "clear" cases. We did not attempt to perform such statistically justified, but medically intolerable, pre-screening. Therefore, in the biometric sense of the word, our results are "contaminated" by the low efficiency of iontophoretic treatment of such patients. In spite of having included such diagnostically and statistically ambiguous cases, the P value shows high statistical significance in favour of the iontophoretic treatment.

Another point which merits consideration is the fact that not only "fresh" cases but also patients with case histories longer than 3 months responded favourably to the iontophoretic treatment. This confirms the earlier reports in which the original paradigm of the iontophoretic treat-

Table I

Results of the Vinca iontophoretic treatment of the PHN patients

| Initials | Age | Sex F=female M=male | Duration of pain before starting the treatment (in months) | Diagnosis and localization l.d=right side l.s=left side | Number of sessions | Vinca alkaloid used (Vcr=Vincristine) | Relative percentage of pain alleviation |
|---|---|---|---|---|---|---|---|
| PJ | 71 | F | 24 | PHN Th2-4 l.d. | 18 | Vcr | 28 |
| MJ | 69 | F | 2 | PHN C4-Th2 l.d. | 25 | Vcr | 34 |
| BJ | 80 | M | 1 | PHN C4-Th1 l.s. | 20 | Vcr | 34 |
| ES | 75 | F | 13 | PHN Th2-6 l.d. | 20 | Vcr | 34 |
| KI | 49 | M | 6 | PHN C4-8 l.d. | 15 | Vcr | 34 |
| SJ | 76 | M | 4 | PHN Th10-12 l.d. | 21 | Vcr | 34 |
| CI | 65 | F | 1 | PHN Th3-5 l.d. | 10 | Vcr | 38 |
| VE | 68 | F | 33 | PHN S2-3 l.s. | 20 | Vcr | 38 |
| BP | 57 | F | 2 | PHN Th5-11 l.s. | 25 | Vcr | 38 |
| KL | 67 | M | 3 | PHN Th2-6 l.d. | 25 | Vcr | 39 |
| KA | 42 | F | 12 | PHN V1-2 l.d. | 25 | Vcr | 43 |
| TG | 78 | M | 24 | PHN Th3-7 l.s. | 14 | Vcr | 43 |
| HJ | 67 | F | 15 | PHN Th2-10 l.s. | 24 | Vcr | 45 |
| LI | 62 | F | 1 | PHN Th4-6 l.s. | 25 | Vcr | 45 |
| KF | 64 | M | 2 | PHN Th7-11 l.s. | 25 | Vcr | 50 |
| AL | 52 | F | 1 | PHN Th6-10 l.s. | 14 | Vcr | 50 |
| RP | 60 | F | 1 | PHN V1-C2 l.s. | 10 | Vcr | 50 |
| FI | 60 | F | 1 | PHN C4-Th8 l.d. | 25 | Vcr | 56 |
| KA | 41 | F | 26 | PHN V1-3 l.d. | 23 | Vcr | 58 |
| MJ | 75 | F | 1 | PHN Th12-L3 l.s. | 15 | Vcr | 60 |
| BJ | 81 | M | 2 | PHN L5-S3 l.s. | 25 | Vcr | 60 |
| BI | 75 | F | 18 | PHN Th5-8 l.d. | 14 | Vcr | 63 |
| RT | 68 | M | 8 | PHN L2-S4 l.s. | 30 | Vcr | 63 |
| KL | 75 | F | 3 | PHN Th4-6 l.d. | 25 | Vcr | 63 |

Table I (cont.)

| Initials | Age | Sex F=female M=male | Duration of pain before starting the treatment (in months) | Diagnosis and localization l.d=right side l.s=left side | Number of sessions | Vinca alkaloid used (Vcr=Vincristine) | Relative percentage of pain alleviation |
|---|---|---|---|---|---|---|---|
| TI | 68 | M | 1 | PHN Th5-8 l.d. | 17 | Vcr | 70 |
| SA | 86 | M | 3 | PHN C8-Th12 l.s. | 21 | Vcr | 70 |
| LM | 57 | F | 3 | PHN Th12-L2 l.s. | 20 | Vcr | 70 |
| SJ | 77 | F | 1 | PHN C3-4 l.d. | 10 | Vcr | 70 |
| SI | 76 | M | 10 | PHN V1-2 l.d. | 26 | Vcr | 75 |
| JA | 65 | F | 3 | PHN C3-Th6 l.d. | 17 | Vcr | 75 |
| BJ | 85 | M | 1 | PHN C4-8 l.s. | 23 | Vcr | 78 |
| SG | 65 | M | 24 | PHN V1-2 l.s. | 19 | Vcr | 86 |
| ML | 68 | F | 2 | PHN L3-4 l.d. | 24 | Vcr | 90 |
| BJ | 77 | F | 1 | PHN Th2-6 l.s. | 25 | Vcr | 100 |
| FJ | 76 | M | 4 | PHN C3-Th6 l.s. | 20 | Vcr | 100 |

ment was strictly observed /5, 6, 17/ and contradicts those of Layman et al. /18/ who, though using Vinca alkaloids, changed the classic paradigm, and, by using their formula, could not observe any alleviation of chronic PHN pain in cases where pain had already persisted for more than 3 months.

Our results support earlier notions that PHN affects mainly patients over the age of 50 years /12, 13, 21, 27, 32/ and that the majority of the patients affected by this disease are women (Table I). Also evident is the fact, as it has been emphasized earlier, that at least 10 sessions are needed for a successful iontophoretic treatment of PHN /6, 9, 24/.

## DISCUSSION

"It is exceedingly difficult to treat PHN, which fairly often leads to suicidal attempts that, if successful, obviously conclude all the therapeutical efforts" /26/.

The "gate of pain", as described by Melzack and Wall more than two decades ago /22/ gave rise to the technique of transcutaneous stimulation (the device is called "pain killer") in the therapy of PHN. Unfortunately, the results obtained by this technique, if any, were very short-lived /23/. The classic trial of structure-function-therapy in chronic pain syndromes has been developed in the course of the last decade by Csillik and Knyihár-Csillik /5, 16/, based upon the structural and functional plasticity of the primary nociceptive analyser.

In addition to the accompanying biomedical dimensions and their closely related psychological aspects, autochthonous chronic pain syndromes also pose serious social and financial problems. Not only the costs of medical treatment, but also the loss in work hours have to be taken into consideration. According to statistical evaluations, in the United States alone, 2 million people become incapacitated for short or long periods because of pain and costs for compensation benefits, hospitalization expenditures and, losses sustained in the work force, are estimated to run between U.S. $ 2.5 billion ($2.5 \times 10^9$) and 20 billion per annum /15/.

The results of the present study, evaluating the efficiency of Vinca iontophoretic treatment on 14 male and 21 female PHN patients prove the practicability of this technique. In contrast to non-treated patients, none of treated ones were considering suicidal ideas. In addition to obviously cured individuals (i.e. where the alleviation of pain was >70%) the amount

of pain-killing drugs could be drastically reduced even in patients showing no spectacular improvement.

Accordingly, the use of Vinca iontophoresis can be emphatically recommended in PHN patients and, by extrapolation, also in other genuine, autochthonous chronic pain syndromes. A few words of warning, however, seem appropriate at this point:

(1) First, the cause of pain has to be carefully determined, not only by the neurologist, but also by a comprehensive medical examination. Symptomatic pain should never be treated; only meaningless, excruciating, autochthonous chronic pains should be treated.

(2) In the case of PHN, the Vinca iontophoresis should be started as early as possible, i.e. if pain is still present 4 ... to ... 6 weeks after conclusion of th eruptive state.

(3) Depending on the responsiveness of the patient, the iontophoretic sessions should number at least 10; on the other hand, if the pain is refractory, there is no good reason to continue treatment beyond one month.

(4) The pain state of the patient should be estimated each day before treatment. Although the use of the multicoloured scale is highly recommended, a simple analogue scale can also be used /1/.

(5) Strictly adhering to the parameters of iontophoresis outlined above, we have never met supersensitivity or allergic dermatitis. However, if such a case would occur, a few days of intermission, and the use of a hydrocortisone unction, would be used to cure the hyperaemic or allergic changes. Most importantly, and due to the simplicity of the iontophoretic technique, the patients do not have to be bedridden and the entire treatment can, and should, be organized on an out-patient basis.

Since we are reporting here on the results of the last two years' treatments only, the follow-up period is very short, indeed. During this time, relapses ranging from 3 months to 2 years, were not observed. However, on the basis of experiences obtained on earlier cases /7/ our patients seem to have a good chance to be relieved of chronic pain for good. Anyway, we are planning to conclude this present study with a catamnestic evaluation after a five-year follow-up period.

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# MYASTHENIA GRAVIS: FAMILIAL OCCURRENCE
# A STUDY OF 1100 MYASTHENIA GRAVIS PATIENTS

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Eleven-hundred myasthenia gravis cases observed by the author in a period of 37 years are reviewed. The ratio of familial incidence was 4.23%. Transitory (neonatal) myasthenia in new-born babies should be separated from the familial cases. In familial myasthenia gravis both maternal and paternal line can occur. The majority of the cases are similar to the generalized, acquired myasthenia gravis, still there are some myasthenic familial congenital patients, too. Some rare instances are reported, among them a unique family with six sisters suffering from myasthenia gravis. Genetic line and HLA antigens' role are dealt with. Observation of familial myasthenia cases may contribute to the knowledge of the immunologic and clinicopathologic background of the disease.

Keywords: myasthenia, familial occurrence, thymectomy, thymus pathology

## INTRODUCTION

Familial myasthenia gravis (FMG) has been known for a long time /13, 28, 29/; the ratio of its occurrence in great casuistics is between 1.2 and 4.2% /20, 22, 23, 36/. In a period of 37 years, we observed and followed up 1100 myasthenia gravis (MG) cases. Of these 47 (4.23%, a rather high incidence) were FMG cases. Review of these cases is the subject of this study. We wish to report on a few special cases and on a family unique in the literature.

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Abbreviations: myasthenia gravis: MG, familial myasthenia gravis: FMG, acetylcholine receptor: AChR

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## PATIENTS AND METHODS

All the 1100 patients dealt with in this study were examined in our Neurological Department; their diagnosis was established according to the known criteria discussed by us /36/, among others. Transitory (neonatal) MG was diagnosed on the basis of anamnesis or on examination in gynaecologic departments. Diagnosis, observation and treatment of infants took place in co-operation with the Neurological Department of the Pál Heim Children Hospital, Budapest /39/. The observation and follow-up of the infant patients was in every case long-lasting, even of the infants who suffered from transitory (neonatal) MG. We investigated the clinical course of FMG cases, in operated cases also the muscle and thymus pathology, therapeutical response and in certain cases, the HLA antigens /11, 12, 38/.

## RESULTS

Forty-seven cases (4.2%) were of familial occurrence. Transitory (neonatal) MG was observed in 11 infants of 10 myasthenic mothers. Heredity in the familial cases is shown in Table I. In the mother-child or father-child relation daughters and sons occurred without appreciable difference. Among siblings the male : female ratio was 4:2. Out of the 25 cases of MG in siblings, we observed childhood MG in 8 instances. In the cases of MG in cousins, the maternal line was dominant in all the cases.

Table I

Heredity of familial myasthenia gravis cases

| | No of cases | per cent |
|---|---|---|
| Mother and child: | 8 | 17 |
| Father and child: | 6 | 13 |
| Brothers and sisters: | 25 | 53 |
| Cousins: | 8 | 17 |
| Total | 47 | 100 |

All the clinical forms of MG occurred in the FMG cases among the parents, siblings and children. Still, the dominant form was the generalized, benign MG, with the exception of childhood MG cases (congenital, local, non-progressive clinical forms).

The time of onset and the clinical form of the second-generation cases cannot be considered characteristic. The interval between the onset of MG of parents and that for their children was between 9 and 21 years, while that of siblings and cousins between 12 and 26 years. The only exception is in this respect the congenital (or later progressing) MG of siblings which manifested itself in general at an interval of 2–11 years /39/.

Transitory (neonatal) MG cannot be considered familial. In these instances the MG of the mother (no matter, known or not known), would not be hereditary, but it can develop through diaplacentar infiltration of the mother's anti-AChR antibodies. Still, this condition of the infants can be treated quite well (if recognized!), can be cured, and the symptoms will never return during the life of such infants /36/. We mention as a very important item of our experience on transitory (neonatal) MG cases that in four cases of infants' transitory MG, the mothers' disease had not been known before the affected infant was born. In one case of the mother's MG was complicated with rheumatoid arthritis. In another case both infants of the myasthenic mother were born with transitory (neonatal) MG: the first one before the correct diagnosis of the mother's disease, the second one one year after the mother's successful thymectomy.

Thymectomy was performed in 23 of the FMG cases, in the remaining 24 cases the treatment was conservative. Thymus activity (as shown by the germinative centres) in the operated cases is presented in Table II. The therapeutic response did not show any remarkable difference from other cases of MG, with the exception of local (ocular) congenital MG, which seemed to resist therapy. In these congenital cases no thymectomy was performed.

Table II

Operated cases. Thymus activity. Histological classification according to the germinal centres

| | No of cases |
|---|---|
| Persistent thymus | 3 |
| Hyperplasia I | 4 |
| Hyperplasia II | 7 |
| Hyperplasia III | 7 |
| Thymoma | 2 |
| Total | 23 cases |

Some specially interesting familial cases:

1. A 47 years old Arabian man was operated in England on account of generalized MG and chest tumour. Half a year later he developed a very serious myasthenic respiratory crisis with bulbar symptoms. Therefore — one year after the first operation — he was operated again (dr. J. Molnár, Hungary). A very active thymus (hyperplasia of IIIrd degree with germinal centres) was removed, but no tumour tissue was found. The patient recovered. Two years later, his sister's disease was diagnosed as MG in this Department. Her operation was similarly successful. No tumour, only a very active thymus was found.

2. Being aware of the rapid, respiratory death of a 12 years old Libyan girl, we observed her two brothers, 7 and 8 years old; both suffered from congenital (oculo-skeletal) non-progressing MG. In these cases conservative treatment was successful. Neither the parents, nor the other members of the family suffered from any muscle disorder.

3. Three children of gipsy parents — who have been cousins — suffer from congenital MG. At present the children are 7, 4 and 2 years old. Conservative therapy seems to be efficient at the present time. — In another gipsy family, two of the three children suffer from congenital (local) MG.

4. In a further family, the disease of a 61-year-old man was diagnosed as MG in our Neurological Department. Similar symptoms directed our attention to his brother, 7 years older, whose bulbosceletal MG was also recognized in our Department. These patients represent the oldest familial cases of the literature. Conservative and cytostatic immunosuppressive treatment of both brothers resulted in a very good remission which lasted more than 5 years.

5. The huge mediastinal tumour of a 16 years old girl was operated (Dr. I. Besznyák). On the next day a very serious myasthenic respiratory crisis developed. The MG of the girl had not been known before. Her crisis resulted in death on the fourth day in spite of a very intensive care. The tumour was a Hodgkin granuloma. This case was published as a provoked (but not caused) case of MG /37/. Seven years later the patient's brother, a 27 years old man, was examined by us on account of mediastinal mass. MG was not to be diagnosed. A big mediastinal tumour was removed. Histologically the tumour proved to be a malignant epithelioma with marked lymphatic reaction. Due to infiltration of the left upper lung section, the operation was not successful: the patient died one year later, without signs of MG.

6. In a family, originating from healthy parents, five brothers and the oldest sister are healthy, while the other six sisters are sick. Out of them, the three younger sisters had to be operated (Dr. J. Molnár), a further one will be operated in the next future, while the other two sisters' MG can be considered — at present — latent, provokable, but not manifest MG, a condition that needs hardly any therapy. It is interesting that the severity of the myasthenic symptoms is inversely related to the age of the sisters. The two youngest sisters were in a crisis-endangered state. In this family neither the healthy nor the sick members showed a characteristic HLA antigen pattern, therefore some other factor(s) (bound to sex) had to be supposed which could explain this unique and specific susceptibility /11, 36, 38/.

## DISCUSSION

Familial occurrence of a disease has become a specially important question in relation to the general and specific susceptibility which could include among others, genetical and immunological relations. The familial (and transitory) occurrence of MG has been known for several decades. Similarly, the immune or autoimmune research of the disease has been followed for more than two decades, thus the meeting point between the genetic aetiology and the immune pathomechanism has been given as a matter of course. Concerning this point of view, the familial occurrence of MG and its accumulation would be an important question in spite of the fact that dealing with this question has hardly gone beyond registration of cases. In this respect the myasthenic process of two female twins (being 20 years old) is of interest; their disease started at the same time and thymectomy resulted in both cases in a very good success /3/. In another case of identical twins, MG manifested itself at a five-year interval. Although the thymus showed follicular hyperplasia in both cases, the therapeutical responsiveness was different /1/.

In our casuistics oculo-sceletal, non-progressing MG developed in one member of a female identical twin; the other member did not develop MG during 21 years of observation, even no symptoms of latent MG have been provocable by curare /36, 37/. The following question may be raised: Does the close relationship play a part in the gipsy family mentioned above, whose three children suffer from congenital (hitherto non-progressing) form of MG?

MG was observed in two brothers, both of whom had thymoma and partly identical HLA antigens /24/. The investigations into the genetic susceptibility determined by HLA antigens have led to equivocal results /4, 6, 11, 12, 16, 21, 25, 26, 27, 30, 35, 38/; very few of them could be related to FMG. Ten members of four families are known in connexion with this question /25, 26/: The HLA-AS was not characteristic. On the other hand, in another study HLA-B8 alleles were found in the brothers of a patient with MG /31/. — Hokkanen et al. /15/ conclude on the basis of an electrophysiological examination (i.e. on that of "jitter phenomenon") the existence of some "familial" factor which could have some role in the pathomechanism of the disease. The occurrence of some HLA antigens can partly be characteristic of relatives of myasthenic patients who suffer from immunological disorders other than MG /17/. In the above-mentioned family in which 4 manifest and

further 2 provocable MG cases were demonstrated among the 12 family members, neither the parents or the healthy members of the family, nor those with MG showed the characteristic occurrence of HLA-B8 and DRw3 antigens /11, 38/. With regard to this unique family, we agree with Pirskanen /25/ and Simpson /32/: The HLA-B8 antigen — although occurring in higher levels — does not indicate a specific susceptibility to MG; it can be a factor in the overall susceptibility to immune disorders /11, 38/.

The problem of FMG may be influenced, or even complicated, by the fact that in certain MG cases manifested in infancy or in childhood as congenital MG, familial accumulation can be recognized. It is well known that quite a few instances of MG or myasthenic syndrome have been published in the past decade, and these syndromes hardly differ from each other from the clinical point of view. On the other hand, many data concerning the pathomechanism, morphology of the muscle and the synaptic end-plate, biochemistry and electrophysiology seem to be different in these syndromes /2, 5, 8, 9, 14, 18, 19, 33, 34, 40/. All these myasthenic syndromes have been reviewed by Engel, Engel et al. /7, 8, 9, 10/. In all these syndromes one can hardly find common or characteristic pathogenic, electrophysiological, morphological pathomechanisms, only the clinical picture seems to be similar and hardly distinguishable: it is in general related to the extraocular muscles, and — in a moderate way — to the sceletal ones, non-progressing in its character, sometimes resembling myopathy, sometimes combined with myopathy. In the pathogenesis, however, pre- and post-synaptic pathomechanisms can equally occur, which can be characterized by decrased ACh-release, inhibition of its mobilization, by a genetic damage or decrease of post-synaptic receptors, muscle alteration or by the underdeveloped state of the pre-synaptic apparatus.

As mentioned above, these congenital MG forms could hardly be differentiated /36/. Still, we would like to identify a new form: An MG process congenital at the beginning and stationary, non-progressing, local for years, may show a rapid or subacute progression between 6 and 12 years of age, and may develop similarly to generalized childhood or acquired adult MG, with all the therapeutical consequences, which have been known in the treatment of acquired MG /36, 39/. This is a very rare form of FMG, still it can occur, as it did in two consecutive cases of ours.

In conclusion, the genetic, electrophysiological, clinical and morphological background of FMG is far from being clarified. On the other hand, the review of familial cases, the data of these families have essentially

contributed to the up-to-date clinico-pathological understanding of MG, to the comprehension of its genetical backgrounds and, to some degree, to the understanding of the polymorphism observed in the immunology and electrophysiology of the disease.

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ENDOCRINOLOGY

# DETECTABILITY OF THYROID ANTI-MICROSOMAL ANTIBODIES, CHANGES IN THYROID-STIMULATING IMMUNOGLOBULINS (TSI) AND THYROTROPIN-BINDING-INHIBITING IMMUNOGLOBULINS (TBII) DURING METHIMAZOLE TREATMENT OF GRAVES' DISEASE PATIENTS

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The prognostic value of determination of different antibodies in Graves' disease patients is questionable. The authors simultaneously assessed the generation of cAMP, the TSH-receptor binding inhibitory assay and the detectability of anti-microsomal antibodies by indirect immunofluorescence. The tests were performed before, during and after methimazole treatment. During a 12 months' medication all 22 patients became euthyroid. Six months after withdrawal of the drug, 15 patients were still euthyroid (Group A); 7 relapsed (Group B). Patients showing enhanced activities by all three methods, relapsed (5 out of 7 cases of Group B). The results indicate that simultaneous determination of TSI, TBII and anti-microsomal antibodies are of high prognostic value for relapses. These data should be taken into consideration for the further therapy.

Keywords: TSI, TBII, anti-microsomal antibodies, Graves' disease

## INTRODUCTION

It has generally been accepted that immunoglobulins directed against different constituents of the thyroid gland play a decisive role in autoimmune thyroid diseases /1, 34, 35/. Each has been postulated to be an antibody directed against a component of the thyroid cell membrane. They can be detected in different ways. Some methods are based on their ability to stimulate thyroid function (thyroid stimulating immunoglobulins-TSI), others on their ability to bind to the thyroid cell membrane and prevent

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Abbreviations: immunofluorescence: IF; fluoresceinisothiocyanate: FITC

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the binding of labelled thyrotropin (thyrotropin binding inhibiting immunoglobulins-TBII) /2, 11, 13, 23, 27–29/. Antibodies against different thyroid structures can be visualized by immunofluorescence (IF) /20, 21, 22, 25, 30, 31/, but their precise relation to TSI and TBII has not been clarified so far.

The measurement of thyroid-related antibodies is important during the treatment of Graves' disease; their elimination is in favour of clinical remission, while if they are existing for a long time, their withdrawal is followed by a recurrence almost invariably /3, 4, 6, 19, 24, 27, 32/.

The purpose of the recent work was to investigate the detectability of TSI and TBII, as well as anti-microsomal antibodies, before, during and after methimazole treatment of Graves' disease patients, in relation to the clinical course of the disease.

## PATIENTS AND METHODS

The diagnosis of Graves' disease was based on conventional clinical and laboratory data. Triiodothyronine uptake test, triiodothyronine-RIA, thyroxine-RIA, TSH-RIA, triiodothyronine suppression-test, thyrotropin-releasing hormone test, $^{131}I$ scintiscan and uptake were determined in each case. The results supported hyperthyroidism of Graves' disease. The possibility of Hashimoto's thyroiditis was excluded by fine-needle biopsy. All patients, except one were female. Mean age: 29.6 years.

Blood samples were taken before beginning of the treatment and during treatment at 3, 6, 9 and 12 months, as well as 6 months after methimazole had been withdrawn. All patients were treated with methimazole for 12 months. Tranquillizers and Propranolol were administered as justified. The clinical and hormonal status was thoroughly controlled in each period.

Determination of TBII activity:

TBII was determined by a radioreceptor assay (TRAK-assay, Henning) according to the original prescriptions. TBII activity was expressed according to the corresponding binding activity of TSH. Binding activities exceeding 10 U/l TSH equivalents were regarded as positive.

Assessement of cyclic-AMP:

Surgically removed human thyroid slices were processed as prescribed by Brown et al. /5/. A commercially available cAMP-kit (Amersham) was used. Activities higher than 150% of the control values were regarded as positive.

Immunofluorescence (IF) tests:

Indirect IF was carried out on thyroid cyst or neoplasm surgically removed from the surroundings of normal thyroid tissue as described previously /25, 30, 31/. Frozen sections were incubated with patients' immunoglobulin at 1:10, 1:20, 1:40 and 1:80 dilutions. Reincubation was made with fluorescein-isothiocyanate-(FITC) labelled anti-human IgG (Hyland). The localization and feature of IF was evaluated as previously described /30, 31/.

## RESULTS

All patients had become euthyroid by the end of the 12 months' therapy. Fifteen patients (Group A) remained euthyroid clinically. Their euthyroidism was proved by hormone measurements six months after the end of the therapy. Seven patients (Group B) showed relapse of hyperthyroidism.

### Group-A patients

Before treatment, 13 patients had elevated TSI levels; 12 of them showed enhanced TBII activites. By IF 14 cases proved to be positive for anti-microsomal antibodies. The number of patients positive for TSI and TBII diminished gradually during the treatment. Three patients remained

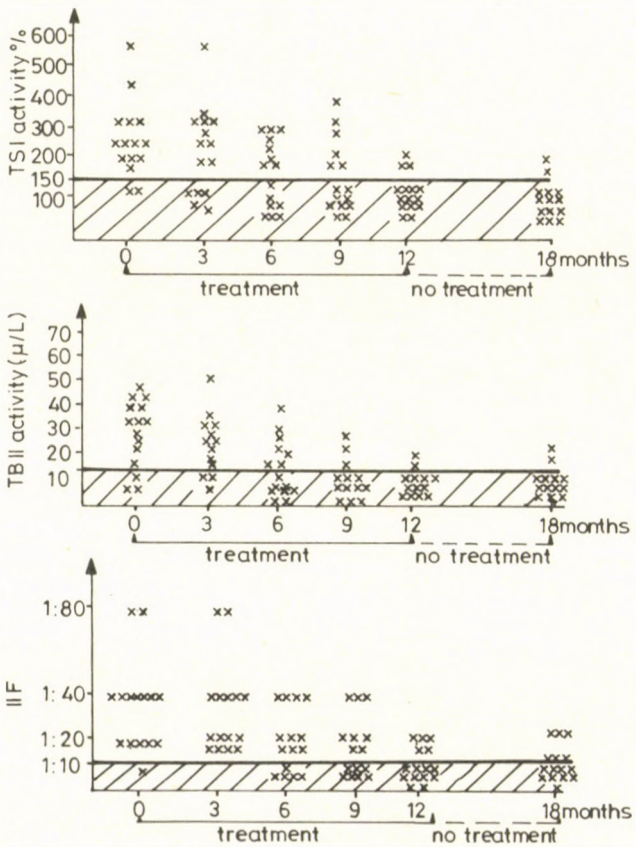


Fig. 1 Patients' Group A. TSI, TBII and IIF before, during and after 12 months' methimazole treatment

positive for TSI, two others showed enhanced TBII activity. The cases with positive indirect IF for anti-microsomal antibodies diminished in number, but at the end of the 12th month, 5 cases showed persistent positivity. Two out of them were positive for TBII and TSI another only for TSI (Fig. 1).

Group-B patients

Before treatment, all of the 7 patients showed enhanced TBII activity. Anti-microsomal antibodies were detected in each case. The TSI activities were elevated in all but one patient. During the period of treatment the activities of TBII and TSI changed, 5 out of 7 patients were negative for TBII 6 months after the beginning of methimazole administration. At the same time 3 patients out of 5 with negative TBII showed diminshed TSI activity. The detectability of anti-microsomal antibodies showed only slight

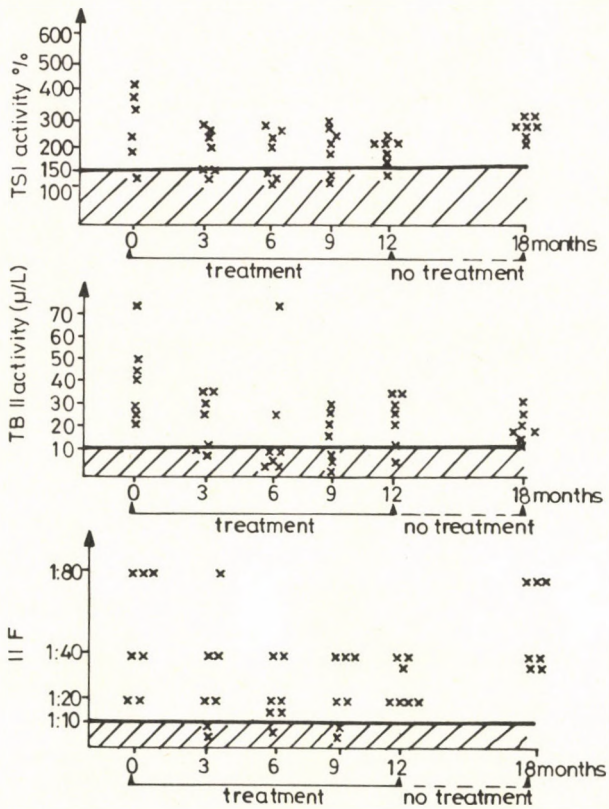


Fig. 2 Patients' Group B. TSI, TBII and IIF before, during and after 12 months' methimazole treatment

alterations during the treatment, two patients proved to be negative 3 months after the beginning of methimazole therapy, but both became positive 3 months later. The one became negative after 6-month therapy, the other after 9 months. All patients had become positive by the end of the 12-month therapy for anti-microsomal antibodies; one of them was negative for TBII and another for TSI. Six months after withdrawal of methimazole all patients were positive for TSI, TBII and anti-microsomal antibodies (Fig. 2).

## DISCUSSION

We assessed parallel the TSI and TBII activities and attempted to detect anti-microsomal antibodies in patients with Graves' hyperthyroidism undergoing methimazole treatment. In accordance with literary data /3, 4, 12, 14, 18/ there was a good correlation between the TSI and TBII activities before methimazole administration. Anti-microsomal antibodies were demonstrated in the majority of the cases. Out of our 22 patients, seven showed a relapse (Group B) 6 months after the therapy had been ceased. One case showed normalization of TSI, another was negative for TBII, and all cases were positive for anti-microsomal antibodies after the 12-month treatment with methimazole. The results differed significantly from those of Group A. In the latter group, only 3 of 15 cases showed slightly enhanced TSI activity and 2 out of them moderately elevated TBII levels. Strangely enough, 5 cases showed anti-microsomal activity at the end of the treatment and 3 out of them showed slightly elevated TSI levels.

It has been reported that TSI and TBII activities during antithyroid drug treatment may reflect the clinical outcome /3, 12, 14, 23, 32, 33/. However, other investigators insist that TSI or TBII are of less prognostic value /8, 9/. Hörmann et al. /11/ emphasize that the persistence of TSI is more reliably associated with relapses than the persistence of TBII. Our results indicate that persisting positivity of both TSI and TBII during the treatment, even if the patient becomes euthyroid, predicts a relapse of hyperthyroidism, but positivity of either TSI or TBII alone does not necessarily indicate a relapse. Our results are in good accordance with the recent data of Kasagi et al. /12/ and support the concept that these antibodies are highly involved in the pathogenesis of Graves' disease.

Anti-microsomal antibodies, which can be detected by indirect IF, have been found frequently in Graves' disease /20, 30, 31/. The microsomal

antigen is generally believed to be a cytoplasmic glycoprotein associated with the membrane of the smooth endoplasmic reticulum /26/, and being expressed on the outer surface of the thyroid cell as well /15, 16, 20/. Czarnoczka et al. /7/, Hamada et al. /10/, Kotani et al. /17/ have recently demonstrated that thyroid peroxidase and the thyroid microsomal antigen are antigenically related and that the latter is involved in autoimmune thyroid disease. Our results are in favour of this hypothesis; all patients of Group B showed enhanced activities of anti-microsomal antibodies after cessation of therapy, in euthyroid phase. For the individual patient, the persistence of anti-microsomal antibodies alone was not a certain indicator of relapse of hyperthyroidism.

Parallel investigation of TSI, TBII and anti-microsomal antibodies may be of high predictive value for relapse. Persisting positivity of all the three tests predicts relapse of Graves' hyperthyroidism. These patients should be treated for longer period of time with thyrostatic drugs or ablative therapy should be taken into consideration.

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# EFFECT OF LITHIUM ON CHEMILUMINESCENCE OF POLYMORPHONUCLEAR GRANULOCYTES AND MONONUCLEAR CELLS OF PERIPHERAL BLOOD

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Influence of lithium on luminol amplified chemiluminescence activity of leucocytes of human peripheral blood was studied by a continuously recorded system. Lithium had a biphasic effect on mitogen-induced early activation of mononuclear cells, i.e. at concentration of 1.0 mM an increase, at higher concentrations a significant inhibition in photon emission was observed. Activating the mononuclear cells by opsonized Zymosan in the presence of lithium a dose-dependent decrease of chemiluminescence was registered. The respiratory burst of polymorphonuclear granulocytes induced by either mitogen or opsonized Zymosan was significantly inhibited at 2.0 and 5.0 mM of lithium, respectively. Chemiluminescence activity of peroxidase-dependent and independent cell-free system was not influenced by lithium. It was concluded that lithium by accumulating into target organs might have an immunosuppressive and antiphlogistic effect by mean of inhibition of antigen-presenting cells and polymorphonuclear granulocytes.

Keywords: chemiluminescence, mononuclear cells, polymorphonuclear granulocytes, phagocytosis, lithium

## INTRODUCTION

Lithium used successfully earlier for the treatment of manio-depressive illnesses was found to be associated with thyroid abnormalities /2, 32/. Wolff et al. described 102 patients treated with lithium who had about one-third goitre, half had hypothyroidism alone and the remainder exhibited both goitre and hypothyroidism as a result of lithium therapy /32/. Since

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lithium has been shown to interact with organs including kidney, adrenal gland, heart as well as granulocytes and thrombocytes the investigation of distribution of this metal in different tissue sections provided useful and instructive analysis. The highest lithium concentrations was found in the kidney, thyroid and strikingly elevated values in thymus /4, 27/. At serum lithium concentrations in the neighbourhood of 1.0 mM the thyroid gland concentrated it 2.5—5.0 fold and the cortex thymus contained 1.7—2.0 times more than medulla and sera /22/. Although the thymus has not been implicated in the side effects of lithium treatment, these observations have focused the attention on cell-mediated immunity /2, 13/. The different and sometimes incongruous findings suggested an immunomodulating effect of lithium in autoimmune processes /10, 13, 14/. We therefore studied the effect of lithium on in vitro chemiluminescence and transformation of lymphocytes and phagocytes as well as respiratory burst of polymorphonuclear granulocytes (PMN).

## MATERIALS AND METHODS

Mononuclear cells and PMN were obtained from 20 ml peripheral blood healthy volunteers by separations in Ficoll-Uromiro gradient /6/. Of these cells in the upper layers, 85—95% proved viable (85—90% were lymphocytes) and 90—95% of PMN in the lower layer proved to be viable by trypan blue exclusion. Various cellular immunological parameters were determined in the absence or presence of different concentration of lithium chloride (Reanal Fine Chemical Factory, Budapest) dissolved in phosphate buffered saline (PBS), pH 7.2. Viability of mononuclear cells and PMN was between 85—89% in the presence of 1.0—5.0 mM/1 of lithium, since the therapeutic levels of lithium are 0.5—1.5 mM/1.

The lymphocyte transformation test was carried out by Hadden et al. modified method /3, 15/. The lymphocyte cultures were incubated for 72 h at 37°C in humified atmosphere of 5.0% $CO_2$ in air in the presence of 10 µg of PHA-P (Sigma) and were labelled by $^3$H-thymidine (2.0 µCi, Amersham Searle) incorporation. Radioactivity was measured by liquid scintillation method. The results were expressed as a transformation index which was ratio derived from the mean c.p.m. in triplicate experimental tubes containing 10 ug of PHA-P in the absence of lithium divided by c.p.m. in triplicate tubes containing no PHA-P.

The phagocytic activity of PMN was measured as previously described /1/. The concentration of PMN was adjusted to $5 \times 10^{-6}$ cell/ml and 20 µl of the cell suspension was dropped on to a sterile slide to which 20 µl of suspension of opsonized Saccharomyces cerevisiae ($10^{-7}$/ml) was added. The slides were incubated at 37°C for 30 min in a moist chamber. Smears were then made, air dried and stained by May-Grunwald-Giemsa. The phagocytic index was calculated as the ratio of the number of ingested yeast cells to the number of PMN examined. Each set of cultures was set up duplicate and 200 PMN were counted.

Measurement of luminol-dependent chemiluminescence of PMN and mononuclear cells was carried out in the lightproof chamber of Luminometer 1250 (LKB, Wallac, Sweden). In this reaction luminol is oxidized by peroxides and the decay from the excited state to basel is accompanied by the emission of light /30/. Zymosan (Human Chemical Factory, Budapest) was suspended in PBS to a final concentration of 20 mg/ml. Opsonization was performed by adding the required volume of pooled sera from 20 healthy volunteers to the suspension and incubated at 37°C for 30 min. The suspension was centrifuged at 2500 g for 10 min, washed and resuspended in PBS. Luminol (Sigma, St. Louis, Missouri, USA) was dissolved in dimethylsulphoxide (DMSO) (Sigma) at a concentration of $2x10^{-3}$M. A 0.5 ml aliquot cell suspension ($10^{-6}$cell/ml) was preincubated in the presence or absence of different concentration of lithium chloride (Reanal, Budapest) at 37°C for 30 min. The respiratory burst was started by adding to this cell suspension 1.0 ml of medium containing $2x10^{-3}$M luminol and 100 µl of opsonized Zymosan particles. The resulting light output in mV was continuously recorded on a chart recorder (LKB 2210) which had a wide range of sensitivity and variable paper speeds. The reaction mixture was kept at 37°C by water passed from a thermostatically controlled circulator through a polished hollow metal sample holder. A background subtraction control adjusted the instrument to zero before the addition of Zymosan. PHA-P induced chemiluminescence reaction was determined as described above. The mononuclear cells were incubated in the presence or absence of lithium chloride at 37°C for 30 min, then the photonemission was induced by 10 µg of PHA-P and detected a luminol-amplified system /29, 33/.

Measurement of peroxidase enzyme activity in human thyroid membrane and PMN was carried out by chemiluminescence technique /31, 32, 33/. Human thyroid glands were obtained at surgery from patients undergoing thyroidectomy for nodular goitre. Thyroid tissue was homogenized in cold 10 mM trishydrochloric acid (pH 7.5). After centrifugation at 800 g for 5 min at 4°C a crude membrane fraction was sedimented from this supernatant by centrifugation at 15 000 g for 10 min at 4°C. The membrane was homogenized in PBS and stored at 0°C.

Peroxidase of PMN was obtained after disruption of cells by a conventional technique and its activity was determined by luminol-dependent chemiluminescence method /17, 24/. 100 µl of 100 fold diluted thyroid and PMN membrane (0.3 mg/ml) was incubated in presence or absence of different concentration of lithium for 30 min at 37°C. Then it was supplemented with reaction mixture: 0.3 ml of 1.0 M glycerine-NaOH buffer (pH 9.0), 0.3 ml of $10^{-3}$ EDTA, 30 µl of $10^{-3}$M luminol (final concentration $10^{-5}$M) and the luminescence reaction was initiated by injection of 500 µl of $1x10^{-4}$ of $H_2O_2$.

Mathematical analysis: Student't-test was used to make comparison between means.

## RESULTS

Lithium had a biphasic effect on early activation of mononuclear cells induced by PHA-P. At a concentration of 1.0 mM lithium caused a slight increase in photon emission of cells, however, at 2.0 mM and 5.0 mM of lithium a significant inhibition was observed ($P < 0.01$ and $P < 0.001$,

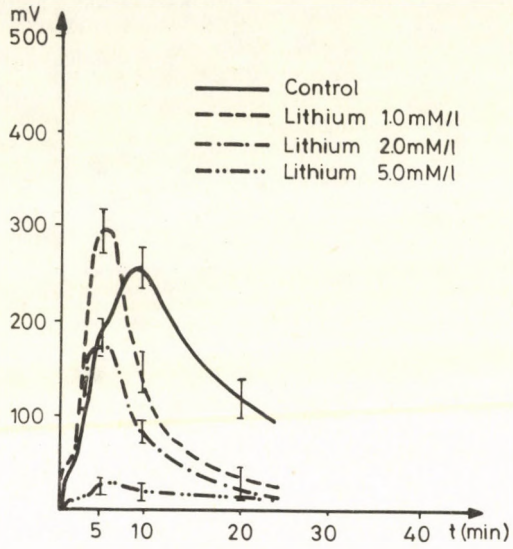


Fig. 1 Effect of lithium on chemiluminescence (mean ±S.E.M.) of mononuclear cells, induced by PHA-P (10 µg/ml). Luminol-dependent chemiluminescence activity of mononuclear cells induced by PHA-P. The ordinate shows the light output in mV, the abscissa the speed of paper (1.0 mm/min). These continuously-registered curves represent the time-dependent process of chemiluminescence. The significance was calculated from automatically registered (in each 10 s) integrated values from ten experiments. This experiment indicates that lithium exhibited a transient increase in chemiluminescence followed by a significant decrease in presence of 2.0 mM ($P < 0.01$) and 5.0 mM ($P < 0.001$) of lithium

respectively) (Fig. 1). In contrast, when mononuclear cells were activated through Fc-receptors by opsonized Zymosan the chemiluminescence activity decreased already at 1.0 mM. At higher concentrations an obvious inhibition was found (at 1.0–2.0 mM $P < 0.05$, at 5.0 mM $P < 0.01$) (Fig. 2). It is noteworthy that the biphasic early activation was observed only mononuclear cells were activated via the mitogen receptor. The respiratory burst of PMN by opsonized Zymosan and PHA the chemiluminescence was slightly different from that of mononuclear cells. The mitogen-induced activation was monophasic and dose-dependent in the presence of increasing concentrations of lithium. At 1.0 mM of lithium a minimal inhibition was observed in chemiluminescence, but 2.0 mM and 5.0 mM concentrations resulted in significant decrease in photon emission ($P < 0.05$ and $P < 0.001$) (Fig. 3).

The opsonized Zymosan induced Fc-receptor-dependent respiratory burst

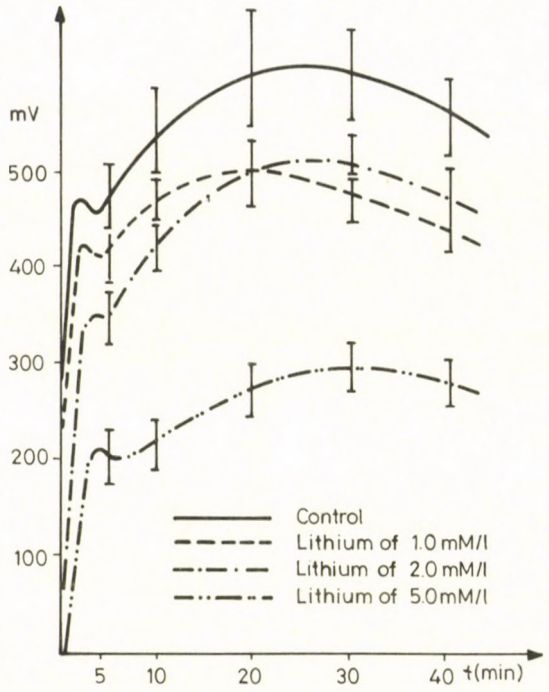


Fig. 2 Effect of lithium on chemiluminescence (mean ±S.E.M.) of mononuclear cells, induced by opsonized Zymosan. Zymosan induces a photon emission from mononuclear cells. The oxygen radicals generated as a result of oxidation of luminol which emit chemiluminescent signal were measured. Concentrations of lithium reduce chemiluminescence significantly compared to controls (at 1.0–2.0 mM: $P < 0.05$, and at 5.0 mM: $P < 0.01$). Comparisons were made between integrated chemiluminescence values from ten experiments. The abscissa shows the speed of paper of 1.0 mm/min, the ordinate the chemiluminescence expressed in mV

was gradually diminished and significant reduction was calculated at 2.0 and 5.0 mM of lithium ($P < 0.05$, $P < 0.01$) (Fig. 4). Since lithium exerted effect on chemiluminescence activity of PMN, therefore, we tested the engulfment of opsonized Saccharomyces cerevisiae in the presence of this cation. In accordance with changes in respiratory burst a dose-dependent significant decrease was observed in phagocytic indices (Table I). $^{3}$H-thymidine incorporation is an accepted test for lymphocyte proliferation, therefore, in the next series of experiments we sought whether not only the early but also late metabolic changes in lymphocytes could be affected by lithium. We measured the PHA-P induced $^{3}$H-thymidine incorporation into

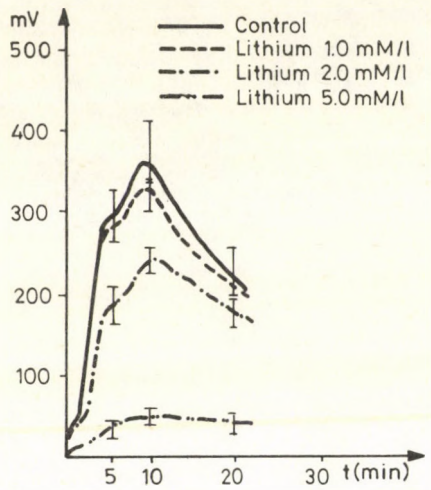


Fig. 3 Effect of lithium on chemiluminescence (mean ± S.E.M.) of polymorphonuclear cells induced by PHA-P (10 μg/ml). The ordinate indicates the light emission expressed in mV, the abscissa the speed of paper (1.0 mm/min), registered the time-dependent chemiluminescence activities in absence and presence of lithium. The comparisons were calculated between the integrated chemiluminescence values from ten experiments. Significant decrease by lithium of 2.0 mM ($P < 0.05$) and 5.0 mM ($P < 0.01$)

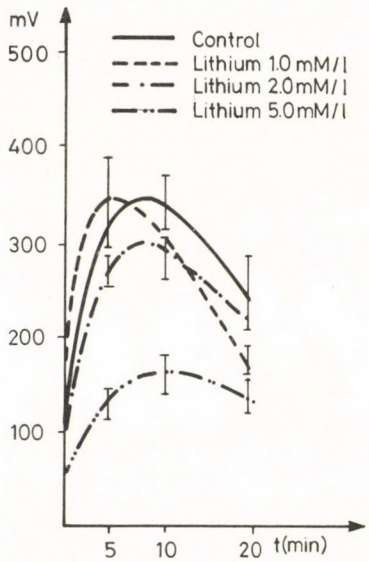


Fig. 4 Effect of lithium on chemiluminescence (mean ± S.E.M.) of polymorphonuclear cells, induced by opsonized Zymosan

Table I

In vitro effect of lithium on phagocytosis of polymorphonuclear granulocytes (n=24)

| | Concentration of lithium (mM/l) | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 5 |
| Phagocytosis index | 2.26 ± 0.39 | 1.96 ± 0.31 | 1.42 ± 0.30 | 1.04 ± 0.28 |
| | | n.s. | $P < 0.05$ | $P < 0.01$ |

n.s. = nonsignificant (mean ±S.E.M.)
$P < 0.05$

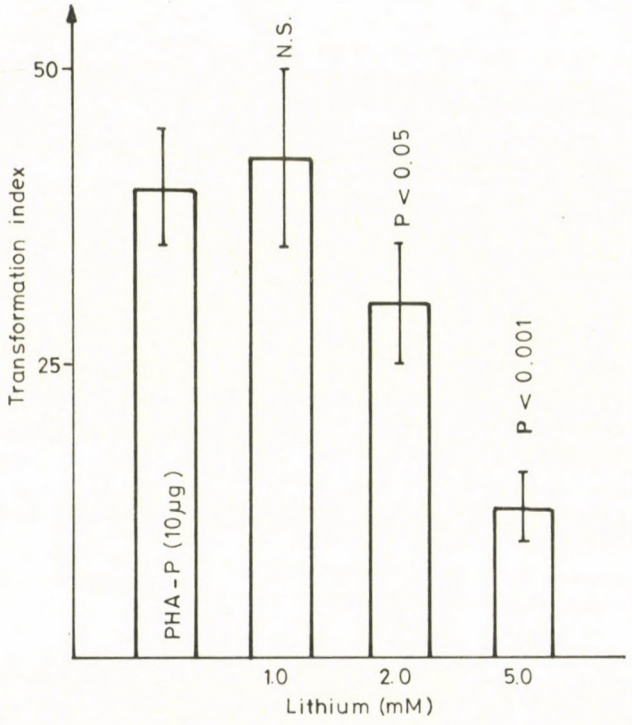


Fig. 5 In vitro effect of lithium on lymphocyte transformation, induced by PHA-P (mean ±S.E.M.)

lymphocytes in presence of lithium and calculated the lymphocyte transformation indices. Our findigs show a biphasic effect of lithium on incorporation of thymidine that resembles the PHA-P induced chemiluminescence. Lithium resulted in an apparent rise at 1.0 mM concentration, by contrast, at larger doses caused a significant decrease in lymphocyte transformation indices (Fig. 5). The peroxidase enzymes have been shown to play an important role in function of both thyroid gland and PMN /3, 27, 31/. We tested, therefore, the effect of lithium on activites of these enzymes. In the presence of lithium at various concentrations any changes in peroxidase produced luminol amplified photon emission was not observed. Furthermore, the peroxidase-independent system which contained $10^{-3}$M of $H_2O_2$ and $2x10^{-5}$M of luminol was also not influenced by lithium. Taken together, these findings seem to support the idea that lithium does not exhibit a free oxygen radical scavenging effect.

## DISCUSSION

Although lithium is an effective therapeutic agent in different disorders the precise mechanism of its action has not been identified /2, 28/. Previously, Jankovič et al. have found that lithium suppressed experimental allergic thyroiditis in rats /14/. On the contrary, Fernandez and Fox have observed in four patients treated with lithium an increased $^3$H-thymidine incorporation into lymphocytes and explained this finding by a selective depression of T lymphocytes /10/. It should be emphasized that this stimulatory effect of lithium restricted to relative low concentrations that accepted as a therapeutic range of this cation in patients' sera but much less than in some organs which are able to accumulate lithium. Our present study provides further evidence for in vitro inhibitory effect of this drug on luminol-dependent chemiluminescence and thymidine incorporation of PMN and mononuclear cells. Although measurement of light emission has been widely used to evaluate the function of PMN and mononuclear cells /3, 30, 33/, similar study concerning the effect of lithium on chemiluminescence has not yet been published. The chemiluminescence of these cells reflects early activation of membrane including increased permeability to cations, amino acid, $Ca^{2+}$ pump and stimulation the membrane bound oxidaze enzymes which are responsible for production of reactive oxygen radicals /7, 8/. Chemiluminescence activity of these cells can be modulated

by influencing of oxygen radical producing enzymes and/or by scavenging these highly active oxygen radicals. It is possible that a drug can be carried out independently /30/. We found that lithium has not influenced the enzyme-independent chemiluminescence reaction, therefore, its scavenging effect can be excluded. The other possibility that the effect of this cation is exerted by inhibition of peroxidase enzymes is unlikely because both thyroid and PMN peroxidase activity remained intact in the presence of high concentrations of lithium. Analysing our experimental data concerning the cellular effect of lithium we have observed a similar characteristic changes in PHA-P induced lymphocytes transformation test as well as in chemiluminescence of mononuclear cells. Undoubtedly, chemiluminescence activity and lymphocyte transformation are not obligatory parallel mechanism because the photon emission indicates the early metabolic processes of membrane, the $^{3}$H-thymidine incorporation test reflects the late mitotic activity of lymphocytes. Observations of Fernandez and Fox concerning the in vivo stimulatory effect of lithium on $^{3}$H-thymidine incorporation are not necessarily in contradiction with our finding. We have also observed that at relative low concentration an enhancement in lymphocyte mitotic activity, however, at 2.0 mM or higher concentrations a significant decrease occurred. Since lithium has not been shown to be cytotoxic at higher concentrations this phenomenon can not be explained by selective destruction of lymphocytes. It is noteworthy that this biphasic effect of lymphocyte activation was observed by PHA-P activation in the presence of lithium. The possible explanation for this phenomenon is the existence of two mechanism of oxygen radicals production. It was found that whereas both chemotactic peptide and mitogen stimulate oxygen radical production, only chemotactic peptide stimulate a rise in intracellular $Ca^{2+}$ /11/. The rise in intracellular $Ca^{2+}$ has been shown to be essential for Fc-receptor-dependent stimulation of oxygen-radical production monitored by chemiluminescence /11, 12, 25, 26/. The influence of lithium on $Ca^{2+}$ pump might be responsible for the increased inhibitory action on Fc-receptor mediated activation and biphasic effect of lithium on mitogen activation of mononuclear cells. Undoubtedly, the subcellular effect of lithium must be in connection with adenylate-cyclase activation /23/. Moreover, the decrease in intracellular concentration of cAMP in lymphocytes has been proved to be the very marker of cellular activation /19/. It must be emphasized that cAMP level in PHA-P activated lymphocytes after a transient rise decrease gradually, furthermore, the cAMP itself can produce an inhibitory activity on mitogen

responses that assumes an important aspect of complexity of lithium /23, 31/. Similarly, a short transient rise in cAMP concentrations is also observed within one minute of stimulation of PMN /18/. Transiency is likely to be a relevant feature in the increase of cAMP concentration, because agents that cause a persistent elevation of the steady-state concentration of this nucleotide also inhibits the metabolic activity of PMN as well /18, 19/. An other pathway posed to explain the diverse lithium actions which based on modulation of second messenger role of diacylglycerol and inositol triphosphate /5/. The key reaction of this membrane activation is a receptor mediated hydrolysis of the phosphoinositides to give two products, both of which may function as second messengers to initiate the signal cascade. Inositol triphosphate seems to act by mobilizing intracellular calcium, whereas diacylglycerol stimulates protein phosphorylation. Lithium prevents the formation of inositol by inhibiting the inositol-1-phosphatase that dephosphorylates the inositol-1-phosphate being recycled or originating from synthesis de novo /5, 25, 26/. In spite of these epxerimental data that can explain the complex effects of lithium on chemiluminescence of mononuclear cells and PMN the precise mechanism of this agent remains to be eluciated. It requires further observation whether this cation might be involved in destruction of membrane bound receptors or intracellular calmodulin-calmodulin acceptor complex /19, 21/. The other aspect of lithium treatment of whether this drug can influence the autoimmune processes in thyroid gland. Since the intrathyroidal concentration of lithium is generally much higher than 2.0 mM, therefore, our present findings allow us to speculate that this cation might be important in immunological remission of autoimmune thyroid disorders. The thyroid antibodies have been shown to be produced mostly in thyroid gland due to imbalance of immunoregulatory cells consequently the high concentration of lithium can interfere with the antigen presenting cells. In order to decide the clinical relevance of our observations a prospective study is needed for monitoring of immune parameters of patients with autoimmune thyroid disorders during long-term lithium therapy.

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# VARIATIONS IN PROLACTIN SECRETION IN HYPER- AND NORMOPROLACTINAEMIA WITH OR WITHOUT GALACTORRHOEA

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The circadian variations and secretory rhythms in prolactin secretion were examined in 10 hyperprolactinaemic and 10 normoprolactinaemic women with or without galactorrhoea in order to establish a clearer picture of this secretion and to find, if exists, correlation between the prolactin level and galactorrhoea.

In the normoprolactinaemic women a rhythmical rise and fall were observed within 20 min, with higher values during nocturnal sleeping; these changes were more marked in the galactorrhoeic group. In the hyperprolactinaemic group the diurnal and pulsation changes were less pronounced, galatorrhoea usually being accompanied by a higher degree of hyperprolactinaemia.

In galactorrhoeic patients with a normal basal prolactin level, a relative prolactin excess may be reckoned with at certain times. A proportion of these women can in fact then be regarded as hyperprolactinaemic. In the hyperprolactinaemic cases without galactorrhoea, a decreased prolactin sensitivity and milk-forming ability of the breasts may be assumed.

Keywords: prolactin, rhythm of pulsation, circadian variation, galactorrhoea

## INTRODUCTION

The secretion of prolactin is subject to certain biological rhythms, such as those present throughout the life span, and circadian and hourly rhythms /3/. Like the other pituitary hormones, prolactin displays a day-night rhythm, its plasma concentrations showing an increase during sleep. Frequent blood sampling also reveals episodic, although apparently

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aperiodic prolactin bursts, occurring during states of both wakefulness and sleep /10/.

The syndrome of inappropriate lactation has been shown usually to be associated with increased circulating prolactin levels /1, 6, 8, 13/. However, only 55.6% of all hyperprolactinaemic cases were found to exhibit galactorrhoea, so that the typical biologic manifestation of elevated circulating lactogen is missing in a high percentage of cases /4/. A considerable number of patients seeking medical advice can suffer from mild galactorrhoea although they have regular menstrual periods and normoprolactinaemia.

Women are generally classified as normoprolactinaemic or hyperprolactinaemic on the basis of two or so serum prolactin determinations. We decided to carry out a series of serum prolactin measurements throughout a 24 h period in order to establish how the prolactin levels vary during the day and also within shorter periods of time, thereby permitting an assessment of the correctness of the diagnosis of normoprolactinaemia or hyperprolactinaemia. A further aim of this work was to examine the existence or absence of a correlation between the serum prolactin level and the occurrence of galactorrhoea.

## MATERIAL AND METHODS

A total of 20 women who were attending our Clinic in connection with infertility counselling were examined. All of them had given their advised consent to the investigations. All examinations conformed in every respect to the requirements of the University Ethics Committee. None of the subjects involved were taking any medication which could affect the prolactin level of the plasma. Prolactinoma was excluded by means of a radiological procedure.

During the preliminary testing relating to the infertility, plasma prolactin levels were measured on two occasions in each woman, always at 10 a.m. The subjects selected for these target investigations were chosen in accordance with the results of the preliminary plasma prolactin testing, and with the presence or absence of galactorrhoea. They were classified as normoprolactinaemic if the mean of the two results was $< 640$ mU/l, and as hyperprolactinaemic if the mean was $> 640$ mU/l. The presence of galactorrhoea was established on the basis of the subjects' symptoms and also by physical examination, milk readily being expressed from the breasts of patients with galactorrhoea.

The subjects were divided into two groups, each involving 10 women. Group 1 contained 10 normoprolactinaemic women, 5 of them with galactorrhoea, while group 2 contained 10 hyperprolactinaemic women, 5 of them with galactorrhoea. The average age of the group-1 subjects was 25.2 years (range, 21–32 years), while that of the group-2 subjects was 27.4 years

(range, 24–33 years). The prolactin levels were determined when the women were in the proliferative stage of their menstrual cycle.

Venous blood samples were taken from all subjects at 2 h intervals for a period of 24 h, and at 20 min intervals during a 2 h period in the morning (9–11 a.m.) and in a 2 h period in the afternoon (3–5 p.m.). These blood samples were utilized for determination of the blood plasma prolactin levels by means of the standard radioimmunoassay method reported by the WHO /14/.

## RESULTS

Figure 1 depicts the results on the normoprolactinaemic women throughout the 24 h, and Fig. 2 the data for the same subjects during the 2 h periods in the morning and in the afternoon. The corresponding data on the hyperprolactinaemic women are shown in Figs 3 and 4. In each figure the upper line relates to women with galactorrhoea, and the lower line to the non-galactorrhoeic group. The data are the means for each of the respective groups.

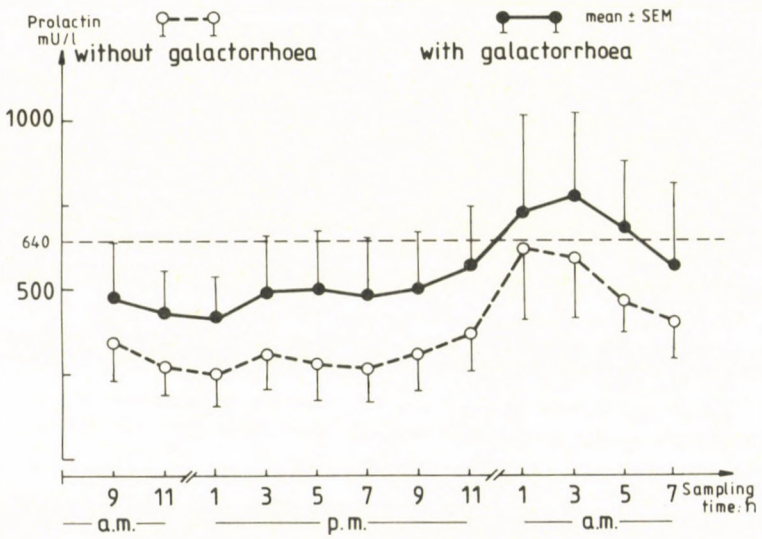


Fig. 1 Circadian variations of prolactin in normoprolactinaemic women without galactorrhoea (n=5) and with galactorrhoea (n=5)

In group-1 women without galactorrhoea, the prolactin level remained within the normal range throughout the day (e.g. 347±105 mU/l at 9 a.m., and 294±98 mU/l at 5 p.m.). The marked increase accompanying nocturnal

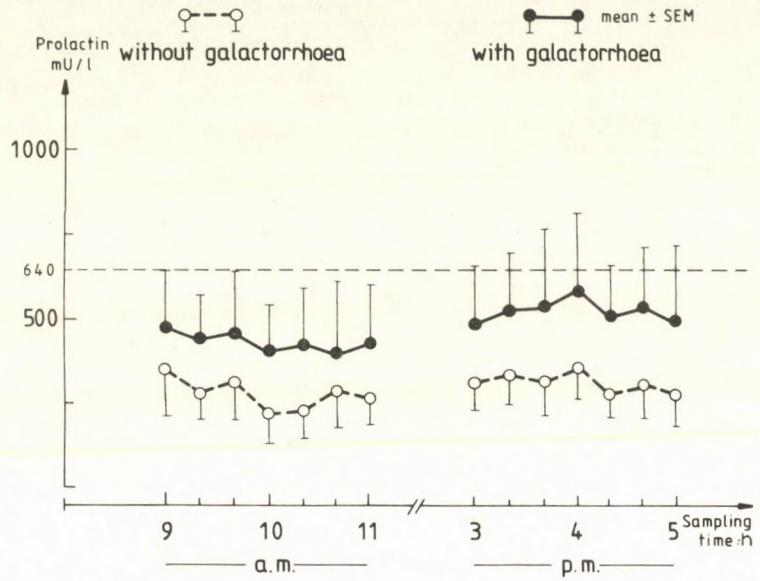


Fig. 2 Secretory rhythms of prolactin in normoprolactinaemic women without galactorrhoea (n=5) and with galactorrhoea (n=5)

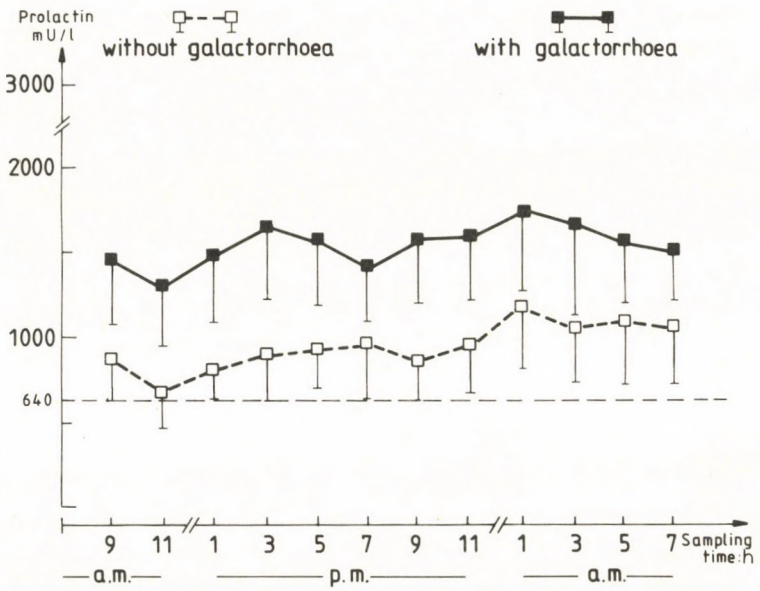


Fig. 3 Circadian variations of prolactin in hyperprolactinaemic women without galactorrhoea (n=5) and with galactorrhoea (n=5) (mean ± S.E.M.)

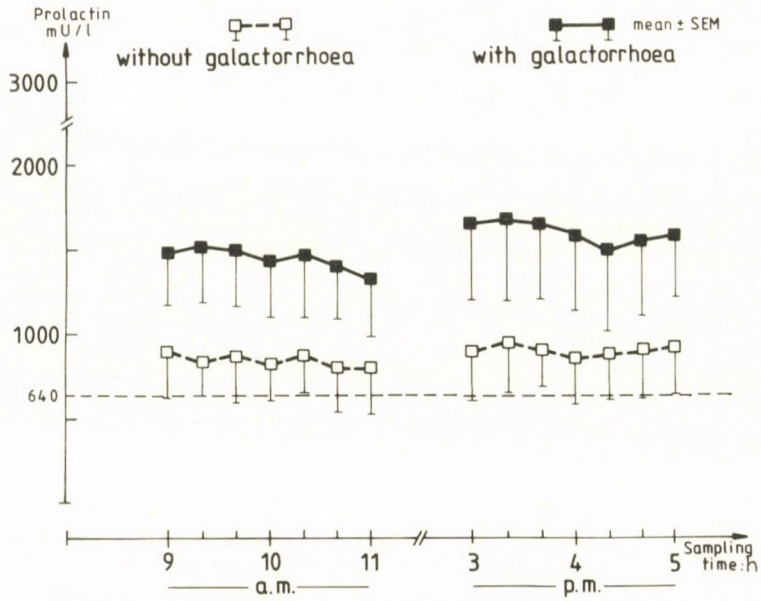


Fig. 4 Secretory rhythms of prolactin in hyperprolactinaemic women without galactorrhoea (n=5) and with galactorrhoea (n=5)

sleeping led to a mean prolactin level close to, but not in excess of, the upper limit of the normal range (e.g. 620±160 mU/l at 1 a.m., and 598±146 mU/l at 3 a.m.). In group-1 women with galactorrhoea, similar variations are to be observed, but the prolactin levels are always much higher than those in the non-galactorrhoeic group (481±114 mU/l at 9 a.m., 503±119 mU/l at 5 p.m., and 737±211 mU/l at 1 a.m.). The short-term changes in the group-1 women reveal only minor rhythmical variations (Fig. 2 and Table I). There is a tendency towards a slight minimum around 10 a.m. (non-galactorrhoeic group: 241±82 mU/l; galactorrhoeic group: 412±123 mU/l), and towards a slight maximum around 4 p.m. (non-galactorrhoeic group: 354±108 mU/l; galactorrhoeic group: 591±144 mU/l). It should be noted that, on the basis of certain diurnal and secretory prolactin levels, some patients in the galactorrhoeic group could perhaps be considered boderline hyperprolactinaemic.

In the group-2 subjects, the prolactin levels are 2–3 times higher than the levels in group-1. At all times throughout the day the prolactin level is above the upper limit of the normal range, even in the cases without galactorrhoea (874±230 mU/l at 9 a.m., 907±237 mU/l at 5 p.m., and 1174±261 mU/l at 1 a.m.). As previously, the galactorrhoeic cases display

Table I

Short-term changes in prolactin secretion

| Sampling time | | | a.m. | | | | | | | p.m. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 9 | 9.20 | 9.40 | 10 | 10.20 | 10.40 | 11 | 3 | 3.20 | 3.40 | 4 | 4.20 | 4.40 | 5 |
| Variation Coefficient % | I/a | (n=5) | 79 | 68 | 79 | 94 | 87 | 81 | 70 | 61 | 61 | 70 | 58 | 63 | 71 | 78 |
| | I/b | (n=5) | 69 | 59 | 76 | 73 | 81 | 91 | 76 | 65 | 63 | 77 | 78 | 60 | 67 | 79 |
| | II/a | (n=5) | 60 | 47 | 64 | 54 | 48 | 63 | 65 | 61 | 58 | 44 | 57 | 55 | 57 | 54 |
| | II/b | (n=5) | 41 | 43 | 44 | 46 | 49 | 44 | 51 | 52 | 55 | 51 | 54 | 64 | 55 | 43 |

I: normoprolactinaemic
II: hyperprolactinaemic

a: non-galactorrhoeic
b: galactorrhoeic

considerably higher prolactin concentrations (1452±342 mU/l at 9 a.m., 1592±374 mU/l at 5 p.m., and 1649±413 mU/l at 1 a.m.) than the non-galactorrhoeic group. The circadian changes are much less pronounced in group-2 than in group-1. Nocturnal sleeping is clearly accompanied by a rise in prolactin level, but this rise is much less marked than that in the normoprolactinaemic subjects. Here, too, there is a minimum in the late morning (11 a.m.: non-galactorrhoeic group: 789±217 mU/l; galactorrhoeic group: 1342±319 mU/l), and tendencies towards slight rises in early and late afternoon (5 p.m.: non-galactorrhoeic group: 847±231 mU/l; galactorrhoeic group: 1582±347 mU/l). The short-term changes in group 2 are much less pronounced than in group-1, the changes being somewhat more apparent in the women with galactorrhoea (Fig. 4 and Table I).

## DISCUSSION

In the adult human, basal prolactin serum concentrations show episodic or pulsatile variations of low amplitude, probably reflecting oscillations of tone in the central control of prolactin secretion /11, 12/. Prolactin secretion is increased during sleep, this occurring independently of the time of day. Waking baseline levels are mostly reached only 1–2 h after waking /5/.

In pathologic states, blunting or absence of the nocturnal prolactin elevation has been reported, but exceptions are possible /2, 7, 9/. The sleep-related peak is missing in cases of galactorrhoea-amenorrhoea, although the episodic day-time variation seems to be preserved /4/. The mechanisms involved in the abolition of the circadian prolactin rhythm are far from being clear. However, indirect evidence exists for an endogenous blockade of dopaminergic mechanisms controlling prolactin secretion. Not only blunting of the prolactin response to chlorpromazine has been reported, but also a reduction in the nocturnal elevation with progressively increasing basal prolactin /9/.

As regards our own results, the normal phenomena were observed as concerns the pulsation and diurnal variations in the prolactin level in group 1, i.e. the normoprolactinaemic women (a rhythmical rise and fall within 20 min with higher values during nocturnal sleeping). In the galactorrhoeic subgroup, these changes were more marked. Higher prolactin levels were often observed at night and (for the secretion pulsation)

particularly in the afternoon. In group 2, the hyperprolactinaemic group, the diurnal and pulsation changes were weaker, the same being true for the galactorrhoeic cases in this group. High prolactin levels ( >2500 mU/l) were in all cases accompanied by galactorrhoea.

Besides prolactin hyperproduction, the hypersensitivity of the milk-forming tissue in the breast (possibly at a receptor level) and other mechanisms governing lactation may play a part in inducing extrapuerperal galactorrhoea. In the hyperprolactinaemic non-galactorrhoeic cases, a decreased prolactin sensitivity or milk-producing capacity of the breasts may be assumed. In galactorrhoeic patients with a normal basal plasma prolactin level, a relative prolactin excess must be reckoned with; this can be established by further examinations (diurnal changes, secretion pulsation, hormone response to physiological stimuli enhancing prolactin production). On the basis of such a prolactin profile, a considerable proportion of the galactorrhoeic patients can be regarded as hyperprolactinaemic.

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# DRUG USE DURING PREGNANCY IN HUNGARY

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The data-set of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980–1985, demonstrates the occurrence and distribution of drug intakes during pregnancy in the so-called negative control pregnant women delivering unmalformed babies. Of 13.882, only 1260 (9.1%) did not mention drug intakes during pregnancy studied. When vitamins, iron and calcium supplements are excluded, this proportion increases to 29.1%. The most frequently used categories of drugs were sex hormones (41.8%) mainly as hormonal support therapy sedatives,hypnotics and other drugs acting on the central nervous system (38.8%), and drugs acting on the cardiovascular system (33.9%).

Keywords: drugs, pregnancy, Hungary

## INTRODUCTION

Among teratogens, drugs are considered one of the most important groups. Recently their teratogenic effect has been exaggerated, therefore, it is necessary to have a Case-Control Surveillance System of Congenital Abnormalities which includes drug ingestions during pregnancy in both malformed babies and controls, i.e., healthy babies. Data of drug use during control pregnancies are being summarized here, because this data-set can review the Hungarian situation.

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## MATERIALS AND METHODS

The Hungarian Congenital Malformation Registry /2/ (HCMR) is a population-based data-set of cases affected by congenital anomaly (CA). The recent total birth prevalence rates of CAs recorded have exceeded 46 per 1000 total births. In Hungary, the Case-Control Surveillance System of Congenital Abnormalities (CCSSCA) was established in 1979 and it has been carried on since 1980 /3/. The aim of CCSSCA is to obtain aetiological information nearly in parallel with the notification of major CAs to the HCMR. First a reply-paid questionnaire with an explanatory letter and lists of drugs and diseases are mailed to the parents of index patients within one month after being notified to the HCMR. (In general, it is within one month of birth.) The questionnaire involves information on drugs taken, and maternal diseases, during pregnancy according to gestational weeks as well as employment, social class and occupational exposure. The drug dosage is not asked because our pilot study in 1979 indicated that the reliability of this information is low. Additionally in general the recommended daily dose is used. In order to standardize the answers, mothers are asked to read the enclosed lists of drugs and maternal diseases before they reply. Furthermore, mothers are requested to send us the prenatal care logbook and every medical document of the child's CA. The prenatal care logbook is a mandatory diary of all prescribed drugs completed by physicians during pregnancy. The response rate was 81% in 1980–1985. Only those cases where medically documented data available are evaluated.

Owing to adequate evaluation two negative controls (i.e., newborns without CA) are matched to every index patient according to sex, birth week, and district of the parents' residence from the national birth registry of the Central Statistical Office. (Accidentally selected malformed infants are excluded from this sample.) Mothers of these newborns get the same reply-paid questionnaire which is sent to mothers of index patients with another explanatory letter. Furthermore, of course, these mothers are also requested to send us the prenatal care logbook and every medical document of their possible diseases and hospitalization during pregnancy. The response rate was 71% in negative control mothers.

Index patients such as those with Down syndrome are evaluated as positive controls.

At the evaluation of the drug intakes during pregnancy there are three technical points. First, gestational time. Three periods are separated: (i) First month; it is before the critical period of CAs, involving two weeks before conception, because pregnancy is calculated from the LMP, and two weeks of the preimplantation period. (ii) The second and third months as the critical period in the majority of major CAs. (iii) The subsequent months of pregnancy. If pregnant women were treated more then once during pregnancy, only the first treatment is evaluated in these cases. If the treatment was longer then one month or there was an overlapping among months, there are two types of the analysis: to evaluate only the first month of treatment, or all months independently. At this analysis, only the first month of drug intakes was taken into consideration. Second, if there was multiple use of different durgs, each drug is coded independently, thus it is possible to separate "pure" and "combined" groups. In this paper both kinds of drug intakes were evaluated. Third, discordances are sought between maternal information in the questionnaire and medical information in the prenatal logbook or discharge summary. Both pieces of information are coded, so it is possible to evaluate them separately. In this study, data of drug intakes were obtained in both

sources. Of course, the evaluation of confounding factors, e.g., maternal disorders during pregnancy and before, maternal age and birth order, socio-economic status, etc. is only performed if necessary. Within this descriptive review of drug intakes in controls, there is no space to demonstrate them.

After the coding of data by trained technicians, drug intakes and maternal diseases are reviewed by a medical teratologist in each case. Two categories of cases are excluded from the analysis: those from whom no answer is received concerning the drug intake during pregnancy, and those with confusing information, e.g., insulin treatment in epileptic women or intake of contraceptive pills in the last quarter of pregnancy. The sum of these two subgroups was about 3% of the cases.

## RESULTS

The data-set of the CCSSCA, 1980–1985 involves 13.882 mothers with unmalformed babies, which comprises the so-called negative control sample. (Within the study period, 22.877 questionnaires were sent to mothers of negative controls. The address was unknown or wrong in 2463 families (10.8%). Of the remaining 20.414 mothers, 14.483 sent back the filled-in questionnaires (70.9%), however, 601 cases (2.9%) were excluded because of lacking or confusing data concerning drug use during pregnancy.) The study period contained 813.796 total births. Thus, the study sample involves 1.7% of all births in Hungary and it can indicate a general view about the distribution of drug use in the pregnant population.

Table I shows the distribution of pregnant women with one or more drug uses during pregnancy. Of 13.882 mothers with unmalformed babies, only 1260, i.e., 9.1% denied the use of any drug during the pregnancy. However, it is worthwhile excluding the usual pregnancy supplements: vitamins, calcium and iron preparations. If doing so, the proportion of no drug intake during pregnancy increases significantly, to 29.1% of the pregnant women with unmalformed babies. The largest subgroup of pregnant women used only one drug, but women with two drugs were near to this figure. 5.4% of pregnant women took more than 5 drugs excluding supplement. During the gestational period, study mothers received an average of 2.7 drugs.

The distribution of the main categories of drugs used during pregnancy in the study sample is summarized in Table II. The most frequently used category of drugs involves sex hormones (in 41.8% of pregnancies) due to the extensive use of progestogens as hormonal support therapy. The second and third categories are sedatives, hypnotics and other medicines acting on the central nervous system (38.8%) and medicines acting on the

Table I

Numerical distribution of drug intakes during pregnancy in Hungary, 1980–1985

| Number of drugs | All drugs | | Drugs excluding vitamins, iron and calcium supplements | |
|---|---|---|---|---|
| | No. | % | No. | % |
| 0 | 1260 | 9.1 | 4042 | 29.1 |
| 1 | 1172 | 8.4 | 2902 | 20.9 |
| 2 | 2245 | 16.2 | 2591 | 18.7 |
| 3 | 2723 | 19.6 | 1774 | 12.8 |
| 4 | 2184 | 15.7 | 1155 | 8.3 |
| 5 | 1669 | 12.0 | 672 | 4.9 |
| 6 | 1095 | 7.9 | 363 | 2.6 |
| 7 | 664 | 4.8 | 211 | 1.5 |
| 8 | 386 | 2.8 | 102 | 0.7 |
| 9 | 262 | 1.9 | 54 | 0.4 |
| 10 or more | 222 | 1.6 | 16 | 0.1 |
| Total | 13 882 | 100.0 | 13 882 | 100.0 |

cardiovascular system (33.9%). The figure for six further categories exceeds 10%. However, the well-established human teratogenic drugs, including some groups of anticonvulsants (e.g., hydantoin, sodium valproate), anticoagulants as warfarin, or antineoplastics were used rarely in pregnancies of mothers having unmalformed babies.

Data for the most commonly used drugs (in >3% of pregnant women) are demonstrated in Table III. These 18 drugs represent 35% of total drug intake during pregnancy involving 518 recorded drugs. Some of them (allylestrenol, promethazine, terbutalin, diazepam, drotaverinum) are used for the treatment of threatened abortions and preterm births, or other high-risk pregnancies. A second group contains two antiemetics (dimenhydrinatum and thiethylperazin) frequently used in early pregnancy. A third group of drugs is connected with the treatment of genital infections (clottrimozal and metronidazol) and of other infections, e.g., antipyretics (acetylsalycilicum and novamidopyrinum), antibiotics (penamecillinum and ampicillin) and other anti-infection drugs (nitrofurantoin). The high proportion of

Table II

Distribution of main categories of drugs used by category in 12,622 pregnancies where at least one drug was taken delivering unmalformed babies in Hungary, 1980–1985

| Main categories | Month of pregnancy I. | II–III. | IV–IX. | Unknown | Total No. | % |
|---|---|---|---|---|---|---|
| Sedatives, hypnotics and other drugs acting on nervous system | 239 | 1048 | 3156 | 450 | 4893 | 38.8 |
| Anticonvulsants | 14 | 6 | 3 | 3 | 26 | 0.2 |
| Spasmolytics, Relaxants | 65 | 342 | 764 | 158 | 1329 | 10.5 |
| Analgetics, Antipyretics | 201 | 437 | 1058 | 442 | 2138 | 16.9 |
| Drugs acting on respiratoric system | 39 | 154 | 419 | 64 | 676 | 5.4 |
| Drugs acting on cardiovascular system | 140 | 524 | 3253 | 355 | 4272 | 33.9 |
| Drugs acting on digestive systems excl. antiemetics | 99 | 168 | 544 | 175 | 986 | 7.8 |
| Antiemetics | 260 | 930 | 316 | 188 | 1694 | 13.4 |
| Antidiabetics, antithyroid drugs | 12 | 2 | 15 | 3 | 32 | 0.3 |
| Antibiotics | 161 | 523 | 1362 | 164 | 2210 | 17.5 |
| Sulphonamides | 34 | 60 | 201 | 26 | 321 | 2.5 |
| Other anti-infectious agents | 39 | 201 | 951 | 123 | 1314 | 10.4 |
| Sex hormones | 289 | 1334 | 3252 | 404 | 5279 | 41.8 |
| Others | 55 | 242 | 887 | 149 | 1333 | 10.6 |

Table III

The most commonly used drugs by gestational period in 12,622 women who took at least one drug during pregnancy in Hungary, 1980–1985

| | Generic name | Trade name | Gestational month I. | II–III. | IV–IX. | Unknown | Total No. | % |
|---|---|---|---|---|---|---|---|---|
| 1. | Allylestrenol | Turinal | 200 | 1136 | 1554 | 201 | 3085 | 24.4 |
| 2. | Promethazine | Pipolphen | 11 | 83 | 1175 | 104 | 1373 | 10.9 |
| 3. | Diazepam | Seduxen | 53 | 249 | 1454 | 169 | 1925 | 15.3 |
| 4. | Terbutalin (sulfuricum) | Brycanil | 116 | 676 | 1417 | 208 | 2417 | 19.2 |
| 5. | Drotaverinum (hydrochloricum) | No-Spa | 51 | 290 | 682 | 136 | 1159 | 9.2 |
| 6. | Penamecillinum | Maripen | 58 | 274 | 612 | 65 | 1009 | 8.0 |
| 7. | Clottrimozal | (Ung.) Canesten | 24 | 160 | 608 | 79 | 871 | 6.9 |
| 8. | Dimenhydrinatum | Daedalon | 137 | 467 | 131 | 71 | 806 | 6.4 |
| 9. | Aminophyllin | Diaphyllin | 19 | 64 | 581 | 63 | 727 | 5.8 |
| 10. | Acido-Acetylsalycillicum + Calcium carbonicum | Kalmopyrin | 38 | 156 | 363 | 92 | 649 | 5.1 |
| 11. | Pholedrinsulfate | Pulsotyl | 35 | 202 | 343 | 53 | 633 | 5.0 |
| 12. | Nitrofurantoin | Nitrofurantoin | 13 | 86 | 444 | 57 | 600 | 4.8 |
| 13. | Ampicillin | Semicillin | 29 | 118 | 400 | 48 | 595 | 4.7 |
| 14. | Clopamidum | Brinaldix | 3 | 15 | 528 | 41 | 587 | 4.7 |
| 15. | Novamidopyrinum + Methasulfonate natrium | Algopyrin | 59 | 103 | 234 | 168 | 564 | 4.5 |
| 16. | O-betahidroxietilrutozid | Venoruton | 18 | 63 | 349 | 22 | 452 | 3.6 |
| 17. | Metronidasol | Klion | 13 | 76 | 262 | 35 | 386 | 3.1 |
| 18. | Thietylperazin | Torecan | 47 | 220 | 92 | 40 | 399 | 3.2 |

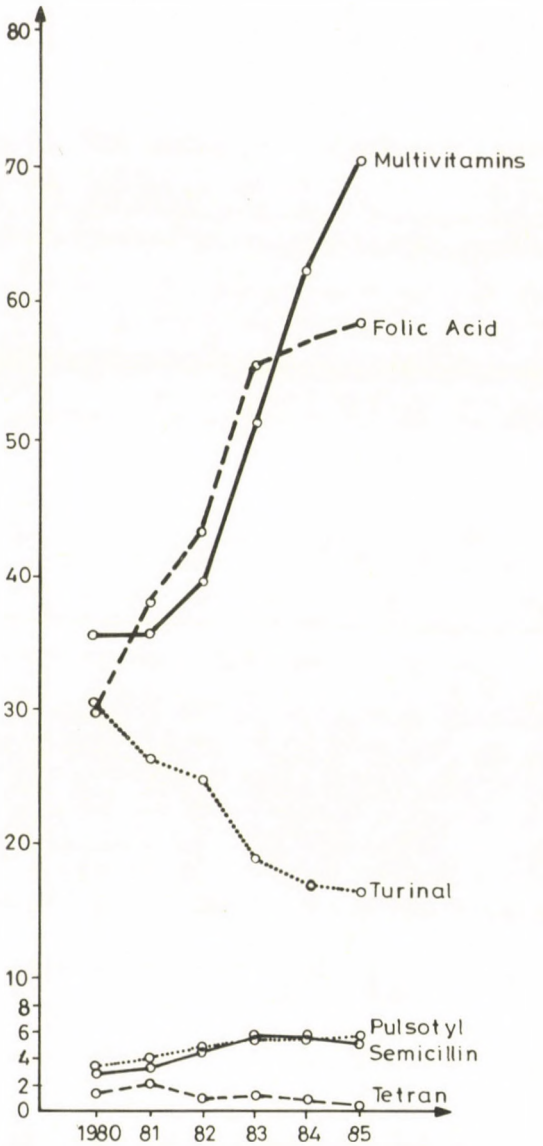


Fig. 1 Annual distribution of some selected drug intakes during pregnancy in Hungary, 1980–1985

drugs acting on the cardiovascular system (aminophyllin, pholedrinsulfate, clopamidum) is surprising.

The annual distribution of drugs used during pregnancy in general shows a balanced pattern in the study period. However, there are some exceptions, e.g., significant increases or decreases of drug uses (Fig. 1). The increasing trend of folic acid and multivitamin is explained by reports on good efficacy of periconceptional multivitamin supplementation in the reduction of neural-tube defects /4/. There is a change within antibiotic use: tetracycline (Tetran$^{R}$) had a decreasing, while ampicillin (Semicillin$^{R}$) an increasing intake of pholedrinsulfate (Pulsotyl$^{R}$). Finally the extremely and probably unreasonable high use of allylestrenol (Turinal$^{R}$) decreased in the early eighties due to recent Hungarian recommendations /5/.

## DISCUSSION

The main purpose of the evaluation of the so-called negative controls within the Hungarian CCSSCA is to provide an appropriate material for case-control analysis. Besides, this control sample can throw light on the pregnant population in Hungary. There are two theoretical problems. (i) This study sample involves only unmalformed infants, while the study population has 6% of cases with CAs including 2.3% of babies with major CAs /6/. In Hungary, about 75% of all CAs and 95% of major CAs are recorded in the HCMR. Additionally, the data of drug use during pregnancy are available in the majority of malformed cases (it means 8011 cases in the period 1980–1985). Thus, it is possible to estimate the drug intakes during pregnancy in the study population using an appropriate combination of cases and controls. (ii) Controls were matched by parents' district and this technique allows for the control of environmental factors but these controls may not be representative of pregnancies in Hungary.

The Hungarian data indicate a high drug intake during pregnancy even if vitamins, iron and calcium supplements are disregarded. More than 70% of pregnant women were treated with one or more drugs. A survey in the United States showed that about 45% of women used at least one drug on prescription, and many more used drugs bought over the counter /7/. The recent paper of Piper et al. /8/ described prescription drug use before and during pregnancy based on data obtained from the paid Medicaid claims of 16 886 Michigan women aged 15 to 44 years who were delivered of a live

infant. Study mothers received an average of 3.1 prescriptions for non-vitamin drugs. A prospective survey in the United Kingdom demonstrated that about 35% of women took drugs at least once during pregnancy, although only 6% used drugs in the first trimester /9/. This excludes iron and vitamin supplements and drugs used during labour. These data indicate a considerable decrease (from 80% to 35%) in the total drug use during pregnancy since the last major survey in the United Kingdom in the mid-1960s and particularly in self-administered drugs (from 64% to 9%) due to the attention paid by the news media to drug-induced CAs /10/. There are significant similarities and differences in the distribution of drugs used during pregnancy in different countries. On the one hand, proportions of non-narcotic analgetics, antibacterial agents and antacids were 12.9% and 12.3%, 10.3% and 11.6%, 7.4% and 7.7%, i.e., within the same range, respectively, in the United Kingdom and in the Netherlands /10/. On the other hand, the Hungarian pattern obviously differs from these figures.

The point is that some countries, including Hungary, have adequate estimations concerning drug intakes during pregnancy. In Hungary, experts have to face the high rate of drug uses among pregnant women and particularly the extremely high level of hormonal support therapy.

The knowledge of the national characteristics of drug intake during pregnancy may explain special patterns of CA-syndromes, e.g., the higher rate of fetal valproate syndromes in certain countries /11/ or a significant increase in congenital limb reduction abnormalities in Hungary, 1975–1978 /12/.

## ACKNOWLEDGEMENT

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C I R C U L A T I O N

# THE CHARACTERISTICS OF THE PERIPHERAL — LIMB — CIRCULATION IN CONGESTIVE HEART FAILURE

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The regulation of the peripheral — limb circulation was investigated in 21 patients suffering from chronic cardiac failure (NYHA stage II and III). In 11 patients the extremital circulation was intact, while 10 patients suffered from peripheral obliterative arterial disease, too (intermittent claudication or rest pain). The control group consisted of 75 subjects with normal cardiac condition. In 35 of the control subjects the peripheral circulation was intact, the remaining 40 suffered from extremital obliterative arterial disease. The limb blood flow was measured by using the venous isotope dilution technique. In congestive heart failure the limb blood flow and the limb oxygen consumption slightly diminished, but remained in the normal range. The limb vascular resistance significantly increased. In patients suffering from intermittent claudication or rest pain, the marked diminution of the limb blood flow and elevation of the vascular resistance was more pronounced in congestive heart failure than in healthy subjects. The pathologically elevated limb vascular resistance decreased and the limb blood flow significantly increased in congestive heart failure on administration of vasodilator drugs. A pathological and mostly reversible increase in extremital vascular resistance is the most characteristic sign of the peripheral circulation in congestive heart failure.

Keywords: cardiac decompensation, limb blood flow, limb vascular resistance, limb oxygen consumption, intermittent claudication

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Abbreviations: LBF: limb — total — blood flow; LVR: limb vascular resistance; $LO_2C$: limb oxygen consumption; MBP: mean blood pressure; CO: cardiac output

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## INTRODUCTION

In chronic cardiac failure, characteristic circulatory changes develop, viz. decrease of cardiac output, increase of venous pressure, slowing down of circulation, prolongation of the circulation time and an increased systemic vascular resistance.

It is very important that on the effect of congestive heart failure not only the circulation, but also its regulation tends to change. The redistribution of the cardiac output between the different vascular areas tends to be modified in the course of cardiac decompensation. Some vascular beds (coronary circulation, cerebral circulation) receive an increased, others (splanchnic vascular area) receive a diminished, fraction of the cardiac output. Abnormalities in the extremital — peripheral — circulation due to cardiac decompensation have often been reported, but the results of the investigations are divergent. A modification of the limb extremital circulation can be expected in any case of congestive heart failure.

The present study was undertaken in order to obtain more insight into the regulation of limb circulation in chronic heart failure. One of the aims of our study was to investigate the peripheral circulation in the normal and the pathological condition of limb circulation.

## PATIENT GROUP, METHODS

The limb circulation was studied in 21 patients suffering from congestive heart failure. According to the NYHA classification the patients were in stage 2 or stage 3 of cardiac decompensation. In 11 patients the peripheral circulation was intact (group A), while 10 patients suffered from obliterative peripheral arterial disease with clinical sign of intermittent claudication or rest pain (study group B). The most important clinical data of the patients are summarized in Table I.

The data of 75 subjects with normal cardiac condition served as control. In 35 cases (20 male and 15 female) the limb circulation was normal (control group A); age: 46.2 (29–64) years.

40 of the control patients (31 male and 9 female) suffered from obliterative arterial disease with complains of intermittent claudication or rest pain (control group B); age: 46.4 (26–64) years.

The limb blood flow was measured by the aid of the venous counterflow isotope dilution technique with double puncturing of the femoral vein /21/, a method apt to correct determination of the total limb blood flow. The limb oxygen consumption was determined from the quotient of the limb blood flow and the arteriovenous extremital $O_2$ difference limb. The oxygen content of the femoral arterial and venous blood was determined in blood samples taken from direct punction of the femoral artery and vein. Cardiac output was measured by application of the dye dilution principle. As indicator Evans blue was used.

Table I

Clinical data of patients suffering from chronic cardiac failure (study group)

| | | |
|---|---|---|
| A: patients with intact limb circulation (group A) | | |
| n = 11, age: 48.5 (32–60) years, sex distribution: | 5 male | |
| | 6 female | |
| cause of chronic heart failure: n = 11 | valvular heart disease: | 7 |
| | ischaemic heart disease: | 3 |
| | dilated cardiomyopathy: | 1 |
| B: patients with obliterative peripheral arterial disease | | |
| n = 10, age: 51.3 (30–67) years, sex distribution: | 8 male | |
| | 2 female | |
| cause of chronic heart failure: n = 10 | valvular heart disease: | 6 |
| | ischaemic heart disease: | 3 |
| | dilated cardiomyopathy: | 1 |

For statistical evaluation of the data the Student's t test for paired and unpaired data was used. Means ± S.D. are shown in the Figures.

## RESULTS

In congestive heart failure the limb circulation tends to be modified. The limb blood flow (LBF) and the oxygen consumption of the limb tissues ($LO_2C$) tends slightly downwards and the limb vascular resistance shown an increase. It is important that the limb blood flow and the limb oxygen consumption almost remain within the normal límits. The normal mean value of the total limb blood flow (calculated for one lower limb) is about 360 ml/min and the limb oxygen consumption is about 14 ml/min /22/. In patients suffering from congestive heart failure (patients group A) we found lower values; limb blood flow = 325 ml, limb oxygen consumption = 13 ml/min. Characteristic was the marked elevation of the limb vascular resistance (LVR) in chronic heart failure. The cardiac output was diminished — as it was expected — in patients suffering from congestive heart failure. The

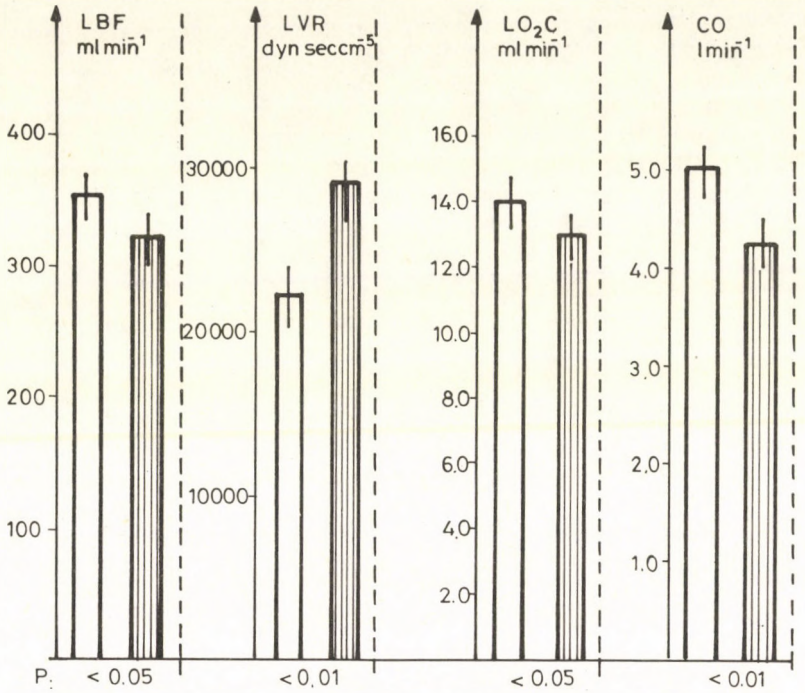


Fig. 1 Alterations of the limb circulation in congestive heart failure. Empty columns represent the values of patients with normal cardiac condition (control group A; n=11), while the hatched ones the data of patients with chronic heart failure (study group A; n=11). LBF: total limb blood flow; LVR: limb vascular resistance; $LO_2C$: limb oxygen consumption; CO: cardiac output; means ±S.D.

changes of limb circulation in congestive heart failure are shown by column diagrams in Fig. 1.

In patients suffering from both cardiac decompensation and peripheral arterial disease (study group B) the deterioration of the limb circulation was more expressed than in patients with only peripheral obliterative arterial disease (control group B) (Fig. 2).

The present data convincing have proved that in congestive heart failure the most characteristic alteration of the extremital circulation is a pathological elevation of the limb vascular resistance (peripheral vasoconstriction). The next question was as to whether the peripheral vasoconstriction due to chronic cardiac failure is reversible. To answer this question, we studied the effect of acute vasodilator therapy on the limb circulation in 10 patients suffering from both obliterative peripheral arterial disease and congestive heart failure (study group B). The vaso-

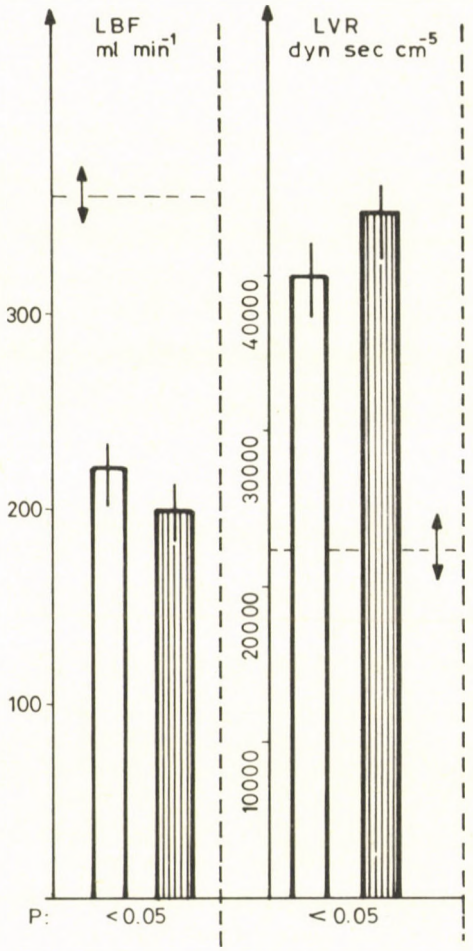


Fig. 2 The effect of chronic heart failure on the limb blood flow (LBF) and limb vascular resistance (LVR) in patients with peripheral arterial disease. Empty columns: patients with normal cardiac condition (control group B; n=10); hatched columns: data of patients with cardiac decompensation (study group B; n=10). The mean normal values (and scatter) of limb blood flow and limb vascular resistance; means ±S.D.

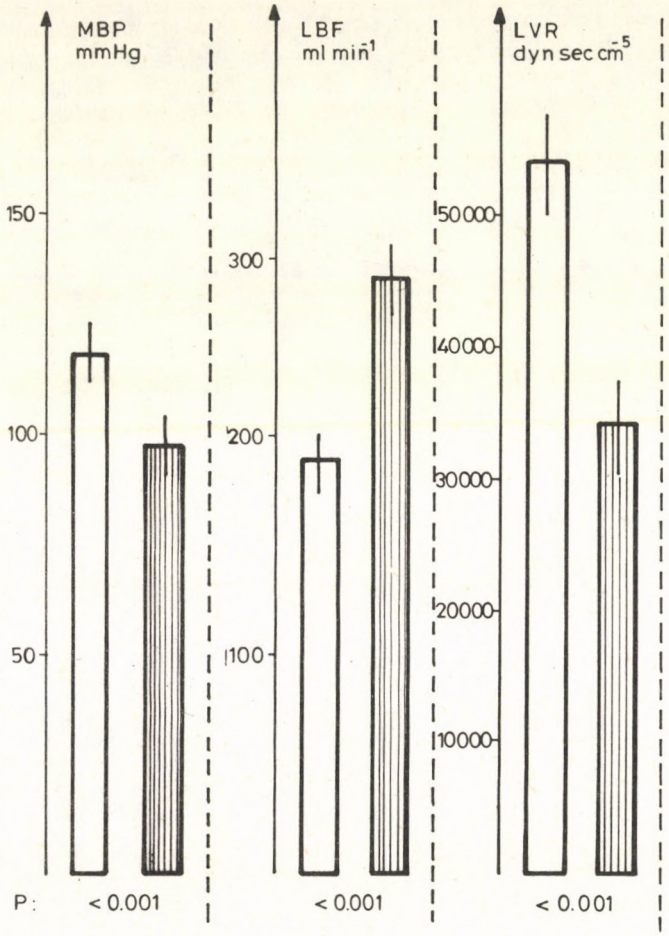


Fig. 3 The effect of acute vasodilator therapy on the limb circulation in patients suffering from both peripheral arterial disease (n=10) and cardiac decompensation (n=10). MBP = mean arterial blood pressure; empty columns: initial values; hatched columns: values after vasodilation therapy; means ±S.D.

dilator agent (nitroprusside sodium 0.3 μg/kg/min or hydralazine 0.2 μg/kg/min) was administered in slow intravenous infusion until a 15–20% diminution in the mean blood pressure (MBP) was achieved. On the effect of acute vasodilatation, despite the drop in blood pressure, the limb blood flow significantly increased owing to the very excessive diminution of the limb vascular resistance (Fig. 3).

There was no difference in this reaction between using nitroprusside sodium or hydralazine as vasodilator agents.

## DISCUSSION

The limb circulation in chronic cardiac failure has been studied by different groups using divergent methods. The results are contradictory. Some authors found a normal limb blood flow and detected normal extremital circulation in basal condition /8, 5, 19, 20, 24, 25/, while other working groups reported on deterioration of the limb circulation on the effect of chronic cardiac decompensation /2, 6, 7, 9, 10, 11, 13, 14, 16, 17, 18, 27–29/. On exercise (reactive hyperaemia) an impaired limb circulation (a lesser increase of limb flow) was established by many investigators /6, 9, 10, 13, 16, 19, 24, 26, 28, 29/. Only few authors found a normal limb circulation on exercise /8, 15, 25/ in chronic heart failure. It is a very important finding that the limb circulation significantly increased in congestive heart failure on the effect of cardiac compensation /7, 16, 18/.

Our data seem to prove that the limb circulation significantly changes in the course of chronic cardiac failure. The limb blood flow and the oxygen consumption of the extremital tissues significantly diminish, but the limb blood flow and the limb oxygen consumption remain within the normal range. In the pathological conditions of the limb circulation, the limb blood flow decreases under 300 ml/min and the limb oxygen consumption under 12.5 ml/min /23/. Values as low as these were only exceptionally observed in our study. The most characteristic modification in the limb circulation on the effect of chronic heart failure is the pathological increase of the limb vascular resistance (Fig. 1). The deterioration of the limb circulation in cardiac decompensation is more pronounced in patients with pathological condition of the extremital circulation. In patients suffering from both congestive heart failure and peripheral obliterative arterial disease, the diminution of the limb blood flow and the pathological

elevation of the limb vascular resistance in chronic heart disease are probably due to an increased vasomotor tone; peripheral vasoconstriction is very pronounced. An alteration of the vasomotor tone, expressed by an increase of the systemic — total — vascular resistance and inclination to peripheral vasoconstriction, was revealed in congestive heart failure /3, 5, 30/. When myocardial failure develops and cardiac decompensation appears, a series of neural and hormonal systems will be activated. An increase of the sympathetic nervous activity /5, 9, 11/, an augmentation of the catecholamine discharge /1, 5, 12/ — increased activity of the renin — angiotensin — aldosterone system /1, 10, 12/ was detected in chronic cardiac failure. The sympathetic overactivity, the sodium and water retention — due to the increased activity renin-angiotensin system — will contribute to increased vasomotor tone in cardiac heart failure. It is remarkable that on cardiac compensation this peripheral vasoconstriction is diminished /3, 5, 30/. In this study we could establish that the pathological elevation of the limb vascular resistance, owing to heart failure, is partly reversible: on administration of vasodilator drugs the limb vascular resistance will markedly diminish and the limb blood flow significantly increase.

Our present results have some clinical implication: 1) In cardiac decompensation the limb circulation will pathologically change. Although, the limb blood flow and oxygen consumption mostly remain within the normal ranges, the capacity of the limb circulation tends to be limited, the reserve of the extremital circulation is diminished. On exercise, the decrease in the vascular resistance (due to pathological increase of the limb vascular resistance) will be reduced and — mostly for this reason — the limb blood flow will not correctly increase. 2) The increase in the extremital resistance represents the most characteristic change of the limb circulation in cardiac decompensation. This pathological increase of the vascular resistance can markedly be diminished by vasodilator drugs. 3) In patients with pathological condition of the peripheral circulation the pathological decrease in limb blood flow is more expressed in cardiac decompensation. The administration of vasodilator therapy seems very favourable in cardiac decompensation in patients suffering from intermittent claudication or rest pain.

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METABOLISM

# CHANGES WITH AGING IN THE BONE MINERAL CONTENT OF THE LUMBAR SPINE AND FEMORAL NECK IN HEALTHY WOMEN IN HUNGARY

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The bone mineral content (BMC) of the lumbar spine and femoral neck was studied in relation to aging in healthy Hungarian women by duel photon-absorptiometric method. The data were compared with North-American and West-European values. The Hungarian values are lower than those for reported from North-America or France, but similar to the BMC values for Sweden. The observed vertebral and femoral bone loss could be well represented by cubic equations. The acceleration of bone loss seems to begin around 40 years. The rate of bone loss was similar to the published values but the decrease in bone loss in the 6th and 7th decades was more suggestive. With regard to the fracture-threshold below which the risk for non-traumatic fractures of vertebrae increases, about 60% of Hungarian women at age 50–59 and about 84% at age 60–69 are considered to be at risk. It is concluded that geographical and habitual differences might be important factors in the development and change of BMC for different populations.

Keywords: bone mineral content, dual photon absorptiometry, vertebrae, femoral neck, Hungarian normals, geographical differences

## INTRODUCTION

Nowadays attention is focused on the clinical syndrome of postmenopausal osteoporosis characterized by nontraumatic fractures at different points of the skeleton, mainly at vertebral sites. The disease occurs mainly in postmenopausal women and concerns a considerable portion of the female population /4/. To recognize the pathological process, it is necessary to

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Abbreviation: BMC: bone mineral content

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know the physiological bone loss with aging. The loss of axial bone, which has a high trabecular content, begins prior to the loss of compact bone, perhaps prior to the menopause /10, 18/. The consequences of this bone loss in elder age could result from a low peak adult value of bone mass, from an increased bone loss around menopause or from an earlier onset of aging loss /5/. These factors and the consequent diminution of bone may be modified by different manners (mealing habits, physical activity, etc.) of populations of different geographical origin.

The diminution of bone tissue can well be estimated by measuring the bone mineral content (BMC). In recent years several methods of quantifying BMC of the axial skeleton have been developed /2, 4, 13/.

Dual photon absorptiometry has become one of the most wide-spread methods /11, 12, 23, 24/. A number of papers have been published about the normal female BMC values of the axial skeleton, measured by this method mainly in the U.S.A. and West-Europe /5, 16, 17, 19/.

To obtain comparable data for the area of Central Europe in which Hungary is situated, we determined the amount of bone mineral in lumbar vertebrae and femoral neck of healthy women aged 20—79 years by dual photon absorptiometry. We were especially interested in the onset and rate of bone loss with aging and around menopause.

## METHODS

### Bone densitometry

BMC of the axial skeleton was determined by dual photon absorptiometry /11, 12, 23, 24/. This technique for measuring BMC is based on measurements of radiation transmission of two separate photon energies (44 keV and 100 keV) through a medium consisting of two different materials, bone and soft tissue. The dichromatic photon beam comes from a 153Gd source. The BMC can be calculated from the ratio of the attenuated photon energies. A bone profile curve is obtained by plotting each scanned point vs. the position over the bone. Points outside the curve on each side are selected to form end points of a base line above which the profile curve is integrated yielding the BMC in units of g/cm (linear-BMC).

By dividing the linear-BMC by the width of vertebrae the bone mineral density (area-BMC) can be calculated in $g/cm^2$. By summarizing the BMC of the region of interest (lumbar vertebrae 2, 3 and 4 in this study) the total BMC is obtained in grams.

Our instrument consists of a Novo BMC Lab 22/a osteodensitometer scanner that is interfaced with a computer that controls the scanning pattern, the point-by-point determination of bone density, the storing of data and calculation of BMC. The long-time precision of the method on

phantom in our hand is better than 1%, the intra-observer and inter-observer coefficients of variation were 2.1% and 4.2%, respectively.

## Normal subjects

We measured the BMC of lumbar vertebrae 2, 3 and 4 on a random sample of 128 healthy women 20–79 years old. We also measured the BMC of the right femoral neck of 93 healthy women 20–79 years old. Age distribution is shown in Table I and II. The women were in good health and not on any medical therapy known to influence calcium metabolism. None of them had a history of severe back pain or fractures. Their smoking habits, milk and coffee consumption, physical activity did not differ from the Hungarian average.

Table I

Lumbar BMC of normal women at age 20–79

| Age groups year | N | Total-BMC $\bar{x} \pm$ S.D. g | Linear-BMC $\bar{x} \pm$ S.D. g/cm | Area-BMC $\bar{x} \pm$ S.D. $g/cm^2$ |
|---|---|---|---|---|
| 20–29 | 12 | 40.6 ± 6.3 | 4.23 ± 0.63 | 0.96 ± 0.10 |
| 30–39 | 22 | 41.9 ± 7.8 | 4.24 ± 0.67 | 0.93 ± 0.10 |
| 40–49 | 20 | 34.3 ± 7.1 | 3.57 ± 0.57 | 0.82 ± 0.12 |
| 50–59 | 30 | 32.5 ± 7.7 | 3.37 ± 0.62 | 0.77 ± 0.13 |
| 60–69 | 22 | 31.3 ± 7.9 | 3.39 ± 0.81 | 0.74 ± 0.12 |
| 70–79 | 22 | 30.3 ± 5.3 | 3.47 ± 0.60 | 0.73 ± 0.12 |

N = number of measured subjects

$\bar{x} \pm$ S.D. = mean ± standard deviation

The results were evaluated for 10-years age groups. Data of premenopausal and postmenopausal women at age 40–59 were compared.

Student's $t$ test was affined to assess difference between group means, linear and polynomial regression were applied to calculate the regression curves.

Table II

Femoral neck BMC of normal women at age 20–79

| Age group year | N | Linear-BMC $\bar{x} \pm$ S.D. g/cm | Area-BMC $\bar{x} \pm$ S.D. g/cm$^2$ |
|---|---|---|---|
| 20–29 | 9 | 2.81 ± 0.30 | 0.90 ± 0.09 |
| 30–39 | 11 | 2.94 ± 0.72 | 0.96 ± 0.13 |
| 40–49 | 16 | 2.63 ± 0.30 | 0.84 ± 0.10 |
| 50–59 | 21 | 2.36 ± 0.33 | 0.72 ± 0.09 |
| 60–69 | 19 | 2.22 ± 0.47 | 0.68 ± 0.12 |
| 70–79 | 17 | 2.03 ± 0.39 | 0.63 ± 0.09 |

N = number of measured subjects

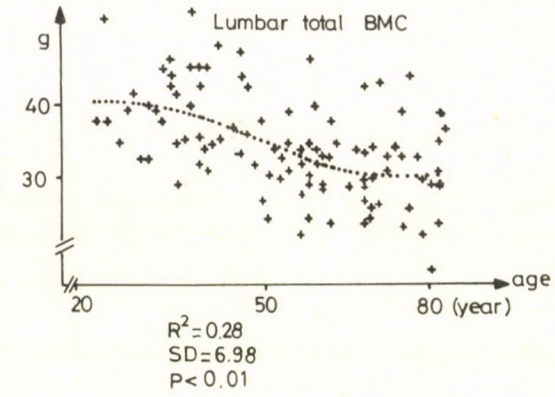
$\bar{x} \pm$ S.D. mean ± standard deviation

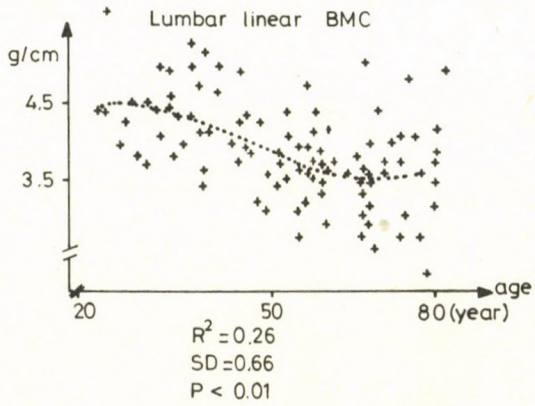
## RESULTS

The mean values corresponding to age groups are shown in Tables I and II. The lumbar BMC decreased significantly but not linearly with increasing age. The change in lumbar BMC of those women who were younger than 40 was significantly different from those of the older one ($P < 0.001$). The diminution of bone with aging is not linear because of this difference. In the case of femoral BMC data the rate of bone loss with aging before and after 40 years does not differ significantly from each other but the decreases of the younger ones are slighter or cannot be observed. The decreases of lumbar and femoral BMC with aging could be represented the best by cubic equations and curves (Figs 1 and 2).

The bone loss in the lumbar vertebrae and femoral neck between 40 and 49 years is 10–12% and 7–8%, respectively, depending on the chosen parameter. After 50, the decrease lessens to the half of the previous values. The overall diminution of bone from young adulthood to senescence (20–79 years) is about 25%.

The effect of menopause on bone loss was studied at age 40–59. The BMC values of women being in the menopause are lower than the values of premenopausal women, however, this difference is not significant (Table III).



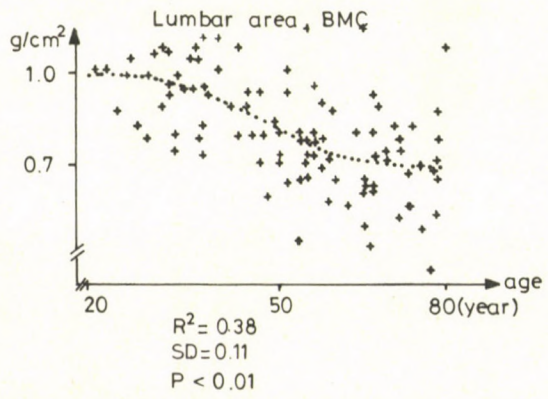




Fig. 1 The decrease of lumbar BMC with age

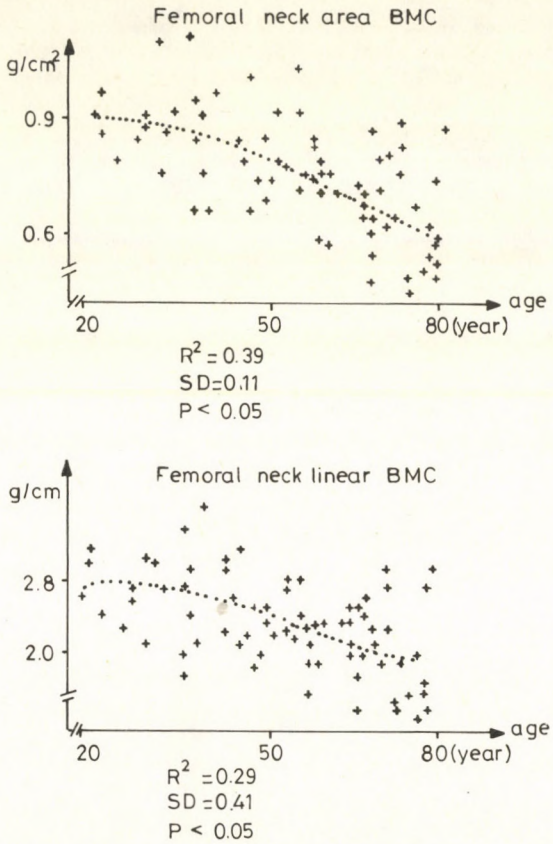



Fig. 2 The decrease of femoral BMC with age

Table III

Lumbar and femoral neck BMC of pre- and postmenopausal normal women at age 40–59

| | | | LUMBAR | | | FEMORAL NECK | |
|---|---|---|---|---|---|---|---|
| Women | N | Age mean $\bar{x} \pm$ S.D. year | Total-BMC $\bar{x} \pm$ S.D. g | Linear-BMC $\bar{x} \pm$ S.D. g/cm | Area-BMC $\bar{x} \pm$ S.D. $g/cm^2$ | Linear-BMC $\bar{x} \pm$ S.D. g/cm | Area-BMC $\bar{x} \pm$ S.D. $g/cm^2$ |
| Premenopausal | 20 | 46 ± 5.1 | 34.2 ± 7.4 | 3.57 ± 0.6 | 0.82 ± 0.12 | 2.55 ± 0.39 | 0.80 ± 0.12 |
| Postmenoapusal | 30 | 53 ± 4.3 | 31.9 ± 6.2 | 3.38 ± 0.6 | 0.76 ± 0.13 | 2.42 ± 0.32 | 0.75 ± 0.09 |
| | | | n.s. | n.s. | n.s. | n.s. | n.s. |

N = number of measured subjects

$\bar{x} \pm$ S.D. = mean ± standard deviation

n.s. = non-significant

## DISCUSSION

A statistical comparison between our data and other normal BMC values /5, 17, 18, 19/ was not possible because of some lacking statistical parameters of those studies. In spite of this, explicit difference can be seen between North-American /18, 19/ and Hungarian BMC values of axial skeleton: the American values are higher than the Hungarian ones. The French BMC values /17/ are slightly lower than those published from North-America. The Swedish normal BMC values are close to ours in every age group. The differences might be due to the different geographical situations, calcium intake, cuisines and habitual physical activity. Data about the effectiveness of calcium intake in prevention of osteoporosis are controversary. A number of studies have indicated that high calcium intake (1000 mg or more per day) can reverse a negative calcium balance and prevent osteoporosis /6, 22/. On the other hand, Riis et al. /21/ reported that even 2000 mg/day of calcium had no effect on the trabecular bone. The ratio of calcium and phosphorus in the food should be 1:1. This ratio may be increased to 1:4 by excess meat intake and consumption of soft drinks. This ratio may result in reduced bone mass /15/. Physical fitness may also be an important determinant of the axial BMC /16/.

The vertebral and femoral bone loss in our study was not linear. Bone diminution was not observed before 40 years; it increased significantly after 40. The exact time of the beginning of the accelerated bone loss could not be determined because of the great individual fluctuations of the measured values. It seems to begin around 40. Our findings corroborate the results of others /5, 7, 17, 18/, although Riggs /18/ raised the possibility that bone diminution would start earlier. In contrast with others /5, 18, 19/, we found that decreases of lumbar and femoral BMC with growing age could be represented by cubic equations. Ribot et al. /17/ also found non-linearity in vertebral bone loss in their study, however, they did not apply any curve of other degree. The turning point of bone loss around 40 indicates the importance of hormonal changes before and during menopause.

The rate of bone loss in our study is similar to the published values /5, 26/ but the decrease in bone loss in the 6th and 7th decades is more significant. Meunier et al. /14/, who studied the diminution of trabecular bone mass in iliac crest biopsy samples, found the value of 43% as the cumulative diminution the BMC of vertebrae between young adulthood

and old age. The overall diminution in women measured by dual photon absorptiometry was 47%; it was found by Riggs et al. /18/. In contrast, the rate of bone loss with aging found by these authors was threefold that was reported by Madsen /9/. Our value, 25%, is also significantly lower. The reason for these discrepancies is unclear.

In a longitudinal study, Smith et al. /25/ found that age-related loss of appendicular bone was an exponential function of bone mass: those with the most bone tissue also had the most rapid bone loss. This observation might be valid for the axial skeleton, and in this way it could be explained why North-American women have higher initial, but more rapidly decreasing, BMC values than Hungarian women.

Riggs et al. /18/ chose the value of 0.965 $g/cm^2$ (as the 90th precentile for vertebral BMC for patients with nontraumatic vertebral fractures) to define a threshold below which the risk for nontraumatic fractures increases. Breaking strength of bone is mainly related to its mineral content. Supposing that a given amount of bone mineral guarantees the same strength of bone independently from geographical situation and the rate of change of aging bone tissue follows the cubic regression we outlined, then at age 50–59 about 60% of Hungarian women are below the fracture threshold, while at age 60–69 about 84% are at risk. The validity of this threshold in Hungary is now being reconsidered. There was no significant difference in axial BMC of pre- and postmenopausal women at age 40–59. There are controversary data in connection with this problem /1, 3, 7, 8, 10, 18/. The cause of this contradiction could be the fact that in cross-sectional studies, such as ours, the small differences could be less apparent than in longitudinal studies. On the other hand, it should be considered that the end of menstruation is only a point in the process of climax and the diminution of bone tissue is rather related to the oestrogen deficiency beginning prior to menopause. Lindquist et al. /8/ found that lumbar BMC was higher in premenopausal than postmenopausal women of the same age, and the rate of bone loss was greater in subjects closer to menopause than in women after menopause. In our study the decrease of lumbar BMC at age 40–49 was twofold greater than at age 50–59. This difference could not be observed at the femoral neck, possibly due to the smaller amount of trabecular bone in the femoral neck. These results suggest that menopause has less of a direct effect on spinal and femoral bone than has been suspected, but the hormonal changes before and during menopause may still be influential. Certainly, we,

too, have to take into consideration the existence of two types of aging osteoporosis as Riggs defined them /20/.

The observed differences between Western and Hungarian populations raise the need of special investigations of populations of different geographical origin in respect of different habits and manners.

## ACKNOWLEDGEMENTS

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# COMPARATIVE STUDIES OF THE ELASTIN CONTENT OF NORMAL AND EMPHYSEMATOUS HUMAN LUNGS WITH SPECIAL REGARD TO ANTITRYPSIN DEFICIENCY

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The elastin content and the amino acid composition of elastin in emphysematous and healthy control lung tissues were comparatively studied. Decreased lung elastin content was found in the emphysematous patients of both normal and reduced trypsin inhibitor activity compared to normal control subjects. In the emphysematous patients with reduced inhibitor level a more significantly decreased elastin level was found than in those emphysematous patients with normal inhibitor level. There was no difference between the two groups in either the amino acid composition of elastin or the ratio of nonpolar to polar amino acid components. There was no significant correlation demonstrable between the age and the elastin content of the lung. This applied to the control group as well as to the emphysematous patients with normal trypsin inhibitor activity. The results indicate that no genetically abnormal pulmonary elastin structure is a predisposing risk factor but the reduced elastin content induced by elastase enzymes leading to changed mechanical properties is responsible for the development of pulmonary emphysema.

Keywords: elastin, lung, antitrypsin deficiency

## INTRODUCTION

Elastin plays an important role in the structure and function of lung parenchyma. Therefore, its destruction is a primary factor in the development of pulmonary emphysema. The studies of experimental emphysema have shown that only enzymes with elastolytic properties will produce the physiologic and morphologic changes of emphysema /11, 18/. According to published data, the elastin content in lung have varied widely (1.3 to 47 percent of dry lung weight), probably as a result of different methods of analysis,

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nonuniform sampling of lung and variable ages of subjects /2, 4/. There are different results and there is a disagreement concerning the changes and the amount and composition of elastin in the lung of normal controls and emphysematous patients. In this paper comparative studies on the crude connective tissue and elastin content, as well as the amino acid composition of the normal and emphysematous lung parenchyma are reported.

## METHODS

The diagnosis of emphysema was based on clinical, functional and morphological results. Elastin determination was performed in lung tissues obtained during lung surgery. Twenty non-emphysematous patients were operated for small peripherial round change in the lungs. Twenty eight emphysematous patients were examined, 15 had normal serum trypsin inhibitor capacity. In these cases lobectomy was made for the above diagnosis. In 13 young patients with intermediate antitrypsin values surgery was called for spontaneous pneumothorax or bulla formation. In these cases the degenerated emphysematous areas were excised in the intact lung tissue by a combined surgical procedure /1/. (The operations were performed by Prof. Pál Keszler at the former Department of Chest Surgery of the János Hospital, Budapest.) Elastin and amino acid composition were determined in samples obtained from several sites of the removed specimen, also from parts distant from the changes. The values for materials from various sites did not differ significantly.

Serum trypsin inhibitor capacity (STIC) was determined according to Eriksson's method /6/.

In the lung parenchyma samples the total amount of crude connective tissue, including the elastin content as well as the amino acid composition of elastin was performed according to Fitzpatrick and Hospelhorn /8/ as well as Evans et al. /7/. After washing out and removing blood by filter papers, the lung samples were weighed, then the samples were homogenized with distilled water in an Ultraturrax equipment. It was centrifugated at 200xg at 5°C for 20 min, then the residue was suspended in a 9-fold volume in 1 M sodium chloride. It was then stirred overnight in a cold room to remove all saltsoluble proteins. The following day it was repeatedly centrifugated. The residue was washed with 1 M sodium chlorid and then three times with distilled water to remove the salt. The residue was then freeze-dried and then extracted three times with n-butanol at 0°C, followed by three extractions with acetone at — 10°C to remove lipids. After air-drying, the product was designated crude connective tissue (CCT). Lung elastin was isolated by the method of Lansing et al. /14/ which employs NaOH in order to solubilize all proteins except elastin. The residue of the lipid-free crude connective tissue with 0.1 M sodium hydroxide was placed in a boiling water bath for 45 minutes, then chilled to 5°C and centrifugated for 30 minutes at 2800xg. After this procedure the residue of elastin was washed three times with distilled water until the pH reached 7. The final residue of elastin was suspended in 1 ml water, freeze-dried and weighed. The method does not depend on tissue weight and since results are presented as a ratio, they are not affected by random losses in the isolation procedure. The elastin was not destroyed by the procedure.

The separation of elastin from crude connective tissue by the Lansing

technique and quantitation of amino acid residues from the isolated elastin increases the accuracy of the method. The method can be performed on small amounts of tissue with no loss in accuracy.

The elastin was hydrolyzed in 6 normal hydrochloric acid. Their amino acid composition was determined on Amino Acid Analyzer with type JEOL by means of a column method. The amino acid composition of lung elastin was expressed as amino acid residues/1000 residues. The elastin content are expressed as a percentage of crude connective tissue of lung parenchyma. Nonpolar/polar ratio/Gly+Ala+Val+Pro+Leu+Ile/Lys+Arg+His+Asp+Glu/ was determined.

## RESULTS

The mean elastin proportion expressed as a percentage of crude connective tissue of lung parenchyma was statistically significantly decreased in the emphysematous patients (Fig. 1). The elastin content of lung parenchyma was lower in the emphysematous individuals in each case than that of the normal controls. The mean proportion of elastin was 29.5 percent S.D. $\pm$ 2.3in the group of normal subjects. The mean percent of elastin of patients with pulmonary emphysema was 16.2 S.D. $\pm$ 3.7.

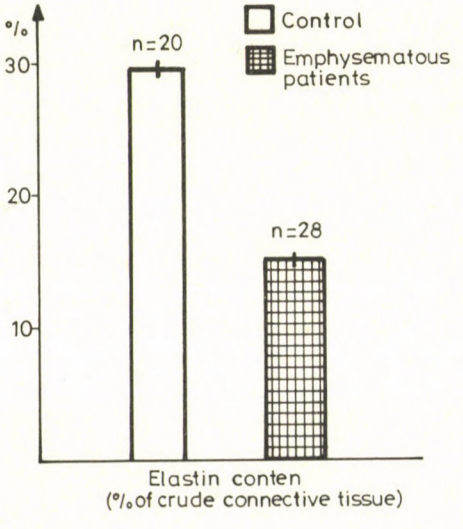


Fig. 1 Mean ($\pm$ S.D.) percentual elastin proportion of crude connective tissue of lung parenchyma

Table I

Correlations between human lung elastin content, serum trypsin inhibitor capacity and age, in emphysematous patients

| Group of normal inhibitor value n=15 | Age (years) | % Elastin $\bar{x}$ ± S.D. | STIC* mg/ml | Group of decreased inhibitor value n=13 | Age (years) | % Elastin $\bar{x}$ ± S.D. | STIC** mg/ml |
|---|---|---|---|---|---|---|---|
| F.N. | 46 | 18 | 1.23 | N.M. | 30 | 16 | 0.52 |
| K.A. | 39 | 19 | 1.20 | B.J. | 27 | 13 | 0.45 |
| Cs.J. | 55 | 16 | 1.26 | L.J. | 22 | 15 | 0.45 |
| D.J. | 54 | 16 | 1.05 | S.P. | 25 | 11 | 0.40 |
| P.L. | 51 | 20 | 1.15 | L.K. | 23 | 11 | 0.41 |
| A.S. | 56 | 21 | 1.19 | T.S. | 30 | 14 | 0.42 |
| V.E. | 47 | 20 | 1.25 | V.B. | 28 | 14 | 0.48 |
| P.K. | 50 | 20 | 1.21 | M.P. | 24 | 12 | 0.42 |
| E.K. | 49 | 19 | 1.24 | T.A. | 26 | 11 | 0.43 |
| B.P. | 32 | 20 | 1.25 | Sz.P. | 21 | 12 | 0.44 |
| K.K. | 46 | 20 | 1.15 | L.P. | 25 | 11 | 0.38 |
| D.B. | 50 | 21 | 1.26 | Z.I. | 29 | 14 | 0.45 |
| T.P. | 48 | 21 | 1.23 | N.E. | 27 | 12 | 0.43 |
| P.L. | 55 | 20 | 1.20 | | $\bar{X}$:25.9 | $\bar{X}$:12.7 | $\bar{X}$:0.43 |
| M.T. | 53 | 19 | 1.22 | | | ±S.D.1.5 | ±S.D.0.03 |
| | $\bar{X}$:48.7 | $\bar{X}$:19.3 | $\bar{X}$:1.2 | | | | |
| | ±S.D.6.4 | ±S.D.1.5 | ±S.D.0.05 | | | | |

r = 0.1934 — P > 0.05 —

r = 0.816 — P < 0.001 —

r = 0.3077 — P > 0.05 —

— P < 0.001 —

— P < 0.001 —

STIC* = serum trypsin inhibitor capacity (mg/ml serum); control value: 1.27 mg/ml

** = control value: 1.25 mg/ml

The values of serum trypsin inhibitor capacity were normal in the control group (mean STIC: 1.26 mg/ml S.D. $\pm$ 0.15). Table I summarizes the correlations between elastin content, serum trypsin inhibitor capacity and age in emphysematous group. A reduced inhibitor level was found in 13 young emphysematous patients (aged 22 to 30), who had been operated for spontaneous pneumothorax or bulla formation. Decreased lung elastin content was found in the emphysematous patients of both normal and decreased inhibitor level. Within this, a positive correlation was observed ($r = 0.816$, $P < 0.001$) between the decreased serum trypsin inhibitor capacity and the lung elastin content. In the young emphysematous patients with reduced inhibitor level a more significantly decreased elastin level was found than in those emphysematous patients with normal inhibitor level, indicating that shift between the elastolytic enzymes and their inhibitors led to a more conisderable decrease in the amount elastin. There was no significant correlation between age and elastin content either in the controls ($r = 0.3925$, $P > 0.05$) or in the emphysematous patients of a normal inhibitor level ($r = 0.1934$, $P > 0.05$).

In the amino acid composition of elastin, cystine and histidine were missing, methionine had an extremely low level; there was an excess of nonpolar amino acids characteristic of elastin. There was no characteristic and consistent difference in the amino acid composition and the nonpolar to polar ratio of elastin between emphysematous and control subjects, in accordance with Chrzanowski et al. /4/. There was no significant difference in the relative proportions of any amino acid between the two groups suggesting that the residual elastin in lung parenchyma of patients with emphysema does not differ from that found in normal lungs despite lung destruction.

## DISCUSSION

A great importance was attributed to the elastic fibers in the development of emphysema already in the last century. The disruption of elastic network in pulmonary emphysema was described by Eppinger /5/ in 1876. In 1888, Virchow asserted that pulmonary emphysema was primarily a change in lung tissue /19/. The characteristic mechanical feature of the emphysematous lung, diminished elastic recoil, was recognized in 1934 as a physiologic result of elastin destruction /13/.

In 1958 Briscoe and Loring /2/ found an incrase with age in human

lung elastin expressed as percent of the dry weight of the sample. An increased elastin level was observed with the advance of age also by Pierce and Hocott /15/, but they did not find any sex difference in the total elastin and collagen contents of the lung parenchyma. Pierce et al. /16/ comparing the lung tissues of emphysematous patients and controls, failed to find any difference either in the elastin or the collagen content or in the collagen/elastin ratio. Similar observations were made by Wright et al. /20/ and Johnson and Andrews /10/. John and Thomas /9/ failed to find any age-dependent change in the elastin of the lung parenchyma, only the amino acid composition changed. The amount of the crosslinking amino acids desmosine and isodesmosine was lower in lung elastin of old subjects than those from younger ones. Bruce and Adamson /3/ did not find any difference between antitrypsin deficient emphysematous patients and controls in the elastin and collagen content and the ratio of collagen to elastin. According to Fitzpatrick /8/ connective tissue from lungs with severe emphysema showed amounts of elastin and collagen similar to those present in normal adult lungs. He reported a significant difference in the amino acid composition of normal and emphysematous lung elastin. He found that elastolytic breakdown products from normal lung protein differed from those obtained from the emphysematous lungs, which contained more cross-linking compounds in smaller polypeptide fragments indicative of greater susceptibility to enzymatic attack. The smallest sized polypeptide fraction showed striking increases in aromatic and basic amino acids, with reduced content of neutral amino acids in patients with emphysema. Fitzpatrick ascribed the difference to a genetic disorder in synthesis or metabolism unique to lung connective tissue in emphysema. Similar observations were published by Keller and Mandl /12/. Chrzanowski et al. /4/ observed first a significantly decreased elastin content in patients with pulmonary emphysema as compared with normal control subjects, but no significant difference was demonstrated in the amino acid composition.

In Hungary, Appel and Keszler /1/ performed comparative histochemical (elastase digestion) studies in juvenile emphysema and that of the aged associated with spontaneous pneumothorax. These histochemical data supported that the idiopathic spontaneous pneumothorax in young age is a special form of the localized emphysema which is not a consequence of congenitally reduced elasticity of the elastic network. Szemenyei /25/ reported on the biochemical differences in the composition of the three different connective tissue fibres of the lung, on the effect of certain chemical sub-

stances, on their polarization optical behaviours as well as on their detection.

Our results indicate that: 1) in contrast to the majority of literary data /2, 10, 15, 16, 20/, the elastin content did not correlate with age; 2) levels of pulmonary parenchymal elastin in emphysematous patients were significantly lower than those in normal control subjects. This confirms the observation of Chrzanowski et al. /4/; 3) it should be pointed out as a recent observation that there was an even lower elastin content within the emphysematous group in case of the intermediate antitrypsin deficient young patients; 4) our investigations have shown that the predisposing factor in the development of emphysema was not an abnormal pulmonary elastin structure but the elastin destruction (elastolysis) due to elastases resulting in decreased elastin content and leading to a change in the elastic properties of the lungs.

Our results indicate, as also demonstrated by animal experiments in our previous studies that elastin is the basic, critical component for maintenance of the mechanical properties and the normal structure of the lung parenchyma. Destruction of elastin fibres is necessary to produce emphysematous lesion. The characteristic physiological abnormality of emphysema is the loss of elasticity, a function that is critically dependent on the integrity of elastic fibers. The pathogenetic mechanism damaging elastin, causing its decrease, is responsible for the development of pulmonary emphysema.

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# STUDIES ON SEMEN OF MALE RABBITS IN STILBESTEROL–INDUCED HYPERPROLACTINAEMIA

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(Received: June 6, 1987)

Seminal volume, motility and sperm cell concentrations and seminal plasma levels of frucose, lactate, pyruvate, citrate and non-esterified fatty acids were determined in the semen of male rabbits treated with a daily dose of 1 mg stilbesterol for 15 successive days, and were assessed in their relation to the reproductive state.

The results revealed reduction in the seminal volume, motility and sperm cell concentration in the treated animals as compared with the findings in untreated rabbits. The fructose level increased while the lactate level followed by pyruvate, citrate and non-esterified fatty acids levels decreased after stilbesterol injection.

Thus, frequent administration of stilbesterol results in changes in both physical and biochemical constituents of semen. The physical characters and chemical constituents measured were discussed on the basis of their vital importance in the reproductive processes.

Keywords: male infertility, estrogens, semen chemistry

## INTRODUCTION

Many studies demonstrated that any alteration in the seminal chemical constituents may affect greatly the fertility potential in males either in animal /13, 19/ or in human being /1, 20/.

Prolactin over-secretion has been reported to be one of the most important causes in decreasing the fertility both in animal /4, 19/ or in

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Akadémiai Kiadó, Budapest

man /2, 6, 20/. It appears to have a similar response to testosterone deficinecy /2/. It was demonstrated that elevation of prolactin has been stimulated by estrogen or its analogue and can be more or less normalized by application of a prolactin secretion inhibitor like bromocriptin /6, 9, 10, 19, 20/.

Considering the above mentioned facts, the object of the present study is, therefore to present some seminal characters and certain biochemical parameters of the semen of rabbits treated with stilbesterol for 15 consecutive days. The semen constituents measured were selected on the basis of their vital importance in the reproductive processes. The importance of prolactin status in relation to fertility necessiated establishment of its level in blood serum in control and stilbesterol injected animals.

## MATERIAL AND METHODS

### Material:

The male rabbits to be worked out were obtained from local farm. These animals were apparently healthy, aged 10 to 12 months and weighing from 1.5 to 2.0 kg. One group of 10 rabbits was i.m injected with stilbesterol (Folone 5, Misr Co.). To each animal a daily dose of 1 mg of stilbesterol was given along 15 successive days. Another group of 10 rabbits was used as control. All the animals were fed on a mixture of ground wheat, barely and maize in addition to green fodder and provided with excess water.

### Methods:

At the end of the fifteen days of the experiment, semen samples were collected from both control and treated animals using the artificial vagina designed by Bredderman and his co-workers /4/ in which, the volume of the ejaculate, % of motile sperms as well as sperm cell counts were determined /4/. Thereafter, the seminal plasma were separated out by centrifugation at 5000 r.p.m. for fifteen min in which fructose /14/, lactate /11/, pyruvate /5/, citrate /16/ and non-esterified fatty acids /8/ were assayed.

After collection of semen samples all animals were slaughtered and blood samples were collected. Serum samples were removed after centrifugation at 3000 r.p.m. in which prolactin levels were assayed using radioimmunoassay method /15/.

Statistical analysis of the obtained data was carried out using the method of Snedecor and Cochran /18/. Mean $\pm$ S.E.M. are demonstrated on the tables.

## RESULTS

In the present study, the obtained data revealed that stilbesterol injection at daily dose of 1 mg for successive fifteen days produced prolactin over-secretion in the treated animals in comparison to its level in the control ones as given in Table I. The prolactin level in the serum of the stilbesterol injected animals (3095±244.5 m mol/ml) was highly significant higher ($P \leq 0.01$) than the corresponding level in the non-injected control group (1612±199.4 m mol/ml).

Table I

Serum prolactin following stilbesterol injection in male rabbits (m mol/ml) ($\bar{x} \pm$ S.E.M; n=10)

| Control untreated animals | Stilbesterol treated animals |
|---|---|
| 1612.00 | 3095.00 |
| ±199.40 | ±244.50** |

**Highly significant ($P \leq 0.01$)

Concerning the seminal characters, there were marked reduction in the volume of the ejaculate, percentage of motile sperm cell concentration in the animals injected with stilbesterol as compared with the findings in the animals of the control group (Table II).

Table II

Volume, motility and sperm count of semen in control untreated and stilbesterol treated male rabbits ($\bar{x} \pm$ S.E.M; n=10)

| | Volume (ml.) | Motility (%) | Sperm count ($10^6$/ml) |
|---|---|---|---|
| Control group | 0.86 ±0.10 | 72 ±3.3 | 147±9.8 |
| Treated group | 0.42 ±0.12** | 15 ±1.8** | 27±2.6** |

**Highly significant ($P \leq 0.01$)

Regarding the biochemical alterations observed in the seminal plasma, Table III showed that the level of fructose in the stilbesterol injected rabbits (37.94±0.51 mmol/l) was highly significantly more ($P \leq 0.01$) than its level in the control on treated animals (24.47±0.63 mmol/l). In contrast to this, lactate and pyruvate levels were significantly ($P \leq 0.05$) less than their levels in control group. The normal levels of lactate and pyruvate were 4.26±0.39 and 0.56±0.03 mmol/l respectively and after stilbesterol injection the levels were 3.05±0.18 and 0.14±0.02 mmol/l, respectively. The levels of citrate and non-esterified fatty acids were highly significantly lower in the seminal plasma of animals given stilbesterol (55.30±0.31 g/l and 0.64±0.02 mmol/l, respectively) than their corresponding levels in the non-treated control group (77.8±0.3 g/l and 0.78±0.03 mmol/l, respectively).

Table III

Seminal biochemical alterations in male rabbits with experimental hyperprolactinaemia and normal control ones

($\bar{x}$ ± S.E.M; n=10)

| | Fructose m mol/l | Lactate m mol/l | Pyruvate m mol/l | Citrate g/l | NEFA m mol/l |
|---|---|---|---|---|---|
| Control-untreated group | 14.47 ±0.63 | 4.26 ±0.39 | 0.56 ±0.03 | 77.80± ±0.30 | 0.78 ±0.03 |
| Stilbesterol-treated group | 37.94** ±0.51 | 3.05* ±0.18 | 0.41* ±0.02 | 55.30** ±0.31 | 0.64** ±0.02 |

*Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$)

## DISCUSSION

In the present investigation, prolactin over-secretion was observed in male rabbits received repeated doses of stilbesterol. This findings coincided with that reported by many authors /9, 10, 20/. It was demonstrated that frequent estrogen administration result in hyperprolactinemic infertility in man /10, 20/ and animals /9/. It was found that estrogens have a dual effect in stimulating prolactin secretion, as they deplete hypothalamic prolactin inhibiting factor (PIF) and stimulate the prolactin-secreting pituitary cells directly /10/.

The harmful effect on semen characters noticed in the present study as shown in Table II is probably due to the fact that the application of estrogens increases the protein-binding capacity and in this way it decreases the biologically active testosterone level in the plasma /2, 3, 6/. On the other hand, it was suggested that prolactin over-secretion appears to have a similar response to testosterone deficiency /2, 3/.

In the present work, the fructose level was inversily proportional to sperm number and motility as there was a marked significant increase in fructose level in treated rabbits than in the normal control ones. This could be explained by the decrease of the fructose utilization in oligospermia and that the apparent lower fructose values in normospermic samples were due to fructose utilization as stated by Schirren /17/.

The present study demonstrated a significant reduction of lactate and pyruvate contents in semen samples of stilbesterol treated rabbits. Moreover, this reduction was proportional to the reduction in the sperm number and motility. These findings were agree in principale with those of Attia and his co-workers /1/ in human infertile semen.

The marked reduction of the citrate levels (Table III) of the seminal plasma of treated animals might be due to defective androgen secretion as stated earlier by Dondero and his co-workers /7/.

A significant decrease in non-esterified fatty acids level was noticed in the seminal plasma of treated animals. Fatty acids appear to play an important role as energy substrate for spermatozoa due to their rapid oxidation /12/. Fatty acids have been shown to support respiration in spermatozoa in the absence of glycolysable substrate /13/. Thus, fatty acids are required in the metabolism of spermatozoa.

It can be concluded that frequent stilbesterol administration results in harmful effect of the physical characters of the semen, elevation of serum prolactin, and marked alterations in the seminal chemical constituents of high vital importance in the reproductive processes.

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BOOK REVIEWS

## Non-invasive Cardiology, 1985

Editors: E. Kékes, L. Matos and L. Mihóczy

Akadémiai Kiadó, 1987.

This volume is based on papers presented at the 4th European Conference on Mechanocardiography, held in Budapest, September 11–14, 1985.

The review papers and lectures provided abundant data on the scientific and clinical aspects of non-invasive methods in cardiology. The volume contains the "classic" techniques – phonocardiography, apexcardiography – and the latest technical achievements, such as colour Doppler methods. Special emphasis is placed on the relationship between direct (invasive) and indirect techniques in the evaluation of cardiac function. The papers were grouped around 11 main topics:

1. Review papers
2. M-mode and 2-dimensional echocardiography
3. Computer analysis of non-invasive data
4. Measurement of drug effects by non-invasive cardiological methods
5. Clinical application of Doppler echocardiography
6. Exercise test using systolic time intervals, mechanocardiography and echocardiography for assessing left ventricular function
7. Mechanocardiography: new methods – clinical application
8. Technology: new technical possibilities – standardization in methodology
9. Systolic and diastolic time intervals
10. Combination of the non-invasive cardiological methods
11. Round table. Mechanical function of the heart.

This book is recommended for all physicians (first of all cardiologists and scientific researchers) involved in the physiological and pathophysiological aspects of recent research as well as in the clinical application of these non-invasive methods.

Veronika Morvai

## Tobacco. A major international health hazard

Editors: D.G. Zaridze and R. Peto. World Health Organization

IARC Scientific Publications No. 74. Lyon, 1986

This volume contains proceedings of an International Meeting held in Moscow, 4–6 June, 1985.

Tobacco smoking is one of the major causes of disease and death today: it may cause cancer, pulmonary obstructive disease and cardiovascular disease. The list of target sites for tobacco-related cancers is impressive: lung, urinary bladder, renal pelvis, oral cavity, pharynx, larynx, oesophagus, pancreas and, possibly, kidney and liver. The evidence, therefore, of the severe consequence of smoking is so compelling and so overwhelming that it is difficult to understand why it has been so difficult to initiate successful preventive measures. The answer is probably two-sided. The first is the difficulty that individuals have in renouncing a habit that has become solidly rooted in their culture and their daily life. The second part of the answer is the interest of governments all over the world in

tobacco-derived income. Many governments, although genuinely concerned in the good health of their citizens, still continue to allow the sale of tobacco and to make money out of it. Of course, in many countries powerful private interests are involved.

Recently, evidence has become available that smokers of cigarettes yielding high levels of tar and nicotine have a greater risk of developing lung cancer than smokers of cigarettes that yield less tar. Tobacco is a mixture containing a large number of chemicals, many of which are recognized carcinogens and/or mutagens. Tar, which results from the pyrolysis of tobacco, contains carcinogenic chemicals. One can therefore assume that, by decreasing the delivery of at least one of the carcinogenic fractions of smoke, a less intense carcinogenic activity of the total mixture may result. Tobacco smoke will, however, clearly continue to be a carcinogen even if it contains less tar.

It is also important to stress that tobacco is carcinogenic not only when it is burned and smoked, but also when it is chewed. Recent advertising in which it is claimed that tobacco chewing is harmless contradicts the very clear, definite evidence that tobacco is carcinogenic when it is chewed. The main sections deal with:

1. Implications and recommendations
2. Tobacco – a major health hazard
3. Disease patterns and smoking
4. Tobacco – spread of the habit and trends
5. Smoking – current research issues
6. Health effects of low-tar, low-nicotine cigarettes
7. Smoking control implementations

Especially great interest have the papers written by Professor R. Doll, S. Eckhardt, C.S. Muir, R. Peto, M. Sorsa and L. Tomatis.

This book is recommended for physicians dealing with prevention, aetiology, epidemiology and clinical aspects of cancer.

Veronika Morvai

August 29th – September 1st 1989. **5th International Congress on Interventional Ultrasound.** Free communications deadline April 1st. Herlev Hospital, Copenhagen, Denmark.
Information: Spadille Congress Service, Sommervej 3, DK – 2730 Hornbaek, Denmark.

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## NEW IPA RESEARCH AWARD

The **International Psychogeriatric Association** is pleased to announce the creation of a new, biennial **Research Award** in psychogeriatrics. This award, which is sponsored by Bayer AG, will be offered every two years for presentation and recognition at each IPA International Congress.

The award will be given for the best original research paper in the field of psychogeriatrics. Any unpublished, original research paper in English will be considered. All papers should be accompanied by an abstract of no more than 250 words.

The 1989 award shall consist of (1) travel and accommodations expenses for presentation of the paper at the Fourth Congress of the International Psychogeriatric Association in Tokyo, Japan, from September 5–8, 1989, (2) a plaque, (3) a cash award to cover expenses during the Congress, (4) an opportunity to publish the paper in the IPA's new journal, International Psychogeriatrics, which will begin publication early in 1989.

Selection of the award winning contribution will be by an international committee: M. Bergener, FRG; G. Cohen, USA; S. Finkel, USA; K. Hasegawa, Japan; T. Nishimura, Japan; B. Reisberg, USA; G.P. Vecchi, Italy; and a Bayer AG representative. Eight copies of the paper and abstract must be submitted no later than March 1, 1989 to Barry Reisberg, M.D., Aging and Dementia Research Program, NYU Medical Center, 550 First Avenue, New York, New York 10016.

The awardee will be notified by June 1, 1989.

Contact: Sanford I. Finkel
312-251-3090

**THE CHALLENGE OF HEALTH**

— The new Role of Sickness Funds —

Internationaler Kongress in Hamburg vom 13. bis 16. Juni 1989

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With its regional strategy "Health for All in the Year 2000" adopted in 1980, the WHO has developed a concept which was subsequently consolidated in the "Ottawa Charter" of November 1986 for the field of health promotion.

Major challenges confronting the promotion and protection of health are apparent in all developed countries with a structured health system — the changing disease spectrum, the increasing health hazard of environmental pollution, and the changing age structure of the population. These factors clearly indicate the limits of medical care and point to the need for sickness funds and health insurance schemes to prepare now for the challenge of the future. The congress will provide ideas, and clarify the essential contribution of sickness funds and health insurance schemes towards an improved policy of health promotion.

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# INFORMATION FOR AUTHORS

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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.

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Examples:

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

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

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IMMUNOLOGY

# EUTHYROID INFILTRATIVE OPHTHALMOPATHY: CLINICAL-IMMUNOLOGICAL CHARACTERISTICS

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(Received: June 22, 1988)

The thyroid hormone titres of the sera of 25 euthyroid infiltrative ophthalmopathic patients was examined, TRH test was performed, and thyroid-stimulating antibodies were studied by membrane receptor assay and TRAK assay. Previously, other diseases causing exophthalmos could be excluded by ophthalmological, radiological examinations, orbital ultrasonography and/or CT. Following TRH administration, 18 out of 25 patients showed abnormal TSH response, 16 of them were TSI – positive. Five of them became hyperthyroid 2–2.5 years later. After TRH administration 7 patients produced normal TSH response, none of them became hyperthyroid in the subsequent 2–4 years follow-up period. In the 7 TRH-negative patients, four were found to have higher hTG and an antithyroid microsome antibody titre. In those patients the fine needle biopsy verified chronic lymphocytic thyroiditis. In three patients there was no evidence of a pathological change of the thyroid gland. Based on our results, the patients could be divided into three groups. The prognostic and therapeutic differentiation of these groups seems to be justified.

Keywords: Graves' disease, ophthalmopathy, euthyroid, TSI, antibodies

Abbreviations: TSH: thyroid stimulating hormone; TRH: thyrotropic releasing hormone; $T_4$RIA: thyroxine-radioimmunoassay; $T_3$RIA: triiodothyronine-radioimmunoassay; TRAK: thyrotropin receptor antibody assay; TSI: thyroid stimulating immunoglobulin; CT: computed tomography; hTG: human thyroglobulin; M: microsoma; EOP: endocrine ophthalmopathy

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## Introduction

Euthyroid (endocrine) ophthalmopathy (EOP) is usually defined as a variant of Graves' disease associated with ophthalmological symptoms but not with hyperthyroidism /13, 23/. In several cases, however, besides the clinical and laboratory euthyroidism, a pathological TSH response can be detected after administration of TRH /3, 4, 19/. In some cases antithyroid antibodies can be demonstrated in the patients' sera /25/. In our previous examinations, infiltrative eye signs were found in a part of the patients with Hashimoto's thyroiditis /9, 12/.

Euthyroid ophthalmopathic patients can later become hyperthyroid. Bartels and Irie observed the development of eye signs prior to the appearance of hypothyroidism in 9 cases out of 117 endocrine ophthalmopathic patients. Eye changes developed, however, only in 48 of them parallel with the hyperthyroid symptoms; 5 patients were found to be permanently euthyroid /1/.

Based on earlier data in the literature, neither the pathogenesis, course and prognosis nor consequently the therapy of euthyroid ophthalmopathy appear to be homogeneous. For differentiation of the various pathogenetic and prognostic groups, the results of the clinical-immunological examinations of 25 euthyroid patients with infiltrative ophthalmopathy having been examined at our thyroid care unit are summarized.

## Patients and Methods

In the period between 1982 and 1986, a total of 226 untreated patients with Graves' disease were examined and cared at our policlinic. Infiltrative eye symptoms were detected in 108 patients (47.8%), of whom 25 proved to be euthyroid at the moment of examination and subsequently for at least two years (23.1%). The mean age of euthyroid ophthalmopathic patients (10 males and 15 females) was 42.9 years.

The follow-up time was 2 to 4 years; the patients were controlled every 2–3 months. For determining the functional state of the thyroid gland, the following assays were performed: $T_4$-RIA (Izinta, Budapest) and $T_3$-RIA (DOTE KIL kit). The TRH tests (TRH, Berlin, Chemie) were performed as follows: blood was collected for TSH-RIA (Byk-Mallinckrodt) assaying, then 20 min after i.v. administration of 200 ug TRH blood was repeatedly collected for TSH examination. The TRH test was considered to be positive if the basal TSH titre was $<$ 2.0 mU/l and the value examined 20 min later was $<$ 3 mU/l. $T_3$ suppressive test, $^{131}I$ uptake and thyroid scintigraphy were carried out in some cases.

Detailed ophthalmological examination of our patients (vision, fundus, exophthalmometry according to Hertel, Hess' screen, slit lamp

examinations) was made in each case. During the radiological examination a two-dimensional cranial X-ray, X-rays of the sella turcica, Rhese's exposure were performed. In order to exclude intracranial tumour and vascular abnormalities, neurological examination was carried out.

Ultrasonography of the orbit was performed in each case on euthyroid ophthalmopathic patients, in 16 cases also computed tomography was made. Based on these studies, a clinical picture causing exophthalmos could safely be excluded.

The patients' eye signs were classified according to the criteria of the American Thyroid Association (ATA) after Werner /24/. On suspicion of thyroiditis and in nodular goitre, fine needle biopsy and cytological examination were made.

For examining the thyroid-stimulating immunoglobulins (TSI), initially thyroid membrane receptor assay was applied based on the description of Smith and Hall /17/. Subsequently, the standard TRAK (Henning, Berlin GmBH) was employed.

Each patient's serum was examined for human thyroglobulin (hTG) and antithyroid microsome antibody titre according to Boyden by haemagglutination /2/. Based on our standard laboratory values, the test was considered positive if agglutination occurred in a serum dilution 1 to 32 or still greater.

## Results

Unilateral eye symptoms were found in 5 of the 25 euthyroid patients with infiltrative ophthalmopathy, and bilateral ophthalmopathy in 20 patients. Symmetric alterations were found in 14 patients and there was a difference in the severity of eye symptoms in 6 cases. Clinical and immunological data are shown in Tables I and II.

The TRH-TSH stimulation test was positive in 18 patients. In five of them hyperthyroidism developed in the follow-up during an average period of 2–2.5 years. Thirteen patients remained euthyroid during the observation period of 2 to 4 years.

Seven patients gave a normal TSH reponse to administration of TRH. These patients remained euthyroid and TSI could not be detected, in the follow-up period.

There were only two out of the 18 TRH positive patients in whom TSI could not be detected.

Pathologically elevated hTG and anti-M serum antibody titre was noted in five patients: in one of them TSI, too, could be demonstrated. The results of the fine-needle biopsy of the thyroid gland of four patients verified chronic lymphocytic thyroiditis.

**Table I**

Clinical data of euthyroid ophthalmopathic patients

I. TRH-positive cases

| No. | Initials | Sex | Age (years) | TSI | hTG | M | ATA classification | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | right side | left side |
| 1. | BL | male | 48 | neg | neg | neg | 2c,3b,4b | 2c,3b,4b |
| 2. | BJ | female | 41 | pos | neg | neg | 2b,3a,4b | 0 |
| 3. | DM | male | 49 | pos | neg | neg | 2b,3b,4a | 2b,3b,4a |
| 4. | ZK | female | 41 | pos | neg | neg | 2a,3a,4b | 2a,3a,4b |
| 5. | HJ | female | 51 | pos | neg | neg | 2a,3b,4a | 2a,3b,4a |
| 6. | GB | male | 54 | neg | neg | neg | 2b,3a,4b | 2b,3a,4b |
| 7. | VM | female | 32 | pos | neg | neg | 2b,3b,4c | 2b,3b,4b |
| 8. | VF | female | 38 | pos | neg | neg | 2b,3b,4c | 2b,3b,4c |
| 9. | HA | male | 45 | pos | neg | neg | 2b,3b,4c | 2b,3b,4c |
| 10. | KL | female | 53 | pos | neg | neg | 2b,3a,4b | 2b,3a,4b |
| 11. | ZK | male | 48 | pos | neg | neg | 2a,3a,4b | 2a,3a,4b |
| 12. | LG | female | 43 | pos | neg | neg | 2a,3a,4b | 2a,3a,4b |
| 13. | EA | male | 61 | pos | neg | neg | 2b,3b,4a | 2b,3b,4a |
| 14. | TS | female | 27 | pos | neg | neg | 2b,3b,4a | 2a,3a |
| 15. | BD | femal | 53 | pos | 1:32 | 1:32 | 2b,3a,4b | 2a,3a |
| 16. | DZ | female | 27 | pos | neg | neg | 2b,3a,4b | 2a,3a |
| 17. | PJ | female | 51 | pos | neg | neg | 2b,3b,4b | 2b,3b,4b |
| 18. | SZ | female | 44 | pos | neg | neg | 2a,3b,4a | 2a,3b,4a |

**Table II**

Clinical data of euthyroid ophthalmopathic patients

II. TRH-negative cases

| No. | Initials | Sex | Age (years) | TSI | hTG | M | ATA classification | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | right side | left side |
| 1. | LJ | female | 33 | neg | neg | neg | 0 | 2b,3b,4a |
| 2. | TK | male | 42 | neg | 1:64 | 1:32 | 2a,3a | 2b,3b,4a |
| 3. | SL | male | 53 | neg | neg | neg | 2c,3c,4c,5a,6b | 2c,3c,4b, 5c,6a |
| 4. | IL | female | 31 | neg | 1:64 | 1:32 | 0 | 2a,3b,4b |
| 5. | HJ | male | 40 | neg | 1:64 | 1:128 | 2a,3a,4a | 2b,3a,4b |
| 6. | BB | male | 32 | neg | 1:32 | 1:64 | 0 | 2b,3a,4b |
| 7. | PJ | female | 35 | neg | neg | neg | 0 | 2b,3a,4b |

## Discussion

Based on the examination data of the recent 15 years, Graves' disease can be included in the organ-specific autoimmune diseases /10, 16, 22/. There is evidence of pathological processes both in the cellular and humoral immune reactions /15, 20, 21/.

Immunological factors are of great importance also in the development of endocrine ophthalmopathy. Cellular sensitization can be detected against retrobulbar antigens and antibodies specifically reacting with the antigens of the eye muscle and of the connective tissue /7, 8, 11, 14, 22/. Based on their studies, Solomon et al. consider ophthalmopathy an independent entity /18/. Studying 57 euthyroid ophthalmopathic patients, Tamai et al. found a close correlation, on the basis of their results, between euthyroid Graves' ophthalmopathy, hyperthyroid Graves' disease and Hashimoto's thyroiditis and they considered them to be undifferentiable prior to the development of the full-blown disease /19/.

Our results partly contradict the data of Kasagi et al. who observed a significant increase of cAMP titre in both euthyroid and hyperthyroid EOP, in FRTL-5 thyroid cell culture, using the patients' sera as stimulator /6/. According to their latest investigations, as opposed to TSAb (Thyroid Stimulating Antibodies) titre, the TBII (Thyrotropin Binding Inhibiting Immunoglobulin) titre is significantly lower in euthyroid Graves' ophthalmopathy, so it is assumed that the lower level of TBII, of the genuine stimulator, is responsible for euthyroidism /5/. Contrary to Solomon et al., they have arrived at the conclusion that the development of Graves' ophthalmopathy is correlated with the appearance of thyroid-stimulating antibodies, and the development of hyperthyroidism also depends on the mass of and radioiodine uptake by the thyroid gland /5, 18/.

In view of our own results, the following can be established:

1. In diagnosing euthyroid infiltrative ophthalmopathy, besides the determination of the titres of thyroid hormones, it is necessary to perform TRH and/or $T_3$ – suppressive tests and tests for thyroid-stimulating antibodies. In a part of the TRH- and TSI-positive euthyroid patients, hyperthyroidism can be expected to develop (Group I).

2. In a part of the TRH-negative euthyroid ophthalmopathic patients, chronic lymphocytic thyroiditis (Hashimoto's thyroiditis) can be detected, therefore these patients should be observed for later developing hypothyroidism and, if necessary, they should receive substitutive therapy (Group II).

3. In a smaller proportion of patients suffering from EOP, no change indicating thyroid disease can be detected. In these cases, if a space-reducing process, vascular disease or, cyst can be excluded by CT and/or orbital ultrasonography, then besides orbital granulomatosis, ocular myasthenia or myositis, autoimmune infiltrative ophthalmopathy may be a possible diagnosis (Group III).

Attempts should be made to differentiate the three patient groups by the aforementioned diagnostic tests, TSI examination, the assessment of antibody titres as well as by cytological examination of the thyroid gland, since different prognoses can be expected in the individual diseases and different therapeutic procedures should be considered in the above cases.

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# CLINICAL SIGNIFICANCE OF CIRCULATING IMMUNE COMPLEX ASSAY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Circulating immune complexes (IC) were assayed in 65 patients with systemic lupus erythematosus (SLE), 34 patients with rheumatoid arthritis (RA), 40 patients with progressive systemic sclerosis (PSS), 35 patients with chronic glomerulonephritis (GN) and 30 healthy controls. Immunoglobulin components of PEG-precipitated IC from 10 patients with SLE were also determined. Cryoglobulins isolated from 11 patients with SLE were assayed for their IC-like activity. The effect of corticosteroid treatment on IC levels were also studied in SLE patients with nephritis. IC levels significantly increased in all groups but those of SLE patients were the highest values. The SLE patients with nephritis had higher levels than those without renal involvement. IgG- d IgM- but no IgA-components were found in 100% and, 90%, respectively, of IC preparations. IC-like activity of cryoglobulins were found to correlate with disease severity and appeared to be characteristic of clinical manifestations. Corticosteroid therapy significantly decreased IC levels, however, related to the entire patient group, the mean of IC level was still higher than that of healthy controls. The degree of IC level decrease (as percentage), but not absolute values, appeared to be significant in assessing disease activity. The clinical significance of IC determination and IC activity of cryoglobulins are discussed.

Keywords: Systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, glomerulonephritis, immune complex

Abbreviations: IC: immune complex; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; PSS: progressive systemic sclerosis; GN: glomerulonephritis

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## Introduction

The pathogenetic significance of immune complexes in SLE has been widely accepted. In an attempt to find the clinical application of IC assay, various tests have been evaluated /3, 13, 26, 39/. Raised levels of IC in SLE patients were found by many investigators /10, 13, 25, 29, 33/. In respect to clinical significance, a relation of IC determination with other immunological parameter in SLE was revealed by some authors /4, 22/. Many authors focussed on the defect in clearance of IC mediated by complement-dependent solubilization mechanism /5, 6/, by the degradation via macrophages and the reticuloendothelial system /19, 24/ and by the mechanism of IC disposal through the erythrocyte C3b receptor /30, 36/. In SLE, tissue damage often results from the formation and wide spread deposition of IC /2, 17, 27, 28, 32, 37, 40/. With the exception of a small number of studies /34/, correlation between the changes of IC level and clinical activity has been suggested /1, 4, 9, 25, 33, 38/. Diagnostic and prognostic value of the determination of circulating IC as well as their significance in monitoring disease activity of SLE has been investigated /7, 8, 21, 22, 41/.

Despite numerous advances in SLE, the clinical usefulness of IC assays has not been entirely clear and their role in monitoring therapeutic decisions should be justified. In this study, we investigated the levels of circulating IC in patients with SLE and other autoimmune disorders. THE IC-like activity of cryoglobulins isolated from SLE sera as well as immunoglobulin components of IC preparations obtained from SLE patients' sera were determined. The effect of corticosteroid treatment on IC levels of SLE patients was also studied.

## Patients and Methods

### Patients

Sixty five patients with an established diagnosis of SLE, fulfilling the ARA criteria /35/, 34 patients with RA, 40 patients with PSS, 35 patients with GN, and 30 age- and sexmatched healthy controls were included in the study. All the SLE patients were active and divided into two groups: patients with nephritis (n = 31) and without renal involvement (n = 34).

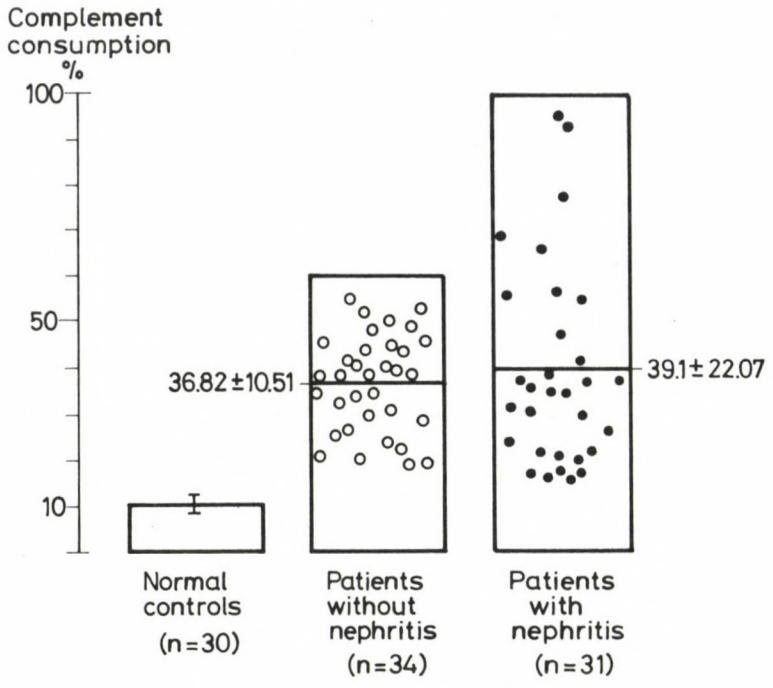


Fig. 1. Distribution of IC levels of SLE patients (% complement consumption; mean ± SD value is established for each patient group)

**Table I**

Circulating IC levels of SLE patients with and without nephritis

| Subjects | | (n) | IC level (mean ± SD) |
|---|---|---|---|
| a) | SLE without nephritis | (34) | 36.82 ± 10.51 |
| b) | SLE with nephritis | (31) | 39.10 ± 22.07 |
| c) | RA | (34) | 21.61 ± 7.50 |
| d) | PSS | (40) | 18.40 ± 8.30 |
| e) | GN | (35) | 14.99 ± 6.30 |
| f) | Healthy control | (30) | 10.50 ± 1.50 |
| P: | a-b | not significant | |
| | a-c | $< 10^{-9}$ | |
| | b-c | $< 10^{-4}$ | |
| | b-d | $< 10^{-5}$ | |
| | b-e | $< 10^{-7}$ | |
| | b-f | $< 10^{-9}$ | |
| | c-f | $< 10^{-9}$ | |
| | d-f | $< 10^{-8}$ | |
| | e-f | $< 10^{-4}$ | |

### Determination of circulating IC

IC in the serum samples were heat-inactivated by incubation at 56°C for 60 min (in some cases with EDTA) was determined by using the complement consumption assay according to the method of Johnson et al. /18/ with modifications /14/.

### Separation of cryoglobulins

Blood samples from SLE patients were immediately incubated at 37°C until clotting. The serum was separated by centrifugation at 37°C and kept at 4°C for 72 h. Cold precipitates were isolated by centrifugation and washed with cold 0.9% NaCl solution and dissolved in warm saline solution. Complement consumption of the amount of cryoglobulin corresponding to 0.1 ml of the original serum was determined.

### Isolation of IC and analysis of immunoglobulin components

IC were precipitated with 3% polyethylene glycol (PEG) solution in borate buffer (pH 8.3) according to the method of Creighton et al. /11/. IgG, IgA and IgM components of IC preparations were identified by double diffusion method using agarose gel. Analyses were performed by antisera specific for IgG, IgA and IgM.

### Chemicals

Haemolysin (rabbit anti-sheep red blood cell) was obtained from Humán (Hungary). Antisera directed against human IgG, IgA and IgM were purchased from Oxford Laboratories (USA). PEG (m.w. 6000) was obtained from Merck (F.R.G.). All the other reagents were obtained from Reanal (Hungary).

### Statistical methods

Significance was calculated by the t-test. Mean ± SD are shown on the tables.

## Results

### IC level in SLE patients

The data are summarized in Table I. The IC levels were much higher in both SLE groups than in the controls. SLE patients with nephritis tended to have somewhat higher levels as compared to the other SLE patients, however, the difference is not significant. Regardless of the distribution of IC levels of SLE patients, those with nephritis had a wider range (Fig. 1).

**Table II**

Immunoglobulin components in the IC preparations of sera from SLE patients

| | Immunoglobulin component | | |
|---|---|---|---|
| | IgA | IgG | IgM |
| No. of + cases | 0 | 10 | 9 |
| No. of − cases | 10 | 0 | 1 |

**Table III**

IC-like activity of cryoglobulin preparations from sera of SLE patients

| Serum inactivation | (n) | IC-like activity (mean ± SD) |
|---|---|---|
| EDTA | (7) | 27.14 ± 24.55 |
| 56°C | (4) | 32.17 ± 15.50 |

The difference is not significant

By analysing 10 IC preparations from 10 SLE patients' sera, IgG was found in 100% and IgM in 90% of cases, whereas no IgA component was revealed (Table II).

IC-like activity was found in all cryoglobuline isolated from 11 SLE patients (Table III). The difference between the heated and EDTA-treated samples was not signficant.

Circulating IC in patients other than SLE

In order to find the diagnostic value as well as other clinical significance of the IC determination, we measured the serum IC levels of some patients suffering from other autoimmune diseases. The IC levels of patients with RA, PSS and GN were also signficantly higher than those of healthy controls (Table I). However, these values were much lower than those of SLE patients without treatment. They were similar to the IC levels of SLE patients after corticosteroid therapy (Table IV), except the cases of GN whose IC levels were still lower than those of treated SLE patients (P 0.05).

**Table IV**

Effect of corticosteroid treatment on IC levels of SLE patients with nephritis

| Patients | | (n) | IC level |
|---|---|---|---|
| a) Before therapy | | (22) | $44.43 \pm 23.75$ |
| b) After therapy | | (22) | $19.69 \pm 9.93$ |
| c) Control | | (30) | $10.50 \pm 1.50$ |
| P: a-b | $< 10^{-5}$ | | |
| b-c | $< 10^{-4}$ | | |

**Table V**

Rate of reduction of IC levels under corticosteroid treatment in SLE patients with nephritis (in %, mean $\pm$ SD)

| Progression | | (n) | Reduction |
|---|---|---|---|
| a) Remission | | (17) | $59.51 \pm 14.85$ |
| b) Death | | (5) | $14.22 \pm 24.45$ |
| P: (a-b) | $< 0.01$ | | |

Effect of corticosteroid treatment on IC levels

In SLE patients, the IC levels were determined after a three-week course of corticosteroid treatment with total dose for each patient: 650 mg of prednisolone. The IC levels of all patients but not of those progressed fatally, were clearly decreased (Table IV). These patients tended to a new remission. 40.9% of the patients in this group had normal IC values. However, when the whole group was seen, the mean IC level after therapy was still significantly higher than that of controls. Figure 2 shows the individual diversity of decrease of IC under corticosteroid effect. Figure 3 exhibits the IC values of 5 SLE patients with fatal progression and those of 3 SLE patietns with remission. The IC levels before therapy of the latters were much higher than those of the formers. However, in marked contrast to these, the patients reaching remission displayed a much greater decrease in IC level under corticosteroid effect. For the whole group, the percentage of decrease in IC level of patients reaching remission was significantly higher as compared to those who died (Table V).

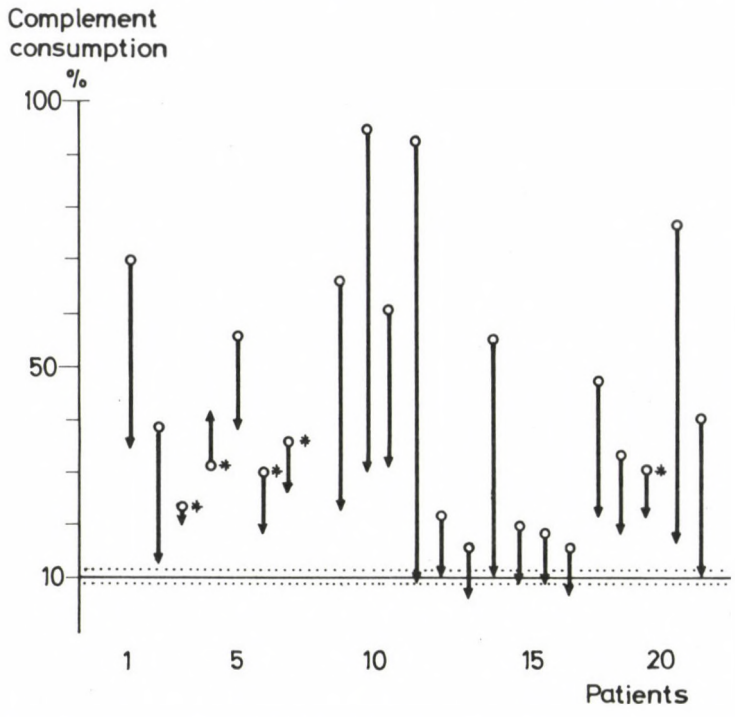


Fig. 2. Rate of reduction of IC levels under corticosteroid treatment. Individual values (% complement consumption; ♀: before therapy, ↓ :after therapy, ∗: fatal cases)

## Discussion

Immune complex levels were elevated in all patient groups. Despite the heterogeneity of the various methods used for determination of circulating IC, these results are consistent with those of many investigators /10, 13, 25, 29, 33/. The high IC levels were not markedly characteristic of renal involvement, though these patients had somewhat higher levels as compared to the other SLE patients. This observation was consistent with the results of Cano et al. /10/. The deposition of IC in various tissues plays an important role in the pathogenesis of SLE. Immune complex deposits in renal glomeruli can arise by deposition of circulating IC or by local formation. While the circulating IC tend to form deposits in the mesangial and subendothelial areas, subepithelial deposits arise mainly by local formation of IC /28/. Immunoglobulin components of IC appeared to be

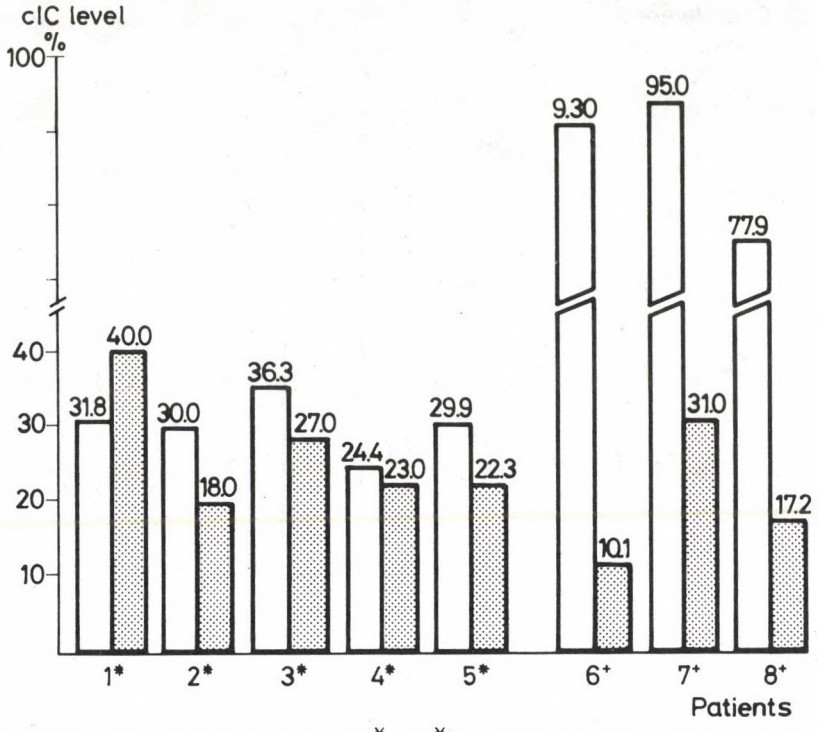


Fig. 3. IC levels of fatal cases ($1^{\times} - 5^{\times}$) and those of reaching remission ($6^{+} - 8^{+}$). Individual values (% complement consumption: ☐ : before therapy, ▨ : after therapy)

important in respect to activation by the complement system, followed by tissue destruction. In this study, IgG was found in 100%, and IgM in 90% of IC preparations. High frequencies of IgG and/or IgM containing IC were found by many authors /15, 16/. Antibody composition, complement-binding activity as well as DNA-protein association in cryoprecipitates from SLE patients have been investigated by many authors and their possibility to reflect properties of IC which affect their tissue localization and pathogeneicity was suggested /12, 31/. By complement consumption assay, we found IC activity in all cryoglobulins, suggesting that these cryoprecipitates contained IC. Furthermore, high IC activity was detected in the cryoglobulins isolated from SLE patients with renal involvement as well as from those with Raynaud phenomenon.

The circulating IC levels of SLE patients were the highest among patient groups in our study. This finding is consistent with the conclusion

of some investigators /13/ and supports the diagnostic value of the determination of IC.

The capability of corticosteroids to decrease IC levels and improve clinical course in SLE has been reported by many authors /8, 9, 20, 25/. In this study, all SLE patients without renal involvement and 77.3% of the SLE patients with nephritis were well controlled by oral prednisolone. 22.7% of these patients failed to respond to therapy, and died. In patients with SLE, we observed that the individual absolute values of IC did not correlate with disease activity. This is in agreement with the findings of Füst et al. /13/ but in contrast with the observations of others /1, 9/. The disagreement may be caused by the different capacity of different methods for detecting IC. The different methods detect different types of IC present in the blood of patients /13/. Furthermore, different ways of IC formation and effects are at least in part responsible for the different manifestations of the SLE /23, 41/. However, when taken the results obtained from prednisolone-sensitive patients together with those from resistant ones, we found that the degree (as percentage) of decrease in circulating IC during treatment was of prognostic significance.

In conclusion, the determination of IC appeared to be significant in the diagnosis and assessing the effect of therapy for SLE. By combining with the measurement for IC activity in cryoglobulins, the detèction of circulating IC may be helpful not only in clarifying the role of IC in the pathogenesis of SLE but also in the clinical practice.

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# A NON-ISOTOPIC SCREENING TEST FOR ANTIBODIES TO TSH RECEPTOR: DOT IMMUNOBINDING ASSAY

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A dot immunobinding assay (DIBA) for thyrotropin (TSH) receptor antibodies is described. The method depends on the detection of antibody binding to highly purified thyroid plasma membrane attached to nitrocellulose solid support by horse-radish peroxidase – conjugated anti-human IgG. The method can detect down to 0.75 mU LATS standard and 1/1000 dilution of Graves' serum or immunoglobulin fraction. The interaction is inhibited dose-dependently by bTSH but not by insulin or human chorionic gonadotropin. The DIBA results show close correlation to those of TRAK (TBII) but not to cyclic AMP generation assay. DIBA is reproducible when tested monthly for 4 months. Sera and immunoglobulins gave virtually the same results. The method has a sensitivity of 90%, validity of 90% and specificity of 80% for both. We have, thus developed a sensitive and reliable method for screening for TSH receptor antibodies which can be performed in routine clinical laboratories.

Keywords: Dot immunobinding assay, antibodies to TSH receptor, thyroid plasma membranes, Graves' disease

Abbreviations: DIBA (Dot immunobinding assay); TRAK (Thyrotropin Receptor Assay Kit); TBII (Thyrotropin Binding Inhibiting Immunoglobulin); ELISA (Enzyme Linked Immunosorbent Assay)

## Introduction

The thyreotoxicosis of Graves' disease is associated with a family of antibodies to the thyrotropin receptor /3, 9, 13, 14/. These include

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antibodies which inhibit the binding of $^{125}$I-TSH to the receptor, antibodies which induced adenylate cyclase, others which induced thyroid cell growth and still others which modulate TSH or stimulatory antibody-related activities /5/. Evidence is mounting that antibodies of different activities bind to separate receptor domains /9/.

The testing of these Graves' IgG activities are technically demanding or require facilities beyond the capabilities of most clinical laboratories. Given the multiplicity of tests for Graves' IgG activity and that none is positive in 100% of patients with active disease, there appears to be a need for a rapid screening test for Graves' IgG. We have developed such a test /8/ and describe here its application to a large series of sera and immunoglobulin from patients with the hyperthyroidism of Graves' disease.

## Materials and Methods

### Buffers

The following buffers were used: 10 mM TRIS-HCl, 1 mM $MgCl_2$, 1 mM EGTA pH 7.4 (TEM); 0.25 M sucrose in TEM (STEM); 50 mM TRIS-HCl, 200 mM NaCl pH 7.4 (TBS); and blocking buffer — 5% bovine serum albumin (BSA) (Sigma) in TBS.

Sera and immunoglobulins were obtained from patients with Graves' disease at the time of diagnosis and before treatment. The diagnosis of Graves' disease was based on established clinical and laboratory criteria /4/. Control sera were obtained from healthy persons with no personal or family history, no obvious sign of thyroid disease and who had normal thyroid function tests. Immunoglobulins for testing were obtained by precipitation with 50% ammonium sulphate.

### Preparation of Thyroid Plasma Membranes

Porcine thyroids were cleaned from fat and connective tissues and stored at $-70^{\circ}$C in 20 gram portions until processed usually within two weeks. Tissue pieces were sliced finely and homogenized with 10 volumes of STEM. The homogenate was filtered through nylon cloths and centrifuged at 1000 x g for 10 min. The supernatant was further pelleted at 4500 x g for 10 min. "Crude" membranes were obtained by centrifugation of the 4500 x g supernatant at 32 000 x g for min. "Purified" plasma membranes (as judged by marker enzymes and electron microscopy) were prepared from the above pellets by discontinuous sucrose-gradient ultracentrifugation using Beckman SW-27 rotor. The pellets were resuspended in 24 ml of 55% sucrose in TEM by homogenization, and equal volumes were transferred into 6 centrifuge tubes. Solutions of 45%, 40% and 35% sucrose in TEM were carefully layered over the membrane suspension in equal volumes. After centrifugation at 113 000 x g for 90 min, the membrane band between the 35–40% gradient interface was collected, diluted with TEM to 0.25 M final sucrose concentration and centrifuged at 113 000 x g for 30 min. The pellets were finally

suspended in TBS, the protein content determined by Lowry et al. /10/. The protein concentration was adjusted to 5 mg/ml with PBS. The yield was usually 2 mg from 20 g of tissue. The entire procedure was carried out at 4°C.

## Dot Immunobinding Assay (DIBA)

The assay /8/ was based on the method described by Hawkes et al./7/. Nitrocellulose filters for immunoblotting (Millipore, Bio-Rad) were used. 5x5 mm rectangular grids were drawn with a pencil on the filter sheet, washed in distilled water by agitation and then allowed to dry. The dried filters were dotted with plasma membrane solution (5 mg/ml in PBS), placing 2 ul drops (10 ug plasma membrane) onto each square. As the drops have dried, the blocking buffer was applied for 15 min. Individual squares were cut out with a scalpel while the filters were still wet. The dried and blocked filters were used for the assays within 4 months.

In the DIBA the filters were placed into individual vials and the blocking step was repeated. The patients' or control sera diluted 1/10 in blocking buffer were allowed to interact overnight at 4°C with the filter squares. The first antibody was removed by aspiration, and the filters washed 5 times (5 min. each) with TBS next morning. The second incubation was carried out with horseradish peroxidase conjugated anti-human goat IgG (HUMAN, Budapest, Hungary) in blocking solution at 1/500 dilution for 2 h. The optimal dilution of each antibody preparation must be tested before use because of variation in quality from batch to batch. The washing step was then repeated. The reaction was developed with 4-chloro-1-naphthol (Sigma) and $H_2O_2$: 1 ml of 3 mg/ml chloronaphthol stock in methanol was added to 5 ml of TBS just before use followed by 20 ul of 30% $H_2O_2$. Positive primary antibodies appear as blue spots on a white background within 2–15 min. When the reaction was complete the filters were washed with distilled water and allowed to dry overnight at 4°C. Serial dilution of MRC Research Standard B 65/122 for human LATS (National Institute for Biological Standards and Control, UK) or positive Graves' sera were used to test the concentration dependence of the reaction.

## Concentration Dependence and Specificity of the Reaction

To test the concentration dependence of the reaction we incorporated into blocking buffer 7.5, 3.75, 2.25, 1.87, 0.75, 0.375, 0.225 and 0.0 mU MRC LATS Standard into the blocking buffer and the following dilution of a positive Graves' serum randomly selected: dilution 1/10, 1/20, 1/50, 1/100, 1/1000 and 1/10000, respectively. To test for TSH binding specificity, a 1/10 dilution of the positive serum and the corresponding ammonium sulfate precipitated immunoglobulins were used in the presence of 1, 10, 100, 1000 and 2000 mU/ml of bTSH (Ambinon, Organon), 1000 mU/ml of hCG (Choriogonin, Gedeon Richter Budapest) and insulin (Gedeon Richter). When tested for specificity of hormone binding the blocking buffer did not contain NaCl, in view of the ability of salt to inhibit TSH binding /2, 12/.

## Reproducibility of the Reaction

Four aliquots each of 10 positive samples of Graves' and 3 control sera and Ig were stored in aliquots at −20°C and DIBA done monthly. The

assay was carried out on the nitrocellulose filter carrying the same plasma membranes for two months and a different plasma membrane preparation in the next two months.

### Determination of Validity, Sensitivity and Specificity

Sixty-eight sera from unselected untreated patients with Graves' disease and 25 sera from healthy control persons were tested. We also examined 60 ammonium-sulfate-precipitated immunoglobulins of Graves' patients and 10 of healthy persons by DIBA. The results obtained with 60 IgG were compared to those of their corresponding sera.

### Cyclic AMP (cAMP) Assay

Fifty-one samples of Ig were tested by DIBA and for their ability to generate cAMP. The latter was done on human thyroid slices using fresh surgical material by the method of Onaya et al. /11/. IgGs were incubated for 120 min in KREBs' Ringer bicarbonate at 37°C in an atmosphere of 95% $O_2$ and 5% $CO_2$. The cAMP content of tissue was measured in ether extract of trichloroacetic-acid precipitate by a $^{125}I$ cAMP kit (Amersham, UK). The results were expressed as pmol/mg of wet tissue. Reactivity of tissue was checked by demonstrating for each batch dose-dependent generation of cAMP by 1, 10, 100 mU/ml bTSH (Ambinon, Organon) and 3 normal sera acted as negative controls. As samples were tested in different runs, they were expressed as per cent of negative controls and $\geq$ 150% taken as cut-off point for a positive result.

### The TRAK Assay

Eighty-seven sera from patients with Graves' disease were tested by TRAK (Henning, West Berlin, FRG). The results are expressed as U/l with results of $\geq$ 10 units being positive.

The qualitative values of DIBA and quantitative measures of the cAMP (%) and TRAK (U/l) assays were compared and analysed by McNemar statistical trial /1/.

## Results

Using the DIBA described above, we found a gradual decrease in colour intensity of LATS standard over the range 7.5 to 0.75 mU. The latter concentration of LATS standard gave a definite positive reaction as did the 1/1000 dilution of the Graves' serum randomly selected for detailed study (Fig. 1) indicating the sensitivity of DIBA.

The interaction of Graves' serum and immunoglobulin in DIBA also appears to be specific. bTSH causes a dose-dependent reduction in DIBA reaction; 10 mU/ml bTSH is associated with 5 times less reactivity than

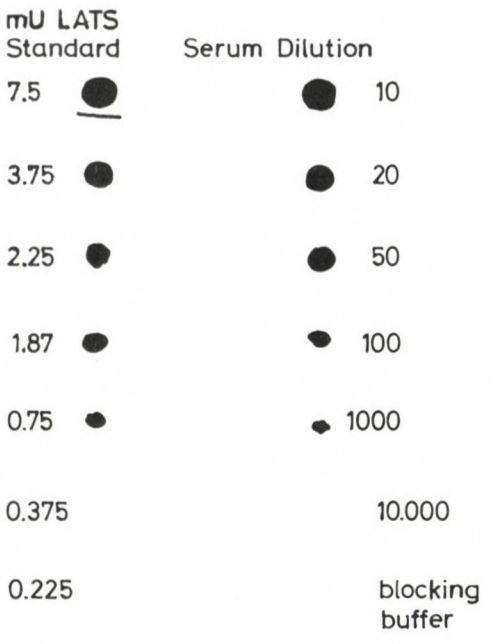


Fig. 1. Results of serial dilution of LATS standard and a randomly selected serum from patients with Graves' disease in a dot immunobinding assay (DIBA). — Up to 1/1000 dilution of serum and 0.75 mU LATS standard gave distinct positive reactions. Blocking buffer (50 mM Tris-HCl, 200 mM NaCl, 5% bovine serum albumin pH 7.4) was used as a blank.

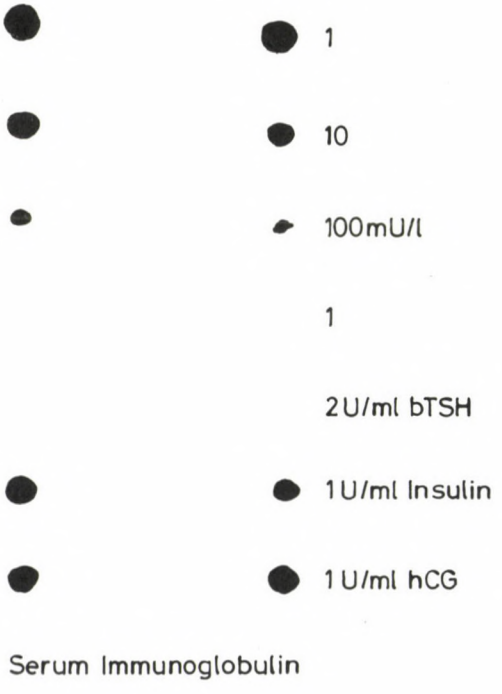


Fig. 2. Specificity of DIBA for TSH receptor antibodies. Interaction of serum and ammonium sulfate precipitate (designated here as immunoglobulin) was dose-dependently inhibited by bTSH. Insulin and human chorionic gonadotropin were without effect.

**Table I**

Dot immunobinding assay (DIBA) for autoantibodies to TSH receptor

| No. | Sex | | 83.03 | 83.04 | 83.05 | 83.06 |
|---|---|---|---|---|---|---|
| 1. | F | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 2. | F | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 3. | M | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 4. | M | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 5. | M | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 6. | F | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 7. | F | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 8. | M | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 9. | F | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 10. | F | Se | + | + | + | + |
| | | Ig | + | + | + | + |
| 11. | M | Se | - | - | - | - |
| | | Ig | - | - | - | - |
| 12. | F | Se | - | - | - | - |
| | | Ig | - | - | - | - |
| 13 | F | Se | - | - | - | - |
| | | Ig | - | - | - | - |

Se = serum; Ig = immunoglobulins; + = positive reactions;
- = negative reactions

**Table II**

Results of Dot immunobinding assay (DIBA) using sera

| | Positive | Negative | Total |
|---|---|---|---|
| Graves' patients | 62 | 6 | 68 |
| Healthy controls | 5 | 20 | 25 |
| Total | 67 | 26 | 93 |

Values of validity: 88.2%, sensitivity:91.1% and specificity: 80.0% were calculated from the data.

**Table III**

Results of dot immunobinding assay (DIBA) using ammonium sulphate precipitates

| | Positive | Negative | Total |
|---|---|---|---|
| Graves' patients | 55 | 5 | 60 |
| Healthy controls | 2 | 8 | 10 |
| Total | 57 | 13 | 70 |

The values of validity: 90.0%, sensitivity: 90.6% and specificity: 80% were calculated.

that with 1 mU/ml and that with 100 mU/ml with 20 times lower colour reaction (read off a dilution curve ran in parallel). One unit of bTSH caused complete inhibition of binding. At 1 unit/ml insulin or hCG strongly positive reactions were still obtained (Fig. 2).

We have tested the stability of the DIBA reactivity of sera or Ig tested monthly for 4 months with two batches of thyroid membranes, each stored for months. The reactivity of the Graves' sera and Ig preparations remained unchanged over the course of the study, even despite change in substrate (Table I).

Sera and Ig from randomly selected patients with Graves' disease were tested in DIBA to establish its value as a screening test for Graves' IgG. Sixty-two out of 68 patients' sera (91.2%) and 5 of 25 control sera (20%) were found to be positive. Given these figures, we can calculate that for sera DIBA has a validity of 88.2%, specificity of 80% and sensitivity of 91.1% (Table II). Fifty-five of 60 Graves' and 2 of 10 control Ig preparations tested were positive for DIBA, giving validity, sensitivity and specificity values of 90%, 91.6% and 80% (Table III). These figures for both sera and Ig qualify DIBA for a screening test.

DIBA results were found identical in case of 60 sera and Ig tested in parallel $\chi^2_{(1)} = 0$; $P > 0.05$ by McNemar trial /1/.

No relationship was found between antimicrosomal or antithyroglobulin antibody titres and DIBA or TRAK assay results (not shown).

When the results of DIBA and TRAK tests were compared we found that of the DIBA – negative sera none was positive by the TRAK assay but among the DIBA-positive sera 11 were negative by TRAK (Fig. 3). This means that, relative to TRAK, DIBA yielded 12% false positive rate (Fig. 3). The McNemar trial yielded an $\chi^2_{(1)} = 0.94$ ($P > 0.05$).

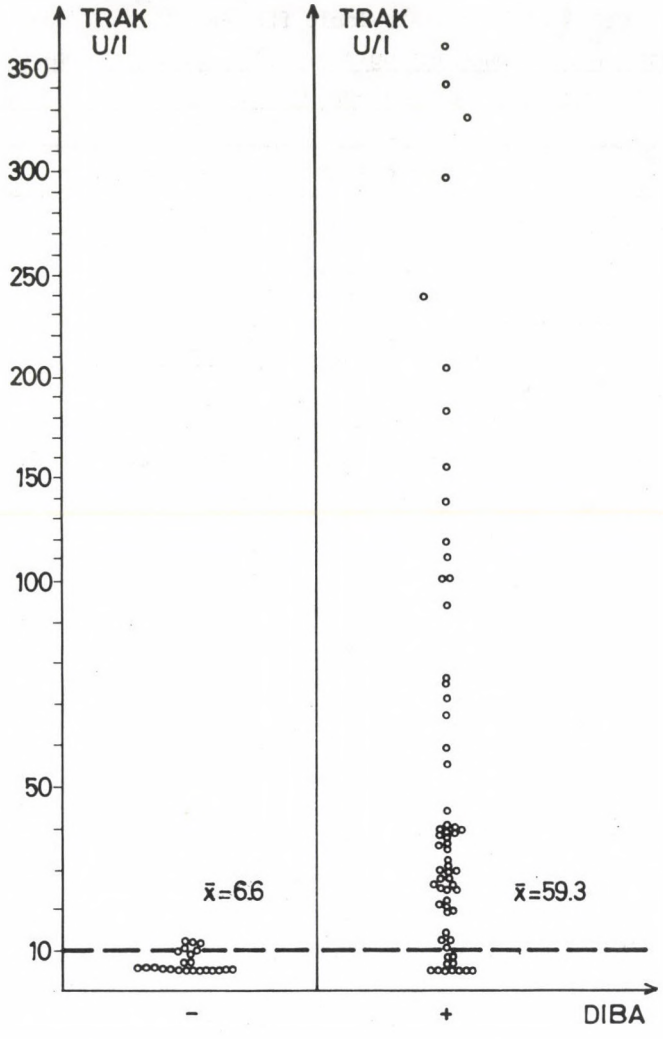


Fig. 3. Comparison of the results of DIBA and thyrotropin-receptor assay kit (TRAK). 23 out of 87 Graves' sera were negative by the DIBA, and their TRAK mean value was 6.6 (range: 5—12.3); 64 out of 87 sera gave positive results by DIBA, and their mean value by TRAK was 59.3 U/l (range: 5—360). McNemar trial gives no significant difference between the methods. The left panel includes DIBA-negative sera and that on the right positive sera

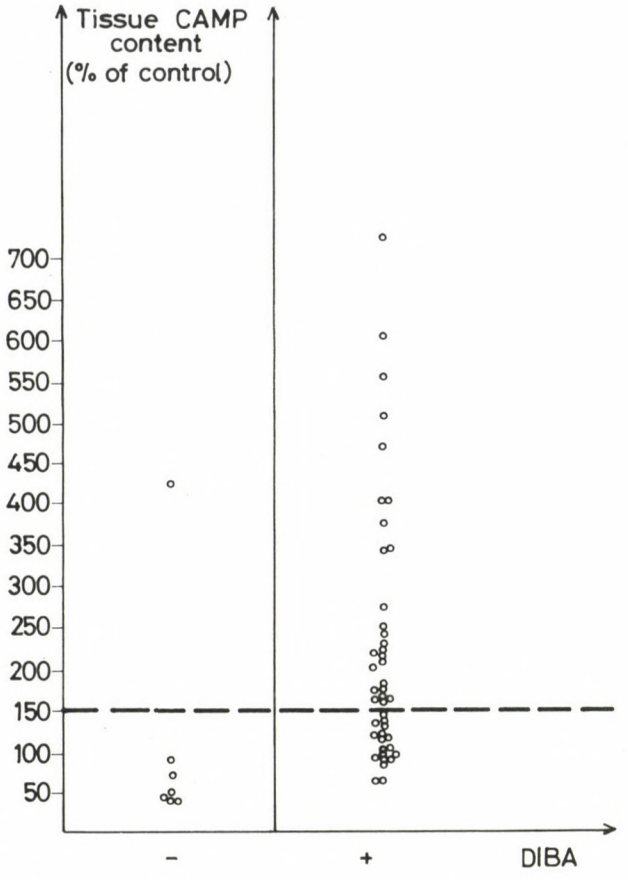


Fig. 4. Comparison of the results of the DIBA and cAMP generation test. 51 samples of Igs of untreated Graves' sera were tested. Serum samples from 7 patients were negative and 44 positive by the DIBA. We found 1 positive case in cAMP generation test among the DIBA-negative patients. There was significant difference between the two methods by McNemar trial. — As in Fig. 3, DIBA negative cases are on the left panel and positive cases on the right

DIBA was positive in higher proportion of Ig tested than was the cAMP generation test. Only 26 of 44 (59%) DIBA-positive Igs were also positive by the cAMP generation assay. In addition, one patient strongly positive by the biological assay was DIBA-negative (Fig. 4). The two methods were significantly different by McNemar trial: $x^2_{(1)} = 13.47$, $P < 0.001$.

## Discussion

Anti-TSH receptor antibodies are clinically useful in forecasting remissions of hyperthyreoidism of Graves' disease after therapy with anti-thyroid drugs, predicting neonatal hyperthyroidism in the offspring of mothers who had or had had Graves' disease and in the investigations of unilateral exophthalmos in clinically euthyroid patients /4, 14/. Their place in the diagnosis of hyperthyroid Graves' disease is more limited. We describe in this study a practical screening test for these antibodies which show excellent measures of specificity, sensitivity and reproducibility. The DIBA has the following advantages: 1) because isotopes are not used, it can be performed in a routine clinical biochemical laboratory, the substances have much longer "shelf" life than any of those used in the existing assays for Graves' IgG; 2) it is, therefore, cheap; 3) it can be performed in small batches as the clinical need arises, at the same time avoiding wide variation in the storage time of samples collected for testing, 4) as there appears to be no essential difference between serum samples and ammonium-sulphate precipitates (used here despite its shortcomings /14/ because of clinical applicability), serum samples can be tested after dilution, 5) the titre of the antibody can be determined after serial dilution of the serum samples, a distinct advantage over other established assays, which allows for the prospective monitoring of change in titre of antibody during the course of therapy etc. Specificity of the assay can be easily investigated by change in colour reaction with the pre-incubation with one set of filters of a crude commercial bTSH preparation. Indeed, an avenue for improving the quality of the test would be reading the dot with and without 10–100 mU bTSH/ml, using an ELISA reader. The seemingly high positive rates among healthy controls may thus be minimized.

While our DIBA was being tested, Gardas and Rives /6/ published a highly sensitive ELISA for antibodies directed to thyroid membranes, distinct from those for microsomal or thyroglobulin antigens (as in the present assay). The ELISA was, however, not influenced by bTSH even at high concentrations, which limits its value as a test for TSH receptor antibodies. This difference is probably related to the use of highly purified thyroid plasma membranes as antigens in the DIBA.

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METABOLISM

# THYROID FUNCTION IN SEVERE "NONTHYROIDAL ILLNESS". LONGITUDINAL STUDIES IN HAEMATOLOGICAL PATIENTS

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It is known that in severe nonthyroidal illness the regulation of thyroid function, the distribution and metabolism of thyroid hormones may change. The present study aimed at clarifying whether a change in the function of the pituitary-thyroid axis can be detected in an approximately homogeneous group of haematological patients, and how it is correlated with the various phases of the disease and with the therapeutic result. Studies were performed on patients with chronic and acute myelogenous leukaemia: serum levels of total thyroxine and triiodothyronine, free thyroxine and triiodothyronine, reverse triiodothyronine and thyrotropic hormone were determined. Apart from a few cases, there was no dysfunction of the pituitary-thyroid axis in chronic leukaemic patients being in the remission phase. However, the peripheral thyroxine metabolism may be altered. The longitudinal studies on acute myelogenous leukaemic patients indicate that, with the progression of the disease, serum TSH and thyroid hormone levels were reduced in a part of the cases and it is not justified to assess the free serum thyroxine level by an analogue-tracer method in this disease. The examinations have revealed that the various phases of the clinical picture as well as the therapeutic results considerably influence the function of the pituitary-thyroid axis. It seems reasonable to consider these findings in the other severe nonthyroidal illnesses as well.

Keywords: Thyroid function, nonthyroidal illness, haematological patients, pituitary-thyroid axis

Abbreviations: NTI = nonthyroidal illness; $TT_4$ = total thyroxine; $TT_3$ = total triiodothyronine; $FT_4$ = free thyroxine; $FT_3$ = free triiodothyronine; $rT_3$ = reverse triiodothyronine; TSH = thyreotropic hormone; AML = acute myeloid leukaemia; CML = chronic myeloid leukaemia

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## Introduction

It is known that both in acute and chronic nonthyroidal illnesses (NTI) the regulation of thyroid function, thyroid hormones' production, transport and distribution within the organism as well as their metabolism may alter and affect the serum thyroxine ($T_4$) and triiodothyronine ($T_3$) levels /7, 13, 14, 17, 21, 25, 26/. According to previous data, thyroid function test results are often ambiguous. Their assessment is made still more difficult by the following factors: (i) the studies performed so far were made in various diseases; (ii) serum TSH and thyroid hormone levels were assessed in various phases of even the same disease; (iii) the results of investigations were influenced by drug (e.g. glucocorticoid, dopamine) therapy; (iv) the methods of examination were often different and inadequate.

Our study aimed at finding out whether a pathological change could be revealed in the function of the pituitary-thyroid axis in an approximately homogeneous group of haematological patients, and how it was correlated with the therapeutic results in a given case.

## Patients and Methods

The studies were first performed on 48 non-medicated patients with chronic myelogenous leukaemia (CML) in remission. Subsequently, the examinations were extended to 24 patients with acute myelogenous leukaemia (AML). The latter group was treated with cytostatics (daunorubicin, vincristine, cytosine arabinoside, 6-thioguamine) after admission. However, they received neither dopamine nor glucocorticoid therapy. The patients were followed up to their clinical remission or death. Blood samples were collected twice weekly. Female patients, aged 20 to 60, with no endocrinological or severe internal disease, receiving no anticoncipients, were used as controls.

For serum determinations the following kits were used: total $T_4$ ($TT_4$, RK-12 Isotope Institute, Hungarian Academy of Sciences; normal value: 70—155 nmol/L); total $T_3$ ($TT_3$, RK-11 Isotope Institute, Hungarian Academy of Sciences; normal value: 1.2 — 3.0 nmol/L); free $T_4$ ($FT_4$): a SPAC-ET $FT_4$ (Mallinckrodt, $FT_4$ assay based on $TT_4$ + $FT_4$ fraction tests; normal value: 8.0 — 23.0 pmol/L); b Amerlex M $FT_4$ (Amersham; analogue — tracer method; normal value: 8.7 — 23.2 pmol/L); reverse — $T_3$ ($rT_3$; Serono; normal value: 15—75 nmol/L); supersensitive TSH (IRMA-mat TSH; Mallinckrodt, normal value: 0.3 — 3.0 mU/L).

## Results

Figure 1 shows our observations on CML patients. Examined by SPAC-ET, the serum $FT_4$ level exceeded the upper limit of the normal range in only two of the 48 cases; in another two the $FT_4$ level was pathologically reduced. The TSH levels seemed to be similar: the serum TSH was pathologically low only in two of the 48 cases; in three, its value exceeded the upper limit of the normal range. On the contrary, the serum $FT_3$ level proved to be lower than the lower limit of the normal range in 10 out of the 48 cases, while the $rT_3$ concentration was markedly increased in 9 cases. Similar results were obtained in the 24 patients sent to us with the diagnosis of AML. The serum hormone assessments of the individual patients are presented in Fig. 2. The serum $FT_4$ and TSH levels could be considered normal, apart from one case each, however, the $FT_3$ level corresponded, in the majority of cases to the lower limit of the normal range and in 7 cases it even proved to be pathologically reduced; the $rT_3$ level was high in 6 cases.

Table I demonstrates the number of AML patients whose serum thyroid hormone and TSH levels changed during their stay in the hospital. In the period of observation, with progression of the disease, the hormone levels

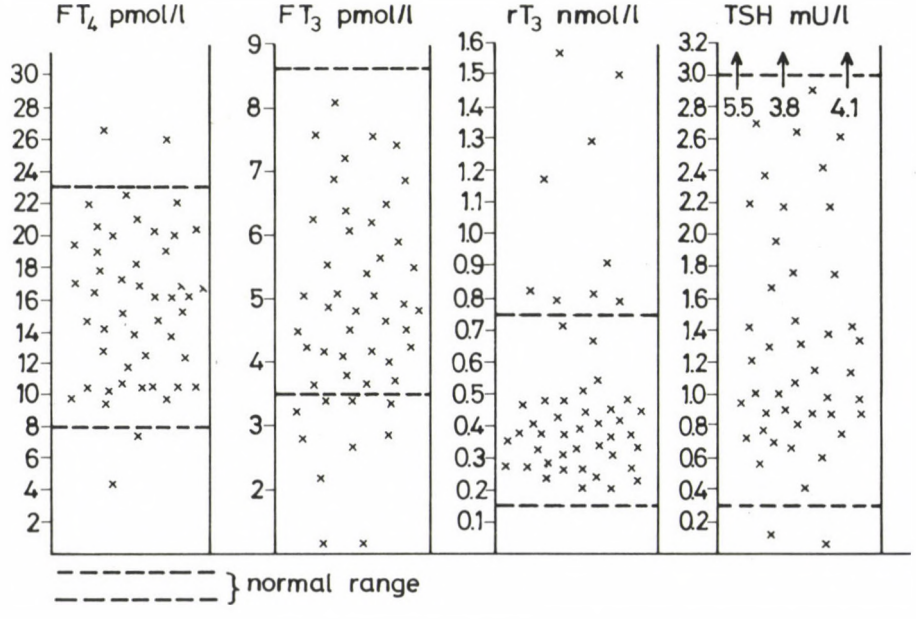


Fig. 1. Serum $FT_4$, $FT_3$, $rT_3$ and TSH levels of patients with AML (n=8)

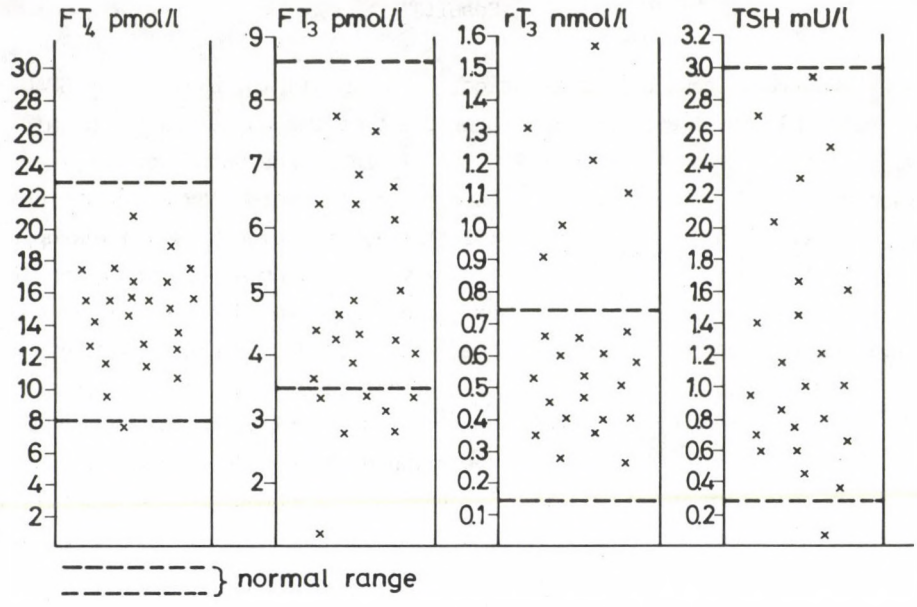


Fig. 2. Serum $FT_4$, $FT_3$, $rT_3$ and TSH levels of patients with AML (n=24)

**Table I**

Data on the longitudinal study of AML patients. The numbers of patients are presented whose serum hormone level fell to a pathological range during the observation

(initial number of patients: 24)

| Method | No. of patients |
|---|---|
| $TT_4$ | 7 |
| $FT_4$ (SPAC-ET) | 4 |
| $FT_4$ (analogue tracer) | 12 |
| $TT_3$ | 11 |
| $FT_3$ (analogue tracer) | 13 |
| TSH (IRMA) | 7 |

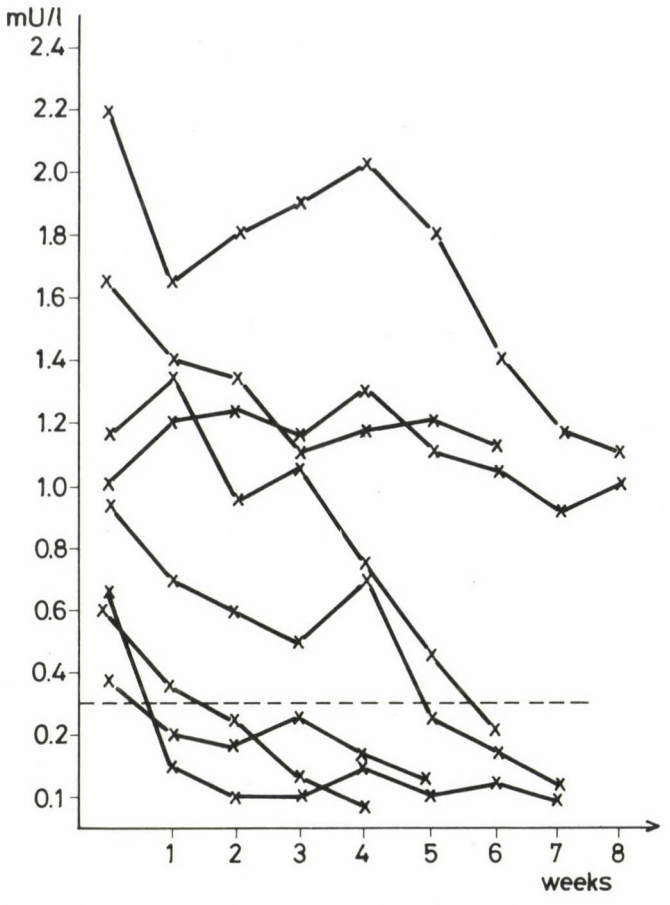


Fig. 3. Longitudinal studies of patients with AML. Note the change in serum TSH level with progression of the disease

decreased below the limit of the normal range. This particularly applied to the $FT_4$ and $FT_3$ test results obtained by the analogue – tracer method.

Figure 3 shows the change in the TSH concentrations in cases of poor prognosis, those terminating in death. The data indicate that in 5 out of 8 cases, the initially normal serum TSH concentration fell to a pathological range.

On clinical remission of the two patients with AML presented in Fig. 4, there was an increase both in the TSH and $FT_4$ levels. In one of the cases, TSH concentration even exceeded the upper limit of the normal range. On repeated relapses, the serum TSH levels considerably decreased again in both patients; this event was followed in one of them by decrease of the $FT_4$ level.

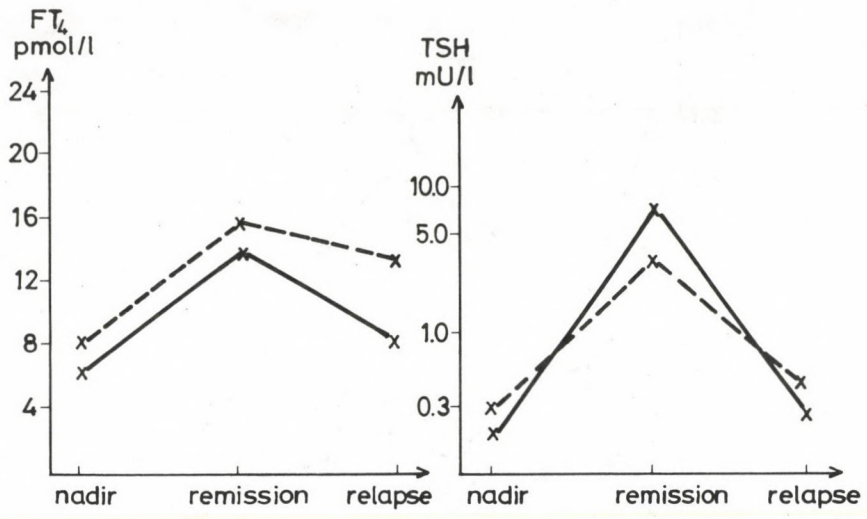


Fig. 4. Change in the serum TSH and $FT_4$ levels of two patients with AML in clinical remission and in relapse

## Discussion

Thyroid function studies have already been performed on haematological cases /18, 26/. However, the serum TSH and thyroid hormone levels were assessed in patients having undergone bone-marrow transplantation, in those who had been treated — besides a large-dose whole-body irradiation followed by cyclophosphamide and immunosuppressive treatments — by the TSH secretion inhibitor dopamine as well as glucocorticoid. All these therapeutic procedures can influence, beside the underlying disease, the pituitary and thyroid functions. Our present study aimed at answering the following questions: (i) Can the changed function of the pituitary-thyroid axis be detected in CML patients in remission? (ii) Data were collected in AML patients having not received dopamine and glucocorticoid therapies, to show whether the results of TSH and thyroid hormone tests were correlated with the various phases of the disease and the therapeutic response during a prolonged period of observation.

Studying the serum $FT_4$ concentration of patients with CML in remission by SPAC-ET, we found its value in the majority of cases, to be identical with that of the controls. On the contrary, similarly to the other NTIs, the serum $FT_3$ level often proved to be lower than the normal value (so-

called low-$T_3$ syndrome). This can partly be ascribed to the fact that in NTI the $T_4$ uptake by the liver is reduced and so less than the normal amount of $T_4$ for $T_3$ production is available in this organ /14, 16, 22, 24/. The other cause of the low $T_3$ level is that in NTI the 5'-deiodinase enzyme activity is decreased and accordingly, there is less $T_3$ production from $T_4$ in the peripheral tissues /11/. This can, to some extent, be considered an adaptation mechanism of the organism, for as a result of the low $T_3$ concentration, energy demand and protein catabolism are reduced. Another metabolite of $T_4$, the $rT_3$ is considered to be inactive from the hormonal point of view. Its serum level is higher than normal in most of the severe NTIs; renal failure seems to be the only exception /5, 6, 20, 28/. In a part of the haematological patients the serum $rT_3$ level increased conspicuously, most probably due to the reduced 5'-deiodinase activity and the consequentially slowed down $rT_3$ metabolism.

The recently widely used supersensitive TSH assay is of great diagnostic importance. Authors disagree as to the serum TSH level demonstrable by this method in NTI; some of them found normal values /8/, others found in the majority of cases, values lower /2, 4/ or higher than normal /9/. The divergent views can be attributed to the fact that the TSH assays were performed in various phases of NTI. The TSH levels of our CML patients in remission proved during their observation period to be predominantly normal, with few exceptions. All these indicate that, apart from some exceptions, no considerable dysfunction of the pituitary-thyroid axis occurred in these patients, though in a part of the cases, the peripheral metabolism of $T_4$ had already changed. Our data support the latest results in NTI obtained by Faber et al. /8/.

Excepting a few cases, the serum $FT_4$ and TSH levels of the patients admitted with AML did not differ from the normal. The $FT_3$ and $rT_3$ levels of these patients were essentially in concert with the levels usual in NTI: in a part of the cases the $FT_3$ concentration was reduced with an increase in the $rT_3$ level. These differences correspond to the results obtained in chronic leukemia and most probably the causes can be the same.

Subsequently, longitudinal studies were performed in AML patients appearing to be euthyroid clinically. Comparing the two $FT_4$ assays used by us, we can state that with the progression of the disease the analogue tracer method reveals pathologically an appearance of low serum $FT_4$ level considerably more frequently than the SPAC-ET procedure. The false low values obtained with the former method may be explained by changes in the

organism (e.g. accumulation of $T_4$ binding inhibitors, decreased serum albumin levels, etc.) changes that influence the evaluation of this procedure unfavourably /7, 23/. In concert with Wenzel et al. /27/, it can be concluded that it is not justified in NTI to assay the serum $FT_4$ level by the analogue-tracer method. Our further studies indicate that, with progression of the disease, the peripheral metabolism of $T_4$ tends to change and, as a consequence, there is a pathological decrease in the serum $FT_3$ concentration.

Our observations on patients suffering from AML indicate that the progression of the disease may be accompanied by a decrease in the serum TSH level. One of the causes can be that in NTI, the production and secretion of cortisol, dopamine, growth hormone, opioid as well as other peptides increase due to stress and all these inhibit TSH secretion /15, 19/. On the other hand, the extent of local $T_4 \longrightarrow T_3$ conversion may also be altered and this, too, may lead to reduced TSH secretion. Our patients did not receive dopamine or glucocorticoid therapies, so the TSH secretion-inhibiting effect of these drugs cannot be considered. The applied cytostatic treatment can be assumed to exert an effect on the pituitary-thyroid function. Nevertheless, this is challenged by the observation that all patients have been given cytostatic treatment, the serum TSH level decreased, however, only in cases with a poor prognosis. These data have disclosed that in our patients the decreased serum TSH level can be one of the consequences of the progression of the disease and it may contribute to the decrease of serum $T_4$ concentration, and to the development of a central type of hypothyroidism.

Previously, the longitudinal studies of Kaptein et al. /12/ and Böttger et al. /3/ revealed that in NTI with a deterioration in the patient's condition the serum thyroid hormone level also decreases. This finding was extended by our studies to the change of serum TSH concentration. Our further findings confirm the earlier observations of Hamblin et al. /10/ as well as Wehmann et al. /26/, according to which the decrease in TSH secretion in NTI may be reversible, moreover it can be increased to such an extent in remissions following a severe state, that the amount of the hormone can exceed the upper serum level of the normal range. The latter finding may account for a higher than normal TSH level in a part of patients with NTI.

Our observations on individuals suffering from acute myelogenous leukaemia indicate that the various phases of this disease markedly in-

fluence the function of the pituitary-thyroid axis and on assessing serum hormone levels, this must be taken into consideration. This finding may probably be extended to other severe nonthyroidal illnesses, too.

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# LIPID AND LIPOPROTEIN MEASUREMENTS IN A NORMAL, ADULT CUBAN POPULATION

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An attempt was made to determine the normal reference values of lipid- and lipoprotein levels (cholesterol, triglycerides, cholesterol in high- and low-density lipoproteins) in a selected, apparently healthy, Cuban population. Results were expressed as mean, and various percentiles of measured values; two ratios: $Risk_1$ (LDL-C/HDL-C) and $Risk_2$ (TC/HDL-C) were also calculated.

Approximately 40% of the subjects aged 20 to 30 years had cholesterol values above 200 mg/dl. Females had significantly higher cholesterol HDL-C values than males, wherease the concentrations of LDL-C and LDL were higher in males. $Risk_2$ ratios were elevated in males.

A correlation was shown between lipid levels and age. There was a strong negative correlation between HDL-C and relative body weight. It is suggested that obesity might be an individual risk factor in the population studied.

Keywords: Lipid, lipoprotein, serum, cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, normal adult population

Abbreviations: TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL: low density lipoprotein as beta-lipoprotein; $Risk_1$: LDL-C/HDL-C; $Risk_2$: TC/HDL-C

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## Introduction

Atherosclerotic cardiovascular disease is the leading cause of death in most of the developed countries /22, 31/. In the last decade myocardial infarction was the leading cause of death in Cuba, too: ischaemic heart diseases, cerebrovascular accidents and peripheral vascular diseases are responsible for about 50% of all deaths /12/.

Epidemiological studies have established a number of risk factors, such as, cigarette smoking /14/, hypertension /13/, hypercholesterolaemia /4/, hypertriglyceridaemia /4/, a family history, and obesity /3/, factors predisposing to premature atherosclerosis. It has been shown that the serum HDL concentration is negatively correlated with the development of the atherosclerotic process /20/.

The purpose of this study was to measure the values of TC, TG, HDL-C, LDL-C and LDL in a normal adult Cuban population in order to obtain the normal reference values for screening the predictive risk factors of ischaemic cardiovascular disease.

## Patients and Methods

A total of 515 apparently healthy subjects (264 males and 251 females), ranging in age from 20 to 39 years, were selected for the analysis of lipids and lipoproteins.

A survey was made in order to obtain information regarding age, sex, body mass, height, smoking habits (number of cigarettes per day and smoking history), diet histories and alcohol habits. Patients who were suffering from diseases that might affect blood lipid concentrations and oral contraceptive users were excluded from the study.

Blood was drawn into plastic centrifuge tubes after overnight fasting, serum was separated by centrifugation (2500 r.p.m. for 5 min).

Total cholesterol was assayed using the ferric chloride method described by Zlatkis in 1971 /32/. Standardization was carried out with Preciset (Boehringer-Mannheim, BRD) as standard and Precinorm U (Boehringer-Mannheim, BRD) as control.

Triglycerides were assayed in the acetylacetone colorimetric system /5/. Calibration was accomplished with tripalmitin (Sigma, St. Louis, Mo., USA) as standard and Precilip R (Boehringer-Mannheim, BRD) as control.

Low-density lipoprotein (beta-Lipoprotein) was determined by specific precipitation of the LDL by dextran sulphate in the presence of calcium ions at pH 9.0 /28/.

HDL-C was measured by the phosphotungstic acid and $Mg^{+2}$ precipitation of Apo-B containing lipoproteins described by Lopes-Virella et al. /17/. The specificity of the precipitation was confirmed by checking the concentration of Apo-B; under the conditions of the assay no Apo-B protein was detected in the supernatant. This was checked by rocket electrophoresis.

LDL-C was calculated according to the Friedewald formula /6/:

$$\text{LDL-C} = \text{TC} - \frac{\text{Triglycerides}}{5} - \text{HDL-C (mg/dl)}$$

Results were expressed as mean $\pm$ S.D. The univariate statistical analysis was performed including one-way analysis of variance to evaluate overall differences among groups.Student's t test was used to compare pairs of groups. Selected percentiles (5th, 25th, 50th, 75th, and 95th) were calculated for lipid and lipoprotein concentrations in males and females. Variance analysis and parametric regression analysis were made. The correlation coefficients (r) were obtained to describe pairwise relationships between lipids and lipoproteins.

## Results

Table I and Table II show the values of TC, TG, LDL, HDL-C, LDL-C, $Risk_1$ and $Risk_2$ for males and females. Triglycerides and $Risk_1$ tended to be higher in males than in females although the differences were not significant. Total cholesterol was significantly higher in females as compared to males. LDL values were higher in males than in females. HDL-C was significantly higher in females, but males exhibited higher values for LDL-C than females. In males and females, TC mean levels were within 2-3 mg/dl of median levels (50th centile). For TG there was a 2-5 mg/dl difference between median and mean levels. There was a 1-3 mg/dl difference between mean and median levels for LDL, HDL-C, LDL-C, and VLDL-C. In $Risk_1$ and $Risk_2$ there was a 0.12—0.80 difference between median and mean values.

Figures 1 and 2 display the distribution of TG and TC levels. The distribution of TG concentrations appears to be very similar in males and females, but the histrogram shows a shift to the higher values in females.

**Table I**

Mean, 5th, 25th, 50th, 75th, 95th centiles for lipid and lipoprotein concentration (mg/dl) in males (264)

| | Mean ± S.D. | 5th | 25th | 50th | 75th | 95th |
|---|---|---|---|---|---|---|
| Total cholesterol | 173 ± 44 | 138 | 153 | 170 | 220 | 248 |
| Triglycerides | 111 ± 33 | 57 | 93 | 105 | 134 | 154 |
| LDL | 504 ±113 | 367 | 401 | 505 | 636 | 680 |
| HDL-C | 45 ± 8 | 30 | 33 | 43 | 48 | 55 |
| LDL-C | 114 ± 37 | 50 | 67 | 113 | 133 | 151 |
| $Risk_1$ (LDL-C/HDL-C) | 3.8± 0.7 | 1.22 | 2.07 | 2.97 | 4.47 | 5.05 |
| $Risk_2$ (TC/HDL-C) | 4.7± 3.6 | 2.75 | 3.67 | 4.55 | 5.25 | 6.10 |

Number of subjects in parentheses

**Table II**

Mean, 5th, 25th, 50th, 75th, 95th, centiles for lipid and lipoprotein concentration (mg/dl) in females (251)

| | Mean ± S.D. | 5th | 25th | 50th | 75th | 95th |
|---|---|---|---|---|---|---|
| Total Cholesterol | 189 ± 33*** | 145 | 160 | 187 | 225 | 250 |
| Triglycerides | 105 ± 34 | 53 | 79 | 103 | 135 | 150 |
| LDL | 469 ±111** | 350 | 400 | 467 | 600 | 650 |
| HDL-C | 51 ± 12*** | 39 | 44 | 50 | 56 | 63 |
| LDL-C | 100 ± 36** | 53 | 79 | 98 | 132 | 145 |
| $Risk_1$ (LDL-C/HDL-C) | 2.5 ±0.3 | 1.49 | 1.96 | 2.27 | 3.39 | 4.26 |
| $Risk_2$ (TC/HDL-C) | 3.4 ±2.0* | 2.73 | 2.96 | 3.06 | 5.32 | 6.00 |

Number of subjects in parentheses

Significance between males and females (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

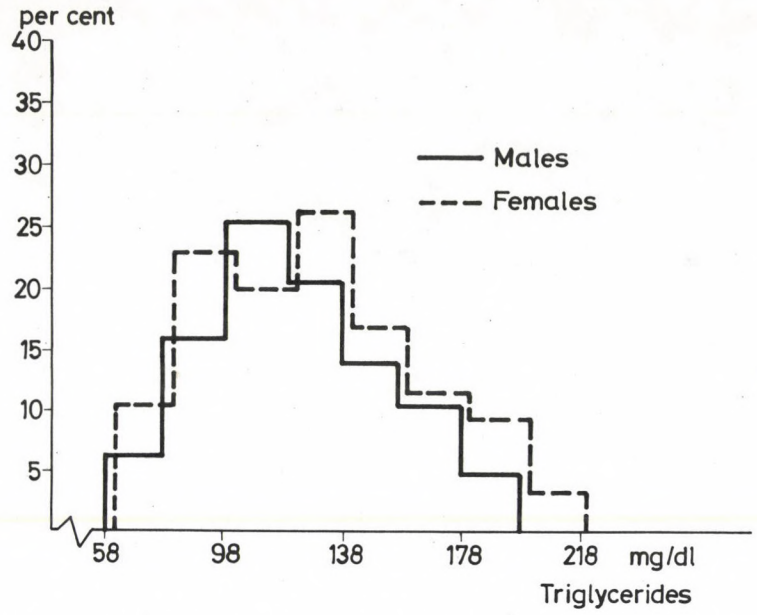


Fig. 1. Distribution of triglycerides in relation to sex

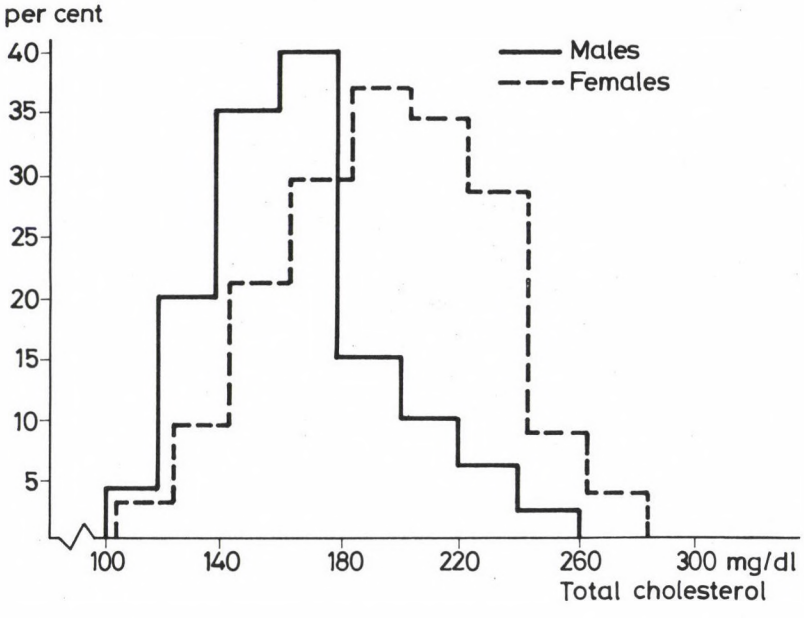


Fig. 2. Distribution of total cholesterol in relation to sex

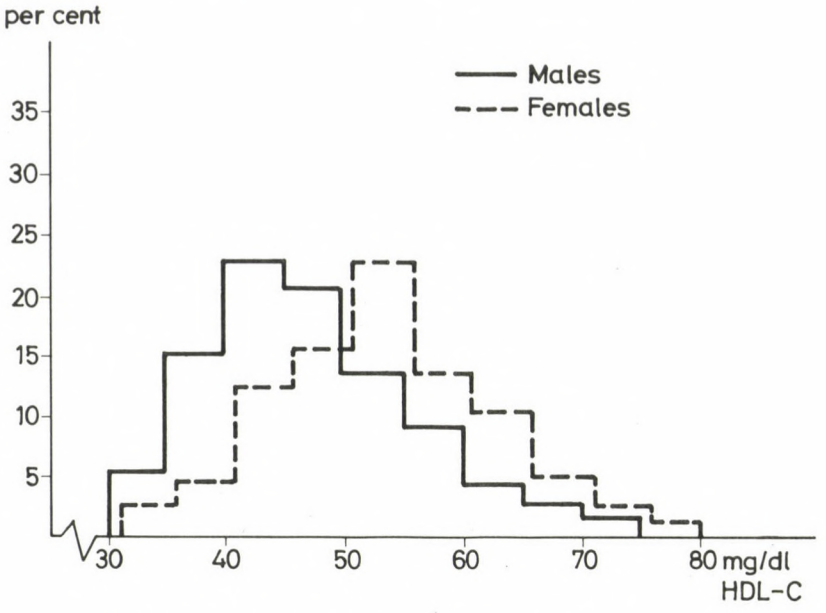


Fig. 3. Distribution of HDL-C in relation to sex

HDL-C appears to considerably vary with sex, female population has an elevated level of HDL-C (Fig. 3).

All measured parameters correlated positively with age in males. In females, a significant correlation was shown for TC, LDL and LDL-C (Table III).

Table IV shows the regression analysis of HDL-C, $Risk_1$ and $Risk_2$ to relative body weight. The relative body weight is the ratio of actual body weight/ideal body weight (calculated according to Broca). A significant negative correlation was found between relative body weight and HDL-C in both males and females. Apositive correlation was found between the risk parameters ($Risk_1$ and $Risk_2$) and the relative body weight.

## Discussion

Levels of TC in a normal adult Cuban population were significantly higher in females than in males. This result differs from that reported by Kostner et al. /15/ for a London population and from various Australian population studies /18/, but is similar to those reported by Ononogbo /21/, Lindgren et al. /16/, and Gidez et al. /9/. Heiss et al. /11/ in 1980

**Table III**

Age trends in serum lipids and lipoproteins in a normal adult Cuban population

| | Males (264) | | | | Females (251) | | | |
|---|---|---|---|---|---|---|---|---|
| | r | $t_c$ | F | P | r | $t_c$ | F | P |
| Total cholesterol | 0.35 | 2.69 | 7.28 | $<0.01$ | 0.21 | 2.64 | 6.38 | $<0.01$ |
| Triglycerides | 0.21 | 2.85 | 8.13 | $<0.01$ | 0.03 | 0.39 | 0.14 | N.S. |
| LDL | 0.31 | 4.22 | 17.84 | $<0.001$ | 0.29 | 3.73 | 13.91 | $<0.01$ |
| HDL-C | -0.20 | -2.63 | 7.20 | $<0.01$ | 0.02 | 0.29 | 0.08 | N.S. |
| LDL-C | 0.24 | 3.24 | 10.54 | $<0.001$ | 0.17 | 2.12 | 4.50 | $<0.04$ |
| $Risk_1$ | 0.30 | 4.11 | 16.38 | $<0.001$ | 0.13 | 1.60 | 2.56 | N.S. |
| $Risk_2$ | 0.36 | 5.03 | 25.38 | $<0.001$ | 0.10 | 1.33 | 1.77 | N.S. |

Number of subjects in parentheses

N.S. Not significant

**Table IV**

Correlation between HDL-dependent values and relative body weight in males and females

| | Males (264) | | | | Females (251) | | | |
|---|---|---|---|---|---|---|---|---|
| | r | $t_c$ | F | P | r | $t_c$ | F | P |
| HDL-C | -0.29 | -3.32 | 15.41 | $<0.001$ | -0.22 | -2.80 | 7.86 | $<0.01$ |
| $Risk_1$ | 0.29 | 3.94 | 15.54 | $<0.001$ | 0.36 | 4.75 | 22.57 | $<0.001$ |
| $Risk_2$ | 0.36 | 4.93 | 24.34 | $<0.001$ | 0.42 | 5.55 | 32.01 | $<0.001$ |

Number of subjects in parentheses

reported mean TC values about 180 mg/dl for males and 173 mg/dl for females. Mean serum TC level of women in the USA was 188 mg/dl, and of men 193 mg/dl /25/. From the comparison of the percentiles it is clear that about 25–30 % of the investigated population has a cholesterol level above the "desirable" 200 mg/dl. The same is true for a USA population (75th percentile level in males was found at 228 mg/dl; in females at 216 mg/dl) /25/.

The mean values of TG did not show significant differences between the male and female groups. Lindgren et al. /16/ found significant sex differences between the mean values of TG (TG levels were higher for males than for females), no differences were reported by Kostner et al. /15/.

The LDL levels show a sharp rise from the age of 12 years in both sexes; the rise weakens between age of 25 and 35 years, and the levels decrease in the sixth decade in males. Mean values in females, however, continue to rise from about the 55th year onwards /10, 28/. It has been suggested that the difference in lipoprotein levels during the reproductive age is due to a controlling effect exerted by the sex hormones.

The well-known higher HDL-C levels in females compared to males /1, 19, 29/ was also observed in our population. HDL-C values higher than 50 mg/dl were seen in most of the females studied, contrasted to the 45 mg/dl found in males. Our results are very similar to those reported by Wahl et al. /27/ and by Heiss et al. /11/.

As seen in Table I, LDL-C values are significantly higher in males than in females. Again, about 25–30 % of the subjects investigated has an LDL-C level above the "desirable" 130 mg/dl level. In the USA the 75th value: females: 137 mg/dl, males: 138 mg/dl /25/. According to the recommendation of the National Consensus Conference /25/ about 1/4 of the population aged 20–39 years requires special medical attention in Cuba in order to prevent ischaemic cardiovascular diseases.

Several epidemiological studies have confirmed the importance of the TC/HDL-C ratio in predicting coronary heart disease /26, 30/. Swanson et al. /24/ found that this ratio facilitates the differentiation between subjects with or without cardiovascular diseases. In cardiovascular diseases this ratio can be about 6.27 as reported in the Framingham Study /30/ and in an Israel Study /7/.

The present results are similar to those reported by others /2, 9/. We observed a significant positive correlation between lipids and lipoproteins with age. In males the levels of HDL were negatively correlated with age, indicating that, in males the risk of cardiovascular diseases

could increase with the age. On the other hand, in females only TC, LDL and LDL-C correlated, positively, with age.

It was shown previously that obesity was associated with elevated serum lipid and decreased HDL-C levels /3/. We found a close correlation between relative body weight and HDL-C, in both males and females.

Experts in the USA and Europe have worked out recommendations for the primary prevention of ischaemic heart disease. Their opinion is that cholesterol values should not exceed 200 mg/dl /23, 25/.

From this point of view about 40% of the patients investigated in this study requires special medical attention. It is believed that in Cuba obesity is an important risk factor and it may be associated with pathological HDL-C levels and $Risk_{1-2}$. In evaluating coronary risk during a prospective study in Cuba the determination of predictive lipid and lipoprotein variables (including HDL-C and risk ratios) is recommended.

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# LIPID AND LIPOPROTEIN PROFILES IN CUBAN CHILDREN OF THREE AGE GROUPS

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We determined the lipid and lipoprotein levels in a selected group of apparently healthy adult Cuban subjects in a previous paper /27/. In this paper the basic lipid variables (TC, TG, HDL-C) in 271 healthy children are published. LDL-C levels were also calculated.

A small, but continuous, rise was found in the TC level between 0 and 14 years in both sexes. The rise of TG was accompanied by HDL increase in girls but by LDL increase in boys. This phenomenon might explain the augmented susceptibility of men to ischaemic heart disease. Children at "high risk" should be identified (in case of positive family history of ischaemic heart disease) by cholesterol determinations, the borderline of the pathologic cholesterol levels seems to be very similar to that found in the USA, 170–190 mg/dl in the age group between 0 and 14 years.

Keywords: Lipid, lipoprotein, serum, cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, children of three age groups

Abbreviations: TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol

## Introduction

The determination of total plasma cholesterol and lipoprotein-cholesterol levels is very important because of their predictive role in

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the risk of coronary heart disease /3, 17, 24/. The rate of progression of atherosclerosis is directly related to serum cholesterol and LDL-C level /12, 30/. However, serum cholesterol and LDL-C vary greatly in relation to age, and their contributions show striking diversity among different populations /9, 10, 23/.

Recent population studies of schoolchildren have characterized paediatric risk factors for adult coronary heart disease, including variables of lipid and lipoprotein metabolism, reflecting the aggregate of environmental and genetic factors /6, 8, 32/.

The aim of this study was to measure the basic lipid and lipoprotein levels in a group of Cuban schoolchildren in order to obtain probable basic reference values.

## Subjects and Methods

271 healthy children (135 boys and 136 girls) between 0 and 14 years of age completed the study. The subjects were divided into three age groups: 0–4 years old (56 boys and 46 girls), 5–9 years old (35 boys and 40 girls) and aged 10–14 years (44 boys and 50 girls). Each group was submitted to medical examinations to reveal possible diseases related to lipid metabolism and medication that might affect blood lipid concentrations.

Sampling: Blood specimens were obtained from children who had fasted for at least 12–16 h. Serum was separated from the cells by centrifugation (2500 r.p.m. for 2 min).

Total cholesterol was measured using the ferric chloride method described by Zlatkis /33/. Standardization was carried out with Preciset (Boehringer-Mannheim, BRD) as standard and Precinorm U (Boehringer-Mannheim, BRD) as control.

Triglyceride was assayed in the acetyl-acetone colorimetric system /13/.

HDL-C was measured after phosphotungstic acid and $Mg^{2+}$ precipitation as described by Lopes Virella et al. /22/.

LDL-C was calculated according to the Friedewald formula /14/:

$$\text{LDL-C} = \text{TC} - \frac{\text{TG}}{5} - \text{HDL-C (mg/dl)}$$

Results were expressed as mean $\pm$ S.D. Student's paired t test was used.

## Results

A small, statistically not significant, but consistent, increase in TC level was observed between 0 and 14 years in both sexes.

An elevation was found in HDL-C at age 4-5 years in girls, and an increase of LDL-C in 9-10 years old boys.

The increase of total cholesterol level in boys was caused by the LDL-C elevation. In girls, on the contrary, the total cholesterol increase was associated with HDL-C elevation (Table I)

**Table I**

Profiles of plasma lipid and lipoproteins in Cuban children

| Age, years | Total cholesterol | Triglycerides | HDL-C | LDL-C[b] |
|---|---|---|---|---|
| Males | | | | |
| 0 - 4 | 159 ± 29[a] | 79 ± 36 | 40 ± 12 | 105 ± 33 |
| 5 - 9 | 163 ± 34 | 64 ± 35 | 45 ± 18 | 105 ± 36 |
| 10 -14 | 165 ± 36 | 81 ± 30 | 41 ± 11 | 113 ± 30 |
| Females | | | | |
| 0 - 4 | 151 ± 28 | 101 ± 58* | 32 ± 11* | 100 ± 33 |
| 5 - 9 | 153 ± 23 | 65 ± 30 | 44 ± 13 | 97 ± 25 |
| 10 -14 | 160 ± 27 | 98 ± 40* | 44 ± 15 | 98 ± 28* |

[a]Mean ± S.D., mg/dl; LDL-C[b] was calculated according to Friedewald /14/.
*Significant difference ($P < 0.05$) between boys and girls

## Discussion

Coronary artery disease in adults has strong associations with increased LDL-C and decreased HDL-C levels /2, 15, 19, 20, 21, 25/. Recently a relationship of clinical risk factors to vascular lesions in children (and young adults) has been confirmed.

Total cholesterol and LDL-C in serum are strongly associated with the extent of aortic fatty streaks. Higher values of triglycerides were observed (115 mg/dl versus 71 mg/dl) for serum triglycerides, too /26/.

Children at "high risk" should be identified primarily by carefully obtained family histories. A family history of hypercholesterolaemia or premature coronary disease of parents, grandparents or first-degree relatives should alert the physician to assay blood cholesterol /11, 29/. A vast number (65–75 %) of children with positive risk factors does not have a parental history of cardiovascular disease; this observation underlines the importance of measuring serum lipids and lipoproteins in children as predictors of disease /5, 8, 21/.

Results obtained from our investigations are very similar to those described by other authors in American population /1, 9/.

The problems of nromal reference values are complicated with the fact that dramatic changes of lipoprotein concentrations in serum occur during sexual maturation, establishing adult patterns of greater concentrations of LDL-C and decreased concentrations of HDL-C /7, 31/. Age-related changes are generally believed as a physiologic phenomenon, however, in populations with a low incidence of ischaemic heart disease the changes are minimal after early childhood /8/.

The increase of LDL-C and the small decrease of HDL-C in boys may reflect the changes seen in puberty.

Our data indicate that the "borderline" levels of intervention proposed by the Consensus Conference /11/ are very similar (170–190 mg/dl for total cholesterol) in the Cuban population and the guidelines of screening and intervention are also valid for this country.

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# ALOPECIA AREATA AND BISALBUMINAEMIA (A CASUAL COINCIDENCE)

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(Received: September 29, 1988)

Bisalbuminaemia, the heterozygote manifestation of an autosomal dominant condition, is detected by electrophoresis, when two distinct albumin bands are separated. Approximately 50 cases with bisalbuminaemia have been published worldwide. This phenomenon is very rare in Central Europe. — A 17-year-old Hungarian girl, admitted for an alopecia areata of bacterial origin (tonsillitis), was found after routine electrophoresis to have bisalmbuminaemia. The albumin variant was of the "slow type". The ratio of the two components was 1:1. Immunoelectrophoresis with anti-albumin serum showed only one precipitation band in the albumin region. — The rarity of bisalbuminaemia appeared to justify publication.

Keywords: Bisalbuminaemia, heredity, rarity in Central Europe

## Introduction

Human serum albumin has particular genetic interest because, like haemoglobin, it is abundant in blood, easily detected in a variety of electrophoretic systems, and subject to extensive incidental screening in clinical laboratories. Nevertheless, appreciation of the diversity and frequency of albumin variation has developed slowly.

Bisalbuminaemia (paraalbuminaemia, alloalbuminaemia) was first described by Scheurlen /5/ and its familial occurrence demonstrated by Knedel /3/. The anomaly has since been recognized in individuals and

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families of ethnic groups from all over the world and at least 23 genetic variants have been distinguished /8/.

Bisalbuminaemia is the heterozygote manifestation of a group of electrophoretic albumin variants, all apparently inherited by an autosomal co-dominant mechanism.

Bisalbuminaemia is detected when two distinct albumin bands are separated. They are not usually differentiated by ultracentrifugation and on immunoelectrophoresis. Several methods — for example dye binding, thyroxine binding and heat stability — have been used as aids to classification of the polymorphs, but any complete solution will await the full structural elucidation of the albumin.

The literature of these cases have been reviewed by Melartin et al. /4/ and their classification attempted by Tárnoky et al. /7/. They exist as rare traits in families of Western European descent but are common in American Indians and Eskimos. Approximately 50 cases with bisalbuminaemia have been published all over the world. The first Hungarian bisalbuminaemic case was published by Donhoffer et al. /1, 2/.

## Clinical and laboratory findings

A 17-year-old Hungarian girl, admitted for alopecia areata of bacterial origin (tonsillitis), was found on routine electrophoresis (barbitone buffer, pH 8.6) to have bisalbuminaemia (Fig. 1). The albumin variant was of the "slow type". The ratio of two components (stained with amino black) was 1:1. Immunoelectrophoresis with whole antihuman and anti-albumin sera showed only one precipitation line in the albumin region (Fig. 2). A new two-dimensional (crossed) agargelelectrophoretic method (first run is the electrophoretic separation of proteins and the second one is the precipitation with anti-albumin serum) showed a double-curved precipitationline of albumin (Fig. 3). The mother's serum did not show a bisalbuminaemia (Fig. 1). The father's and grandparents' sera were not available. Other laboratory data: blood group: "B" Rh positive. HLA: A 2.26, B 7, Cw 4. Immunoglobulins: IgG: 11.6, IgA: 2.4, IgM: 1.5, C3: 0.7 g/l.

The first bisalbuminaemic case was published by Donhoffer et al. /1, 2/ in Hungary: a patient and her two children were affected. The presented case is the second Hungarian one. This phenomenon is very rare in Central Europe. — Weitkamp et al. /8/ summarized the results of bis-albuminaemic cases: 23 different types of albumin variants have been distinguished by comparative starch-gel electrophoresis. Albumin B, the most

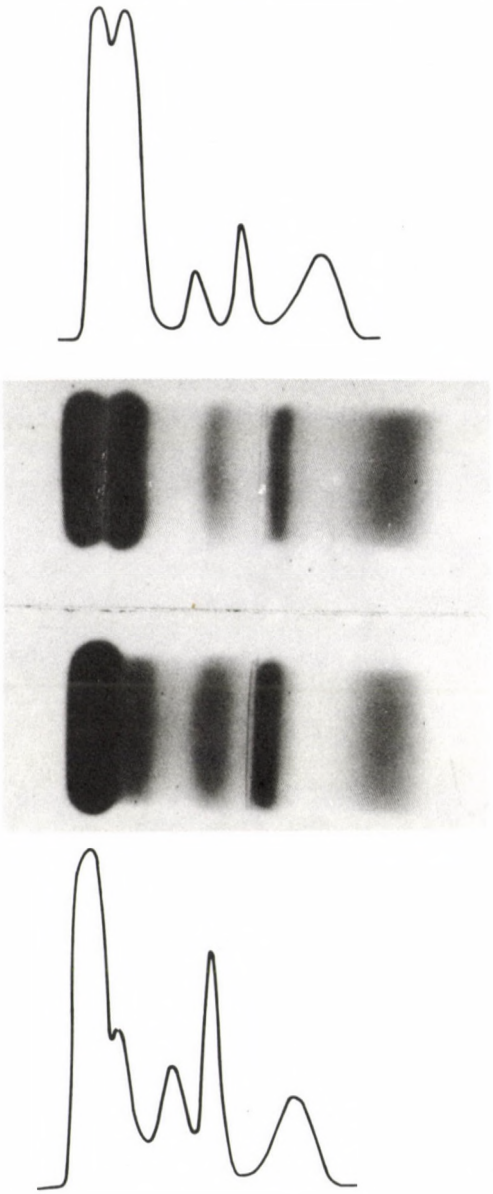


Fig. 1. Agar gel electrophoretogram: the patient with bisalbuminaemia (above), and her mother with single albumin (below)

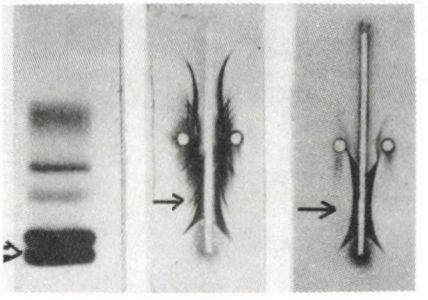
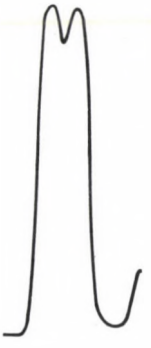



Fig. 2. Agar gel electrophoretogram: bis-albuminaemia (double arrow). Immuno-electrophoretograms: one precipitation-line of albumin (single arrow, immunsera: anti-human polyvalent serum = AHS, and anti-albumin serum. "Human" Budapest)



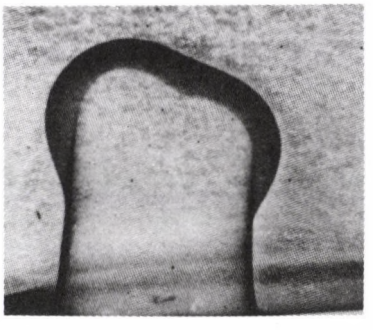


Fig. 3. Crossed immunoelectrophoretogram: immunserum: anti-human albumin ("Human", Budapest) (below): the method is a combination of a simple gel electrophoresis and electro-immunodiffusion; (above: the insert picture of double albumin of patient)

common European variant and possibly the product of a single allele, has a frequency of less than 1 in 1000 and yet is widely distributed among Europeans of different ethnic origin /6/.

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# LIFE EVENTS AND SEIZURE FREQUENCY IN EPILEPTICS: A FOLLOW-UP STUDY

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On the basis of 2227 examinations of 272 epileptic patients the connection between the seizure frequency and life events of negative or positive emotional nature was analysed. Negative life events went with a deterioration, positive factors with a decrease of seizure frequency in most cases. Results of antiepileptic serum measurements suggest that emotional factors exert their effect by influencing the patient's compliance. Supposed intrapsychic pathomechanisms of this connection are briefly discussed. Authors' results stress the importance of psychic care of epileptics simultaneously with medical treatment.

Keywords: Life events, epileptics, seizure frequency, intrapsychic pathomechanisms

Abbreviations: I: time interval between two examinations; P: psychogenic effect of a life event (negative or positive); NP: negative change in psychic state caused by a life event; PP: a positive change in psychic state caused by a life event

## Introduction

Life events, changes in circumstance of life, can play an important role in the development of all chronic illnesses. They are factors of extreme importance in epilepsies in several respect. (i) Epilepsy can have its own psychopathological symptoms; (ii) the evaluationof psychogenic pseudoseizures occurring besides, or instead of, real epileptic attacks

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constitute one of the most difficult problems in epilepsy care /10, 21, 22, 49/.

The importance of emotional elements affecting the epileptic process and some psychogenic factors involved in the induction of epileptic seizures are mentioned in nearly all reviews dealing with epilepsy and its borderland. However, objective measurement and statistical analysis supporting this connection are still lacking. This strange situation is also reflected by the fact that in the Handbook of Neurology, page 272. Vol. 15 (Epilepsies), the possible psychogenic aetiology of epilepsy is quoted without any further explanation /53/. Just in the opposite are the efforts to originate the manifestations of epilepsy from the patient's life history /6/ or to deduce them from a psychosomatic model /30/.

In our recent long-term follow-up study the possible connection between life events and seizure frequency has been analysed in a quite large epileptic population.

## Material and Method

The role of particular life events was evaluated by 2227 examinations of 272 outpatients (159 males and 113 females, age 18-24 years, mean 20 years) originating from the Epilepsy Department of the National Institute for Nervous and Mental Diseases, Budapest, Hungary. The patients observed for less than half a year were excluded. Fig. 1 shows the distribution of the numbers of examinations per patient.

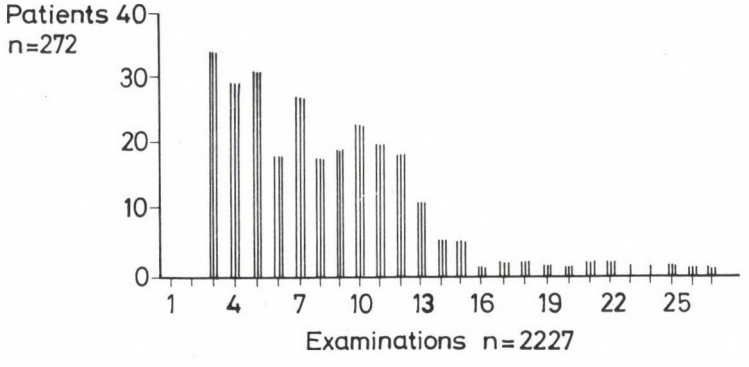


Fig. 1. Distribution of the patients by the nubmer of examinations during follow-up

Each interval (I) between two examinations (I = 16-12 weeks, mean 13 weeks) was clinically evaluated. Seizure-free periods (a) as well as "decreased" (b), stagnating (c) and "increased" frequencies (d) were distinguished.

"Decreased and increased" seizure frequency means changes by minimally two attacks compared to the preceding I. Also changes of medication, results of serum level measurements, and inexactly followed medical advices ("non-compliance") were registered. Data were registered in a standardized outpatient documentation /40/.

Supposed psychogenic effects (P) of the given life events were grouped as follows:

A) Lasting negative changes in psychic state (NP) were presumed to take place upon the effect of the following life events:
   1) mainly exogenic psychic exhaustion
      a) in the family: death or illness of relatives or closer acquaintances, accident, serious or chronic illnesses, etc.
      b) unexpected financial difficulties, legal issues, divorce, etc.
      c) changes at home or at the working place with strong emotional consequences (new job, reorganization, moving to a new flat, change of personnel or in the family, etc.)
   2) mainly physical exhaustion: newly-organized shift work, extra work, building or renovation in progress, etc.
   3) psychodynamic factors: intrapsychic or environmental conflicts mentioned by the patient spontaneously.

B) Positive changes in psychic state (PP) caused by the following life events:
   1) mainly psychic effects
      a) in the family: engagement, marriage, planned pregnancy, successful childbirth, birth of grandchild, etc.
      b) in working place improving circumstances, appointment, premium, reward, etc.
   2) mainly physical effects
      new flat, desired release from working place (new job with less problems, retirement, etc.), passed examinations, obtained qualification, etc.
   3) events omitted from points 1 and 2 of paragraph B caused elimination of negative factors mentioned in part A.
   4) psychodynamic factors mentioned by the patient.

Our list is based on the "Life Event Scale" elaborated by Holmes and Rahe /15/, modified by Paykel /33/ and adapted for the Hungarian population by Tringer and Veér /51/.

Items dealing with events accompanied by material changes in the patient's surroundings, in personal connections or in his/her life style were only taken into consideration. These, so to say well-assessable events evoke acute emotions of similar directions (either negative or positive) in every person (their possible paradoxical psychodynamic effects come to the foreground only later).

Emotional consequences of certain life events (disputes, controversies etc.) depend on the patient's personality and occasional psychopathological symptoms /8/; their interpretation may even be influenced by the personality of the examiner /32/. In view of their problematic nature, these life events were not evaluated. We could not apply the "dependent-independent" list of life events constructed by Jacobs and Myers /18/ either.

The importance of particular events taking place mainly intrapsychically is unanimously supported by daily routine and by literary data. These events were only taken into consideration when they were given account of spontaneously by the patient and were evaluated form the same aspect as

reported (e.g.: crisis in marriage, decisions, arrangements for divorce, etc. See also paragraphs A.3 and B.4 before).

## Results

I. Connection between life events and seizure frequency

Figure 2 shows the distribution of the clinical picture during the investigated 2227 Is. The rather high number of seizure free Is reflects a global efficacy of the antiepileptic treatment. The distribution of Ps is seen in Fig. 3. The prevalence of NPs is remarkable. Figure 4 demonstrates the interrelation of the seizure frequency and psychogenic effects of life events (NP and PP) in 601 of the registered intervals.

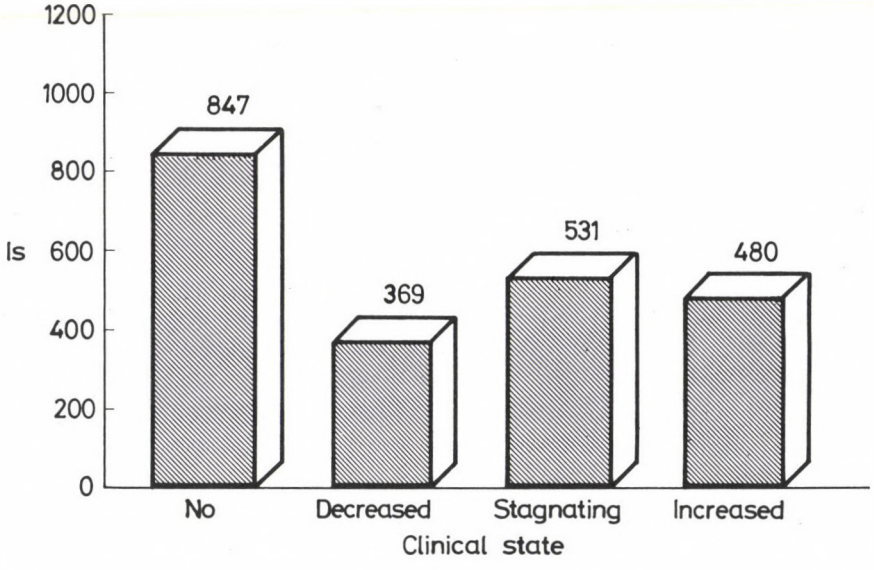


Fig. 2. Number of seizures (no, decreased, stagnating, increased – definitions in text) in 2227 Is compared to the preceding I

II. Serum level measurements

Antiepileptic serum level measurements were made in 378 cases. The values were within the therapeutic range in 160 cases, in 102 cases they were below, and in 116 above, the therapeutic range. In case of combined treatment the change in the measured results was judged according to the alteration in the serum level of one component. Few measurements were made in IS with Ps. Distribution of such Is is seen in Fig. 5

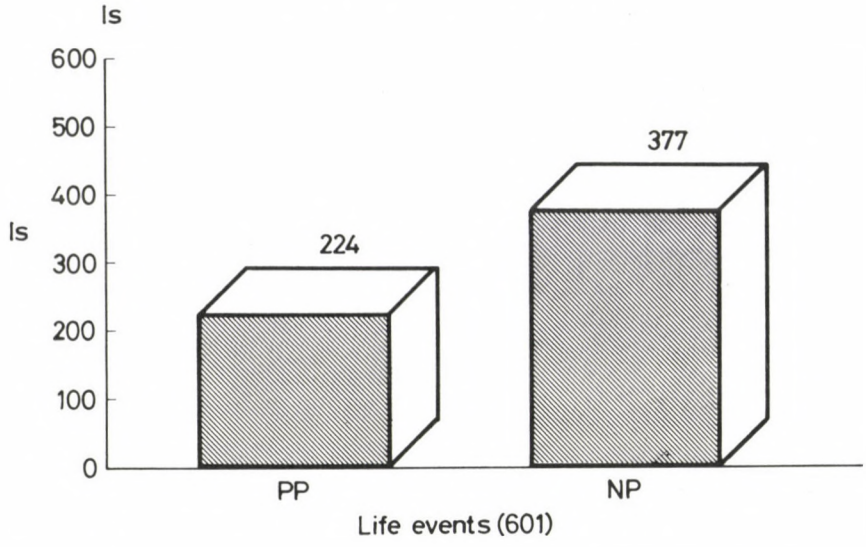


Fig. 3. Occurrence of PPs and NPs during the follow-up

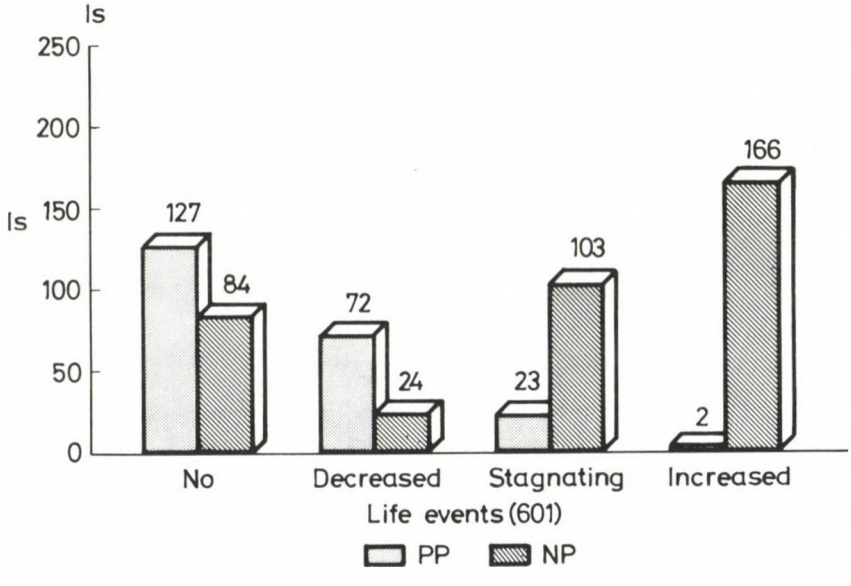


Fig. 4. Distribution of the clinical picture in Is with PPs and NPs

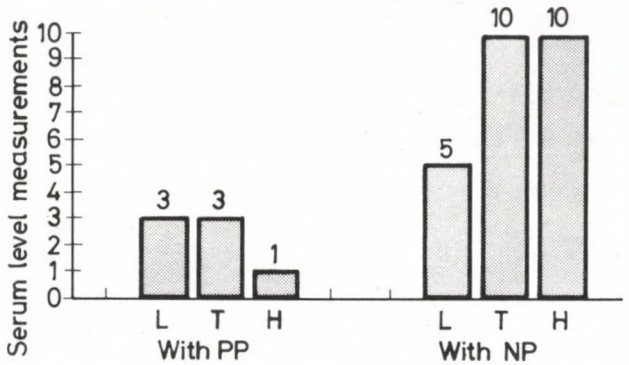


Fig. 5. Results of serum level measurements in Is with PPs and NPs

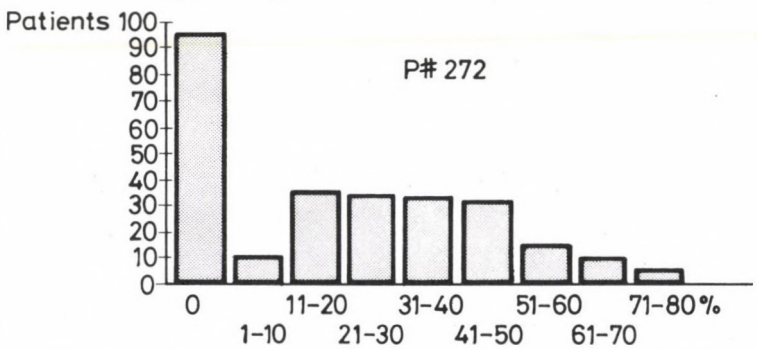


Fig. 6. Distribution of the population according to the probability of the occurrence of Ps. (Characterized by the number of all Ps and Is for each patient, expressed in percentage)

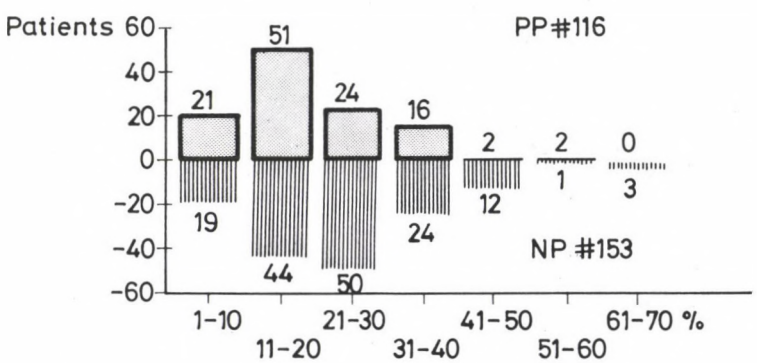


Fig. 7. Distribution of the population according to the probability of the occurrence of PPs and NPs, expressed as in Fig. 6

III. Life events and "non-compliance"

"Non-compliance" was found in almost 10% (in 191 examinations) of all Is. In 24 Is P occurred, 2 with PPs and 22 with NPs.

IV. Probability of the occurrence of P

After the global analysis of Is, the incidence of P, PP and NP was investigated in the 272 patients. Probability of their occurrence for a given patient can be calculated as the ratio of the total number of the given life event to the total number of Is:

Probability of P, PP, NP = $\frac{\text{all Ps}}{\text{all Is}}, \frac{\text{all PPs}}{\text{all Is}}, \frac{\text{all NPs}}{\text{all Is}}$ for a given patient.

The probability of all Ps is shown in Fig. 6, that of NP/PP in Fig. 7. During the whole study period 96 patients had no P, 119 no NP, 156 no PP.

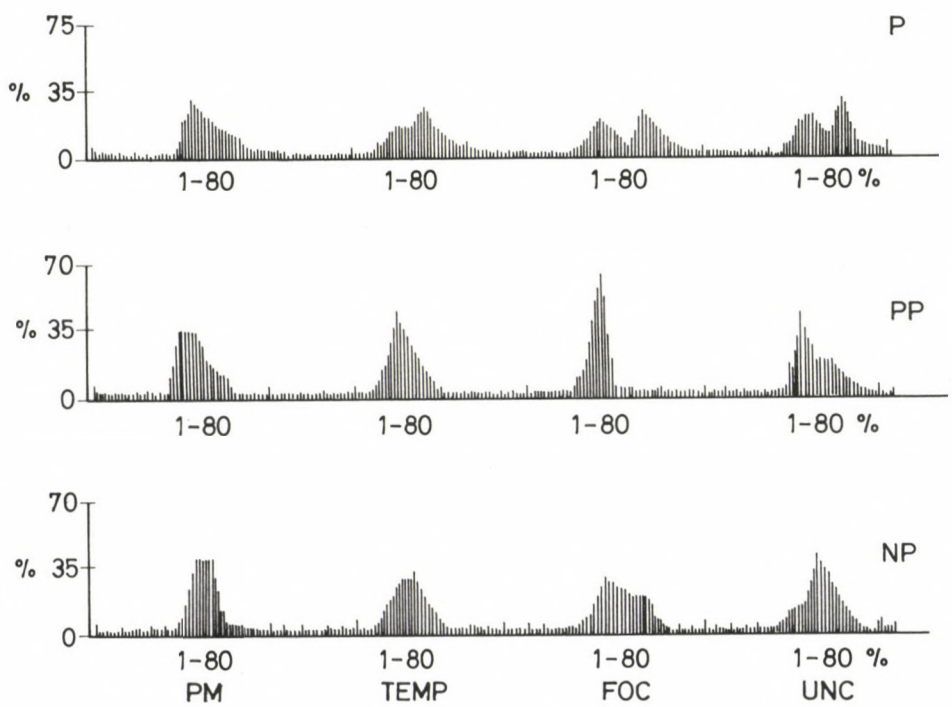


Fig. 8. Probability of the occurrence of Ps, PPs and NPs in different types of epilepsy. Horizontal percentages as in Fig. 6. Vertical axis: Percentage of patients in the different epilepsy groups

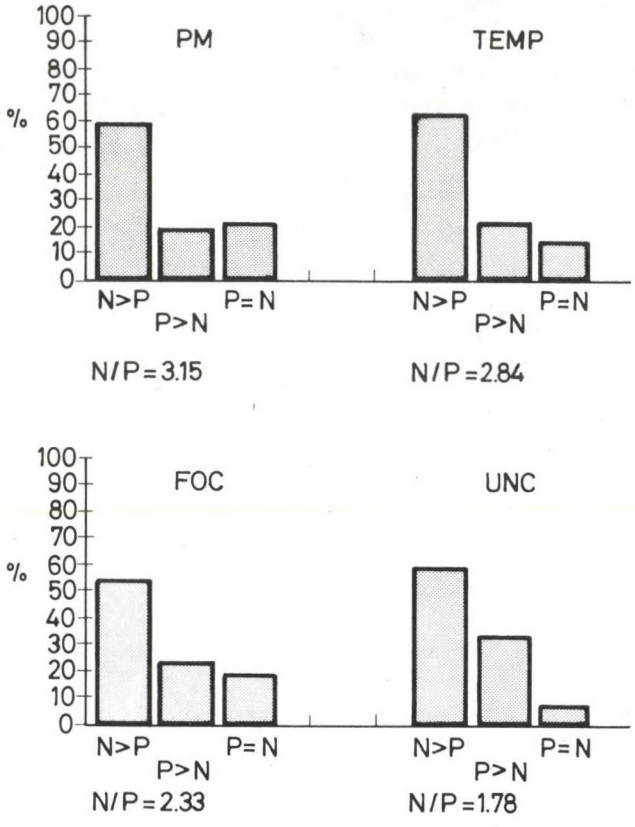


Fig. 9. Ratio of patients with more NPs than PPs (N P) and with more PPs than NPs (P N) and with equal number of PPs and NPs in different types of epilepsy. Vertical percentages and abbreviations as in Fig. 8. Numbers at the bottom: Ratio of patients with NP and PP predominance (N/P)

V. P and the type of epilepsy

Distribution of our population by the type of epilepsy was as follows:

| | |
|---|---|
| primary generalized | 53 cases |
| focal, not temporal | 43 cases |
| temporal lobe | 133 cases |
| undetermined | 43 cases |

Small histograms of Fig. 8 show the distribution of our patients by the type of epilepsy and the probability of Ps, NPs and PPs. Places of the highest peaks may indicate the most characteristic values for the probability of Ps, NPs and PPs according to the type of epilepsy.

In the different types the ratio of NPs/PPs was also studied. Prevalence of NPs was found in all groups but the ratio of NPs/PPs was different. The curves in Fig. 9 show the number of patients with prevalence of NPs and PPs, respectively, and that of patients who experienced both types of Ps in equal numbers. The fractions at the bottom indicate the ratio of patients with predominance of NPs and PPs, respectively.

## Discussion

Interrelation between the changes of psychic state accompanying life events and the symptoms of chronic diseases are well-known by patients and doctors alike. In spite of this, an exact quantitative assessment of this connection is rather difficult and prognosis is in most cases impossible. Registration and recalling of life events is not free from subjective elements /19, 56/ and the intensity of stress connected with life events is also the function of individual circumstances /5, 16/. We, too, met the difficulty of evaluating subjective factors. Several of our patients reported a change in the intensity or duration of their seizures connected with life events. Their data were not evaluated because in these cases the emotional work-up of feelings regarding seizures seemed altered.

1) Observing only the fluctuation in seizure frequency (Fig. 2), a high number of stagnating or worsening states (in nearly 1000 Is) is conspicuous. This fact surely does not remain emotionally unnoticed by the patients. It can, however, be supposed theoretically that the relative inefficiency of the treatment affects the next I through psychogenic dimensions, and spoils the result of the treatment. (For clearing this mechanism, performance of a sequence analysis is planned.)

2) The distribution of Ps contains an important information. Epileptics experience are three times more frequently negative than positive life events. This ratio surely reflects an unfavourable societal judgement of the disease. The epileptic is faced with a variety of unsolved psychosocial problems. Medical professionals managing epileptics know well the exaggerated opinions prevailing on epilepsy and the epileptic person either in forensic medicine /17/, or at evaluation of working /9, 12, 34/ and driving ability /38/. These exaggerations interfere with the social adjustment of the epileptic patients /4, 44/. Extreme emphasis placed on biological factors of the disease may unduly spoil the possibilities of practically

independent social development /3, 43/. The psychic effects of epilepsy may insidiously get integrated into the patient's self concept and in the balancing mechanism of his/her family and personal life /40, 44, 50/.

Loss of feeling of health, experience and pathologic emotional effect of therapy resistance can affect the patient's self image and weaken the psychic protective mechanisms /35/. In consequence of them suicides and neurotic decompensation occur 4-5 times more frequently among epileptics than in general /12, 20, 29/.

3) Figure 4 shows a tendency-like correlation. In seizure-free or improved states mainly PPs occur while stagnation or deterioration of the clinical picutre is accompanied by the predominance of NPs. Of course, causality of this tendency cannot be decided in our study, though, as it was already mentioned, even a positive "feed-back" mechanism may be thought of. In epileptic care it is beyond doubt that in a poor emotional state a deterioration of the clinical picutre can be expected and vice versa: deterioration of the seizure control will probably be associated with a bad psychic condition.

4) It deserves attention that subtherapeutic antiepileptic serum levels were found much more frequently in Is with NPs than in general. This implies the possibility that NP may lead to an increase in the number of seizures through non compliance /36, 37, 45/. This mechanism appears to be supported by our following case report:

S.L., a 30-year-old male patient. His father had several generalized convulsions of grand mal type of uncleared origin in his twenties. At the age of 14 years the patient had an "idiopathic" facial nerve palsy. He was 17 when in a period of frequent alcohol abuses a right hemiparesis and a series of grand mal seizures suddenly occurred. He was urgently observed and, because of a progressive intracranial space-occupying process, left temporoparietal craniotomy was performed. (Only a local purulent tissue was removed.) Diagnosis of the final report was circumscribed encephalitis. He was completely seizure-free until the age of 24, whereafter his seizures recurred. Several times in a month, occasionally in series, he had complex motor automatism together with a slight loss of consciousness. EEG records indicated only a local theta activity over the left temporal region. A therapy-resistant stagnating state persisted for years.

At the beginning of his care at our Department, polytherapy with four antiepileptic components was significantly reduced, whereupon he reported worsening of his seizure frequency. Antiepileptic serum level

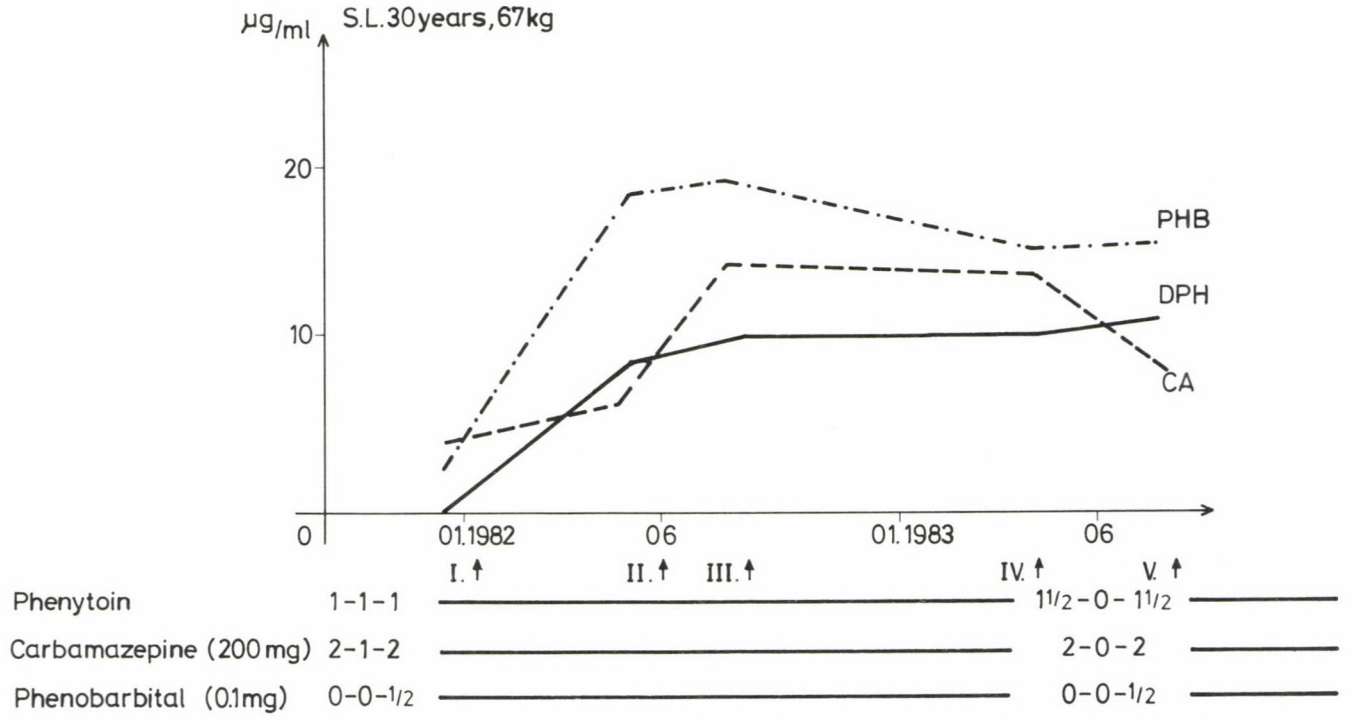


Fig. 10. Changes of antiepileptic serum level in patient SL

measurements showed low concentrations of each component (Fig. 10) though the patient stated to have taken the prescribed doses regularly.

Psychic exploration revealed a serious family situation. His wife had left him a year before his admission because, as she said, she was unable to live with an ill man. She was seduced by his colleague. The custody of their 3-year-old son was awarded to the mother. She refused to let him take the child with him for the weekends because of the danger of seizures. He could therefore fetch his son only in somebody's company (mostly accompanied by his parents). We have then provided him with a medical certificate which enabled him to see his son alone. On these occasions he usually talked to his ex-wife in private. These disputes upset him and his clinical state deteriorated. A second measurement of his antiepileptic serum concentration revealed a slight increase of the barbiturate level into the therapeutic range (Fig. 10.II). The patient reported to have taken of his mother's sedative pills at the times of his nervous periods.

His seizures gradually decreased in frequency and intensity and he stated to have achieved the best epileptiological state he ever had. Beside unchanged medication all serum levels rose to the therapeutic range (Fig. 10.III). The good clinical state appeared lasting. He had a girl friend, but maintained good relation also with his ex-wife. Control measurements showed unchanged antiepileptic serum levels (Fig. 10.IV).

5) An analysis of the data of inadequate taking of drugs confirms the above correlation. In patients with PPs non-compliance was registered in 2, while in patients with NPs in 22 cases!

6) In almost two thirds of our patients P occurred during the follow-up (Fig. 7). Their majority gave account of Ps in 20—50 % of all the Is (at about every third examination). The occurrence of NPs was far more frequent, in several patients it was characteristic of the whole management (Fig. 7).

PPs occurred only in 116 patients so that the other 156 persons i.e. 57% of the whole population met no positive events during the follow-up! These data clearly demonstrate the situation in which the feeling of health may disappear and give place to feeling of stigmatization. An unexpected experience also appears to support his statement: Self-image, expectations for the future and connections with friends and parents of young epileptics in a relatively balanced clinical state are more conflictuous and more problematic than of those with a more serious epilepsy /14/. This paradoxical constellation is to be taken into consideration in the daily routine, too (as the following case report shows):

S.T. 24-years-old female patient. She had no epileptogenic noxa either in the family or at birth, or in early childhood. At the age of 14 years a febrile state developed with permanent bifrontal headache. On the third day of her illness she had convulsive seizure with adversion to the right side. On the basis of CSF investigation and EEG alterations a para-infectious encephalitis was established. During an 8-week hospitalization she had complex partial seizures with speech arest alternating with disturbed speech intentions, and postictal tenebrosity. Her neurological status normalized, but seizures kept recurring, once or twice in a month.

Antiepileptic treatment with primidone was instituted, but the drug caused strong side-effects (skin rash). Two months later the febrile state recurred. Cerebral abscess was excluded, and the acute phase of her illness got terminated. When first treated at our Department with hydantoin-sultiam combination, her seizures decreased in frequency to one attack per month, occurring usually during night sleep. She pursued her studies at the secondary school to which she had been admitted before her illness, two years earlier. She is an only child, overprotected by her elderly parents. After about one year antiepileptic medication her seizures disappeared, but at the control examinations she continued to report on permanent nervous tension, and on fear of renewed seizures. Probably because of being older than her class mates, they did not admit her to their company, and she was unable to establish closer friendly contacts. She could not discuss her problems at home, and her parents were trying to put restrictions on her on account of her illness. Although she had no longer fits, she still did not feel healthy. We organized conversations with her parents to reveal the patient's intrapsychic conflicts. We suggested her to go out more frequently, and were trying to persuade the parents to regard their daughter as healthy and to release her from restrictions.

She had been seizure-free for 3 years even in the period before taking her final examination at the secondary school. Although the right posterior slow wave focus persisted on the EEG (Fig. 11), a reduction of her antiepileptic doses was considered.

It was at that time that she unexpectedly attempted suicide by ingesting about 200 antiepileptic tablets. She recovered consciousness only after two days. Five days later she had a generalized grand mal seizure. From this time on she had complex partial seizures at monthly intervals in spite of treatment with phenytoin-sulthiame, then with carbamazepine-sulthiame, and later with carbamazepine monotherapy. Beside this incomplete-

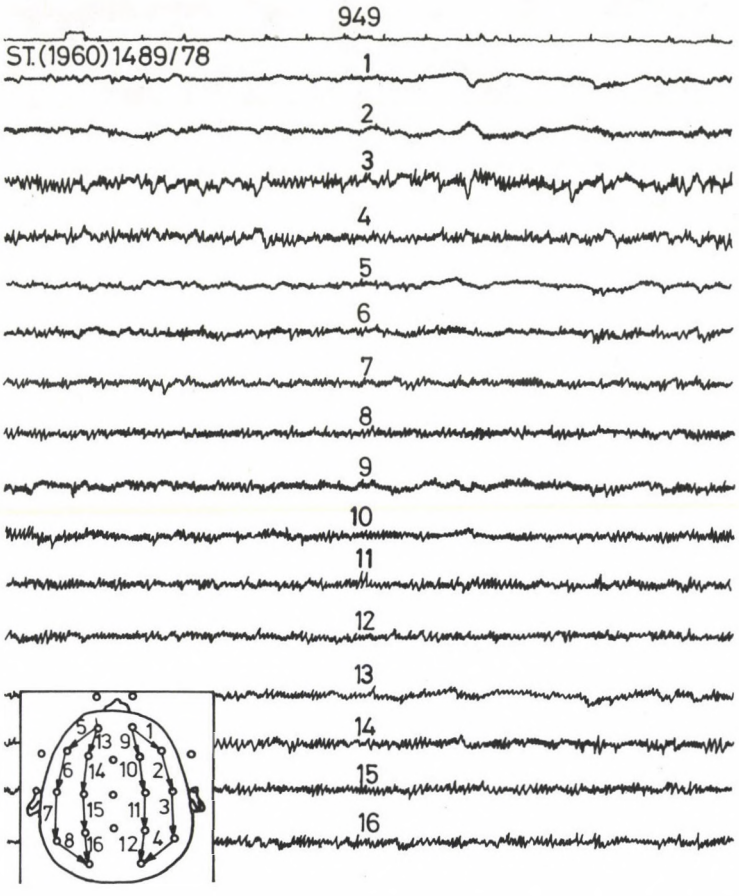


Fig. 11. Interictal EEG of patient S.T.

ly controlled epileptologic picture the patient's psychic state appeared to have improved. She passed her final examinations, learned a good trade, and established a lasting partnership.

7) Occurrence and distribution of Ps in different types of epilepsy seemed to be a not less important issue. But an analysis of our data revealed no difference between types of epilepsy. The various types could be better distinguished on the basis of the NP/PP ratio (Fig. 9). Among the patients suffering from generalized epilepsy the predominance of NPs over PPs was 3.15. This ratio was 2.84 in temporal lobe epilepsy, 2.33 in focal, not temporal and 1.78 in the undetermined group. The highest incidence of generalized type might be somewhat unexpected because this form is clinical-

ly the most benign type. The "psychogenic malignancy" of benign epilepsies was referred to above. Here we should like to draw attention to another characteristic feature of this mechanism. The seizures, as the main symptom of the disease, – especially in case of an easy drift or of a rare occurrence – may remain unnoticed by the environment so that the epileptic patient appears healthy. However, the risk that seizures are witnessed by others, the fear from injury, the experience of temporary loss of sensory control, inability to get protection against them, and living permanently on the border between perfect health and serious disability ("marginality") render the psychic work-up of the symptoms almost impossible.

We have tried to assess the role of psychogenic factors influencing seizure frequency by a statistical investigation. We have found a prevalence of negative emotional effects which may probably be a consequence of the psychosocial and intrapsychic tensions of the epileptic person /31, 48/.

Negative events increasing frustration might be in strong correlation with the deterioration of the clinial picture (increased seizure frequency).

Causality of this correlation has not been cleared in our study, but some data (as the results of antiepileptic serum concentration measurement and inadequate adherence to prescribed dosage) may imply a seizure-evoking effect of the negative emotional events by interfering with the patient's compliance. This possibility has already been analysed quantitatively.

Rimpau /41/ claims the activation of seizures on the basis of intrapsychic crises, Barker /2/ treated absences with psychotherapy successfully. Similar case reports have been published /14, 27/.

Rutgers /43/ achieved even better results by resolution of the social problems: seizure frequency decreased in 60% and antiepileptic medication was reduced in 14% of his population. The excellent case reports of Aird /1/ demonstrate the difficulty in the recognition of emotional factors, which usually are hidden among other provocative elements (alcohol, sleep deprivation, etc.) /54/.

Special, hitherto uncleared, psychologic mechanisms have come into operation in experiments when a decrease of seizure frequency was produced by reapplication of EEG-biofeedback training /22, 24, 47/. In a study reported by Matsuoka /28/ an experimental stress situation ("neurophysiological EEG activation") was created for studying the seizure-activating role of emotional tension. The experimentally activated anxious state unambiguously evoked paroxysmal EEG pattern in 25 of his 445 patients.

The main purpose of our study was to prove the connection of the above-mentioned factors without clearing their pathomechanism. Statistical analysis of our results unanimously indicates a correlation between the frequency of epileptic seizures and the patient's psychic state (primarily the level of emotional tension). The occurrence of psychogenic effects was registered in two thirds of the patients, at every 2nd or 3rd examination. In case of negative emotional effects deterioration of the clinical state (seizure frequency) might be expected.

Based on our results — in agreement with others /1, 7, 12, 13, 25/ we can state that without treatment of the emotional background even the up-to-date medicinal treatment alone will fail to produce optimal result in the management of epileptic symptoms.

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LABORATORY

# TRIACYLGLYCEROL AND GLYCOGEN CONTENTS IN THE HUMAN GASTRIC MUCOSA: EFFECT OF HISTAMINE $H_2$, MUSCARINIC AND GASTRIN RECEPTORS BLOCKADE

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Triacylglycerol and glycogen contents in the gastric mucosa were studied in 20 patients with duodenal ulcer before and after two-week treatment with cimetidine, pirenzepine or proglumide. Determinations of both metabolic substrates were performed in mucosal slices taken from gastric corpus and antrum during endoscopic examination: the results were expressed per mg of protein. Concentrations of triacylglycerol were significantly elevated after treatment with any of the blocking agents: in the gastric corpus by 88% after cimetidine application, 69% after pirenzepine and 102% after proglumide; in the antrum by 76, 59 and 132%, respectively. No significant changes in the mucosal contents of glycogen were observed. It is concluded that an increase in the mucosal concentration of triacylglycerol can be connected with an inhibition of the gastric acid secretion following treatment with receptor-blocking drugs.

Keywords: Gastric mucosa, triacylglycerol, glycogen, pirenzepine, cimetidine, proglumide

## Introduction

Triacylglycerols and glycogen are probably the basic endogenous sources of energy for acid secretion in the amphibian gastric mucosa /1/. Chacin et al. /5/ found that carbohydrates were the preferential substrates supporting secretory activity of the human gastric mucosa. On the other

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hand, lipids have been shown to be effective energy substrates for acid secretion in the amphibian and canine stomach /8, 10/. Recent data obtained in vitro have suggested that the metabolic requirements of the acid-secreting parietal cells demanded a combination of substrates like glucose, oleate, lactate, D-3-hydroxybutyrate, L-isoleucine, L-valine and acetoacetate /15/. These discrepancies can be related to variable experimental conditions or species differences.

In the present work we examined the effect of muscarinic, gastrin and histamine $H_2$ receptors' blockade on concentrations of triacylglycerol and glycogen in the human gastric mucosa. We have supposed that inhibition of acid secretion might be accompanied by an increase in content of the mucosal metabolic substrates.

## Material and Methods

The studies were done on 20 patients (15 males, 5 females) 19–71 years old (average age 42 years) with duodenal ulcer confirmed at subsequent endoscopy. The patients were divided into three comparable groups:
1. six patients were treated with cimetidine (Tagamet) – 1000 mg daily p.o.;
2. eight patients received pirenzepine (Gastrozepin) – 100 mg daily p.o.;
3. six patients were treated with proglumide (Milid) – 1200 mg daily p.o.

Endoscopy examinations were performed using fiberogastroscope GIF Q Olympus before, and after 2 weeks' treatment with blocking agent. Two h before the second endoscopic examination patients had received: cimetidine – 200 mg i.v. (1st group), pirenzepine – 10 mg i.m. (2nd group) and proglumide – 400 mg i.m. (3rd group). Gastric mucosal slices (3–5 samples) were taken from antrum and corpus.

Gycogen concentration in the mucosal samples was estimated immediately after receiving material according to Carroll et al. /4/. Triacylglycerols were isolated from the homogenized mucosal slices by Carlson's methods /3/ and determined according to Galletti /6/. Protein was estimated according to Lowry et al. /7/.

Results were evaluated statistically, using the paired Student's t test, accepting statistical significance at the $P < 0.05$ level. Mean $\pm$ S.D. are shown on the Figures.

## Results

As shown in Table I and Fig. 1, mean mucosal concentrations of triacylglycerol were significantly increased after treatment with cimetidine, pirenzepine and proglumide – respectively by 88, 69 and 102% (corpus) and 76, 59 and 132% (antrum), compared with the pretreatment values.

**Table I**

Triacylglycerol and glycogen concentrations in the human gastric mucosa before and after treatment with receptor-blocking drugs

| Blocking drug | Triacylglycerol nM/mg protein | | Glycogen nM glucose/mg protein | |
|---|---|---|---|---|
| | Corpus | Antrum | Corpus | Antrum |
| Cimetidine (n=6) | | | | |
| before treatment | 30.17 ± 12.17 | 33.83 ± 12.67 | 321.33 ± 45.17 | 344.6 ± 35.67 |
| after treatment | 57.00 ± 26.67 | 59.66 ± 22.83 | 326.50 ± 58.19 | 323.00 ± 36.83 |
| P < | 0.01 | 0.05 | NS | NS |
| Pirenzepine (n=8) | | | | |
| before treatment | 37.17 ± 17.50 | 29.33 ± 12.00 | 332.83 ± 56.83 | 334.50 ± 55.33 |
| after treatment | 63.00 ± 19.67 | 46.67 ± 16.67 | 345.83 ± 57.67 | 365.83 ± 73.00 |
| P < | | 0.01 | NS | NS |
| Proglumide (n=6) | | | | |
| before treatment | 22.00 ± 5.33 | 25.17 ± 10.50 | 314.00 ± 52.17 | 390.33 ± 78.67 |
| after treatment | 44.50 ± 11.83 | 58.50 ± 25.16 | 325.67 ± 64.17 | 353.17 ± 64.00 |
| P < | 0.01 | 0.01 | NS | NS |

n = number of patients

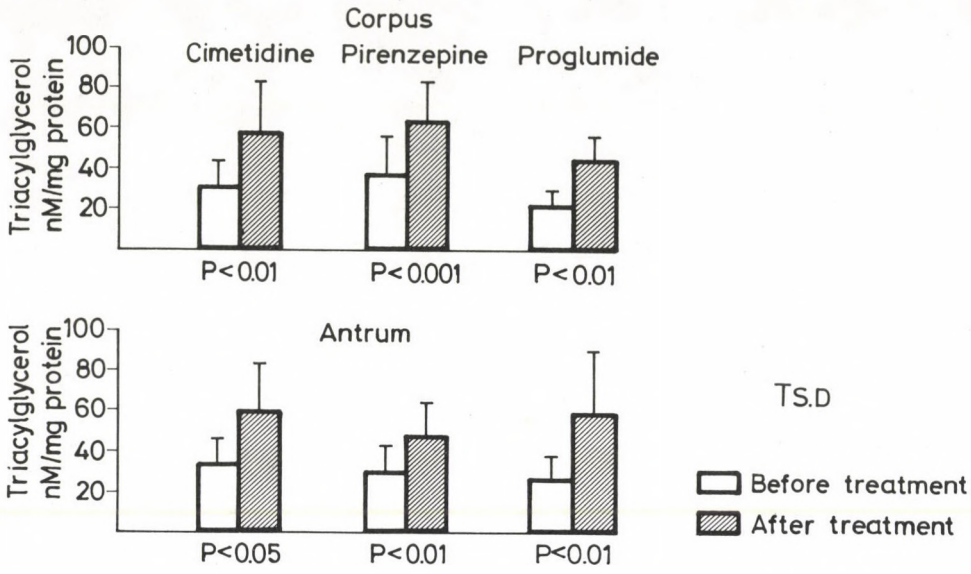


Fig. 1. Triacylglycerol concentrations in the human gastric mucosa before and after treatment with cimetidine (n=6), pirenzepine (n=8) or proglumide (n=6). Mean ± S.D., n = number of patients

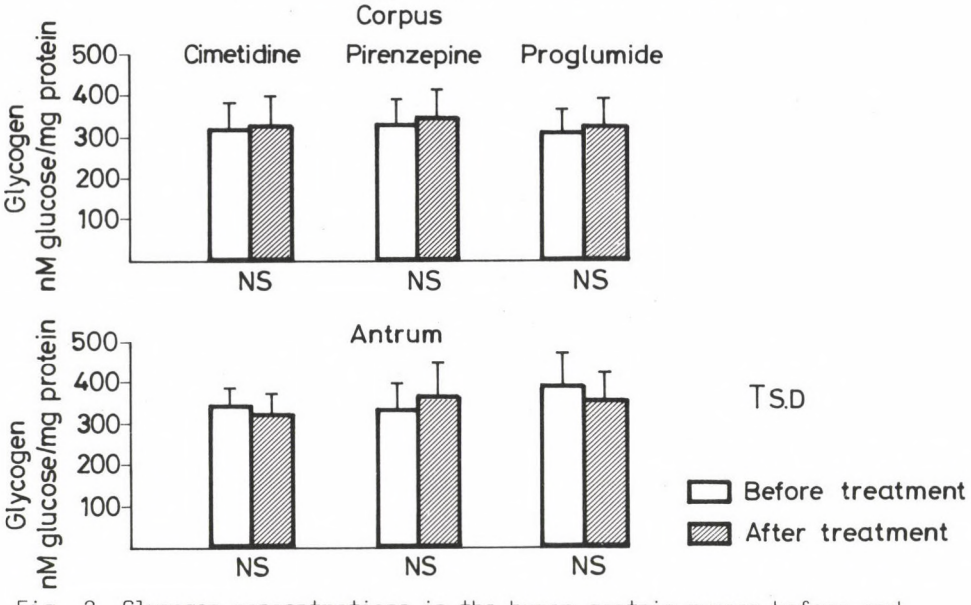


Fig. 2. Glycogen concentrations in the human gastric mucosa before and after treatment with cimetidine (n=6), pirenzepine (n=8) or proglumide (n=6). Mean ± S.D., n = number of patients

The mean concentrations of glycogen, both in the gastric corpus and antrum, estimated before and after treatment with receptor-blocking drugs were not significantly different (Table I and Fig. 2).

## Discussion

Receptor-blocking drugs (cimetidine, pirenzepine and proglumide), applied to our patients for two weeks and given parenterally as a bolus two hours before control examination, caused a significant increase in the mucosal concentrations of triacylglycerols. According to literature /12, 13, 16/ the doses of drugs were entirely sufficient for the evident reduction of gastric acid secretion. Therefore, we supposed that the observed increase in the mucosal content of triacylglycerol can be connected with inhibition of gastric secretion. The results of the present study are in accordance with our earlier findings that concentrations of triacylglycerol in the gastric mucosa were significantly higher in subjects with achlorhydria than in patients with gastric hypersecretion /11/.

Alonso et al. /1/ concluded that triacylglycerol and glycogen were the endogenous sources of energy for gastric secretion. They observed a decrease in mucosal concentrations of both metabolic substrates during prolonged incubation of the amphibian gastric mucosa. Sarau et al. /14/ found that the glycogen content in fundic mucosa of anaesthetized dogs fell by 20% after stimulation with histamine while glycolytic intermediates increased by 160–180 %; contemporarily, glycerol concentration increased by 100 %. Also Martinez and Chacin /8/ observed an increasing glycerol content in the toad gastric mucosa during incubation with histamine. Nalivaiko /10/, studying contents of lipids and glycogen in the canine gastric mucosa in conditions of histamine or carbocholine stimulation, documented that lipids were the main sources of energy for gastric acid secretion. Chacin et al. /5/, on the other hand, suggested that carbohydrates were the essential metabolic substrates in the human stomach.

Our results support the preferential role of lipid substrates in the gastric secretion. However, the lack of changes in glycogen concentrations before and after treatment with receptor-blocking agents could be caused by great lability of glycogen stores in the tissue, especially in the ischaemic conditions during biopsy procedure /9/. Based on recent investigations with isolated parietal cells /15/, we presume that gastric acid secretion

requires a combination of various lipid and carbohydrate intermediates.

Menguy et al. /9/ observed simultaneous occurrence of severe glycogen deficiency and mucosal cellular necrosis during experimental stress ulceration in rats. Bilski et al. /2/ reported a profitable role of mucus lipids produced by the gastric mucosa. We propose that an increase of the metabolic substrate contents in the human gastric mucosa following treatment with receptor-blocking drugs, can be protective against mucosal lesions.

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# KINETIC PARAMETERS OF SERUM AND LUNG TISSUE ANGIOTENSIN-CONVERTING ENZYME IN PATIENTS WITH LUNG CANCER

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Angiotensin-converting enzyme activity and its kinetic parameters were determined in both serum and lung tissue samples obtained from 17 patients with lung cancer and 21 control subjects, regardless of ethical base. The specific activities for cancerous and normal lung tissues were 67.2 ± 27.3 (mean ± SD) U/g protein and 28.5 ± 5.4 U/g protein, respectively ($P < 0.001$). Serum enzyme activity, on the other hand, was found to be higher in controls (198 ± 42 U/L) than in patients (237 ± 68 U/L) ($P < 0.01$). The effects of chloride and zinc ions, pH and temperature on the enzmye activity were investigated as kinetic parameters in both serum and tissue samples.

Keywords: Angiotensin-converting enzyme, lung cancer, tissue enzyme activity.

## Introduction

Angiotensin-converting enzyme (ACE, EC 3.4.15.1), a zinc-related peptidase, contains one atom of zinc per molecule /2/. It converts angiotension I into angiotensin II. In addition, it plays an important role in regulation of the vascular system /4, 12/. ACE is known to be of clinical, diagnostic and prognostic value /1/.

ACE is a membrane-bound enzyme /18/. It is stored on the luminal surface of vascular endothelial cells of the lung /6/ and, essentially

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produced in the lung /3, 15/. This study presents serum and lung tissue ACE activity and some of in kinetic parameters in patients with lung cancer and control subjects.

## Materials and Methods

### Tissue and serum samples

Cancerous tissues were obtained from 17 patients with definite lung cancer during operation, and normal tissues from 21 patients with no malignant lung and operated because of other causes. The tissue samples were immediately carried in ice-colded cases and stored at -70°C up to the test day. The sera obtained at the same time were stored -25°C.

### Tissue extraction

The tissue samples were trimmed and homogenized in a homogenizer with five-fold volume of cold buffer (50 mM potassium phosphate, pH 8.3). The homogenate was solubilized at 4°C for one hour with triton X-100 (0.2% v/v) /19/. Then, it was centrifuged at 10,000 g for 2 h. The clear supernatant was dialysed at 4°C for 24 h against the extraction buffer.

### Determination of ACE activity

Activity was determined by the method of Neels et al. /8/, using hippurylglycylglycine (HCG) as substrate. Specific activity was defined as U/g protein of clear supernatant. Protein was determined by Lowry's method /7/. One unit ACE activity was defined as an enzyme content which yields 1 micromole hippuric acid/min/liter of serum.

### Optimum pH and temperature

The pH profile of serum and tissue ACE, from 5.0 to 10.0, was assayed in different media (0.1 M borate, 0.1 M acetate, and 0.1 M Tris-HCl buffer). The effects of temperature (4, 25, 37, 55°C) on enzyme activity was investigated.

### The effect of chloride ions

Chloride, up to 100 to 1000 mM, was added to samples for 1 h and ACE activity was determined in the samples.

### The effect of zinc ions

As in the case of chloride ions, the samples were incubated with different concentrations of zinc ions, from $10^{-3}$ to $10^{-10}$M, for 1 h, then subjected to activity determination.

**Table I**

ACE activity in human lung tissue and serum

| | ACE activity | | | | Decrease in ACE activity (in % of that measured at 37°C) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control* | | Patients** | | Control | | | Patients | | |
| | (mean ± S.D.) | | (mean ± S.D.) | | 4°C | 25°C | 55°C | 4°C | 25°C | 55°C |
| Tissue[a] | 28.5 | 5.4 | 67.2 | 27.3 | 87 | 38 | 34 | 90 | 71 | 47 |
| Serum[b] | 298 | 42 | 237 | 68 | 87 | 45 | 39 | 89 | 39 | 35 |

a: U/g protein, lower than that of patients ($P < 0.001$)

b: U/L, higher than that of patients ($P < 0.01$)

*n = 17; **n = 21

## Results

The statistical evaluation (Student's t-test) of the results is shown in Table I. The findings show that the patients with lung cancer had higher ACE activity in their lung tissue and lower activity in serum than those of the control group. However, the optimum pH (8.0–9.0) and temperature (37°C) are the same for both kinds of samples of both groups (Figs 1 and 2).

It has been shown that an increase of chloride ion concentration is followed by a significant increase in enzyme activity. In the presence of 600 mM of chloride ions ACE activity in normal serum increased 2.37 times

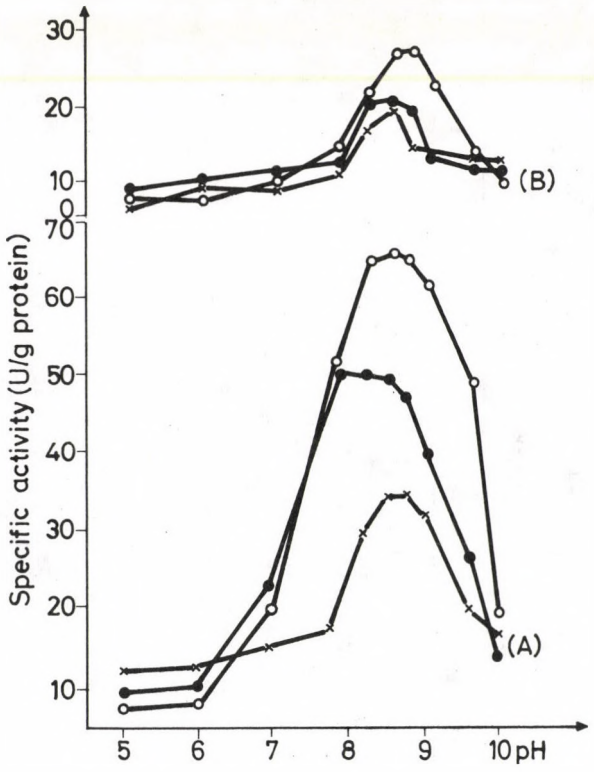


Fig. 1. The pH profile of ACE activity in human lung tissue (A: Cancerous (n=21), B: Normal (n=17), (o): 0.1 M sodium borate buffer, (●): 0.1 M potassium phosphate buffer, (x): 0.1 M Tris-HCl buffer)

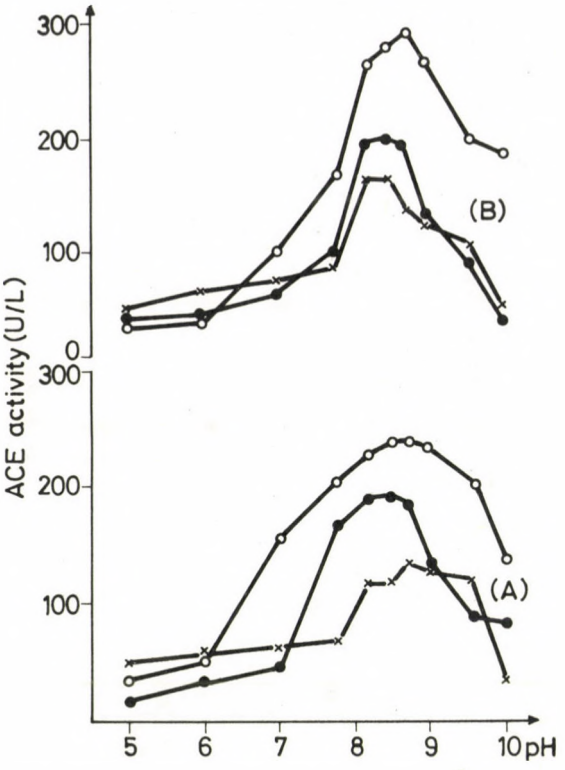


Fig. 2. The pH profile of ACE activity in human serum (A: Cancerous (n=21), B: Normal, (o): 0.1 M sodium borate buffer, (●): 0.1 M potassium phosphate buffer, (x): 0.1 M Tris-HCl buffer)

and in patients' serum 2.26 times. These increases at the same 600 mM of chloride were 2.16 and 4.75 times in tissue of control and patients, respectively (Fig. 3).

Figure 4 shows that an excess zinc ion concentration results in an increase in ACE activity of only patients' serum.

## Discussion

Our findings show a significant decrease in serum ACE activity of patients with lung cancer when compared with that of control group. This is consistent with previous studies /9, 13, 16/. Conversely, the tissue obtained from patients had higher ACE activity per gram protein than in

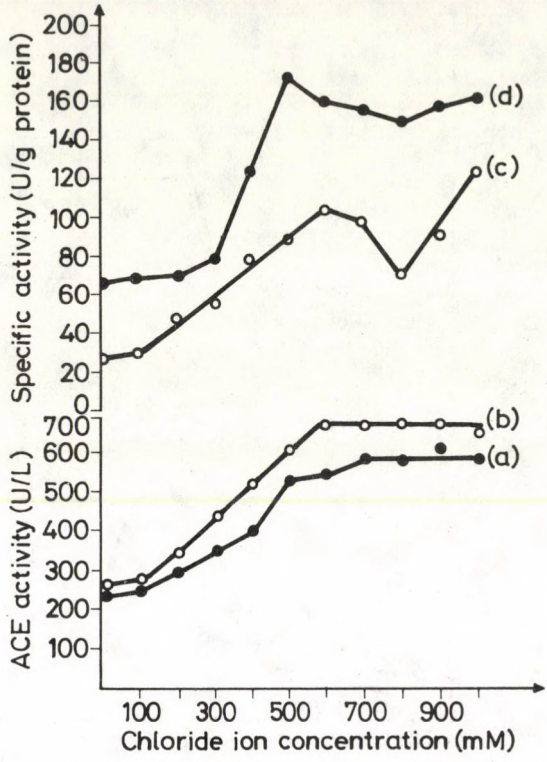


Fig. 3. The effect of chloride ions on ACE activity in human serum: a: Cancerous (n=21), b: Normal (n=17) and in human lung tissue, b: Normal (n=17), d: Cancerous (n=21)

control group. A previous study /15/ reported a specific activity for healthy subjects, which is also consistent with our results. However, we could not encounter any study on cancerous lung tissue with respect to ACE activity. This high ACE activity in patients may be related to inability to release the enzyme from the tissue into the blood stream. This idea is supported by our findings that these patients have low levels of ACE in their serum.

The optimal pH and temperature was practically the same in tissue and serum. This agrees well with other studies /17, 19/.

It is well known /14/ that ACE activity is closely related to halides, especially to chloride /10/. This is true for both normal and cancerous samples.The activation caused by chloride is associated with its binding to the lysine residue of the enzyme. The higher activity in tissue than in

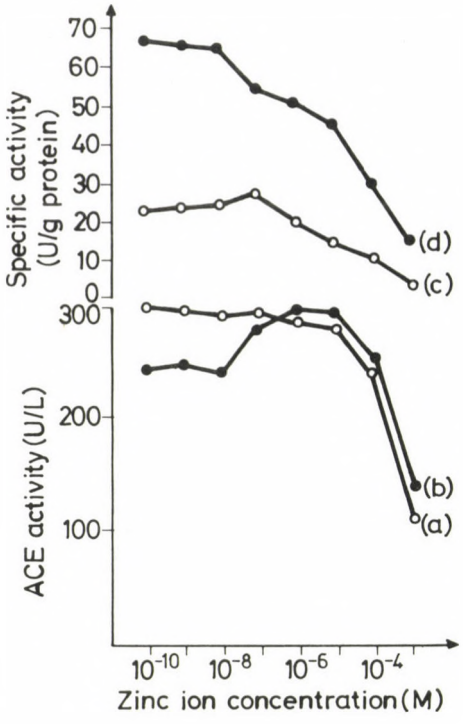


Fig. 4. The effect of zinc on ACE activity in human serum: a: Normal (n=17), b: Cancerous (n=21) and in human lung tissue, c: Normal (n=17), d: Cancerous (n=21)

serum is unrelated to chloride but associated with high enzyme content. On the other hand, the increase in ionic strength and "salting out" event may be responsible for decline in activity at the presence of 600–800 mM chloride.

ACE is activated by many cations, such as $Zn^{2+}$, $Mn^{2+}$, and $Co^{2+}$. The activation by zinc is predominant. On the other hand, that the excess zinc concentrations exert an inhibitory effect is interesting (Fig. 4). Since serum ACE is low in lung cancer patients, an overt rise in activity is to be seen when zinc is added to serum. Moreover, it might be speculated that low activity levels in serum of these patients is associated with their low serum zinc concentrations. This idea is supported by a study /12/ on rats. The present results seem to agree with the assumption that zinc therapy of lung cancer may be taken into consideration.

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# ALTERED FILTRABILITY OF WHITE BLOOD CELLS AFTER MYOCARDIAL INFARCTION

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Abnormal white blood cell rheological behaviour has been implicated as a cause of blood flow disturbances under conditions of ischaemia and reduced perfusion pressure. Accordingly, we have tested the mechanical properties of white cells following myocardial infarction by measuring the rate at which suspension of these cells cause plugging of Nuclepore filters. The number of clogging particles in a standard white cell suspension increased by the third day after infarction but subsequently decreased to the control levels. Since white cells can cause blockage of narrow blood vessels, it is assumed that such changes in cellular properties may influence the eventual extent of infarction.

Keywords: Filtrability of white blood cells, acute myocardial infarction

Abbreviation: CP: clogging particle

## Introduction

The flow properties of the blood have been widely investigated with regard to the pathophysiology of myocardial infarction. It has been found, for example, that red cell filtrability is decreased soon after a myocardial infarction and that the magnitude of the red cell abnormality may be of prognostic clinical significance /4/. However, the rheological properties of white blood cells have not been investigated, although it has been found

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that neutrophilic granulocytes become "activated" after infarction in man /7/, and that the areas of experimentally induced infarcts are smaller in dogs if the animals have previously been depleted of neutrophils /9/. In experimental ischaemia of the dog myocardium, white cells accumulate preferentially in the ischaemic tissue, and capillary blocking by these cells has been proposed as a major cause of incomplete return of blood flow following a period of ischaemia /6/. In order to investigate the possible role played by altered white cell rheology in myocardial infarction, we have used a new blood filtrometer /5/ to study the ability of white cells to pass through narrow pores (diameter = 8 um) from patients at fixed intervals after myocardial infarction.

## Patients and Methods

Ten male patients aged 60 to 83 years were studied within the first 24 h following a myocardial infarction and on the third and tenth day after infarction; three patients were additionally tested on the second, fourth and fifth days after infarction. The diagnosis of infarction was made on the basis of clinical symptoms, characteristic ECG changes and elevated serial cardiac enzymes in the blood. Ten outpatients aged 58 to 82 years with previously proven ischaemic heart diseases for at least 5 years but with no documented history of myocardial infarction served as a control gorup. For each of these control patients blood was taken on one occasion only. Apart from opiate analgesia in the first 24 h, drug therapy in the infarct group did not differ from controls, with patients in both groups receiving combinations of beta-blockers, diuretics and nitrates.

Venous blood samples were taken into plastic tubes and anticoagulated with EDTA. After centrifugation at 2500 g for 10 min, the plasma was discarded and the buffy coat was aspirated with a plastic pipette and diluted in phosphate buffer (pH 7.3 and osmolality 300 mOsm/kg) to give a final total white cell concentration of $1.0 \times 10^9$/l as measured by a Coulter S plus IV counter. The white cell suspension was analysed using the St. George's Blood Filtrometer /5, 3/. In this apparatus, a small volume (65 µm) of white cell suspension flows through a Nucleopore filter (8 µm pore diameter) under a fixed pressure of 4 $cmH_2O$. The rate of flow is measured as a function of time decreases as the pores in the filter become clogged by cells unable to pass through them. Theoretically, the rate at which the flow changes is proportional to the concentration of clogging particles (CP) in the suspension. The experimental results were expressed as CP (in this case single white blood cells) per ml of the white cell suspension. In order to eliminate the effect of changing granulocyte counts in the postinfarction period, the data were subsequently adjusted by dividing CP/ml by the proportion of granulocytes in the white cell suspension. Results were finally expressed as CP/ml of standard granulocyte suspension.

Concentrations of lactate dehydrogenase isoenzyme specific to heart muscle were determined with Sigma diagnostic kit (Sigma Chemical Co., St. Louis, MO. USA) in patients' sera.

## Results

The mean number of clogging particles (CP/ml) on the first day of myocardial infarction was by 20% higher than in the control group, but this difference was not statistically significant. However, by the third day after infarction CP/ml increased by a further 50% (Fig. 1) and was significantly different from the control or day-one values. Subsequently the CP/ml decreased, so that by the tenth day the value was significantly lower than on the third and was approximately equal to the value obtained on the first day (all statistical significances were: $P < 0.01$, Student's t-test).

There was a significant positive correlation between the maximum concentrations of heart-muscle-specific lactate dehydrogenase isoenzyme in patients' sera and the values of CP/ml measured on the third day of myocardial infarction ($r = 0.601$, $P < 0.05$).

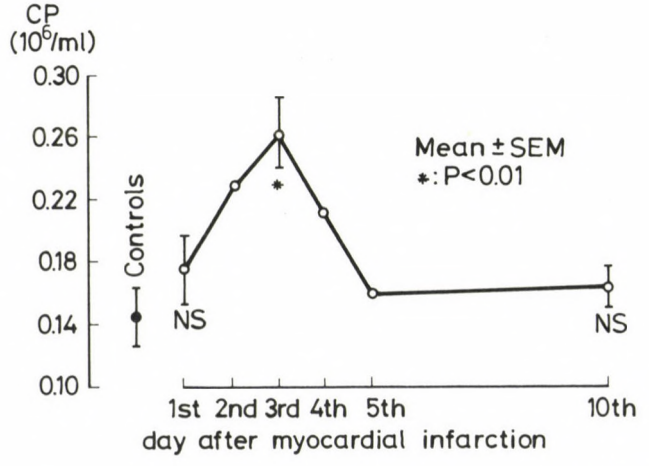


Fig. 1. Changes in the number of clogging particles (CP, $10^6$/ml) for white blood cell suspensions containing $1 \times 10^9$/ml cells following myocardial infarction (n=10 where SEM are shown, otherwise n=3)
NS = non significant

## Discussion

White blood cells are much more resistant to deformation than red cells and, even in the normal circulation, white cells (especially granulocytes) intermittently cause capillary plugging /1/. Such plugging has been found to be greatly increased under experimentally induced conditions of

reduced blood flow, e.g., in acute haemorrhagic shock /2/ and ischaemia of the dog myocardium /6/, and that is believed to perpetuate the abnormal flow patterns. This plugging could be a consequence of reduced local perfusion pressure required to propel white cells through narrow vessels. It is also assumed that when blood flow is reduced and white cells are retarded in narrow vessels they become retained by adhesion to the vessel wall.

The circulating granulocytes and monocytes become stimulated primarily by the activated complement components. This process is generated by the necrotizing muscle cells' proteins, especially by fragments of mitochondria /8/. The consequences of white cell stimulation can be detected in the circulation and locally in the heart muscle, too. The activated granulocytes and monocytes show enhanced degranulation and increased production of oxygen-free radicals which substances can cause further damage to the marginal zone of myocardial infarction /7, 9/. The activated complement fragments also change the mechanical properties of white blood cells. They become more sticky and less deformable causing impaired microcirculation especially at low perfusion pressure.

Changes in the mechanical properties of the white cells could also give rise to vessel blockage and alteration of blood flow. The present study suggests that after myocardial infarction the granulocytes become progressively less deformable over the first three days and hence they cause increasing blockage of Nuclepore filters. After this period, the tendency to clog the pores decreases. The mechanical properties of white cells appear to alter after myocardial infarction. These alterations could arise, for example, if granulocyte activation /7/ was accompanied by a decrease in cell deformability and/or cell adhesiveness and could be of significance in determining the impaired reperfusion, the extent of local tissue damage and eventual infarct size. This hypothesis is partly supported by the significant positive correlation found between the maximum concentration of lactate dehydrogenase isoenzyme in patients' sera and the values of CP/ml measured on the third day of treatment. Further studies are needed to assess the clinical effects of impaired white cell deformability after myocardial infarction.

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Acta Medica Hungarica, Vol. 46/2–3, pp. 213–217 (1989)

BOOK REVIEWS

## Screening for cancer of the uterine cervix.

Editors: M. Hakama, A.B. Miller and N.E. Day, IARC Scientific Publications No. 76. International Agency for Research on Cancer, Lyon, 1986.

This paperback book is a result of IARC and UICC collaboration "having a great pedagogic value by describing the organizational requirements for screening and giving large epidemiological basis for evaluation of different screening policies".

The book provides as much information as possible, it is abundanty illustrated by tables, excellent diagrams and some photomicrographs. Relevant bibliography is provided.

The book is readable and easy to understand. It consists of two parts. The first part is entitled "IARC Collaborative Study on the Evaluation of Cervical Cancer Screening Programmes". Detailed information is given from British Columbia, Canada, Denmark, Iceland, Italy, Norway, Scotland and Sweden. Tables summarize cases of invasive squamous cell cervical cancer by age, after one, two, or more negative smears. Risk after negative smears is discussed according to the incidence of invasive cervical cancer at age 30–64 years, by commulative number of negative smears and the time elapsed since the last negative smear.

It is stressed that in formulating any screening policy, the benefit in terms of reduction in risk obtained from successively shorter screening intervals must be balanced against the cost of the increased number of screening tests required to prevent an invasive cancer.

The second part of the book, "UICC Project on Cervical Cancer Screening", includes the following chapters: Effect of screening on incidence of cancer of cervix; Effect of screening on mortality from cancer of the cervix; Evaluation of the impact of screening. It is emphasized that screening has a role in the reduction of both morbidity and mortality from cervical cancer. Reasons that some screening programmes fail to control cervical cancer,namely, failure to reach the population at risk, lack of sensitivity of cervical screening, infrequently repeated screening, inadequate management of abnormalities detected, ineffective treatment, are discussed in detail.

The chapter entitled "Information requirements for cervical cancer screening programmes", one of the most essential parts of the monography, gives clear-cut standardization, adaptable for computer. There is a need for a systematic approach to the problems of inadequate standardization and conceptional errors in order to improve patient care, to monitor the programmes and for research. Mathematical models are also given.

Mathematical models and natural history in cervical cancer screening emphasize that the picture that emerges is one of a complex preclinical history. There is a clear indication of regression of carcinoma in situ, as well as an age-dependence of transition probabilities and duration of disease status. The chapter entitled "Diagnosis and Management of Cervical Abrnomalities" offers a very useful practical histopathological classification of precursors of carcinoma of the uterine cervix as well as indications of biopsy, cervical curettage and, finally, the possible treatments of cervical abnormalities, as conisation, hysterectomy, radiotherapy, laser treatment and cryosurgery. A very important part of the monograph describes the organization of screening in technically advanced countries, organization and results of cervical cancer in the German Democratic Republic and

organization of screening programmes in developing countries. There is a short report about screening for cancer of the endometrium and ovarian cancer. I highly recommend this very informative book to gynaecologists, oncologists, pathologists and cytologists.

ÉVA MAGYAR

IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Tobacco Smoking. Vol. 38. pp. 421. IARC, Lyon, France 1986. ISBN 92 832 1238 X (soft cover edition).

Tobacco was brought from America to the Old World by Columbus in 1492. Its use spread to many countries during the sixteenth century. The total world production in 1982 reached 6.7 million tons. During the twentieth century, both the number of smokers and the number of cigarettes sold increased until the 1960s. By the early 1980s, some countries had recorded falls in total consumption, total sales and smoking rates. Therefore, this volume should be of special interest to researchers as well as practitioners.

A very short "Note to the Reader" in the Preface deals with the meaning of "carcinogenic risk". The next, also short, chapter presents the participants. The "Preamble" lists the IARC monographs programme on the evaluation of the carcinogenic risk of chemicals to humans.

The proper volume consist of six Chapters and two Appendices. The first chapter is a general introduction: smoking and public health. When tobacco was first introduced into Europe, smoking was recommended for medicinal purposes. But its value soon became controversial. One of its notable opponents, James I, King of the UK, published in 1604 a treatise entitled "A Counterblaste to Tobacco" anonymously. The different causes of death related to smoking can be divided in four groups. The first one is the most important: lung cancer, ischaemic heart disease, respiratory heart disease, aortic aneurysma, peripheral vascular disease and chronic obstructive lung disease.

The next Chapter pays attention to the worldwide use of smoking tobacco, including production and trade, manufacture and usage and, fianlly, smoking and public health considerations.

It is very interesting that in international comparison, percentage of filter cigarettes in the total production of cigarettes increased in the last two decades. Hungary is in the middle of this list: the rate of filter cigarettes increased between 1972 and 1982 from 50% to 80%.

Each of these chapters is well-indexed and every one of them is followed by a very good summary. Chemical analysis of tobacco smoke showed that it contains more than 3800 constituents. The agents in the mainstream include carbon monoxide, benzene, hydrogen cyanide, volatile and nictoine-derived N-nitrosamines, nicotine, phenols, aromatic amines, polynuclear aromatic compounds and polonium-210, among others a potential endogenous formation of N-nitrosamines after smoke inhalation is also reported. The analysis of these agents in sidestream smoke is considered to be important.

The fourth chapter details biological data relevant to the evaluation of carcinogenic risk to humans including the carcinogenicity studies in animals. Tests of cigarette smoke condensates (CSC) with Salmonella typhimurium have been very intensive and showed mutagenicity. But here is some discrepancy between carcinogenicity and mutagenicity in the experiments of different authors. The evidence concerning an increased prevalence

of morphologically abnormal sperm in smokers is conflicting. More data are required before any conclusion can be drawn.

The most important part is the one that summarizes the findings concerning the human epidemiological studies. On the base of these investigations, there is sufficient evidence that inhalation of tobacco smoke as well as topical application of CSC cause cancer in animals. There is also sufficient proof that tobacco smoke is carcinogenic to humans. Malignant tumours in the upper digestive tract and respiratory tract are causally related to the smoking of different forms of tobacco. The occurrence of malignant neoplasms in the bladder, renal pelvis and pancreas are also causally related to smoking of cigarettes.

A. BAJTAI

Early detection of occupational diseases.

World Health Organization, pp. 272. Geneva, 1986.

Detection as early as during the reversible phase is a contemporary and progressive aspect of the control of occupational diseases. The book was aimed at being a guide for factory doctors and health professionals to early recognition, diagnosis and treatment of occupational diseases. Besides being addressed to health professionals at various levels, it is intended to facilitate the organization of occupational health services at the workplace. It will help countries to introduce effective health monitoring of working population and to obtain adequate information on occupational exposure and its adverse effects on health. The information collected in this book is based on research carried out by WHO Collaborating Centers in more than thirty countries.

Occupational diseases are caused by exposure to harmful chemical and biological agents and physical hazards at the workplace. Occupational diseases affect a considerable number of people, particularly in the rapidly industrializing countries; in many cases they are severe and disabling. However, there are factors which make them easily prevented; their causal agents can be identified, measured and controlled; the population at risk is usually easily accessible and can regularly be supervised and treated. The initial changes can be reversible if they are diagnosed and treated promptly, therefore, the early detection of occupational diseases is of primary importance.

The first part of book deals with the principles of early detection of occupational diseases. The second part describes the various occupational diseases and discusses approaches for their early detection and control. (The discussed occupational diseases corresponds to the list which was adopted by the International Labour Conference in 1980.) The third part of book contains discussion of physiological systems altered by occupational diseases, and describes clinical and laboratory tests for their early detection. The final part presents biological and environmental methods for the assessment of exposure to occupational hazards.

Considering the growing tendency of chemical and biological contamination in living environment, the presented information seems to be important not only for the occupational health services, but also for every doctor in practice.

Bertalan VARGA

Pathology of Tumours in Laboratory Animals. Vol. I. Tumours of the rat. Parts 1 and 2. Editor in chief. V.S. TURUSOV. World Health Organization. International Agency for Research on Cancer. (Reimpression of IARC Scientific Publications No. 5 (1973) and No. 6 (1976). Lyon, 1987.

There has been increasing recognition of the necessity for a standardized terminology in the field of human and experimental pathology to facilitate comparative studies in different countries and institutions. In this context the WHO is producing a series of monographs on the standardization of the histological classification of tumours, not only those of man, but also those in species most commonly used in cancer research.

This monograph, dealing with tumours of the rat and written by 42 excellent animal experimental and human pathologists, was prepared in close collaboration with the WHO and with the intention of providing a service for experimental pathologists. The book is divided into 24 sections on 320 pages, with 779 figures. In all chapters the following scheme has been adapted by the contributors: normal structure of the organ in question, morphology and classification of tumours, spontaneous neoplasms, principle methods of induction of tumours and comparative aspects.

The style and format make reading easy. The monograph is an illustrated reference book on the subject of tumours of laboratory rats. The classic features of the various tumours of all organs and tissues are well illustrated and the black-and-white photographs (macro-, micro- and electron micrographs) are of good quality. An outstanding feature of the book is the very complete and up-to-date bibliography.

I consider it an extremely useful volume and believe that it should be included in the libraries of all experimental pathologists and all of the human pathologists occasionally involved in histopathological evaluation of experimental carcinogenesis studies.

G. KENDREY

Lipofuscin — 1987: State of the Art. Editor: Imre Zs. Nagy. Publisher: Academic Press. Budapest (1988)

The volume contains the proceedings of an international symposium dealing with age pigment research. This was the second meeting of a series of symposia on the very up-to-date problem dealt with by an international workshop consisting of the leading scientists in this interdisciplinary field. As the preceding meeting on "Age pigments, Biological Markers in Aging and Environmental Stress" held at Vico Equense (Naples, Italy, 1985) chose Hungary for the next Conference, this was held in Debrecen, 26—30 August, 1987. It was organized by the F. Verzár International Laboratory for Experimental Gerontology (V.I.L.E.G.) Imre Zs. Nagy, the editor of the Proceedings is the head of the Laboratory. The volume contains in full length the papers of all the 21 invited lectureres as well as 2-page summaries of the poster presentations and the comments recorded during a plenary poster discussion. The lectures cover both the theoretical and clinical apsects of our present knowledge on the topics of age- and disease-related formation of lipopigments, presenting also the most modern developments in the area of instrumentation and experimental manipulation of the age pigments. The papers presented in 5 sessions dealt with the theory and cellular mechanisms of lipofuscin formation, characterization of biological autofluorescent

products, lipofuscin in various animal tissues, experimental manipulations on lipofuscin, and disease-related problems of pigment accumulations. The last chapter deals with the poster session and its general discussion. All the papers give ample reference to the most recent literature, completely evaluated and updated to 1987. The volume is concluded with a key word index and an author index. The list of participants reveals that 36 scientists from 11 countries and 4 continents have contributed to give an in-depth survey and interdisciplinary overview of our current state of knowledge on the topics of age- and disease-related formation of lipopigments. The volume is of particular value to all those interested in this field.

J. FEHÉR

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*Acta Medica Hungarica* is published under the auspices of the Hungarian Academy of Sciences. Manuscripts and editorial correspondence should be sent to the editorial office: H-1450 Budapest 9, P.O. Box 67.

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

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

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

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

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

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

Examples:

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

*Tables*

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

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# PANCREATOLOGY, PAST, PRESENT AND FUTURE*

HENRY T. HOWAT[1]

(Received: February 27, 1989)

It has been said that nowadays more things happen in a year than happened in a decade in the 19th Century and in a score of years in the 18th Century. (I regret I have been unable to trace the origin of this aphorism.) If the number of medical papers produced and of new journals introduced is taken as a criterion, the pace at which medical science increases is exponential.

## The Past

Pre-1940. Prior to 1900, with a few exceptions, papers on the anatomy and physiology of the pancreas belong to the domain of the medical historian (Schadewaldt, 1964).

It is almost 60 years to the day that I entered the Faculty of Medicine of St. Andrews University as a medical student. Five years later, in 1933, now a newly qualified doctor, on my first night of duty as a house surgeon, I admitted 13 cases to the receiving surgical unit. Of these, three were suffering from acute necrosis of the pancreas; all were confirmed at laparotomy. During the depression of the 1930s, though acute pancreatitis often precipitated by acute alcoholic debauch was relatively common, chronic pancreatitis was a rare disease. We were taught this was a painless entity, which not infrequently affected women.

At that time, corroborative evidence of pancreatitis was scanty. In acute pancreatitis the urinary diastase content of a casual specimen of urine was estimated by Wohlgemuth's iodometric method, while in chronic

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*20th Meeting, European Pancreatic Club, Budapest, Hungary. 29-31 August, 1988

[1]Co-founder and first President of the European Pancreatic Club, Manchester, U.K.

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Akadémiai Kiadó, Budapest

pancreatitis the diagnosis was established by inspecting the faeces for neutral fat and by demonstrating neutral fat (Sudan III) and undigested meat fibres microscopically in the specimen.

1940-1960. The postwar decades were the era in which pancreatic function tests were developed for use in man. Secretin was used as a pancreatic exocrine stimulant, initially by French workers and later by Lagerlőf /3/, who introduced a double-lumen duodenal tube to separate duodenal from gastric secretions. The secretin test was popularized by USA workers including David Dreiling, who is present today. In the 1950s, we added cholecystokinin-pancreozymin (CCK-PZ) as a specific enzyme stimulant, which enabled us to assess pancreatic and gall-bladder function simultaneously in man. Even today, the search continues for the ideal simple non-invasive test of pancreatic function in chronic pancreatitis and cancer of the pancreas which can be applied before gross structural changes are manifest.

At that time, the demonstration of intraductal calculi, at best by tomography, was the only available direct radiological evidence of chronic pancreatitis. The finding of glycosuria or a diabetic glucose tolerance test provided corroborative evidence of advanced chronic pancreatitis or cancer of the pancreas.

The development of the electron microscope proved of great value in understanding acinar cell function. Palade (1962) studied the ultrastructure of pancreatic acinar cells and hence the synthesis, transport and excretion of the pancreatic digestive proteins into the duct lumen. Scrutiny of the proceedings of the European Pancreatic Club over the years reveals the debt we owe to these early workers using this technique. In the past 25 years, great advances have occurred in the understanding of the synthesis of proteins.

1960. Clinicians had long looked forward to the day when the pancreas could be visualized. In the 1960s, radio-isotope scanning of the pancreas, using $^{75}$Se-selenomethionine, permitted a visual display of the normal pancreas for the first time. By the end of the decade, it was being superseded by other methods as was selective angiography, developed about the same time, which is now reserved for use in specific situations, such as prior to elective surgery for pancreatic cancer.

1970. ULTRASONOGRAPHY. Ultrasonography has reached such a degree of

resolution as now to provide an essential tool in the investigation of biliary and pancreatic disease. The use of real time scanning has improved diagnosis and facilitated the development of other techniques, intra-operative ultrasound, fine-needle biopsy of pancreatic tumours, diagnostic percutaneous pancreatography and the therapeutic aspiration of pseudocysts of the pancreas.

COMPUTED TOMOGRAPHY (CT), introduced by Hounsfield in 1972 has developed technically to such a degree as to provide an image of upper abdominal and retroperitoneal structures. Contrast-enhanced CT adds greatly to the investigation of pancreatic lesions.

Ultrasound and computed tomography are complementary: CT is of particular value in obese patients, sonography in spare patients. Magnetic resonance imaging (MRI) at its present stage of development offers little advantage over CT in the diagnosis of pancreatic disease.

The development of long lateral viewing flexible duodenoscopes led to endoscopic retrograde cholangiopancreatography (ERCP), a technique which permits radiological delineation not only of the biliary but also of the pancreatic ducts, demonstrates the presence of strictures or stenosis, the presence of calculi in the ducts, differentiates cancer of the pancreas from chronic pancreatitis and, when indicated, permits pancreatic biopsy. Though less universally used in diagnosis than a decade ago, due to improved results obtained by sonography and CT, there has been a development of the endoscope in therapeutic techniques of papillotomy and palliative biliary drainage in pancreatic cancer by stent insertion.

A common feature of most of these advances in our study of pancreatic disease is that they have followed the application of inventions by pure scientists in the fields of physics, chemistry and bioengineering.

## The Present

In viewing the present, I do not intend to deal only with the pancreas, but with two topics of importance to all scientific and medical research workers.

## Finance

Medical research, whether basic or applied, is now inadequately funded. From the mid-1940s the talented young research worker could rely on being backed financially according to his needs. Up to the mid-1960s the Medical Research Council's budgets in the UK for civil projects (according to Sir James Gowans /2/) grew exponentially (as did the output of scientific and medical papers), then the rate of growth fell to zero in the late 1970s and now funding became linked with "gross domestic product".

This had led to two main consequences:

1. Research funds are increasingly sought from industry and charities.
2. Both basic and applied medical research are being directed to the larger and stronger institutions where resources are concentrated and the worker has access to expensive capital equipment and to the revenue resources required to maintain and operate these: the point is also argued that he can call on the support of talented colleagues of differing disciplines. The path of the gifted young scientist seeking to prove a hypothesis is increasingly difficult. It is a pity when we remember the truism which applies not only to scientists, but philosophers, economists, politicians or whom you like, that few over the age of 40 have produced an original idea of any great quality (Vide addendum ).

## Evaluation of medical research

Earlier I spoke of the proliferation of medical papers. At present, there is a growing cult of "evaluating research performance" through paper counts, citation rates and other indicators. Initiated by bibliometricians, an emerging profession is increasingly applying what some of those evaluated consider to be at present naive, indeed dubious techniques. Their conclusions appear in the journal "Scientometrics". The proceedings of the recent conference on "The evaluation of Scientific Research" held at the Ciba Foundation in London will be published early in 1989 by Wiley of Chichester.

There is a fear that crude quantitative assessments of medical papers may influence the funding of medical research and institutions, and that the more valuable and subtle qualitative assessment we all make when we read a published paper will be ignored.

## The Future

I had hoped to have sufficient time to mention some of the topics of concern to myself:

1. The classification of pancreatitis.
2. The anatomical, functional and pathophysiological inter-relationships of the external pancreas not only with the islets of Langerhans but also the liver and biliary tract.
3. The risks for the clinicians of overspecialization in a limited field such as the pancreas.
4. The problems of management of chronic pancreatitis and cancer of the pancreas and how they may be approached.

I am loathe to make any prophecies for the future. Even Cassandra, the illegitimate daughter of Priam, beloved of Apollo and granted by him the gift of prophecy, was not believed by her father when she correctly forecast the fall of Troy. I content myself with one prediction. Apart from pancreatic transplants, in 20 years time surgery will play little part in the management of pancreatic disease. I console myself that I shall not be here in 2008 to learn from my surgical friends whether my prophecy has proved wrong. Indeed, few if any of you who attend the meeting the European Pancreatic Club in 2008 will recall anything I have said today.

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Addendum (from "The Independent" of Monday. 13th February, 1989).

The Department of Education has increased the funding of basic research (in the U.K.) for ensuing financial year by £127m. Part of the allocation to the Royal Society, £2m, will fund 30 more University research fellowships and part will buy specialized equipment and materials.

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# CHRONIC CALCIFYING PANCREATITIS: EPIDEMIOLOGY AND CURRENT CONCEPT OF THE LITHOGENESIS*

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The exact aetiology of chronic calcifying pancreatitis is unknown; several factors that lead to the development of this well-defined disease have been identified. Epidemiologic studies and careful analysis of nutritional data played an important role in precising the risk represented by alcohol consumption and dietary habits, and characterized the geographical distribution of the disease. At the same time, biochemical modifications of the pancreatic juice were described in alcoholics; later on, a new family of pancreatic secretory protein, the so-called "Pancreatic Stone Protein" was discovered. While its secretory form (PSP S2-5) prevents calcium crystal formation from the supersaturated pancreatic juice, its partially degraded form (PSP S1) is insoluble and probably the main protein of intraductal and intra-acinar precipitates. Recent studies have confirmed that in chronic calcifying pancreatitis patients the mRNA encoding the synthesis of PSP S2-5 is decreased, and the protein is diminished both in the zymogen granules and in the pancreatic juice.

Keywords: chronic calcifying pancreatitis; epidemiology; pancreatic stone protein

## Introduction

Chronic calcifying pancreatitis is a pathological entity defined by a group of features: lobular, patchy distribution of the lesions, important lesions of the ductal epithelium and constant presence in the duct lumina of numerous protein precipitates /15, 30/. Although the morphology of

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Abbreviations: CCP = chronic calcifying pancreatitis; PSP = pancreatic stone protein

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chronic calcifying pancreatitis (CCP) is well-characterized and similar in every case, the aetiology of the disease is multiple and its pathogenesis is not exactly known. Our purpose in this review is to review recent results focusing on epidemiologic and biochemical data.

## Chronic calcifying pancreatitis of different aetiology

### I.) Alcoholic chronic calcifying pancreatitis

It was shown that there was an exponential relationship between the quantity of alcohol consumption and the risk to develop CCP, without any statistical threshold for alcohol toxicity /9/. On the other hand, the majority of heavy drinkers never develop CCP. Alcoholic etiology is the most frequent cause of CCP; this form of the disease is observed world-wide/36/. In the last epidemiologic studies, alcoholic aetiology was almost exclusive in Marseille and in Ivory Coast /38/, dominant in Brasil /5/ and in Hungary /2/, but only exceptional in Kerala state of India /11/. Other nutritional factors may predispose to CCP, but their effect is less than that of alcohol: the risk is higher with increasing protein intake; it is increased also by low fat diet and even higher by a high fat intake /9/. These nutritional data are true in the case of any geographical group studied /36/.

Ethanol has multiple actions on the pancreas: it is metabolized by the pancreatic acinar cells /4, 10/, stimulates, or oppositely, inhibits the pancreatic secretion in a dose-dependent manner /27/ by a nervous pathway. The inhibitory effect of ethanol is transmitted by the vagus, involves at least one nicotinic and one muscarinic receptor in dogs /27/, or the alpha adrenergic nerves in mice /3/. The stimulation is mediated by the intrinsic nervous elements of the pancreas: dopaminergic or muscarinic in dogs /27/ and peptidergic in mice /3/. During chronic alcohol consumption, the inhibitory effect was suppressed in mice /3/, dogs /39/ and humans /31/, unmasking the stimulation induced by even the highest alcohol concentrations. This leads to an increased protein concentration in the pancreatic juice of chronic alcoholics /33/ with higher viscosity and more frequent protein plugs as consequence. At the same time, in humans, bicarbonate and citrate secretion is decreased /33/, as well as the concentration of secretory trypsin inhibitor in the pancreatic juice; fatty deposits and some slight fibrosis appear in the pancreas even in asymptomatic alcoholic patients /29/. However, these modifications can be observed without any

pancreatic disease and there is no proof that they play a decisive role in the pathogenesis of alcoholic CCP.

II.) Tropical pancreatitis

CCP develops in young people without any alcohol consumption in the medical history. Aetiologic role was attributed to manioc (Cassava) consumption. This form of CCP is largely dominant in Kerala /11/, but exceptional in Ivory Coast, in spite of the fact that kwashiorkor /38/ and consumption of manioc (Cassava) is as widespread as in India. No relationship being proven between these presumed aetiological factors and tropical pancreatitis, the name "Cassava-pancreatitis" is thus unjustified. Two factors seem to be associated with tropical pancreatitis: very low-fat diet — probably associated with low protein intake —, and protein- and fat undernutrition in the patients parents.

III.) Hypercalcaemic chronic calcifying pancreatitis

Hypercalcaemia stimulates pancreatic protein secretion. Longlasting hypercalcaemia induces similar modifications in the pancreatic juice of humans /14/ and dogs /28/ as chronic alcohol consumption: increase in protein concentration and viscosity, frequent protein plugs. However, CCP of hypercalcaemic origin is rare, 3 cases were observed in Marseille and only one in Szeged during a ten-year period. On the other hand, CCP is a complication of hyperparathyroidism only in about 10% /8/.

## Chronic calcifying pancreatitis is a lithiasis

The normal clinical evolution is the formation of calcifications visible on plain films of the abdomen, generally after several years of the disease history /1/. It is known that these calcifications are intraductal calcium salt calculi. Therefore, the disease — at least in its advanced stages — is a lithiasis. A morphometric ultrastructural study /42/ has shown that the earliest lesion was formation of protein precipitates in the acini and duct lumina. The ultrastructural aspect of these fibrillar precipitates and their transformation into calculi has been described by the groups of Kern /19/ and of Harada /16, 17/. Therefore, even in its earliest stages, CCP, the most frequent inflammatory disease of the

pancreas, can be considered a lithiasis. This prompted us to study the eventual biochemichal modifications of the pancreatic juice and led to the discovery of a new family of proteins, the PSP or Pancreatic Stone Protein.

## PSP: Biochemical characteristics and its physiological function

The PSP was isolated at first from the pancreatic calculi in a partly denatured form /24/. Later on, the original molecule PSP S 2-5 was purified from non-activated pancreatic juice /6/. PSP S 2-5 is a group of glycoprotein molecules with a molecular weight of 16-19000 /7/. In spite of this heterogenity, due probably to different glycosylation patterns, only one amino acid chain is synthesized by the acinar cell which contains the specific mRNA encoding the PSP /12/. This specific mRNA molecule was purified and its sequence was identified /12, 13/. PSP S 2-5 is a glycoprotein, containing a 144-amino-acid polypeptide, different from any known enzyme. PSP S2-5 is stored in the zymogen granules of acinar cells /20/ and is secreted in parallel with the pancreatic enzymes /25/. Under the action of a small quantity of active trypsin, PSP S2-5 is hydrolysed in a long, 133-amino-acid C-terminal peptide, PSP S1, and a short, N-therminal 11-amino-acid peptide /7/. PSP S1 is not glycosylated, not soluble in water and precipitates as soon as it is formed. The sugars are thus probably carried by the N-terminal 11 amino acids.

PSP S2-5 prevents the precipitation of calcite from a saturated solution /7/ and prevents the crystal growth. PSP S1 has no action on the calcium salt crystal formation, which suggests that the activity is carried by the N-terminal 11-amino-acid chain.

The secretory molecule PSP S 2-5 is thus a glycoprotein of 16000 to 19000 molecular weight, soluble at physiological pH, active in preventing the calcium salt precipitation and crystal growth, and it is present in the non-activated pancreatic juice. It represents about 10% of the pancreatic secretory protein. The PSP S1 produced by the tryptic hydrolysis of PSP S 2-5, has a molecular weight of 14000, it is inactive on the calcium carbonate crystal formation, not glycosylated, insoluble at physiological pH. It is probably the main protein of intraductal protein plugs.

The PSP originally extracted from stones is present in the pancreatic stones, independently of the aetiology of CCP (alcoholic or nutritional) /21/. It is the sole protein component of the radiolucent core of partially

calcified stones /23/. Its amino acid composition is similar to that of PSP S1, but — in contrast with PSP S1 — it gives several bands on isoelectric focusing /21/. In addition, it is active in preventing calcium crystal formation /21/.

## PSP in pancreatic diseases

With a semiquantitative method, PSP was found to be strongly decreased in the zymogen granules in acinar cells in chronic pancreatitis /20/.

In the pancreatic juice, the same difference was shown during the first experiments using polyclonal antibodies with radial immunodiffusion technique of Mancini: PSP level was markedly decreased /25/ in pure pancreatic juice of CCP patients if compared to controls, to alcoholics without pancreatic disease and even to patients with obstructive pancreatitis. These results were later on confirmed by Elisa technique using the same polyclonal antibodies. However, neither the group of Schmiegel /40/ nor our laboratory /32/ was able to confirm these differences in experiments in which a monoclonal antibody prepared in Marseille was used. This antibody recognizes the inactive C-terminal fragment of PSP.

The mRNA specific for PSP was found to be markedly diminished in patients with CCP /13/, thus this decreased PSP synthesis was confirmed on the level of genetic control of protein synthesis, their storage in the zymogen granules and — with some contradictory results — on the secretory level.

## Pancreatic lithogenesis

The first stage of lithogenesis is a double phenomenon, viz. (1) presence in the juice of calcium carbonate crystals which are also found in normals but larger and more numerous in chronic pancreatitis patients, and /2/ presence of protein plugs built up of PSP S1. This double mechanism of pancreatic lithogenesis persists in the late stages of mature calculi generally composed of calcium carbonate and of different degraded forms of PSP, one of which is the first PSP isolated in our laboratory, and other more degraded forms. These disorders are generally associated but they may

be separated: pure calcium carbonate calculi or calculi of degraded PSP without calcium may occur. Therefore, one has to explain separately the precipitation of protein and that of calcium. As most secretory fluids, pancreatic juice is supersaturated in calcium /22/, in consequence, it is necessary to assume the existence of a calcium stabilizer, as in the urine /26/ or in the saliva /18/. PSP S being able in very low concentration to prevent calcium salt crystallization and the crystal growth, should be considered the stabilizer, or one of the stabilizers. The decrease of PSP in patients with CCP very probably explains the formation of calcium crystals. Some other factors, such as citrate decrease, could also play a part.

The precipitation of protein is more difficult to explain. Ultrastructural studies have shown that it occurs in the early stages of the disease. The precipitates are built up of PSP S1 which is a degraded form of secretory PSP S 2-5. PSP S1 could be formed by tryptic hydrolysis of PSP S 2-5, but it should be considered that precipitates were observed in the acinar lumina as well as in ducts. It would be necessary to assume that trypsin or similar proteases are activated in the acinar lumen, but this explication seems to be improbable. Another possibility would be that in CCP patients, besides a decreased biosynthesis of PSP an abnormal, less soluble molecule is synthesized. Nevertheless, there is no data supporting this latter hypothesis.

## Conclusion

Although our knowledge about the chronic calcifying pancreatitis progressed in the last years, many questions have remained unanswered. Thanks to a multidisciplinary clinical research, the histology of CCP is well-defined by morphologic, morphometric studies, some specific causes were identified by epidemiologic works, experimental studies resulted in a better understanding of the molecular basis of the pathogenesis /34, 35/.

As it is shown above, CCP develops only in the minority of alcoholics, hypercalcaemic or undernourished people. It is thus a logical imperative to assume one or some factors which represent a predisposition. Hereditary forms of CCP /41/ are rare, and no convincing evidence has been shown on the role of some HLA groups /36/. The newly-discovered PSP family is a candidate to be the predisposing factor or one of these factors. Secretory forms of these proteins (PSP S 2-5) are the most potent stabi-

lizers of the pancreatic juice supersaturated in calcium. They can be considered to be a strong protective factor against the development of a pancreatolithiasis, if they are present in sufficient concentration and with normal function. As it is described above, a marked unequivocal decrease in PSP synthesis and storage was demonstrated in the acinar cells from CCP patients. The contradictory results in pancreatic juice can be explained either by technical difficulties or with the fact that PSP S1 measured by the monoclonal antibody, is not involved in the stabilization of the pancreatic juice. It was thus demonstrated that the protective secretory PSP molecules are quantitatively decreased in chronic pancreatitis. On the other hand, if PSP S1 the insoluble, inactive, partially degraded form of the secretory PSP is present in the pancreatic juice, it precipitates and facilitates the development of chronic calcifying pancreatitis.

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# INTERRELATION OF SECRETORY AND TROPHIC RESPONSES IN THE EXOCRINE PANCREAS*

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This paper was addressed to know whether early events in mitogenesis (activation of the $Na^+/H^+$, activation of ornithine decarboxylase and formation of cyclic AMP) are involved in pancreatic cell proliferation and mediate secretory process. The AR4-2J cell line was used. Analogues of amiloride inhibited cell proliferation but had no effect on amylase release. Activation of ornithine decarboxylase was triggered via a CCK B receptor type not involved in pancreatic secretion. Inhibition of cyclic AMP was not involved in inhibition of cell proliferation caused by somatostatin. Specific effectors might be related either to the secretory or to the trophic pathway. Another possibility is that multiple receptor sub-classes are linked to specific pathways.

Keywords: pancreatic, secretion, growth, biochemical interrelations, cellular level, cell culture

## Introduction

The pancreas has been largely used as a model system for studying fluid and protein secretion. Factors regulating the secretory response of the pancreas have been identified. They are CCK peptides, bombesin peptides, cholinergic agents, secretin/VIP peptides for stimulating, and somatostatin for inhibiting, pancreatic secretion. Intracellular mechanisms mediating secretory process have been characterized especially on isolated pancreatic acinar cells. The cyclic AMP-adenylate cyclase system has been postulated

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Abbreviation: CCK: cholecystokinin; EGF: epidermal growth factor; ODC: ornithine decarboxylase; SMS: somatostatin

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to be a rate-limiting factor in the regulation of fluid secretion /11/. Two functionally distinct processes trigger enzyme secretion after binding of the secretagogues to receptors: the one is activation of adenylate cyclase, leading to cellular cyclic AMP formation and increased turnover of phosphatidyl inositol, mobilization of cellular calcium, activation of phospholipid-dependent, calcium-sensitive protein kinase /14/.

The pancreas is also able to grow and to regenerate. Factors regulating this effect have been identified mainly in in vivo experiments. Trophic effects have been clearly demonstrated for CCK and its analogue caerulein. An analysis of the sequence of the events caused by caerulein indicated that stimulation of fluid and trypsin secretion appeared first, followed by hypertrophy and, finally, hyperplasia. However, it is less clear whether other secretagogues induce trophic effects. Bethanechol does not increase DNA synthesis, secretin is much less effective than caerulein and the effect of bombesin has been attributed to CCK release. Somatostatin is an antitrophic factor, largely supposed acting indirectly, through CCK release inhibition.

Moreover, polypeptides, so-called Growth Factors, which are not specific gastro-intestinal peptides, have been shown to contribute to the control of pancreatic growth, with more subtle or no secretory capacities. They may potentiate enzyme secretion. Also, steroid hormones increase pancreatic growth in the fetal rats, probably by regulating amylase gene expression, along with insulin.

These first set of results, based on in vivo experiments, might indicate that pathways mediating the pancreatic secretory response are different from those mediating the trophic response, either at the receptor level or (and) at the intracellular level. Elucidating this question is important, since the distinction would enable us to design drugs to block or to stimulate cell proliferation selectively.

The experiments presented here are aimed at studying the relation between trophic and secretory responses at the cellular level. The pancreatic cell line AR4-2Jcell was used. The involvement of the following intracellular events in cell proliferation: intracytoplasmic alkalization via the $Na^+/H^+$ exchanger, cyclic AMP, and activation of ornithine decarboxylase, will be studied and related to pancreatic secretory capacity.

## Materials and Methods

### Materials

Amiloride and analogues were a kind gift from Dr. E. Cragoe from Merck, Sharp and Dhome Laboratories, West Point, Pennsylvania, USA.

SMS (Dphe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr(ol) was a kind gift from Dr. Harris and Marbach from Sandoz Laboratories, Basel, Switzerland.

### AR4-2J cell culture

Cells were grown in Dubelcco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), penicillin, streptomycin, amphotericin B, and L-glutamine. Cells were routinely plated at $2x10^5$ cells/ml in 80 $cm^2$ flasks or 16 mm diameter multiwell plates.

### Growth assay

After plating, and cell attachment overnight, the medium was changed to serum-free DMEM. Following a 24 h serum starvation, the medium was supplemented with 1) 10% FCS, 2) various combinations of factors in the absence of serum for somatostatin experiments, 3) 10% dialysed FCS, 25 mM $Na^+$ concentration, for amiloride experiments. For DNA synthesis assay, 0.1 µCi $^3$H-thymidine was added to cells at specified times and incubation was continued for 1 h. The medium was removed, cells were rinsed, and radioactivity was counted as usual /12/.

### $^{22}$Na uptake

AR4-2J cells were equilibrated for 20 min in a Na-free medium in the presence of various concentrations of amiloride or analogues. Uptake experiments were performed in a 25 mM $Na^+$ medium containing 1 µCi/ml of $^{22}$Na, 0.5 mM ouabain, and the same concentrations of amiloride (or analogues) during 1 min at 37 °C.

### Ornithine Decarboxylase (ODC) assay

ODC activity was measured on cytosolic extracts from AR4-2 cells as described /8/. Briefly, after a two-hour incubation with the agents tested, lysed cells were ultracentrifuged and the supernatant incubated with pyridoxal phosphate, $^{14}$C-ornithine and L-ornithine. Enzyme activity was performed by measuring the liberation of $^{14}CO_2$.

## Results

### Effect of amiloride and analogue on $^{22}$Na uptake and cell proliferation in AR4-2J cells

The sodium-proton antiporter was stimulated by nigericin. Amiloride and analogues, which are specific blockers of the exchanger, were used to measure the uptake of sodium via this pathway.

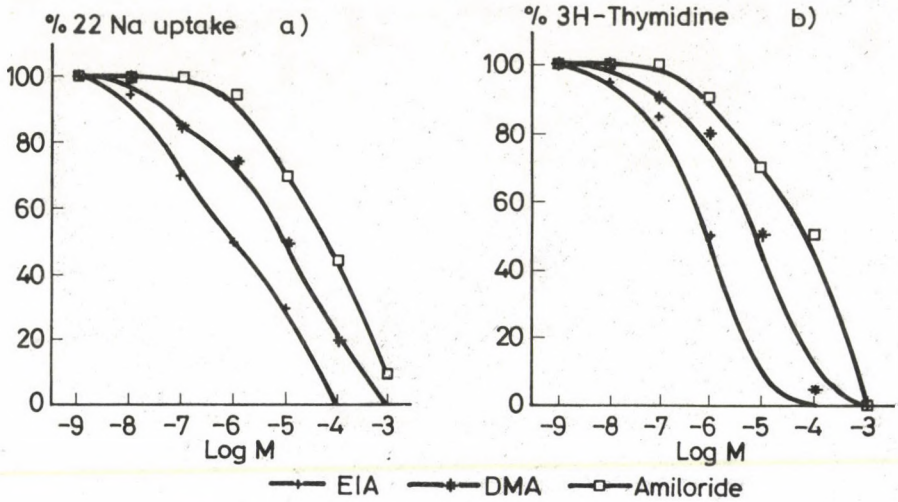


Fig. 1. Effect of amiloride and analogues on $^{22}Na$ uptake (Fig. 1/a) and $^{3}H$-thymidine uptake (Fig. 1/b) in AR4-2J cells. The analogues tested were amiloride (open squares), 5-(N-dimethyl)-amiloride (DMA) (stars), 5-(N-ethyl, 5-N-isopropyl)-amiloride (EIA) (crosses). Points are means from 3 separate experiments. Results are expressed as the percentage of maximal incorporation obtained in the absence of inhibitors

The amiloride inhibition of the initial rate of $^{22}Na$ uptake was concentration-dependent. Fig. 1/a shows the dose-response curves for inhibition of the initial rate of sodium uptake. Concentration for half-maximal inhibition was, respectively, for 5-(N-ethyl-N-isopropyl)amiloride (EIA): 1 $\mu$M < 5-(N,N-dimethyl) amiloride (DMA): 10 $\mu$M < amiloride: 90 $\mu$M. To study the effects of the blockade of the sodium-proton antiporter on cell proliferation, the action of amiloride and analogues were studied on the $^{3}H$-thymidine uptake stimulated by dialysed serum. Fig. 1/b shows the dose-response curves for inhibition of thymidine uptake. Concentration for half-maximal inhibition was, respectively, for EIA: 1 $\mu$M < DMA: 10 $\mu$M < amiloride: 80 $\mu$M.

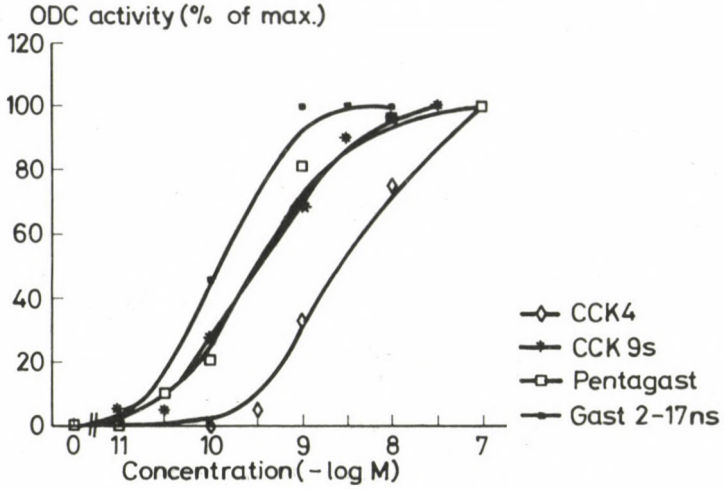


Fig. 2. Effect of various CCK/gastrin peptides to increase ornithine decarboxylase activity in AR4-2J cells. Points are means from 4 separate experiments
CCK 9S: sulphated CCK9; Pentagast: pentagastrin; Gast 2-17 ns: desulphated gastrin 2-17

## Effect of CCK peptides on ornithine decarboxylase activity in AR4-2J cells

CCK peptides stimulated ODC activity by about 2.5 fold. Fig. 2 shows the dose-response curves for stimulation of ODC activity. Concentration for half maximal stimulation was, respectively, for desulfated gastrin 2-17: 0.1 nM<CCK9: 0.25<nM pentagastrin: 0.4<nM CCK4: 6 nM.

## Effect of pertussis toxin on inhibition of cell proliferation caused by somatostatin

Pertussis toxin supressed the inhibitory effect of somatostatin on cyclic AMP formation /13/. Dose-response curve for somatostatin inhibition of EGF (10 nM) stimulation of AR4-2J cell proliferation for 24 h is shown in Fig. 3. Somatostatin (0.1 nM) completely inhibited the effect of EGF. However, pertussis toxin (50 ng/ml), in conditions that suppressed inhibition of cyclic AMP, had no effect on the inhibition of cell proliferation caused by somatostatin (Fig. 4).

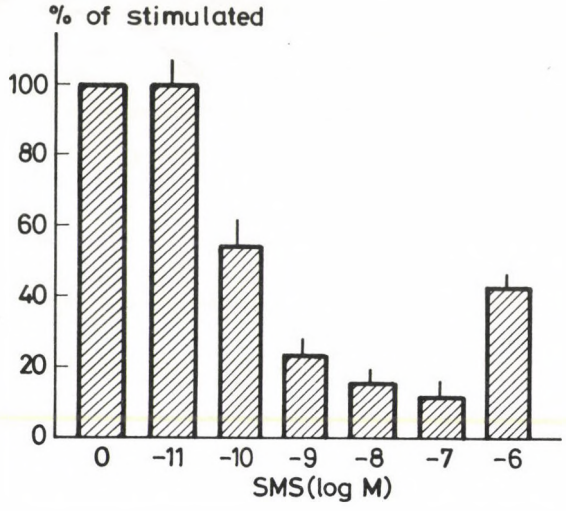


Fig. 3. Effect of various concentrations of SMS on EGF-stimulated $^{3}$H-thymidin incorporation in AR4-2J cells. Results are expressed as percentage of control value obtained with EGF alone. Points are means for 4 separate experiments

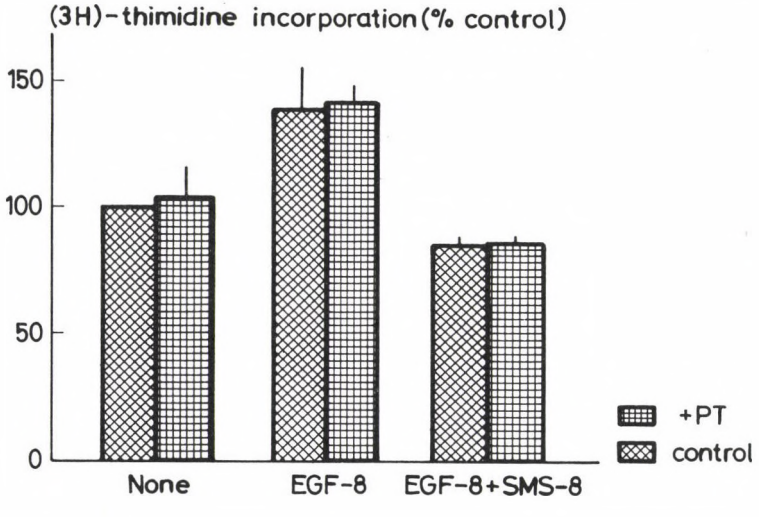


Fig. 4. Effect of pertussis toxin on $^{3}$H-thymidine incorporation in AR4-2J cells in the presence of EGF or somatostatin plus EGF. Bars are means (± SEM) for 2 experiments in quadruplicate

## Discussion

All the pancreatic regulatory agents that act through a common intracellular mechanism calcium/diacylglycerol mediated are secretagogues in vitro. However, they do not stimulate growth in a similar manner. Neither bombesin nor CCK, the receptors of which degrade membrane phosphoinositide, stimulate the growth of AR4-2J cells /12/. An explanation could be that receptors might be coupled to multiple effector systems.

This paper was addressed to know whether the following events: cytoplasmic alkalinization via the $Na^+/H^+$ exchanger, activation of ornithine decarboxylase and cyclic AMP formation, are potentially involved in cell proliferation. Also, the involvement of these events in secretory process will be discussed.

The $Na^+/H^+$ exchanger is the most widely studied membrane mechanism that is involved in the regulation of intracellular pH in vertebrate cells /5/. We have described this system in pancreatic acinar cells /4/ and recently in AR4-2J cells. The existence of this antiporter has been confirmed by another research group /6/. Under the physiological conditions, the system functions as a cell-alkalinizing mechanism and in many cell systems a mitogen-induced rise in pHi is a permissive event for cell proliferation /7/. It was demonstrated that caerulein /4/ and carbamyl choline /3/ caused a cytoplasmic alkalinization. However, amylase release caused by these agents is not invariably associated with alkalinization. Amiloride did not inhibit the amylase release caused by caerulein /1/. The experiments presented here show that amiloride and analogues that did not act directly on tyrosine kinase activity /2/ inhibit the amiloride-sensitive $^{22}Na$ uptake with the same potencies as those with which they inhibit the $^{3}H$-thymidine uptake caused by dialysed serum (Fig. 1). We can conclude that, although inhibition of the antiporter does not affect enzyme secretion, an active sodium-proton exchanger is required for serum-induced proliferation in AR4-2J cells.

Experiments with ornithine decarboxylase show that gastrin and pentagastrin are more potent than CCK to stimulate this activity (Fig. 2). Activation of this enzyme is a limiting step of formation of polyamines which are necessary for cell proliferation in the AR4-2J cell line. Furthermore, gastrin activates a sub-class of CCK receptors, distinct from specific CCK receptors, which could be discriminated by CCK antagonists /9/. Specific high-affinity gastrin antagonists are not yet readily

available. However, CCK-activation of ornithine decarboxylase could not be blocked by specific CCK antagonists /8/, it may be concluded that it is CCK B-receptor-mediated. By contrast, enzyme secretion caused by CCK peptides is CCK-receptor-mediated. Thus, it could be suspected that pathways leading to polyamine-dependent cell proliferation are different from those leading to enzyme secretion.

Cyclic AMP is largely suspected to be the mediator of pancreatic hydrelatic secretion /11/. In pancreatic cells, somatostatin inhibited cyclic AMP /13/ and cell proliferation (Fig. 3). The blockade of cyclic AMP pathway did no interfere with cell proliferation (Fig. 4). Also gastrin inhibited cyclic AMP in AR4-2J cells /10/ while it caused cell proliferation (in preparation). Thus, the hypothesis that intracellular signals leading to hydrelatic secretion are different from those leading to cell proliferation, could be supported.

Finally, it could not be decided whether our results support the concept of multiple effectors-coupled receptors or they could be explained by the existence of multiple receptor sub-classes. Some could be related to secretory pathways, others to proliferative pathways. Antagonists blocking differentially one of those pathways might be conceivable.

## Acknowledgements

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IMMUNOLOGY

# DEFECTIVE PRODUCTION OF INTERLEUKIN-1 AND TUMOUR NECROSIS FACTOR-ALPHA BY STIMULATED MONOCYTES FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Interleukin-1 and tumour necrosis factor-alpha activity by E. coli lipopolysaccharide-triggered monocytes was studied in patients with systemic lupus erythematosus in various stages of activity. Monocytes from both groups of SLE patients produced significantly less tumour necrosis factor-alpha activity than those of age and sex matched healthy controls. However, interleukin-1 activity was only significantly reduced in patients with active stage of the disease. These findings indicate further immunoregulatory disturbances in monocyte function concerning SLE.

Keywords: SLE, tumour necrosis factor-a, interleukin-1

## Introduction

Systemic lupus erythematosus (SLE) is a disease characterized by aberrations of immune regulations /22/, especially T lymphocyte-mediated functions are deranged /7/. Severe abnormalities in monocyte function have also been described /10, 21/. Recently Alcocer-Varela et al. /2/ have shown that monocytes of SLE patients are defective in IL-1 production, and the response of T lymphocytes from the same patients to IL-1 was also found decreased. TNF-a, another protein produced predominantly by stimulated monocytes and macrophages, exerts several biological actions /3/ including effects on immune responses. Therefore, it seemed to be important to investi-

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Abbreviations: IL: interleukin; LPS: lipopolysaccharide; SLE: systemic lupus erythematosus; TNF-a: tumour necrosis factor-alpha

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gate simultaneously the production of IL-1 and TNF-a by activated monocytes from SLE patients with various stages of activity.

## Materials and Methods

### Patients

Twenty three female patients with SLE were included in the study. All patients met the criteria proposed by the American Rheumatism Association for a definite diagnosis /26/. Their age ranged from 18 to 44 years (mean 34.5 years). Nine patients had active disease and were studied prior to initiation of an adequate treatment. The other 14 patients had been in remission and received maintenance oral corticosteroid treatment for 1-2 year (5-10 mg prednisolone daily), which was stopped 48 h prior to cytokine assays. As controls, 15 age and sex-matched healthy volunteers (mean age 32.1 years) were studied.

### Preparation of monocytes

Human mononuclear leukocytes were isolated from heparinized venous blood by Ficoll-Paque (Pharmacia, Sweden) gradient centrifugation. The cells were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Phylaxia, Hungary), 25 mM Hepes buffer (Serva, FRG), 2 mM L-glutamine (Gibco, USA), and antibiotics.

Monocytes were separated by adherence to plastic surface. Briefly, $10x10^6$ cells/ml were seeded into 24-well tissue culture plates (Greiner, FRG) and allowed to adhere for 2 h at 37 $^{o}$C in a humidified atmosphere containing 5% $CO_2$. Cell counts and viability were performed using trypan blue staining. Nonadherent cells were removed by vigorous washing with warm culture medium. The final purified adherent cell preparation contained greater than 96% monocytes according to staining with alpha-naphthylacetate esterase or OKMI antibody (Ortho, USA).

### Triggering IL-1 and TNF-a release

The adherent cells ($1.5x10^6$ cells/ml) were reconstituted with 1 ml/well of the complete culture medium and incubated in the presence or absence of 20 µg/ml lipopolysaccharide (LPS from E. coli 0111: B4, Sigma, USA). After 24 h, cell-free supernatants were harvested by centrifugation to remove cellular debris and filter sterilized using 0.22 µm Millipore (USA) membrane filter. Samples were stored at -20 $^{o}$C until assayed.

### Interleukin-1 assay

Interleukin-1 activity was assessed in the culture supernatants as previously described /16/. Thymocytes from 5 to 7 week old C3H/HeJ mice, were prepared as single cell suspensions and adjusted to a density of $1.5x10^7$/ml in complete RPMI 1640 medium. Thymocytes ($1.5x10^6$ cells/well) were cultured for 48 h at 37 $^{o}$C in 96-well flat bottom microtiter plates (Greiner, FRG) in the presence of 1.0 µg/ml Con A (Pharmacia, Sweden) and test supernatant fluids in humidified atmosphere. After 48 h, the cultures were pulsed and incubated for an additional 18 h with 0.4 µCi/well of $^3$H-thymidine (Chemapol, Czechoslovakia). Cells were collected on filter mats with a multiple cell harvester (Skatron, Norway) and the extent of thymidine incorporation determined by a liquid scintillation counter (Nuclear Chicago, USA). The results were expressed in c.p.m. using the arithmetic mean of quadruplicate cultures. In the majority of experiments the supernatants were diluted 1:2 prior to assay. The proliferation-inducing function has been reported to be a property of IL-1 /8/.

Target cells for TNF-a assay

Target cells derived from the Cincinnati HEp-2 adherent human epipharynx carcinoma cell line (National Institute for Hygiene, Hungary), were cultured in Eagle's MEM containing 10% heat-inactivated fetal-calf serum, 25 mM HEPES, 2 mM L-glutamine, and antibiotics, in 100 ml sterile plastic culture flasks (Greiner, FRG). Serial passage of HEp-2 cell monolayers was performed in 3-5 day periods. After discarding detached, dead cells with the supernatant medium, HEp-2 cells were resuspended from the bottom of culture flasks with 0.5 ml of 0.1% trypsin (Sigma, USA) in TC 199 medium, then washed twice in culture medium. Viability of resuspended targets was evaluated by trypan blue staining and was greater than 99%.

TNF-a assay

TNF-a is considered to be cytotoxic in vitro against a variety of human tumour cell lines /24, 29/. TNF-a cytotoxic activity was measured by the ability of LPS-stimulated monocyte supernatants to cause detachment from the monolayer of $^{3}$H-thymidine labelled HEp-2 target cells as described elsewhere /17/. In brief, resuspended HEp-2 targets ($2.5 \times 10^{3}$ cells/well) were placed into 96-well flat bottom microtiter plates, then labelled with 0.4 µCi/well of tritiated thymidine. To each well aliquots of test supernatant fluids were added and the culture plates incubated for 24 h at 37 $^{o}$C in 5% $CO_2$ atmosphere. Target cells incubated in medium alone were included in each experiment. After 24 h, detached target cells (dead according to trypan blue staining) and test samples having been assayed were removed by washing with warm culture medium. Detachment from the monolayer was used as indicator of cell damage. The remaining adherent HEp-2 cells were frozen at -20 $^{o}$C. After thawing, the content of wells was sucked off onto filter mats with a cell harvester. Incorporated radioactivity was determined by scintillation counting. Results from 6 replicate values were expressed as mean c.p.m. The percentage of cytotoxicity was calculated taking the medium control as baseline, according to the fomrula:

$$\% \text{ cytotoxicity} = 100 \times \left( 1 - \frac{\text{c.p.m. test supernatant}}{\text{c.p.m. medium control}} \right)$$

Using various dilutions of recombinant human TNF-a (NIBSC, England) the assay sensitivity was below 1.0 unit/ml of TNF.

Statistics

All date are given as mean ± S.E.M. of n experiments. Statistical analysis was performed with Student's t test.

## Results

### IL-1 activity of LPS-stimulated monocyte culture supernatants

Figure 1 demonstrates the IL-1 activity of SLE patients and that of healthy controls. Triggered monocytes from nine SLE patients with active disease produced significantly less IL-1 activity than those from normal subjects ($P < 0.01$). The monocyte supernatants obtained from the other

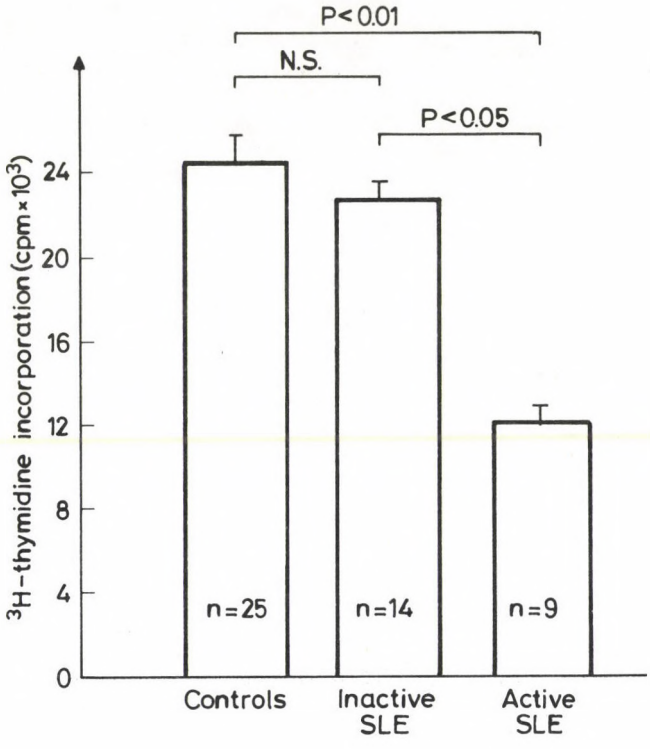


Fig. 1. Production of IL-1 activity by LPS-stimulated monocytes from SLE patients and controls (mean ± S.E.M.)

14 SLE patients with inactive stage of the disease contained almost normal IL-1 activity. Therefore IL-1 activity of patients with active SLE was significantly lower than of those with inactive disease ($P < 0.05$).

## TNF-a activity of LPS-stimulated monocyte culture supernatants

Figure 2 illustrates the TNF-a activity of SLE patients and that of control subjects. We found significantly less TNF-a activity in the supernatants from SLE patients with either active or inactive disease than in those from healthy subjects ($P < 0.001$ and $P < 0.01$, respectively). Moreover, TNF-a activity of SLE patients with active stage of the disease showed a marked decrease when compared to that of patients with inactive SLE ($P < 0.02$).

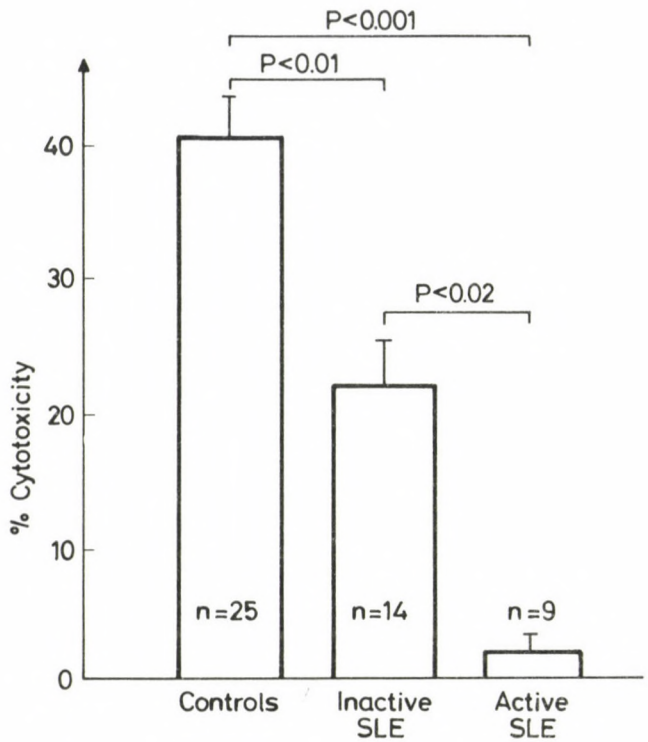


Fig. 2. Production of TNF-a activity by LPS-stimulated monocytes from SLE patients and controls (mean ± S.E.M.)

## Discussion

The present experiments show a marked defect in IL-1 and TNF-a activity in patients with SLE suggesting a decrease in the activity of monocyte helper function. Data concerning decreased IL-1 activity are consistent with earlier observations /2, 14/. Fourteen patients with inactive SLE — but none of the nine with active stadium — had almost normal capacity for IL-1 production. However, all SLE patients, independently of the stage of the disease were defective in TNF-a activity.

In addition to bacterial endotoxins other stimulators are also able to induce TNF-a and IL-1 in vivo, e.g. antigen-antibody complexes /5, 23/ nevertheless, TNF-a itself is known as a potent regulator of IL-1 production /4, 18/. Since a strict correlation of the disease activity with

circulating immune complexes and complement breakdown products can be demonstrated in patients with SLE /25/, further studies have been initiated in our laboratory to elucidate the relationship between the diminished cytokine production and immune complexes.

Our findings of monocyte dysfunction are in good accordance with other reports indicating phagocytic and enzymatic abnormalities in SLE patients /10, 21/.

The expression of IL-2 receptor (IL-2R) as well as the production of IL-2 are thought to require the accessory function of macrophages /28/. The monocyte/macrophage product IL-1 has been implicated to play a major role in the direct activation of T cells through the induction of IL-2 secretion and IL-2R expression /15, 27/.

Recently it has become evident that the other monocyte-derived protein TNF-a is also a pleiotropic mediator exerting profound effects on several biological processes /3/. Among the immunological actions of TNF-a are its ability to induce IL-2R expression /13/ and T cell growth /30/, to stimulate the production of IL-1 /4, 18/, to enhance the proliferation, differentiation and immunoglobulin production of B cells /9, 11/ and to induce macrophage and NK cytotoxicity /19, 20/. These data support the notion that TNF-a is much more than an oncolytic agent and can be considered a potent molecule in the immunoregulation.

Taking the above into consideration it is not surprising that T cells from patients with SLE possess serious defects in the capacity to produce and respond to IL-2 /1, 12, 14/. In turn, the response of T lymphocytes from SLE patients to IL-1 is also markedly altered /2/. It might also be a connection between the impaired NK function /6/ and decreased production of IL-2 and TNF-a observed in SLE.

In conclusion, the present findings of monocyte dysfunction at the level of their production of IL-1 and TNF-a suggest the participation of these cells and their highly refined mediators in the immunoregulatory disturbances of systemic lupus erythematosus.

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# STUDIES ON THE MONOCYTE INTERLEUKIN-1 AND TUMOUR NECROSIS FACTOR-ALPHA PRODUCTION IN PATIENTS WITH ALCOHOLIC LIVER CIRRHOSIS

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Interleukin-1 and tumour necrosis factor-alpha activities by E. coli lipopolysaccharide-triggered monocytes were studied in patients with chronic alcoholic liver disease. Monocytes from cirrhotic patients were shown to have significantly reduced IL-1 and TNF-a activities, compared with that from age and sex matched healthy controls. These findings indicate further immunoregulatory disturbances concerning alcoholic liver cirrhosis.

Keywords: alcoholic liver cirrhosis, interleukin-1, tumour necrosis factor-alpha

## Introduction

Immunological studies in alcoholic liver disease have revealed a wide spectrum of cell-mediated immune defects; especially T lymphocyte functions are deranged /18, 19, 28/. Severe abnormalities in monocyte function have also been described /9, 10/. Recently Yokota et al. /27/ have shown that monocytes from patients with liver cirrhosis and hepatocellular carcinoma are defective in IL-1 production compared to those from control subjects.

TNF-a, another protein produced predominantly by activated monocytes and macrophages, exerts several biological actions in addition to the tumour cell growth inhibition including regulatory effects on immune responses /1/.

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Abbreviations: IL: interleukin; LPS: lipopolysaccharide; TNF-a: tumour necrosis factor-alpha

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Therefore, it seemed to be important to investigate simultaneously the secretion of IL-1 and TNF-a by stimulated monocytes from cirrhotic patients and compare the results to normal adult controls.

## Materials and Methods

### Patients

Twenty-five patients (19 males and 6 females) with histologically proven micronodular cirrhosis were included in the study. Their age ranged from 28 to 49 years (mean 40.7 years). Chronic alcoholism was evident from past history. The mean daily alcohol consumption by the men and the women exceeded 60 g and 30 g respectively for at least 7 years. The general nutritional status of the patients was sufficient: no symptoms of hypovitaminosis or excessive weight loss were seen. None of the patients had any clinical or biological evidence of hepatocellular carcinoma. Markers of current hepatitis B virus infection were absent in all patients' sera. None had received corticosteroids or other immunosuppressive drugs.

Routine liver biochemical function tests including serum bilirubin, aspartate aminotransferase (AST, E.C.2.6.1.1.), alanine aminotransferase (ALT, E.C.2.6.1.2.), gamma-glutamyl transferase (GGT, E.C.2.3.2.2.) and alkaline phosphatase (AP, E.C.3.1.3.1.) were determined in all patients. Data are summarized in Table 1.

Table 1

Biochemical characteristics in patients with alcoholic liver cirrhosis (means ± S.E.M.)

| Parameter | Healthy subjects | Patients with alcoholic liver disease |
|---|---|---|
| Bilirubin μmol/l (2-26) | 11.3 ± 3.8 | 33.4 ± 4.5[a] |
| AST U/l (1-20) | 12.5 ± 4.0 | 72.3 ± 7.2[b] |
| ALT U/l (1-30) | 14.1 ± 5.3 | 83.5 ±10.1[b] |
| GGT U/l (1-25) | 18.7 ± 3.9 | 169.5 ±17.2[c] |
| AP U/l (70-170) | 140.5 ±12.6 | 138.3 ±16.0[d] |

In parantheses, normal values

[a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.001$ vs. controls; [d] not significant

As controls, 20 age- and sex-matched healthy volunteers whose mean age was 42.5 years were studied.

Preparation of monocytes

Human mononuclear leukocytes were isolated from heparinized venous blood by Ficoll-Paque (Pharmacia, Sweden) gradient centrifugation. The cells were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Phylaxia, Hungary), 25 mM Hepes buffer (Serva, FRG), 2 mM L-glutamine (Gibco, USA), and antibiotics.

Monocytes were separated by adherence to plastic surface. Briefly, $10x10^6$ cells/ml were seeded into 24-well tissue culture plates (Greiner, FRG) and allowed to adhere for 2 h at 37 $^{o}$C in a humidified atmosphere containing 5% $CO_2$. Cell counts and viability were performed by using trypan blue staining. Nonadherent cells were removed by vigorous washing with warm culture medium. The final purified adherent cell preparation contained more than 96% monocytes according to staining with alpha-naphthyl-acetate esterase or OKMI antibody (Ortho, USA).

Triggering IL-1 and TNF-a release

The adherent cells ($1.5x10^6$ cells/ml) were reconstituted with 1 ml/well of the complete culture medium and incubated in the presence or absence of 20 µg/ml lipopolysaccharide (LPS from E. coli 0111: B4, Sigma, USA). After 24 h, cell-free supernatants were harvested by centrifugation to remove cellular debris and filter sterilized by using 0.22 µm Millipore (USA) membrane filter. Samples were stored at -20 $^{o}$C until assayed.

Interleukin-1 assay

Interleukin-1 activity was assessed in the culture supernatants as described previously /20/. Thymocytes from 5 to 7 week old C3H/HeJ mice were prepared as single cell suspensions and adjusted to a density of $1.5x10^7$/ml in complete RPMI 1640 medium. Thymocytes ($1.5x10^6$ cells/well) were cultured for 48 h at 37 $^{o}$C in 96-well flat bottom Microtiter plates (Greiner, FRG) in the presence of 1.0 µg/ml Con A (Pharmacia, Sweden) and test supernatant fluids in humidified atmosphere. After 48 h, the cultures were pulsed and incubated for an additional 18 h with 0.4 µCi/well of $^{3}$H-thymidine (Chemapol, Czechoslovakia). Cells were collected on filter mats with a multiple cell harvester (Skatron, Norway) and the extent of thymidine incorporation determined by a liquid scintillation counter (Nuclear Chicago, USA). The results were expressed in c.p.m. using the arithmetic mean of quadruplicate cultures. In the majority of experiments the supernatants were diluted 1/2 prior to assay. This proliferation-inducing function has been reported to be a property of IL-1 /7/.

Target cells for TNF-as assay

Target cells derived from the Cincinnati HEp-2 adherent human epipharynx carcinoma cell line (National Institute of Hygiene, Hungary), were cultured in Eagle's MEM containing 10% heat-inactivated fetal calf serum, 25 mM HEPES, 2 mM L-glutamine, and antibiotics, in 100 ml sterile plastic culture flasks (Greiner, FRG). Serial passage of HEp-2 cell monolayers was performed in 3-5 day periods. After discarding detached, dead cells with the supernatant medium, HEp-2 cells were resuspended from the bottom of culture flasks with 0.5 ml of 0.1% trypsin (Sigma, USA) in TC 199 medium, then washed twice in culture medium. Viability of resuspended targets was more than 99% as evaluated by trypan blue staining.

TNF-a assay

TNF-a is considered to be cytotoxic in vitro against a variety of human tumour cell lines /25, 26/. TNF cytotoxic activity was measured by the ability of LPS-stimulated monocyte supernatants to cause detachment from the monolayer of $^{3}$H-thymidine labelled HEp-2 target

cells as described elsewhere /21/. In brief, resuspended HEp-2 targets ($2.5 \times 10^3$ cells/well) were placed into 96-well flat bottom microtiter plates, then labelled with 0.4 μCi/well of tritiated thymidine. To each well aliquots of test supernatant fluids were added and the culture plates incubated for 24 h at 37 °C in 5% $CO_2$ atmosphere. Target cells incubated in medium alone were included in each experiment. After 24 h, detached target cells (dead as shown by trypan blue staining) and test samples having been assayed were removed by washing with warm culture medium. Detachment from the monolayer was used as indicator of cell damage. The remaining adherent HEp-2 cells were frozen at -20 °C. After thawing, the contents of wells were sucked off onto filter mats with a cell harvester. Incorporated radioactivity was determined by scintillation counting. Results from 6 replicate values were expressed as mean c.p.m. The percentage of cytotoxicity was calculated taking the medium control as baseline, according to the formula:

$$\% \text{ cytotoxicity} = 100 \times \left(1 - \frac{\text{c.p.m. test supernatant}}{\text{c.p.m. medium control}}\right)$$

Using various dilutions of recombinant human TNF-a (NIBSC, England) the assay sensitivity was below 1.0 unit/ml of TNF.

### Statistics

All data are given as mean ± S.E.M. of n experiments. Statistical analysis was performed with Student's t test.

## Results

### Interleukin-1 activity of LPS-stimulated monocyte culture supernatants

Figure 1 demonstrates the IL-1 activity of patients with micronodular cirrhosis and that of healthy controls. Triggered monocytes from cirrhotic patients produced significantly less IL-1 activity than those from control subjects ($P < 0.05$).

### Tumour necrosis factor-alpha activity of LPS-stimulated monocyte culture supernatants

Figure 2 illustrates the TNF-a activity of cirrhotic patients and that of normal subjects. We found significantly less TNF-a activity in the supernatants obtained from the cirrhotic group than in those obtained from the healthy subjects ($P < 0.001$).

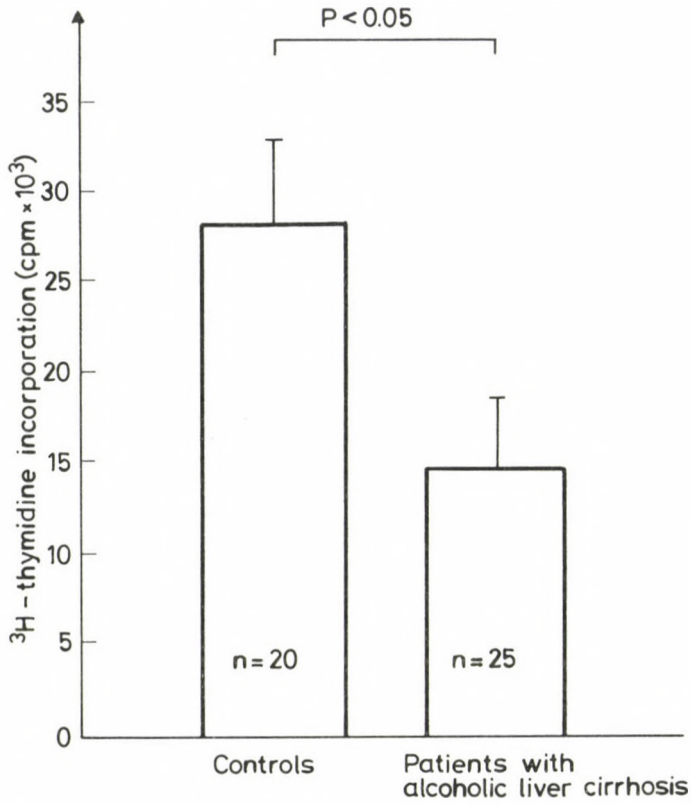


Fig. 1. Production of IL-1 activity by LPS-stimulated monocytes from cirrhotic patients and controls
I
Mean + S.E.M.; n = number of patients

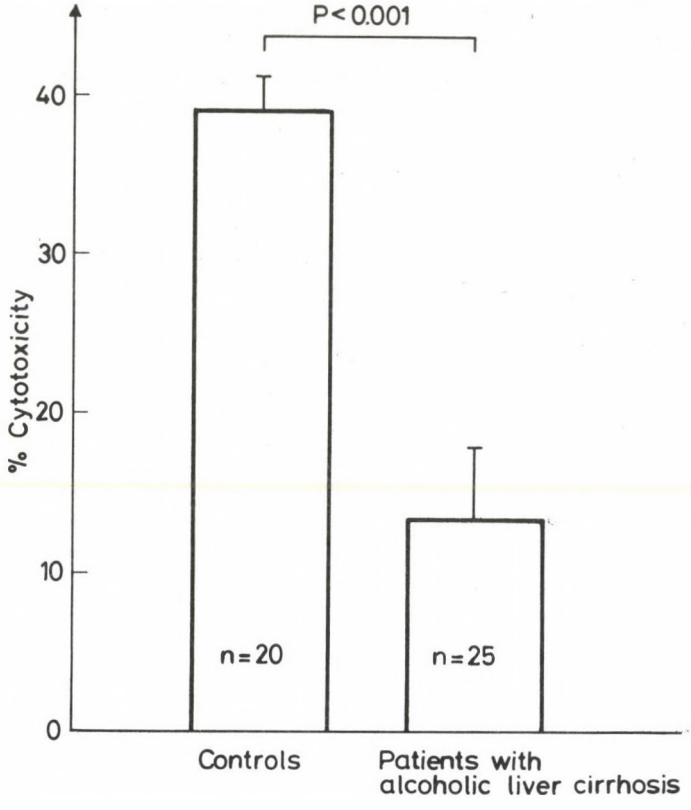


Fig. 2. Production of TNF-a activity by LPS-stimulated monocytes from cirrhotic patients and controls
Mean + S.E.M.; n = number of patients

## Discussion

The present experiments prove a marked defect in IL-1 and TNF-a activity in alcoholic patients with liver cirrhosis, suggesting a decrease in the activity of monocyte helper function. Data concerning decreased IL-1 activity are consistent with earlier observations /23, 27/. Our findings of monocyte dysfunction are in good accordance with other reports indicating phagocytic, killing and enzymatic abnormalities in cirrhotic patients /9, 10/.

Besides the impaired secretion of IL-1 and TNF-a from monocytes the diminished production of these cytokines may also be related to the increased secretion of inhibitory factors of IL-1 and TNF-a activity. Prostaglandins are well-known inhibitors of monocyte IL-1 and TNF-a secretion /12, 13/.

Indeed, in their study Yokota et al. /27/ have found elevated activity of suppressor monocytes, i.e. increased production of prostaglandin $E_2$ in patients with liver cirrhosis and hepatocellular carcinoma, indicating another possible explanation of the simultaneously detected low IL-1 activity.

The depressed cellular immunity often seen in patients with alcoholic liver disease, especially with cirrhosis is characterized, among others, by an impaired lymphocyte proliferative response to various mitogens /11, 14/. In addition, T cells from cirrhotic patients possess a considerable defect in the capacity to respond to IL-2 (Gy. Műzes et al., unpublished). However recent studies have documented that both IL-1 and TNF-a are implicated to play a positive regulatory role in T cell activation and proliferation /8/. IL-1 and TNF-a share a remarkable number of activities including an ability to enhance T cell IL-2 receptor expression and proliferative responses to IL-2 /15, 17, 24/. Moreover, TNF-a has been shown to stimulate IL-1 production by monocytes /3/. These data support the notion that in cirrhotic patients the decreased production of both cytokines may be in part responsible for the reduced lymphocyte transformation.

Patients with alcoholic cirrhosis have an increased risk of developing hepatocellular carcinoma /4/ and cancers elsewhere than in the liver /2/. Previous reports indicated that monocytes represent one of the principal mechanisms of host defense againts malignancies /6/. TNF-a and, to a lesser extent, IL-1 are considered possible mediators of monocyte cytotoxicity for tumour cells /5, 16/. Furthermore, TNF-a and IL-1 are known to induce macrophage cytotoxicity /22/. In this way cirrhotic patients with depressed IL-1 and TNF-a activity may be more prone to develop hepatocellular carcinoma.

In conclusion, the present findings of monocyte dysfunction at the level of their production of IL-1 and TNF-a suggest the participation of these cells and their highly refined mediators in the immunoregulatory disturbances of alcoholic liver cirrhosis. The exact mechanism of diminished cytokine productions, not clarified yet, needs further studies to be determined.

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# IDENTIFICATION OF HEALTH DAMAGE DUE TO ALCOHOL ABUSE; IMPORTANCE OF ALTERATIONS IN CARDIAC FUNCTION AND BLOOD CHEMISTRIES

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Like in any other disease, diagnosis of health damage caused by alcohol abuse can be established all the easier and sooner, the more results of medical examinations are taken into consideration simultaneously. Evidence is presented that a larger proportion (96.4%) of alcoholics suffer from compromised cardiac function (the classification function uses 6 variables out of the measured 21 than from disorders of liver function and the metabolism (87.7%) (the classification function uses 12 variables out of the 21). Taking into consideration all the 41 variables (using only 12) the power of discrimination is 99.2% (discriminant analysis).

Keywords: alcohol, cardiac function, blood chemistry profiles, discriminant analysis

## Introduction

Alcoholism, despite its significance in causing health damage, is diagnosed in a frequency that does not reflect reality /5, 13/. Thus, it is an old effort to find markers suitable for an unequivocal demonstration of alcohol abuse, which has nowadays been done by two methods, viz. use of questionnaires specially constructed for this purpose /7, 20, 24/, or clinical laboratory examinations /8, 12, 13, 25/.

The questionnaire method is subjective, the biochemical tests consider only alcohol-induced disorders of the liver and the metabolism. Detection of health damage caused by alcohol is even more difficult as,

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although the mean values of the variables examined may show significant differences, this approach has no diagnostic meaning in individual cases. For example, we have found the following results for liver gamma-glutamyltransferase (GGT) activity, one of the most specific indicators (according to the literature normal value ranges up to 30 U/l),

| | GGT < 30 | GGT > 30 | Total |
|---|---|---|---|
| control | 166 | 25 | 191 |
| alcoholic | 62 | 109 | 171 |

despite the large difference between the averages for the two groups shown in Table I. When several clinical laboratory tests are simultaneously taken into account, the specificity of the detection can be improved /11, 18, 22, 27/, but in that case one of the multivariate statistical methods, e.g. discriminant analysis, is needed /3, 5, 21/.

The complication of questionnaires and joint use of clinical laboratory tests also increases the diagnostic value. The heart in another target organ of chronic alcohol poisoning as shown in the literature by using different approaches. The frequency of cardiovascular damage and death in alcoholics is greater than that of the hepatic damage and death due to liver insufficiency /4/. The cardiovascular mortality of non-alcoholics is surpassed by the proportion of alcoholics dying of cardiovascular disease. According to several authors /9, 10, 19, 23, 29/ this difference is considerable, while others /14/ suggest that it is minimal.

The average lifespan of alcoholics is 8-12 years shorter than that for the total population. Thus, mortality due to cardiovascular disease in the age groups 30 to 50 years, can reach up to 2-3 times the expected cardiovascular mortality /4, 10, 28/, and these fatalities occur at a younger age than those due to liver insufficiency caused by alcohol (45-65 years), /2, 15/.

As symptoms indicating cardiovascular disorder can be observed with marked frequency among alcoholics suffering from hepatic disorder /1, 26/, it is evident that a whole arsenal of tests, including parameters characterizing altered cardiovascular function caused by alcohol, is suitable for diagnosing alcoholism.

Relying upon these findings, we examined to what extent can alcoholics be discriminated from the healthy controls on the basis of (i) the parameters of liver and metabolic disorders caused by alcoholic (ii), the alcohol-induced changes in the cardiovascular function, or (iii) all the parameters of the liver, the metabolism and the cardiovascular system.

These discriminant analyses enable us to compare the discriminative capacity of the changes in liver function and that of the cardiac function. The best discrimination can be expected from the canonical variable obtained by the simultaneous consideration of all the variables, thus the detection of symptomless alcoholism, and the prevention of the subclinical illness it causes, can be made possible.

## Patients and Methods

A total of 171 patients (147 males and 24 females), 22-50 years of age (average: 35.87 years), each under detoxification, were examined. All of the patients were alcohol-dependent, and fitted the diagnostic criteria of chronic alcoholism suggested by the WHO. Each of the patients admitted had consumed at least 75 ml of pure alcohol a day, for a period of five years before admission. Those suffering from clinically confirmed cardiac disease, and those undergoing medical treatment for any sort of diagnosed cardiopathy (those taking drugs) were excluded. Also excluded were the cirrhosis cases, and the patients suffering from gross tremor, hallucinosis or convulsions.

A control group consisted of 191, 19-50 years old individuals (average: 38.23 years): 174 males, 17 females. The members of this group were clinically healthy, and denied to consume alcohol on a regular basis.

During the clinical examinations, height and body weight were measured in each case, and body area was determined. The cardiothoracic ratio (CTR) was calculated on the basis of two-directional chest X-ray pictures, the R-R, PQ, and QRS intervals were measured on a 12-lead electrocardiogram. The heart rate, and, using the Bazett formula, the QT distance corrected to the heart rate ($QT_c$) were calculated.

$$QT_c = QT(IR\text{-}R)^{0.5}$$

On the ECG, the amplitudes of $S_{v1}$, $R_{v5}$, $T_{v2}$, $T_{v5}$ were measured and axis deviation was determined. The TDEV values in the tables are the consinuses of the axis deviations.

Systolic time intervals were calculated by means of registering three curves synchronized. On a six-channel Hellige instrument, with 100 mm/s paper velocity, were registered the ECG lead II, PCG was registered above Erb point and the carotid curve for the right carotid artery. The final values in each case were the means for 5 successive cardiac cycles. The PEP values were calculated by measuring the $QS_2$ and the LVET, and from these values, the PEP/LVET ratio was computed. For each subject clinical haematology and biochemical tests, including enzyme determinations characterizing liver function, were carried out. The blood samples from the alcoholic and control subjects were analysed by the same clinical laboratory. A list of the blood tests is presented in Table 1. A PHAl was used for the haematological tests; the other tests were performed on a CentrifiChem 400 Auto Analyser. Student's t test and discriminant analysis were used as statistical procedures.

## Results

Table I

Variables in alcoholics and controls

| | Non-alcoholic controls n = 191 | | | Alcoholics n = 171 | |
|---|---|---|---|---|---|
| | mean values | S.D. | | mean values | S.D. |
| Age, years | 38.23 | 8.62 | $P < 0.01$ | 35.87 | 6.46 |
| Heart rate, beats/min | 75.98 | 10.70 | - | 76.94 | 12.91 |
| $QS_2$, sec | 0.3850 | 0.0202 | - | 0.3867 | 0.0240 |
| LVET, sec | 0.2915 | 0.0161 | $P < 0.01$ | 0.2762 | 0.0181 |
| PEP/LVET | 0.3212 | 0.0148 | $P < 0.01$ | 0.4006 | 0.0258 |
| R-R, sec | 0.8081 | 0.1122 | - | 0.8018 | 0.1350 |
| PQ, sec | 0.1689 | 0.0192 | - | 0.1727 | 0.0252 |
| QRS, sec | 0.08115 | 0.00478 | $P < 0.01$ | 0.08526 | 0.00849 |
| $QT_c$, sec | 0.4014 | 0.0280 | $P < 0.01$ | 0.4221 | 0.0320 |
| $S_{V1}$, mV | 0.6476 | 0.2645 | $P < 0.01$ | 1.0643 | 0.4175 |
| $R_{V5}$, mV | 1.078 | 0.437 | $P < 0.01$ | 2.053 | 0.631 |
| $T_{V2}$, mV | 0.5387 | 0.2312 | $P < 0.01$ | 0.6415 | 0.2809 |
| $U_{V2}$, mV | 0.06963 | 0.03025 | $P < 0.01$ | 0.08047 | 0.04476 |
| $T_{V5}$, mV | 0.3487 | 0.1631 | $P < 0.01$ | 0.6076 | 0.2374 |
| TDEV1 | -0.1309 | 0.7344 | $P < 0.01$ | -0.4384 | 0.7153 |
| TDEV2 | 0.1573 | 0.7304 | $P < 0.01$ | 0.4113 | 0.7255 |
| Body weight, kg | 76.26 | 12.59 | $P < 0.01$ | 67.32 | 10.51 |
| Height, cm | 171.0 | 7.7 | - | 170.5 | 7.7 |
| Body surface, $m^2$ | 1.899 | 0.186 | $P < 0.01$ | 1.782 | 0.167 |
| Cardiothoracic ratio | 0.4521 | 0.0395 | $P < 0.01$ | 0.4304 | 0.0448 |
| Total protein, g/l | 69.17 | 5.35 | $P < 0.01$ | 66.65 | 6.37 |
| Albumin, g/l | 48.39 | 4.73 | $P < 0.01$ | 45.18 | 7.35 |
| Total bilirubin, µmol/l | 14.02 | 5.19 | $P < 0.01$ | 12.35 | 6.42 |
| Alkaline phosphatase, U/l | 103.0 | 26.0 | $P < 0.01$ | 113.6 | 33.4 |
| SGOT, U/l | 12.49 | 6.31 | $P < 0.01$ | 18.86 | 14.64 |
| SGPT, U/l | 13.40 | 8.24 | $P < 0.01$ | 21.10 | 18.80 |
| GGT, U/l | 19.14 | 11.02 | $P < 0.01$ | 96.85 | 133.49 |
| Na, mmol/l | 142.1 | 1.6 | $P < 0.01$ | 140.4 | 2.7 |
| K, mmol/l | 4.374 | 0.353 | - | 4.440 | 0.495 |
| Ca, mmol/l | 2.466 | 0.139 | - | 2.474 | 0.175 |
| P, mmol/l | 1.012 | 0.208 | $P < 0.01$ | 1.105 | 0.210 |
| Mg, mmol/l | 0.9026 | 0.1737 | $P < 0.01$ | 0.7840 | 0.2339 |
| CPK, U/l | 47.59 | 19.60 | $P < 0.01$ | 60.82 | 62.40 |
| Cholesterol, mmol/l | 6.434 | 0.963 | $P < 0.01$ | 6.955 | 2.000 |
| Triglyceride, mmol/l | 1.412 | 0.686 | $P < 0.01$ | 2.089 | 1.241 |
| Fasting blood sugar, mmol/l | 5.055 | 0.762 | $P < 0.01$ | 4.851 | 0.656 |
| Urea nitrogen, mmol/l | 5.929 | 1.323 | $P < 0.01$ | 4.413 | 0.963 |
| Creatinine, µmol/l | 85.74 | 13.41 | $P < 0.01$ | 75.16 | 13.10 |
| Uric acid, µmol/l | 306.0 | 69.7 | - | 293.2 | 78.6 |
| Haemoglobin, µmol/l | 9.437 | 0.776 | $P < 0.01$ | 8.910 | 0.930 |

Table II

Discriminant analysis based on the variables of liver function

Variables: total protein, albumin, total bilirubin, alkaline phosphatase, SGOT, SGPT, GGT, sex

Canonical correlation: 0.4886

| | Coefficients for canonical variables |
|---|---|
| 1. GGT | 0.00787 |
| 2. Total bilirubin | 0.07787 |
| 3. Total protein | -0.07550 |
| 4. SGPT | 0.02426 |
| Constant | 5.312 |

Classification matrix:

| | Number of cases classified into group | | |
|---|---|---|---|
| Group | Non-alcoholic control | Alcoholic | Total |
| Non-alcoholic control | 158 | 33 | 191 |
| Alcoholic | 65 | 106 | 171 |
| Total | 223 | 139 | 362 |

Table III

Discriminant analysis by the variables of electrolytes in the blood

Variables: Na, K, Ca, P, Mg, sex

Canonical correlation: 0.4423

| | Coefficients for canonical variables |
|---|---|
| 1. Na | -0.3112 |
| 2. Mg | -2.637 |
| 3. P | 1.757 |
| Constant | 44.36 |

Classification matrix:

| | Number of cases classified into group | | |
|---|---|---|---|
| Group | Non-alcoholic control | Alcoholic | Total |
| Non-alcoholic control | 146 | 45 | 191 |
| Alcoholic | 62 | 109 | 171 |
| Total | 208 | 154 | 362 |

Table IV

Discriminant analysis by the variables of the biochemical tests

Variables: Na, K, Ca, Mg, total protein, albumin, total bilirubin, alkaline phosphatase, SGOT, SGPT, GGT, CPK, cholesterol, triglyceride, fasting blood sugar, urea nitrogen, cretinine, uric acid, haemoglobin, sex

Canonical correlation: 0.7528

| | Coefficients for canonical variables |
|---|---|
| 1. Urea nitrogen | -0.4421 |
| 2. GGT | 0.00374 |
| 3. Triglyceride | 0.3283 |
| 4. Na | -0.1752 |
| 5. Creatinine | -0.02046 |
| 6. Total protein | -0.06507 |
| 7. CPK | 0.00496 |
| 8. Mg | -1.032 |
| 9. Cholesterol | 0.1377 |
| 10. Total bilirubin | -0.03395 |
| 11. SGPT | 0.01407 |
| 12. Ca | 1.183 |
| Constant | 29.34 |

Classification matrix:

| | Number of cases classified into group | | |
|---|---|---|---|
| Group | Non-alcoholic control | Alcoholic | Total |
| Non-alcoholic control | 174 | 17 | 191 |
| Alcoholic | 21 | 150 | 171 |
| Total | 195 | 167 | 362 |

In Table I, the means and standard deviations of the examined parameters are illustrated; the significance of the differences between the two groups can be seen. If we want to select alcoholics, only taking into account parameters characterizing liver function (Table II), 27.1% of the examined individuals will not be classified into the correct group. Taking into consideration the parameters of serum electrolytes (Table III)there is a 29.6% error in classification. If all clinical laboratory parameters (Table IV) are considered, the proportion of falsely classified cases is reduced to 10.5%.

Table V

Discriminant analysis by the variables of cardiac function

Variables: age, heart rate, R-R, PQ, QRS, $QT_C$, $S_{V1}$, $R_{V5}$, $T_{V2}$, $U_{V2}$, $T_{V5}$, TDEV1, TDEV2, $QS_2$, LVET, PEP/LVET, body weight, height, body surface, cardiothoracic ratio, sex

Canonical correlation: 0.9144

| | Coefficients for canonical variables |
|---|---|
| 1. PEP/LVET | 41.39 |
| 2. $T_{V5}$ | 1.668 |
| 3. $QT_C$ | 6.966 |
| 4. Cardiothoracic ratio | -4.821 |
| 5. $R_{V5}$ | 0.3689 |
| 6. QRS | 22.19 |
| Constant | -17.35 |

Classification matrix:

| | Number of cases classified into group | | |
|---|---|---|---|
| Group | Non-alcoholic control | Alcoholic | Total |
| Non-alcoholic control | 191 | 0 | 191 |
| Alcoholic | 6 | 165 | 171 |
| Total | 187 | 165 | 362 |

Classification according to parameters characterizing cardiac function is satisfactory from the diagnostic point of view (Table V) as only 1.7% of the examined persons were classified as alcoholic. All the parameters (Table VI) separate the two groups excellently. The fact that only 0.8% of the cases was classified incorrectly renders the method suitable for diagnostic purposes.

Table VI

Discriminant analysis by all of the variables

Variables: age, heart rate, $QS_2$, LVET, PEP/LVET, R-R, PQ, QRS, $QT_c$, $S_{v1}$, $R_{v5}$, $T_{v2}$, $U_{v2}$, $T_{v5}$, TDEV1, TDEV2, body weight, height, body surface, cardiothoracic ratio, total protein, albumin, total bilirubin, alkaline phosphatase, SGOT, SGPT, GGT, Na, K, Ca, P, Mg, CPK, cholesterol, triglyceride, fasting blood sugar, urea nitrogen, uric acid, haemoglobin, sex

Canonical correlation: 0.9324

| | Coefficients for canonical variables |
|---|---|
| 1. PEP/LVET | 38.91 |
| 2. $T_{v5}$ | 1.560 |
| 3. Urea nitrogen | -0.2491 |
| 4. Triglyceride | 0.3001 |
| 5. $QT_c$ | 8.316 |
| 6. GGT | 0.00241 |
| 7. Cardiothoracic ratio | -5.381 |
| 8. $R_{v5}$ | 0.3632 |
| 9: CPK | 0.00435 |
| 10. Body weight | -0.01276 |
| 11. Heart rate | -0.01805 |
| 12. Haemoglobin | -0.1712 |
| Constant | -12.01 |

Classification matrix:

| | Number of cases classified into group | | |
|---|---|---|---|
| Group | Non-alcoholic control | Alcoholic | Total |
| Non-alcoholic control | 191 | 0 | 191 |
| Alcoholic | 3 | 168 | 171 |
| Total | 194 | 168 | 362 |

## Discussion

As expected — on the basis of analysing data of the literature referred to in the introduction, and also of our previous observations /16, 17/ — we succeeded in providing that parameters indicating cardiovascular changes due to alcohol must unavoidably be taken into consideration when creating test-systems for diagnosing alcoholism.

Our results yield evidence that by considering only parameters characterizing cardiovascular changes caused by alcohol, alcoholics and non-alcoholics can be classified into the correct group with a probability of 98%. This accuracy, being unusually high in medical diagnostics, is remarkable because alcoholism, apart from its neurotoxic effect, is at

present diagnosed on the basis of the hepatotoxic and/or electrolyte-metabolism-deranging effect of alcohol. In our present study, considering parameters characterizing hepatotoxicity in 191 control individuals and 171 alcoholics, the decision was correct in 63% of the cases; using the serum electrolyte values, the agreement was 70%. Even with the combination of parameters indicating both hepatotoxicity and toxic damage of electrolyte balance (20 tests altogether), the proportion of correct decisions was 90%, i.e. we did not reach the proportion of correct decisions obtained on the basis of cardiovascular parameters. Using 25 and 31 laboratory tests, respectively, Ryback et al. /21/, Chan et al. /5/ obtained the same results with discriminant analysis. The proportion of correct decisions based on 21 cardiovascular parameters was further improved by using all three kinds of parameters (cardiovascular, hepatic, electrolyte balance) simultaneously.

The canonical variable (Table 6), obtained by simultaneous consideration of all the variables (it is the linear combination of the 12 "original" variables), makes detection and diagnosis of alcoholism possible.

The results obtained with our method, worked out with discriminant analysis, a method suitable for an objective diagnosis of alcoholism, also indicate that alcoholism affects the heart in practically all cases, at least in the form of instrumentally detectable disturbances. This conclusion fits the views according to which the leading cause of death among alcoholics is cardiovascular, by which this group, frequently suffering from other organ-disorders as well, is afflicted years or decades earlier than the rest of the population. Our observation is in agreement also with the fact that alcohol consumption increases the risk of cardiovascular diseases in adolescents and young adults /6/. The effect of alcohol appears behind (or as part of) many cases of "vegetative neurosis" or a number of diseases attributed to psycho-social pathogenic factors caused by psychical, social problems. Since alcoholism, sooner or later, leads to significant organic damage, its recognition is essential for both the individual and his close environment, every for the whole society. With the discriminant analysis, detection of alcoholism can be expected in an early phase when an intervention resulting in complete recovery is still possible.

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# METABOLISM OF GLYCOCONJUGATES IN HUMAN GASTRIC MUCOSA A REVIEW

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Glycoconjugates, viz. glycoproteins, glycolipids and proteoglycans, play an important role in the protection of human gastric mucosa against pepsin and hydrochloric acid. The biosynthesis and catabolism of these compounds in the human gastric mucosa have been studied. We isolated and identified the majority of the intermediates and isolated some enzymes, taking part in the biosynthesis of glycoconjugates in the human gastric mucosa, and have demonstrated that the human gastric mucosa is able to release reducing sugars from glycoconjugates and shows some glycosidase activity.

Keywords: human gastric mucosa, glycoconjugates, glycoprotein biosynthesis, glycoprotein catabolism, exoglycosidases

## Introduction

Three groups of glycoconjugates containing aminohexoses viz. proteoglycans /28/, glycoproteins /9, 10, 11, 18/ and glycolipids /31/, were isolated from the human gastric mucous membrane. Proteoglycans are constituents of the extracellular matrices, whereas glycoproteins are the main components of gastric mucus and, together with glycolipids, constitute cellular membranes. Of the human gastric glycoconjugates the most widely investigated is the mucin forming the mucus gel /9, 10, 11, 32, 33/; less attention is paid to the intrinsic factor, haptocorrin /18/ and other glyco-

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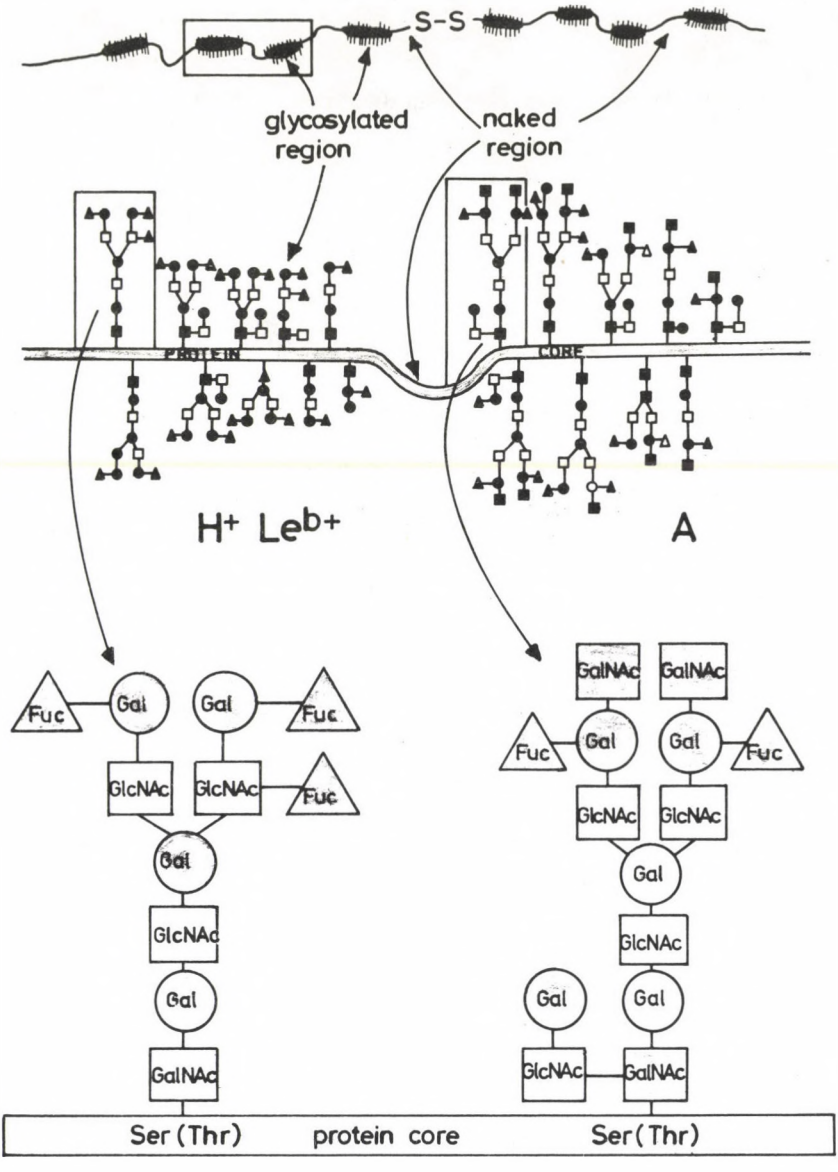


Fig. 1. Schematic view of human gastric mucus glycoprotein showing sugar chains of two different glycoproteins ($H^+$ $Le^{b+}$ and A)
(refs 5, 30, 32, 33)

proteins /17/. Human gastric mucin mainly contains galactose, fucose, N-acetylglucosamine and N-acetylgalactosamine. Of the amino acids serine and threonine were found to constitute the greatest amounts /12, 30, 32, 33/. The sugar chains of secretors are terminated with carbohydrates forming the structures H, A or B, depending on the donor's blood group /5, 30, 32, 33/ (Fig. 1). The intrinsic factor produced by the human gastric mucosa is also of glycoprotein nature /18/. N-acetylglucosaminidase from human gastric mucous membrane is a glycoprotein whose sugar chains have not been elucidated structurally so far /26/. Immunoglobulin A is a further glycoprotein that has been isolated from the human gastric mucosa /34/.

## Biosynthesis of gastric glycoproteins

The biosynthesis of human gastric glycoproteins can be divided into three stages:

a) production of phosphate esters and sugar nucleotides;
b) incorporation of sugars into glycoconjugates;
c) transportation of macromolecules to the sites of destination.

There are three lines of evidence suggesting that phosphate esters and sugar nucleotides are synthesized in the human gastric mucosa: (i) sugar phosphates and sugar nucleotides were identified in the trichloracetic-acid-soluble extracts obtained from the mucosa /13/. Human gastric mucosa contains 67.5% glucosamine and 32.5% galactosamine /41/. There is only 0.46% of free aminosugars and their phosphate esters and 0.24% of aminosugar nucleotides /13, 41/ (Fig. 2); (ii) the gastric mucosa is capable of incorporating radioactive glucose /15, 35/ and galactose /21, 22/ into phosphate esters and glycoconjugates (Fig. 3); (iii) enzymes taking part in the biosynthesis of the phosphate esters of aminosugars /42/, their acetyl derivatives /7, 44/ have been isolated and epimerization of UDP-N-acetylglucosamine into UDP-N-acetylgalactosamine /16/ and mannosamine /14/ has been demonstrated in the gastric mucosa.

The incorporation of sugars into human gastric glycoproteins as O-glycosylation begins when the polypeptide core is attached to t-RNA /38/. Watkins et al. /6/ investigated the incorporation of fucose into glycoconjugates in the human gastric mucosa and noted the activity of three enzymes: 2-fucosyltransferase "Enzyme H" 3-fucosyltransferase and 4-fucosyltransferase "Enzyme Le". Tuppy et al. /6, 28, 36, 37, 40/ found in that tissue the activity of 3-acetylgalactosaminyltransferase "Enzyme A" and

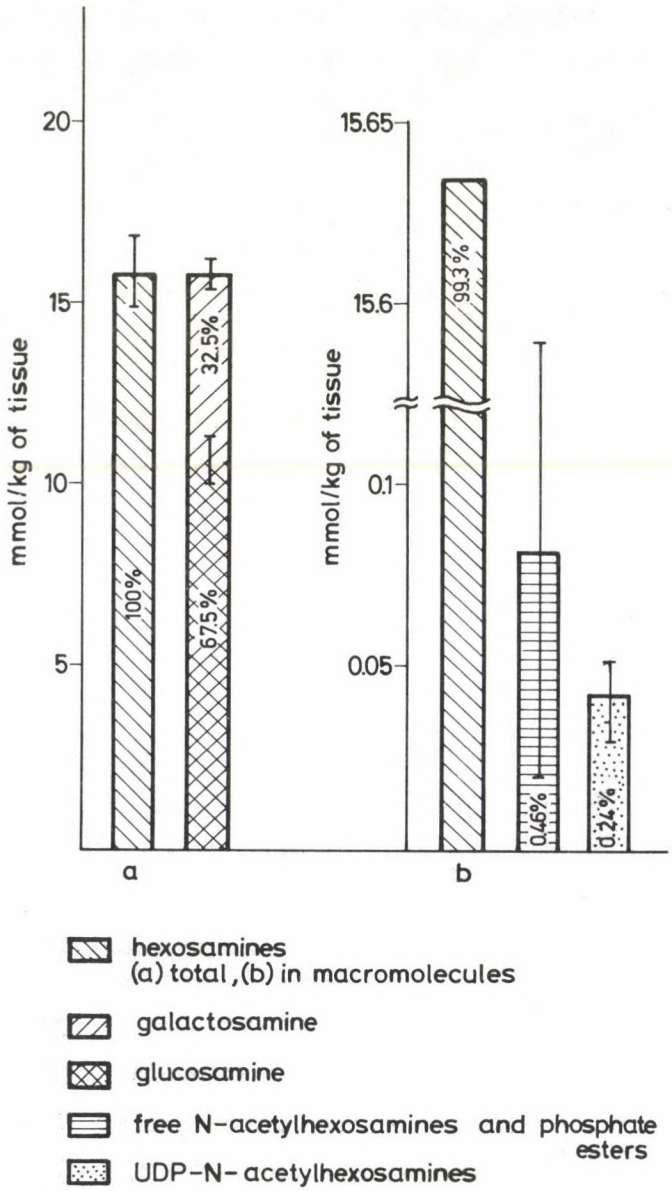


Fig. 2. Aminosugars of human gastric mucosa (refs 13, 41)

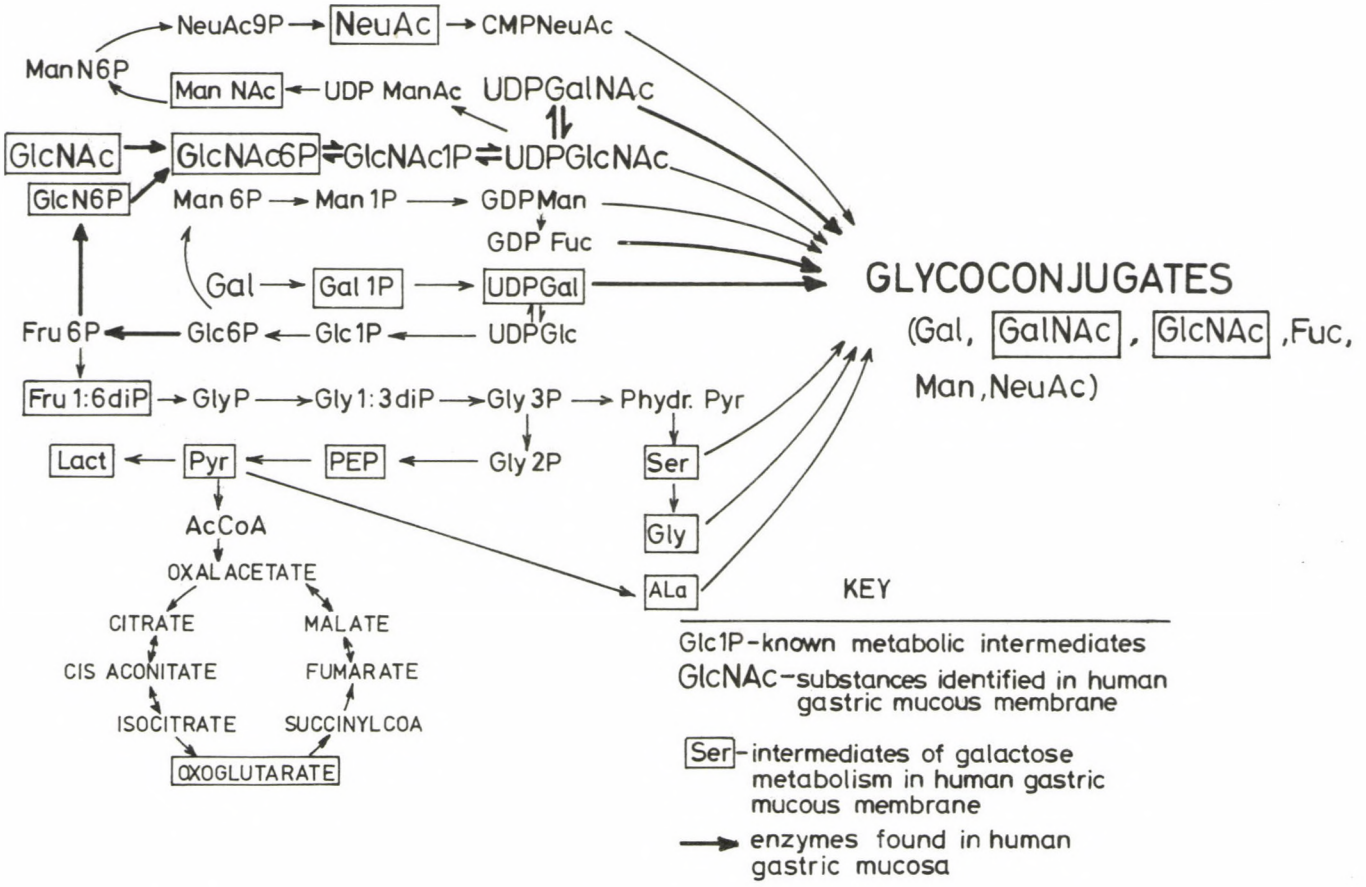


Fig. 3. Metabolism of sugars in the human gastric mucosa (refs, 6, 7, 14, 15, 16, 21, 22, 28, 35, 36, 37, 40, 42)

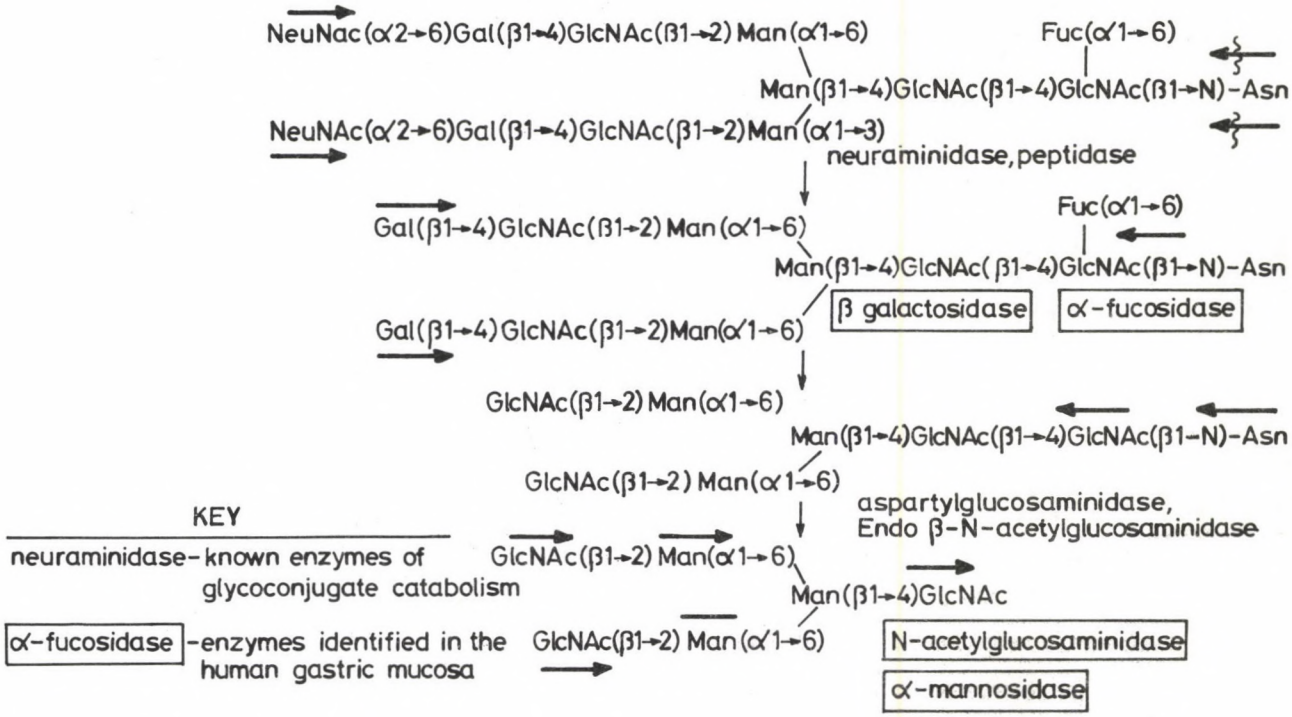


Fig. 4. Catabolism of glycoconjugates in the human gastric mucosa (refs 4, 8, 25, 27, 43, 45, 46)


Fig. 5. Specific activities of glycosidases in the human gastric mucous membrane (43)

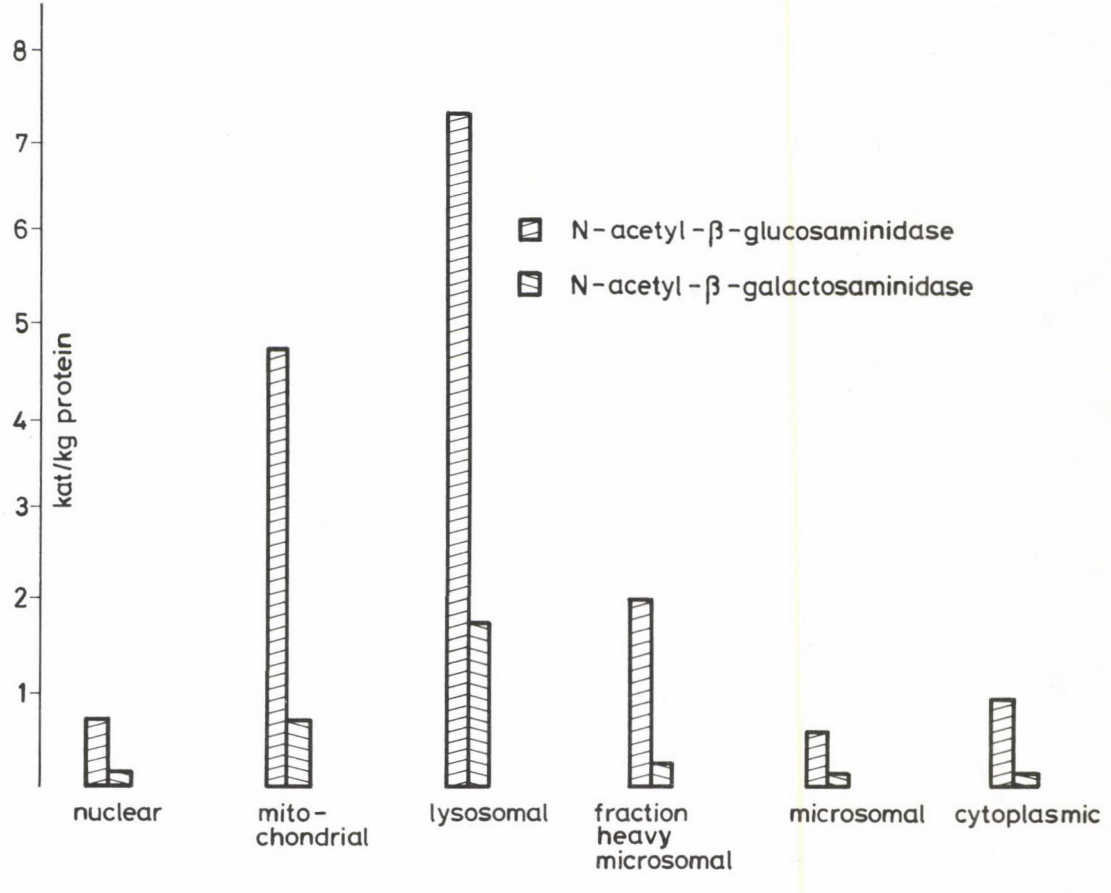


Fig. 6. Specific activity of hexosaminidases in subcellular fractions from human gastric mucosa (43)

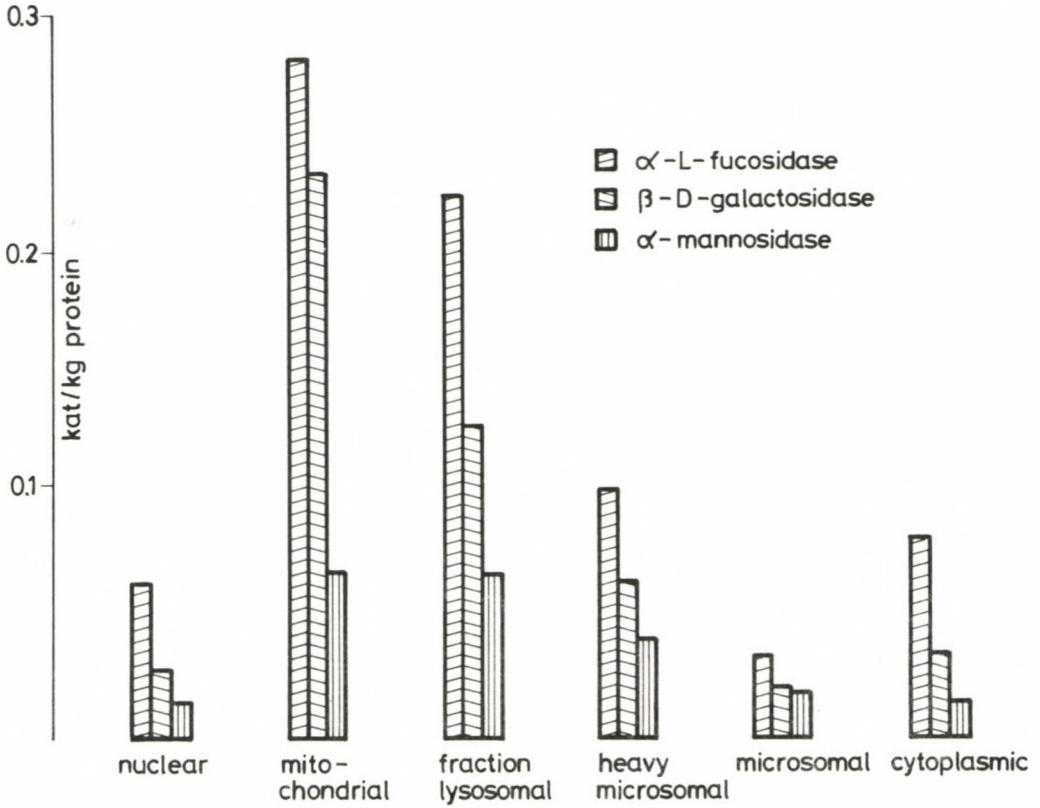


Fig. 7. Specific activities of glycosidases in subcellular fractions from human gastric mucosa (43)

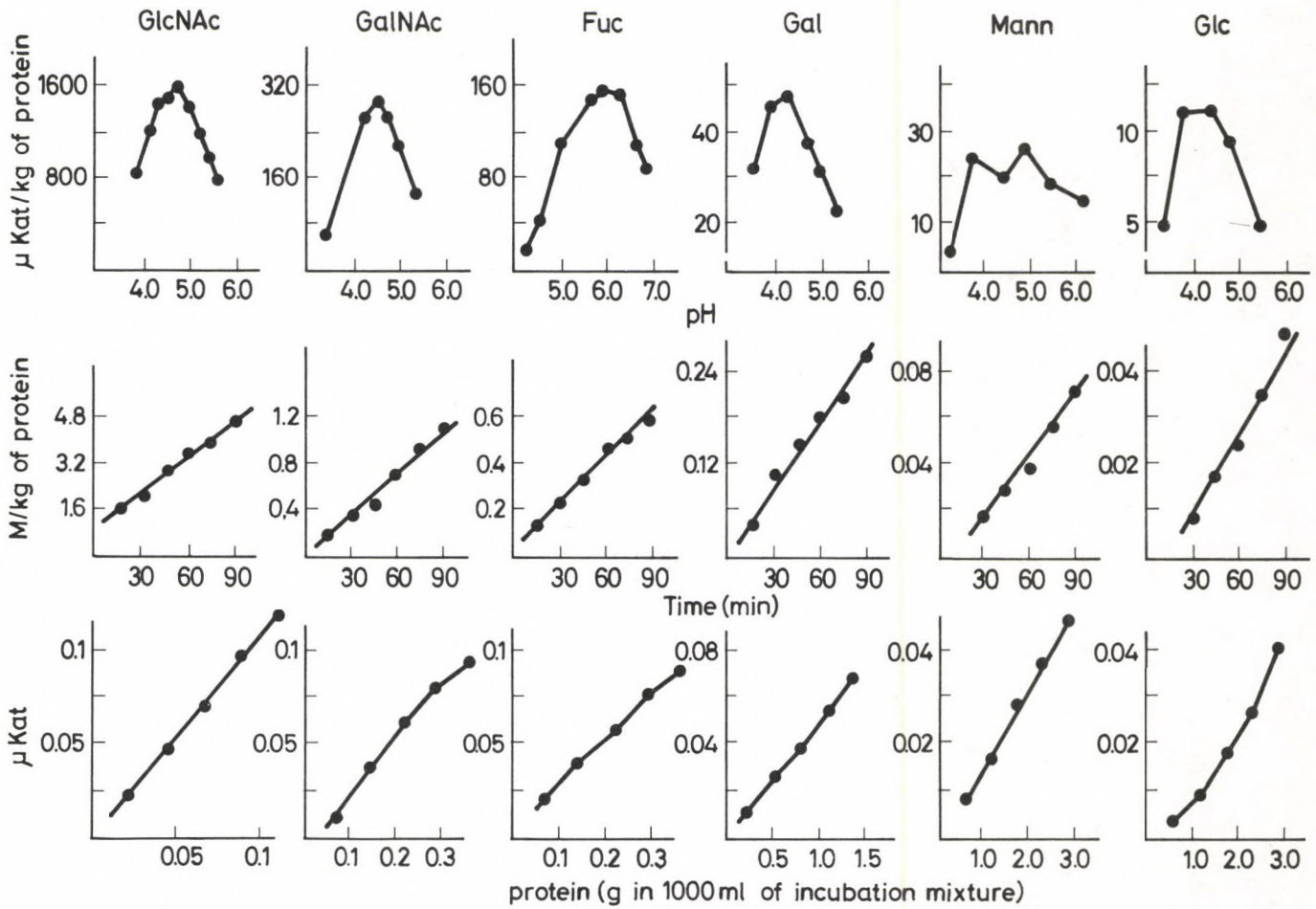


Fig. 8. pH optima, time course and concentration activity graphs of glycosidases from human gastric mucosa (43)

galactosyltransferase "Enzyme B" and observed that human erythrocytes of blood group O (H) human blood group substance H /28/ or hog blood group substance H /37/ can serve as an acceptor for N-acetylgalactosamine. Transferases for N-acetylgalactosamine and galactose have considerable substrate specificity. They can transfer the sugar on the H structure only /28/. Therefore, non-secretors lacking 2-fucosyltransferase activity in the human gastric tissue produce neither H nor A blood group substance /20, 36/.

The glycoproteins of the gastric mucosa are synthesized mainly in the cells of the superficial cylindrical layer of the mucosa /3/. Glycoproteins migrate into the Golgi apparatus and than appear in the mucus granules /24/. The way of migration of mucus glycoproteins in the mucous cells was investigated in the dog gastric mucosa /39/. In the non-stimulated gastric mucous membrane one can observe mostly non-secreting cells. The plasma membrane was not broken and on the apical side of the cells an accumulation of mucus granules was observed. It was found that mucus can be released by exocytosis, apical secretion and cellular desquamation. The majority of the cells secrete by exocytosis. Apical secretion occurs in old cells. After a rapid and single expulsion of the preformed secretion, degeneration of the cell takes place. Desquamation is also observed in old cells but rarely. The quantity and quality of the secreted mucus is subject to nervous and hormonal regulation. Secretin increases glycoprotein secretion in a dose-dependent manner /1/. The same effect was observed after administration of gastrin /2/. Small doses of hydrocortisone increase the biosynthesis of hexosamines and total content of glycoconjugates in the rat gastric mucosa, however, large doses produce opposite changes /23/. Antibodies against gastric mucosa and gastric juice caused a significant decrease in secretion of glycoproteins and hexosamines /19/.

## The catabolism of glycoconjugates in the human gastric mucous membrane

Figure 4 presents the commonly-accepted pathway for catabolism of a glycan of the N-acetyllactosaminic type, with our findings concerning the gastric mucosa shown in its background. We have found that in the human gastric mucosa enzymes capable of releasing the reducing sugars from its native glycoconjugates exist /43/. These results have encouraged us to carry out an examination of several glycosidases in the human gastric mucosa. The results are presented in Fig. 5. N-acetylglucosaminidase was found to be the most active enzyme. The other glycosidases found in the

human gastric mucosa were N-acetylgalactosaminidase, $\alpha$-fucosidase, and $\beta$-galactosidase. Other enzymes detected in the human gastric mucosa were $\alpha$-mannosidase and $\alpha$-glucosidase. The distribution of the glycoconjugate-degrading enzymes in the subcellular fractions of gastric mucosa were found to have the highest levels in the lysosomal fraction (Figs 6 and 7). As shown by Fig. 8 the pH optima were weakly acidic and the reactions were time- and protein-dependent. N-acetyl-glucosaminidase /17/ $\beta$-galactosidase /46/ and $\alpha$-L-fucosidase /45/ extracted from the human gastric mucous membrane have been partially purified. Of the enzymes participating in glycoconjugate metabolism of the human gastric mucosa N-acetylglucosaminidase, N-acetylgalactosaminidase and $\alpha$-fucosidase are the enzymes possessing the highest activity. The other enzymes had much lower activities, therefore, in the gastric bioptic material (4-6 mg of wet tissue) it was possible to determine the activity of N-acetylglucosaminidase /4, 8, 27/ and their isoenzymes /27/. The level of N-acetylglucosaminidase was used as a marker for healing after ischaemic gut injury /25/.

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# SHORT TERM TREATMENT OF TYPE II HYPERLIPOPROTEINAEMIA WITH SILYMARIN

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In a seven-month open clinical study on 14 type-II hyperlipidaemic outpatients, the effects of silymarin (Legalon$^{R}$), an antioxidant and hepatoprotective agent, were investigated. Blood lipid, lipoprotein and apolipoprotein concentrations, as well as liver and renal function parameters were measured. After determining baseline values, patients were treated with 420 mg Legalon daily for three months. After a two-month placebo period, the treatment was repeated with Legalon for a further month. In respect to the serum lipid and lipoprotein concentrations, there were no remarkable changes except that the total cholesterol and HDL-cholesterol levels slightly decreased. At the 12th week, in all cases, the apolipoprotein levels were somewhat decreased compared to the baseline values. By the significant decrease of both apo A-I and A-II values, a decrease of the total structural protein amount of HDL, and thus a relative increase in the proportion of cholesterol in HDL fraction was suggested. There were minor changes in serum protein concentration and liver function tests, but all values remained within the normal range. All of the renal function parameters remained unchanged during both treatments and the placebo periods. An additive role of Legalon in the therapy of secondary hyperlipoproteinaemia resulting from different liver diseases is discussed.

Keywords: type II hyperlipidaemia, antioxidant, silymarin (Legalon$^{R}$)

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Abbreviations: apo A-I: apolipoprotein A-I, apo A-II: apolipoprotein A-II, apo B: apolipoprotein B, HDL: high-density lipoprotein, LDL: low-density lipoprotein

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## Introduction

Recently, a number of studies have indicated a significant role of free-radicals-modified serum low density lipoproteins (LDLs) in the atherogenesis and/or in the progression of atherosclerotic disease /1, 2/. The group of the free-radical reactions, normally, constitutes an important part of the metabolic pathways. During bacterial or viral infections and some of chemical injuries the free-radical reactions take part in the elimination of various toxins or other harmful agents. Under the latter conditions the stimulation of these reactions may lead to an overproduction of free-radicals and their levels may exceed the capacity of the scavenger systems. Some of the liver, lung and heart diseases can be induced and promoted by the excess of radicals /7, 8, 18/. In the latest medical therapy practice a number of antioxidant compounds have been used in the treatment of these disorders /4, 5/.

A beneficial effect of some antioxidant agents on the pathological processes in atherosclerosis have been published /6, 13/. Therefore, investigations either on the antioxidant character of lipolytic and antiatherosclerotic drugs or on the lipolytic effect of antioxidant compounds are of great importance.

Silymarin (Legalon$^{R}$), a purified essence of Silybum marianum (L.) Geartn, has been developed by MADAUS Chem. Co. (Köln, FRG) as an effective substance against Amanita phalloides poisoning /3, 10/. Further investigations have shown that Legalon has antioxidant /9/ and hepatoprotective effects /14/. The compound increases resistance against alcohol intoxication.

In the present study the direct effects of Legalon on serum lipids in hyperlipidaemia (Type II) have been investigated.

## Materials and Methods

### Patients

Fourteen patients (5 men and 9 women) with type II hyperlipoproteinaemia, characterized by Fredrickson typing were included in the study. Mean age of the group was 57 years (range 30-68) and weight 75 kg. Secondary hyperlipidaemia was excluded by routine clinical investigations. The patients suffering from insulin-dependent diabetes mellitus, diseases of kidney and liver, as well as, cancer were also excluded. Two of the 14 patients were found to be of type II.b. Two patients received carbohydrate poor diet.

Medical treatment not influencing lipid metabolism was no excluding criterion. No lipid-lowering drugs had been given for at least six months prior to entry in the study.

Silimaryn (Legalon$^{R}$) was given at a dose of 140 mg, three times a day for three months. After a two-month placebo treatment, the drug was given for a further month. Blood samples were analysed before silymarin administration and in every month. The blood samples were drawn after twelve-hour fasting.

## Analysis

All of the liver and renal function parameters were determined by standard routine clinical laboratory methods. Serum protein was determined by the Lowry method /15/. Total cholesterol, HDL-cholesterol and triglyceride were measured by enzymatic kits, CHOD and Triglyceride-High Performance (Boehringer, Mannheim, FRG), respectively, in an automated instrument. Statistical analysis of data was performed by Student's t test. Mean $\pm$ S.D. are shown in the Tables.

## Results

Legalon administration caused no significant change in body weight and had no side-effects. Until the 12th week, Legalon treatment slightly decreased serum cholesterol: the values varied around baseline level. The concentration of LDL- and HDL-cholesterol did not change. The level of triglyceride remained at the baseline. Furthermore, the atherogen index (LDL-C/HDL-C) somewhat decreased (Table I).

After the two-month placebo treatment an increase in total cholesterol and HDL-cholesterol concentrations was found. Similar increase was seen in LDL-cholesterol.

In consequence of the repeated one month treatments with 140 mg Legalon (three times a day), lipid parameters fixed about the baseline values, except that HDL-cholesterol remained significantly higher. Therefore, by the end of the treatment, the atherogen-index considerably decreased (Table I). At the end of the first treatment period some decrease in cholesterol content of all lipoprotein fractions was found. The apparent controversy between the decreased apo B level and the increased LDL-cholesterol concentration at the 16th week may be ascribed to the change in lipoprotein pattern, thus to the inaccuracy of the Friedewald calculation. At the end of placebo period a mild hypercholesterolaemia was developed, supposedly, due to the disappearance of the effect of the drug. Unfortunately, the investigation of apoprotein content of serum was not possible from the 16th week. During the next treatment period the HDL-cholesterol remained significantly higher than the baseline value, while the total cholesterol decreased again. The atherogen index was beneficially decreased

Table I

Effect of Silymarin (Legalon 140) treatment on serum lipid parameters of type II hyperlipoproteinaemic patients

(n = 14)

| | | Baseline value | 3x140 mg Silymarin | | | placebo | 3x140 mg Silymarin | |
|---|---|---|---|---|---|---|---|---|
| Week | | 0 | 4 | 8 | 12 | 16 | 20 | 24 |
| Total cholesterol (mmol/l) | $\bar{x}$ | 7.86 | 7.5 | 7.75 | 7.24 | 7.68 | 9.18$^{x}$ | 7.73 |
| | s | 1.35 | 1.68 | 1.38 | 1.24 | 1.21 | 1.79 | 1.11 |
| Triglyceride (mmol/l) | $\bar{x}$ | 2.33 | 2.13 | 2.42 | 2.32 | 2.15 | 2.61 | 2.29 |
| | s | 0.89 | 0.84 | 1.02 | 1.01 | 0.85 | 1.37 | 1.62 |
| Se.HDL cholesterol (mmol/l) | $\bar{x}$ | 1.74 | 1.65 | 1.63 | 1.65 | 1.63 | 1.99$^{x}$ | 1.96$^{x}$ |
| | s | 0.49 | 0.48 | 0.46 | 0.36 | 0.37 | 0.55 | 0.86 |
| LDL cholesterol (mmol/l) Friedewald | $\bar{x}$ | 5.38 | 5.47 | 5.54 | 5.08 | 5.68 | 6.69 | 5.32 |
| | s | 0.72 | 1.47 | 1.28 | 1.13 | 1.05 | 1.48 | 0.96 |
| Atherogen-index (LDL c/HDL c) | $\bar{x}$ | 3.59 | 3.44 | 3.68 | 3.29 | 3.49 | 3.36 | 2.71 |
| | s | 1.04 | 1.13 | 1.33 | 1.25 | 1.12 | 1.04 | 0.90 |

n = number of cases
x = average
s : S.D.
x - $P < 0.1$ concerning the differences between means at baseline and different period

Table II

Effect of Silymarin (Legalon 140) on serum apolipoprotein pattern of type II hyperlipoproteinaemic patients

(g/l)

| | Baseline value | treatment | placebo |
|---|---|---|---|
| Week | 0 | 12 | 16 |
| Apo B | 2.17 $\pm$ 0.77$^{*}$ | 2.03 $\pm$ 0.56 | 1.60 $\pm$ 0.28$^{x}$ |
| (n) | 13 | 14 | 13 |
| Apo A-I | 1.55 $\pm$ 0.18 | 1.29 $\pm$ 0.26$^{xx}$ | 1.64 $\pm$ 0.26 |
| (n) | 13 | 14 | 13 |
| Apo A-II | 0.57 $\pm$ 0.12 | 0.41 $\pm$ 0.06$^{xx}$ | 0.53 $\pm$ 0.09 |
| (n) | 14 | 12 | 13 |

P = significance; x = $P < 0.010$; xx = $P < 0.005$; $*$ = mean $\pm$ S.D.; n = number of cases

by the drug treatment in consequence of just a moderate increase in the LDL-cholesterol value.

The concentration of serum apolipoproteins, apo A-I, A-II and B all slightly decreased during the first 12 weeks (Table II). At the week of the treatment the decrease of the protein-lipid ratio in HDL fraction by the expense of apo A-I had a special interest. Formation of an HDL group with larger lipid content has been supposed. Among the routine laboratory parameters a gradual decrease of serum protein was found, but the value remained within the normal range. The slight increase in serum bilirubin level had no clinical significance. No remarkable changes of other parameters were found.

## Discussion

During the last four decades a number of studies have dealt with the formation and inactivation of the free radicals in the body. Some of these radicals control many of biochemical reactions taking place in cells and tissues /12, 14/.

Recently, several publications have revealed a close relationship between a defect of the free-radical metabolism and the development of some diseases including autoimmune diseases, liver diseases, etc. /16, 19/. Many studies have implicated oxygen radicals and lipid-peroxides to investigate the development of atherosclerosis. Hyperlipidaemia is one of the major risk factors for atherosclerosis. There is some evidence that the oxidative modification of low density lipoprotein (LDL) promotes atherogenesis /11/. Parthasarathy et al. showed /17/ that probucol used to lower plasma cholesterol level is a potent inhibitor of the oxidative LDL modification. It is possible that other drugs having antioxidant character may also influence the development of atherosclerosis.

In this study the direct antilipaemic effect of Legalon was invetigated in type II hyperlipidaemic patients. No significant change in lipid parameters were found at the end of the first and second treatment periods, except an increase in HDL-cholesterol. Therefore, the atherogen-index of the patients somewhat decreased compared to their baseline values. Serum cholesterol level was raised within the placebo period, then it decreased again by the end of the second treatment.

From these results a redistribution of lipid constituents of lipo-

proteins caused by Legalon treatment have been suggested. In atherosclerosis, treated with antioxidant, a decrease in plasma lipid-peroxide level and modified LDL concentration was found, which might be a beneficial effect of antioxidant against the rapid progression of the disease.

Szamosi et al. /23/ investigated the level of cholesterol and lipid-peroxides in serum of familial hyperlipidaemic children. They showed that both cholesterol and lipid-peroxide levels were elevated. Beside the increase of intake of natural antioxidant compounds by regular food or diet thought to prevent progression of atherosclerosis, in some cases treatment with antioxidant drugs is necessary. Prolonged treatment with these drugs might favourably influence the atherosclerosis with or without modifying the hyperlipidaemic status of the patients. In the future it seems to be important to study the effect of antioxidants on atherosclerosis and indirectly, on lipoprotein metabolism (type III, IV, V) in a long-term investigation.

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ENDOCRINOLOGY

# ANDROGENS AND BONE MINERAL CONTENT IN PATIENTS WITH SUBTOTAL THYROIDECTOMY FOR BENIGN NODULAR DISEASE

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To investigate the influence of thyroid surgery on the skeleton and different hormones a well characterized patient group of 24 women was selected who had undergone subtotal thyroidectomy for euthyroid benign nodular disease and remained euthyroid after the operation. Bone mineral content was determined in lumbar vertebrae, femoral neck and radius by dual and single photon absorptiometry. The serum levels of calcitonin, dehydroepiandrosterone, dehydroepiandrosterone-sulphate, androstenedione, total testosterone, cortisol and 25-hydroxyvitamin D were measured. A control group was created of 48 healthy female subjects. No reduction in bone mass was observed at the measured sites compared to appropriate controls. Beside normal bone mineral content significantly elevated serum levels of dehydroepiandrosterone (27.4±10.4 vs. 20.8±6.9 nmol/l) and androstenedione (9.3±3.3 vs. 6.6±2.2 nmol/l) were found without any clinical sign of androgen excess. There was no correlation between bone mineral content and these androgen levels. The serum calcitonin levels of all patients were low. With regard to the previously reported interactions among androgen, calcitonin and bone metabolism, our results raise the possibility of a relationship between higher androgen levels and preserved bone mass in these patients, while normal bone mineral content and calcitonin deficiency in these patients does not inevitably indicate that calcitonin does not affect bone tissue in adults.

Keywords: thyroidectomy, osteoporosis, bone mineral content, androgens, calcitonin

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Abbreviations: AD: androstenedione; BMC: bone mineral content; DEA: dehydroepiandrosterone; DEA-S: dehydroepiandrosterone-sulphate; 25-OH-D: 25-hydroxyvitamin D

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## Introduction

Total, near-total or subtotal thyroidectomy has been shown to significantly reduce basal serum calcitonin levels and calcitonin response to calcium load /19, 27, 28/. On the other hand impaired calcitonin reserve has been demonstrated in severe postmenopausal osteoporotic women /24, 30, 33/.

Though calcitonin deficiency seems to be confirmed in thyroidectomized patients, controversial data exist about developing osteopenia in these patients. Reduced bone mineral content (BMC) was found in the radius of totally thyroidectomized patients by single photon absorptiometry /22/. Sianesi et al. /27/ demonstrated lower than normal serum calcitonin levels and decreased BMC of the radius in patients with total or subtotal thyroidectomy while Hurley et al. /19/ found decreased calcitonin reserve and normal BMC in patients with subtotal thyroidectomy.

To study the influence of thyroid surgery on the skeleton a strictly selected group of women was examined who had undergone subtotal thyroidectomy for euthyroid benign nodular disease and remained euthyroid after the operation. The BMC of the axial and appendicular skeleton as well as the serum levels of calcitonin, dehydroepiandrosterone (DEA), dehydroepiandrosterone-sulphate (DEA-S), androstenedione (AD), total testosterone, cortisol and 25-hydroxyvitamin D (25-OH-D) were detected to reveal correlations — if any — between these hormone levels and BMC in the patients.

## Subjects

24 women (mean age: 42 years, range: 33-75 years) who had undergone subtotal thyroidectomy for benign nodular disease (8 with shrink euthyroid multinodular goiter, 12 with benign adenoma and 4 with benign cyst) were examined. Operations were carried out at a mean of 12 years before the study (range: 3-37 years). The patients did not have any other disease and were not on medical therapy known to influence bone metabolism or androgen production. No clinical signs of androgen excess were observed. None of the patients was taking thyroid hormones as replacement therapy. None of them had a previous history of severe back pain or fractures. Alcohol, milk and coffee consumption, calcium intake, smoking habit, physical activity did not differ from the Hungarian average. The patients were euthyroid before operation and all of them were documented as having had normal serum levels of calcium, thyroxine and thyrotropine since their thyroid surgery.

A group of 48 healthy females (mean age: 42 years, range: 33-75 years) carefully matched for body weight, height and habits served as control.

Informed consent was obtained from all subjects.

## Materials and Methods

Axial BMC (lumbar spine 2, 3, 4) and right femoral neck BMC was measured by dual photon absorptiometry with 153-Gadolinium /26, 32/ (Novo BMC Lab 22/a) and was expressed in grams for total BMC of lumbar vertebrae 2, 3, 4 and in g/cm for femoral neck. Peripheral BMC was measured at the midradius and distal radius of the nondominant arm by single photon absorptiometry /8/ (Norland-Cameron BMA 178) and was expressed in g/cm.

Serum DEA, DEA-S, AD, total testosterone, cortisol and 25-OH-D levels were determined by radioimmunoassay methods developed in our institute /3, 4, 5, 12, 13/. To predict the rate of DEA-S — DEA cleavage, the serum DEA to DEA-S ratio was calculated. Serum calcitonin levels were estimated by Byk-Mallinkrodt radioimmunoassay.

Serum levels of T3, T4, TSH were measured by commercial kits. Serum total calcium was estimated by atomic absorption spectrophotometry (normal range: 2.25—2.65 mmol/l), serum phosphorus by colorimetric method (normal range: 0.85—1.20 mmol/l).

Blood samples were taken after an overnight fast and were stored at -20 $^{o}$C until determination.

The statistical methods used were linear regression, t test and analysis of variance.

## Results

The results of bone densitometric evaluations and biochemical data on the patients and controls are summarized in Tables I and II.

Table I

Body-mass index and bone mineral density in patients with subtotal thyroidectomy and controls

| | Patients (n=24) ($\bar{x} \pm$ S.D.) | Controls (n=48) ($\bar{x} \pm$ S.D.) | |
|---|---|---|---|
| Body-mass index | 23.9 ± 1.9 | 24.2 ± 1.5 | N.S. |
| BMC | | | |
| Midradius (g/cm) | 0.85 ± 0.13 | 0.82 ± 0.12 | N.S. |
| Distal radius (g/cm) | 0.92 ± 0.12 | 0.90 ± 0.14 | N.S. |
| Femoral neck (g/cm) | 2.63 ± 0.33 | 2.41 ± 0.29 | N.S. |
| Lumbar spine 2,3,4 (g) | 38.5 ± 5.1 | 36.4 ± 4.9 | N.S. |

Body mass index = weight (kilograms) divided by the square of the body height (meters);
BMC = Bone mineral content;
NS = Non-significant

Table II

Serum values in patients with subtotal thyroidectomy and in controls

| | Patients (n=24) ($\bar{x} \pm$ S.D.) | Controls (n=48) ($\bar{x} \pm$ S.D.) | |
|---|---|---|---|
| Calcium (mmol/l) | 2.44 ± 0.11 | 2.49 ± 0.10 | N.S. |
| Phosphorus (mmol/l) | 1.00 ± 0.10 | 0.99 ± 0.08 | N.S. |
| $T_3$ (mmol/l) | 2.2 ± 0.1 | 2.3 ± 0.1 | N.S. |
| $T_4$ (mmol/l) | 125 ± 21 | 111 ± 27 | N.S. |
| TSH (mE/l) | 2.1 ± 0.3 | 2.0 ± 0.4 | N.S. |
| 25-OH-D (nmol/l) | 92 ± 11 | 99 ± 16 | N.S. |
| DEA (nmol/l) | 27.4 ± 10.4 | 20.8 ± 6.9 | $P < 0.05$ |
| DEAS (µmol/l) | 5.7 ± 2.2 | 5.2 ± 3.0 | N.S. |
| AD (nmol/l) | 9.3 ± 3.3 | 6.6 ± 2.2 | $P < 0.01$ |
| Testosterone | 1.2 ± 0.3 | 1.3 ± 0.3 | N.S. |

$T_3$ = triiodothyronine; $T_4$ = thyroxine; TSH = thyrotropine; 25-OH-D = 25-hydroxy-Vitamin D; DEA = dehydroepiandrosterone; DEAS = dehydroepiandrosterone-sulphate; AD = androstenedione; NS = non-significant

Bone mineral density was not decreased in patients with subtotal thyroidectomy at both appendicular and axial sites. Surprisingly BMC was rather above the control values, however, the differences were not statistically significant (Table I). There was no significant correlation between BMC and the time elapsed since surgery at either axial or appendicular sites (Fig. 1).

Serum calcitonin levels of patients were significantly lower than those of controls (mean ± S.D.) (5.8 ± 2.4 pg/ml and 62.1 ± 11.4 pg/ml, respectively; $P < 0.0001$). Serum DEA and AD levels were significantly higher in subjects with subtotal thyroidectomy. The mean serum DEA level was 33% above that of the controls (27.4 ± 10.4 and 20.8 ± 6.9 nmol/l, respectively, $P < 0.05$). Though DEA/DEA-S ratio was higher in patients, the difference was not significant (5.46 ± 2.68 and 4.63 ± 2.27, respectively). The mean serum AD concentration was 41% higher than in the controls (9.3 ± 3.3 and 6.6 ± 2.2 nmol/l, respectively, $P < 0.01$). No correlation was found between these hormone concentrations and the time elapsed since surgery. Also, no correlation could be shown between either DEA or AD levels and BMC of the patients.

Serum total calcium, phosphorus, 25-OH-D, DEA-S, total testosterone, cortisol, $T_3$, $T_4$, and TSH concentrations showed no significant difference (Table II).

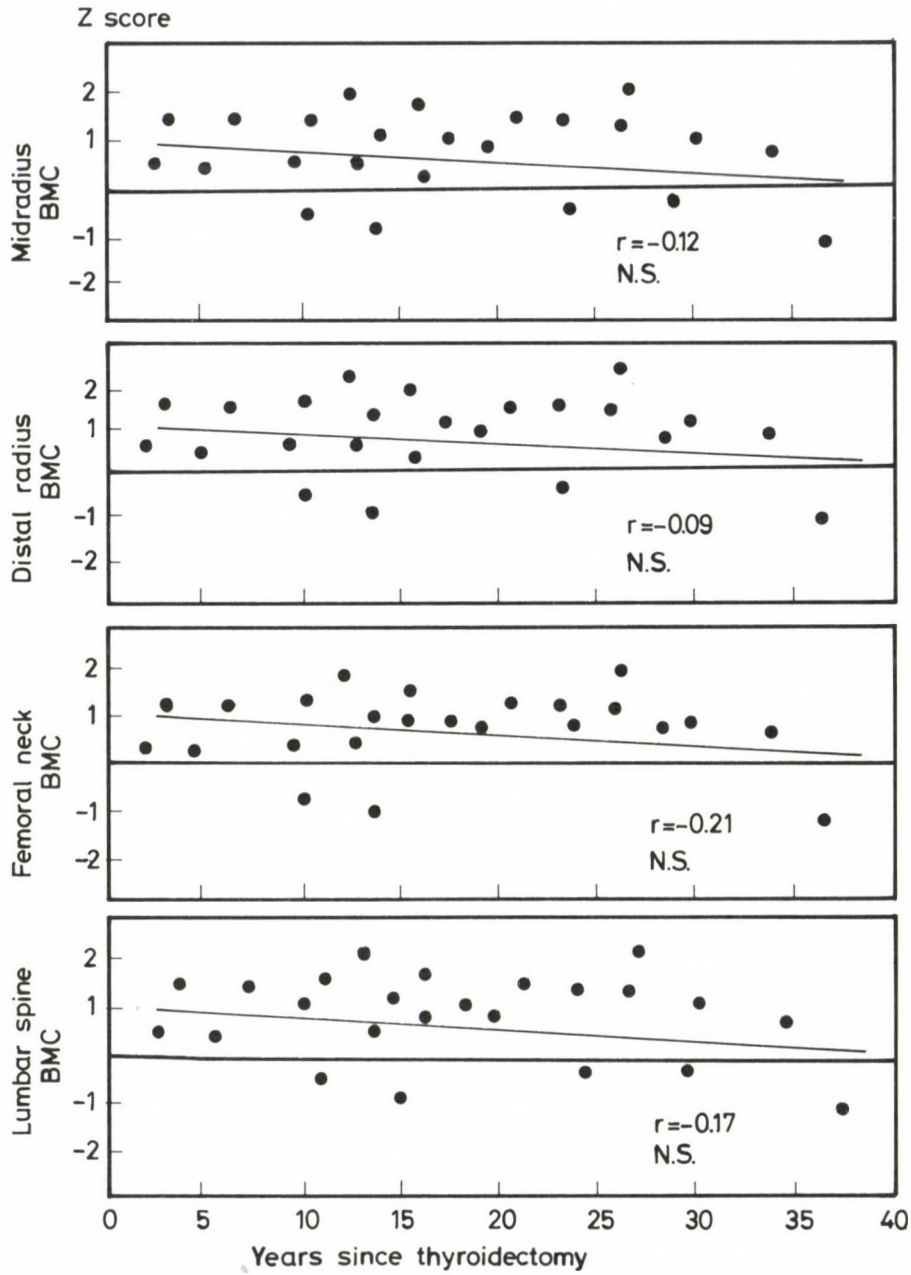


Fig. 1. Bone mineral content in the appendicular and axial sites in relation to time since thyroidectomy

Z-score means the number of standard deviations above or below the appropriate normal mean which is indicated by the horizontal line. The shorter line indicates the regression line for patients' data. NS = non-significant

## Discussion

Lower serum calcitonin concentration in females — approximately only one fourth of those found in males — is well documented /2, 6, 25, 30, 31, 33/. Some investigators suggested that this "failure" can be further aggravated by total or subtotal thyroidectomy /22, 24, 27/ and thus resulting in osteopenia. We found low serum calcitonin levels in our patients. It is well known that alterations in thyroid hormone production may affect bone metabolism /10, 11, 23, 29/, therefore we examined only those patients with subtotal thyroidectomy who were euthyroid before as well as after surgery (at least by clinical and biochemical examinations). In this group we did not find increased bone loss as compared to appropriate controls in spite of their low serum calcitonin levels. Our work corroborate the result of Hurley et al. /19/ who found no reduction in the BMC of such patients. The importance of the selection of patients according to the different diseases of the thyroid and the extent of the operation in these studies has to be emphasized.

Holló et al. /16, 17, 18/ and others /7/ found adrenal androgen deficiency in postmenopausal women, mainly in patients with severe osteoporosis. Besides decreased serum levels of DEA and DEA-S, serum AD is also reduced in osteoporotic females /21/. Kollin et al. /20/ reported elevated androgen levels and increased BMC in premenopausal women with the hyperostosis frontalis interna syndrome and suggested that cranial hyperostosis is only a part of the generalized increase in the BMC of these patients. Androgens may also enhance the sensitivity of bone tissue to calcitonin /16/. These results are in good agreement with the suggested benefit of androgen treatment in postmenopausal osteoporosis /1, 9, 15, 16/. In our study in patients with subtotal thyroidectomy for benign nodular disease, beside normal BMC and low calcitonin levels, significantly increased DEA and AD serum levels were detected without any clinical sign of androgen excess.

Considering the afore-mentioned inter-relationship between androgen and calcitonin metabolism the previously reported calcitonin deficiency after thyroidectomy /19, 27, 28/ might be counterbalanced in our patients by elevated androgen levels. This may prevent bone loss and result in preserved BMC. Nevertheless, we failed to demonstrate any correlation between serum levels of these androgens and densitometric values of our patients. Recent data /14/ suggest a positive correlation between serum free

testosterone and calcitonin levels in men. No correlation was found between serum testosterone levels and BMC in our female patients, however, we measured only serum total testosterone concentrations. The contradiction between low calcitonin levels and normal BMC in subtotally thyroidectomized patients might be explained by an enhancement of calcitonin effect on bone tissue caused by androgens. The normal bone density in these patients does not inevitably indicate that skeletal mass is not affected by endogeneous calcitonin in adults. However, the biological significance of the metabolic and densitometric features found in the present study is not clear, therefore further work is necessary to elucidate the possible relations.

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# PEPTIC ULCER DISEASE IN TROPICAL AFRICA

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Diseases of the stomach, including gastric ulceration, are uncommon in Africa. In spite of this, duodenal ulcer — contrary to the reports a couple of decades ago — is common all over the tropics; there is limited evidence that it is more common in urban areas and that its incidence is increasing further. However, one should borne in mind that accurate incidence rates for diseases which are not immediately fatal, are extremely difficult to obtain in most of the third-world countries.

Keywords: gastric ulcer disease, peptic ulcer disease, tropical, Africa

## Introduction

Reporting the true incidence of peptic ulcer disease (PUD) depends on satisfactory case-history (which is very difficult to obtain where the physician is often so dependent on interpreters) and good diagnostic facilities.

Diagnostic investigations are difficult in many parts of Africa. Usually hospitals in major towns are those having X-ray equipment. The feasibility of performing gastroscopy in a "bush-hospital" has been evaluated in Rwanda by Wierman and Snyder in 1978. They found the method valu-

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Abbreviations: DUD : Duodenal ulcer disease; GUD: Gastric ulcer disease; PUD: Peptic ulcer disease

able and possible to perform, but it is costly, therefore generally unacceptable in most of the African countries /44/.

Considering the above-mentioned problems of diagnosis, correct incidence rates, etc. — the situation of PUD in the tropical Africa seems to be as follows.

## Gastric ulcer disease (GUD)

The incidence of GUD is much lower than that of duodenal ulcer all over the developing world. In fact, although almost a rarity in some countries, it has been reported from all parts of Africa /14/.

It is possible that GUD is more common in East Africa than in West Africa. Generally there is no doubt of its low incidence rate.

Men are involved by GUD more often than women in Africa, it is a disease of the lower social strata; the majority of the patients are in their fifth and sixth decades /29/.

A low incidence of GUD was reported from Nigeria by Konstam /25/. Barton and Cockshott emphasized the rarity of GUD in West Africa /6/. Nwoloko made similar observations in southern Nigeria /29/.

In Uganda, where DUD is common (mainly in the western part of the country), GUD is also unusual /36/. GUD also has been reported as being uncommon in Kenya /21, 32/a/.

When it does occur, there is no difference in the disease from that in the western world /16/, apart from the fact that gastric retention seems to be more common, presumably due to chronicity /4/.

For therapeutic purposes modern drugs (cimetidine, ranitidin) are expensive in most countries, and usually not readily available. Older, less satisfactory therapeutic agents often have to be used, and regimes involving oral antacids and antispasmodics provide symptomatic relief. It is always useful to give up smoking. Large quantities of milk in the diet should be avoided due to the very high incidence of adult lactase-deficiency in most part of the tropics /10/. Complications of GUD usually require surgery.

## Duodenal ulcer disease (DUD)

In both Africa and Asia, there is evidence that DUD has become more common since the beginning of the XX$^{th}$ century /38/, but there are many controversies /37/.

Thirty years ago, DUD was considered as an uncommon disease in tropical Africa /12, 17/.

According to Cook /10/ the main problem was that of recognition and the figures did not reflect the true incidence of DUD; much epigastric pain which is extremely common in Africa, was undoubtedly attributed to intestinal helminths.

Taking into consideration the problems already mentioned, the actual situation regarding to DUD in tropical Africa is the following:

In the western part of the continent there is a high prevalence in Cameroon, Nigeria and perhaps in Ghana. There are also high incidence rates in the Nile-Congo watershed, Rwanda, Burundi, the Lake Kivu area (eastern Zaire), western Tanzania, south-western Uganda and the Ethiopian highlands /1, 6, 18, 24, 25, 33, etc./.

Barton and Cockshott /6/ drew attention to the fact that it was the rural farmer who was usually affected. (Disease of the poorer classes?) There was much fibrosis and stenosis which accounted for the rarity of perforation. They considered DUD as common in Nigeria as in the United Kingdom. They reported a 4.5:1 male-female ratio but it should be remembered that it is often especially difficult for women to get to hospital in rural Africa.

In East Africa, Connel /9/, Vint /41/ and Miller /27, 28/ considered that DUD was unusual. Brainbridge and Trowell /7/, Vassalo /40/ and Wilkinson /45/ expressed the same opinion.

In contrary to the above-mentioned data, Raper /34/ reported on 776 autopsies between 1951 and 1956 in Uganda, and he found peptic ulcers in 15.3% of males and 4.7% of females. The duodenal-gastric ratio was 6:1.

Shaper and Shaper /35/ also considered DUD to be fairly common in Kampala (Uganda) but, interestingly, in 1949 only three peptic ulcers were found in 1020 autopsies in the same town /32/.

Taylor /36/ too, showed the disease to be far from being uncommon in Uganda. It was his impression that the highest incidence was in the tall, thin people of South-West Uganda (presumably the Hamites?). Although there were stress signs in some cases, most came from an unsophisticated rural background.

According to Cook /10/ DUD was a common disease in the late 1960s in Uganda and was not confined to urban population. Surprisingly, members of such tribes (e.g. Hima and Tutsi, etc.) who live largerly on milk and sit for much of their time watching cattle, without any stress situation, often

had the disease, and even young adults and children were frequent sufferers.

Buxton /8/ reported a high incidence rate of DUD in Rwanda and Burundi.

Reports from Nairobi and from other parts of Kenya indicate that DUD is not uncommon there /23, 32, 41/. Miller /27, 28/ had no doubt of the growing incidence of the disease in Nairobi, and this was confirmed by Whittaker /43/. Stenosis was a frequent complication, and the ratio of duodenal to gastric ulcer was 12:1. Similar conclusion was drawn by Lule and Wankya in 1985 /26/. Cross has even reported DUD on Kenyan children /11/.

Grech published data for Tanzania /20/. He reviewed 733 X-ray investigations (barium-meals), of which 191 were PUD, while the vast majority — 170 cases — were DUD.

Baldachin and Palmer /3/, Friedlander and Gelfand /19/ gave evidence that in Zimbabwe DUD seemed to be a predominantly urban disease and was far more common in men, mainly in unskilled workers.

Cook /10/ reported that the disease was common in Zambia, in both urban and rural inhabitants but complications were unusual.

It is a common belief in the literature that genetic factors play a part in the incidence rate. In Uganda there is no doubt that DUD is more common among the Hamites than the Bantu tribes.

In Nigeria there are marked differences in incidence rates between the northern and southern parts of the country /25/. People with blood-group O, and non-secretors of A, B and O blood-group substances are prone to DUD in Nigeria /5, 13/.

As special African causes, various dietary factors have been considered in the aetiology of DUD.

In Nigeria the usual diet is poor in protein and rich in carbohydrate, and it is always bulky. (The staple-foods are rice and tapioca, yam and cassava.)

Spices (mainly pepper and chilli) have been suggested to be important also in the development of duodenal ulcers. Thiamine and riboflavin deficiencies have also been suggested as being important but that has never been proved.

Ascariasis and hookworm disease also seem very unlikely to be relevant.

The disease is doubtless multifactorial (stress, "high fibre" and "refined carbohydrate" hypotheses, specific bacterial infections, (e.g.

Campylobacter pyloridis, which infection seems to be very common in Africa /15, 39/ etc.) smoking,alcohol and caffaine consumption, etc., — similarly to the western world); and at present there is no clear indication that any single factor is involved in the pathogenesis of this complex disease in any part of the tropical Africa.

The presentation of DUD in the tropics is similar to that in the western countries. The main difference relates to the high incidence of stenosis. It is not clear whether the marked fibrous reaction is due to chronicity (and symptoms following that) or has an ethnic basis in Africans, possibly being similar to keloid formation on the skin.

Epigastric pain is extremely common in the population of tropical countries, it is important therefore that DUD is not overdiagnosed. (The casue of this symptom is unclear but it frequently subsides with antacids.)

Pyloric stenosis may be accompanied by severe vomiting leading to severe dehydration,weight loss and (hypokalaemic) alkalosis.

The disease may occur at any time from infancy to the eight decade. (Maximum incidence about 30 years of age.)

The treatment of DUD in the third-world is usually limited by lack of effective therapeutic agents /30/. Carbenoxolone and $H_2$-receptor blockers (cimetidine, ranitidine, etc.) are constly preparations; the local drug-budget can rarely afford them /2, 22, 31/.

For medical treatment the older regimes of antacids and antispasmodics are used. Smoking should be stopped. Dietary restrictions are unnecessary, although frequent meals — if possible — diminish ulcer symptoms. Milk should not be encouraged, because (similarly to GUD) adult lactase deficiency is common, and severe diarrhoea may ensue.

Bed rest is often difficult because hospitals are overloaded with more acute cases. Inevitably therefore, surgery is undertaken at an early stage in many cases. (The ulcer is often very chronic when first seen at hospital, and there is much fibrosis and evidence of obstruction.)

In the so-called western world (among more sophisticated circumstances), PUD (with its well-characterized symptoms) is one of the most common gastrointestinal diseases. The diagnostic facilities and the choice of therapeutic remedies are ample, and since the introduction of $H_2$-recptor blocker drugs into the therapy the sanation of PUD has become a relatively simple task for the health services.

On the contrary, in the underdeveloped or developing countries of the third-world, where numerous difficulties hinder the successful work of

the physician, the disease shows some differences from the well-known, described form(s) of PUD. In this work we wanted to give a short account of the differences that are characteristic of PUD in the tropical Africa.

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LABORATORY

# PLASMA ALPHA-2 GLYCOMICROGLOBULIN IN MONITORING ALLOGRAFT FUNCTION AND IN PREDICTING REJECTION EPISODES IN KIDNEY TRANSPLANTATION (Preliminary results)

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This study reports clinical evaluation of a newly-discovered protein, alpha-2 glycomicroglobulin (A2GM), for monitoring renal function and in the identification and characterization of rejection episodes in kidney transplant recipients. Using a sensitive enzyme-linked immunosorbent assay technique, plasma levels of A2GM were measured prior to, and following, transplantation. There was an initial decrease in plasma A2GM in all patients following transplantation, but the decrease was significantly ($P < 0.023$) greater in patients with initial good function than in those with initial poor function associated with initial acute tubular necrosis or rejection. The decrease in A2GM did not correlate with subsequent graft function and viability. Levels of A2GM were found to be sensitive to changes in renal function and correlated well with creatinine. A sustained rise in A2GM was indicative of rejection. A2GM predicted rejection episodes 48-72 h before any significant rise in plasma creatinine in five of seven rejections studied. It is concluded that plasma A2GM levels may be clinically useful in assessing allograft function and in predicting rejection crises in kidney transplant recipients.

Keywords: Alpha-2 glycomicroglobulin, allograft function, kidney transplantation, rejection episodes

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Abbreviation: ATN: acute tubular necrosis

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## Introduction

Considerable interest has been shown in the use of small or low molecular weight (LMW) plasma proteins in the assessment of graft function, and in the early identification and monitoring of rejection episodes in renal transplant recipients /8, 18/. LMW plasma proteins have the common metabolic fate of being freely filtered at the glomerulus, reabsorbed and catabolized mainly by the lining cells of the proximal tubules /10/. Changes in the glomelular filtration as well as tubular reabsorption, therefore, will alter their plasma levels and urinary excretion and may be useful in monitoring renal allograft function.

Although most of the works on the use of LMW proteins in the assessment of renal function have involved $\beta_2$-microglobulin ( $\beta_2$M) /17, 18/, there is no clear consensus on clinical relevance of this protein in transplantation /15, 16/. Recent reports question the reliability of its measurement in urine /7/; and the endogenous production of $\beta_2$M is prone to significant variations as a result of infection and increased immune activity independent of renal function /16/. Another LMW protein, retinol-binding protein (RBP), has been advocated as an alternative marker for renal function /4, 5, 14/, but a recent work /12/ indicates that RBP may be an unreliable predictor of rejection. This highlights the need for an evaluation of the use of other LMW proteins as indicators of renal allograft function. This study investigates alpha-2 microglobulin (A2GM) for this purpose.

A2GM is a LMW plasma protein (MW 22,000 daltons) recently isolated from urine of patients with tubular dysfunction by DAKOPPATTS Immunoglobulin, Copenhagen, Denmark, and named "Urine Protein 1". It is synthesized in the liver and metabolized by the kidney in a similar manner to other LMW plasma proteins /1, 2, 10/. Evidence has been obtained that A2GM may be sensitive in monitoring renal function /1/. This study reports an investigation into the use of the protein in monitoring allograft function and in the identification of rejection episodes in kidney transplantation. Since variability in urine output and haematuria immediately following transplantation may make the value of urinary protein measurement in the detection of rejection crises meaningless or questionable /13, 19/, only plasma levels of A2GM before and after transplantation were monitored. The diagnostic potential of A2GM as an indicator of acute rejection was assessed by comparing with other clinical parameters for identifiaction of rejection.

## Materials and Methods

Fifteen kidney transplant recipients at the Renal Unit, Guy's Hospital, London, aged between 4 and 52 years were used for this study. Daily plasma samples were obtained from patients before transplantation and for postoperative periods of 5 to 7 weeks. Plasma samples were kept frozen (-40 $^{o}$C) until analysed.

Plasma A2GM was measured by an enzyme-linked immunosorbent assay /1/ and creatinine was measured by Jaffe's (kinetic) reaction on Cobas Bio centrifugal analyser.

Rejection episodes were suspected on the basis of the presence of all or any of the following: pyrexia, graft tenderness and swelling, oliguria or sudden decrease in urine output, increase in blood pressure, and a 24 h rise in plasma creatinine, greater than 20 µmol/l. Rejection and acute tubular necrosis were confirmed by renal biopsy.

## Results

The patients studied were categorized on the basis of graft function as assessed by plasma creatinine and A2GM (Table 1). To assess the initial function of the graft following transplantation, changes in plasma A2GM and creatinine for the patients with good initial function and those with initial acute tubular necrosis (ATN) are compared in Table 2. There was an initial decrease in A2GM and creatinine in all patients, but patients with initial good function had significantly greater decreases in A2GM and creatinine than those with ATN ($w = 71.5$, $P = 0.023$ by Mann Whitney test). These initial changes were however not predictive of subsequent allograft function: some patients had good initial function but subsequently experienced rejection episodes (Table I).

Table I

Representative groups of the graft functions studied*

| Group | Description | No. of patients |
|---|---|---|
| Initial good function | - No subsequent rejection | 5 |
| | - Subsequent rejection | 4 |
| Initial acute tubular necrosis | - Subsequent good function | 3 |
| | - Subsequent rejection | 3 |

*Categorization of graft function was based on plasma alpha-2 glycomicroglobulin and creatinine levels during the first 72 h after transplantation (see also Table II)

Table II

Plasma alpha-2 glycomicroglobulin and creatinine levels following transplantation. (Values given as mean ± S.E.M.)

| Parameter | | Group | |
|---|---|---|---|
| | | Initial ATN+ (n=6) | Initial good function (n=9) |
| A2GM (µg/l) | Initial value | 7292 ± 1393 | 5642 ± 1544 |
| | After 72 h | 2603 ± 487 | 585 ± 87 |
| Creatinine (µmol/l) | Initial value | 1010 ± 136 | 891 ± 75 |
| | After 72 h | 934 ± 183 | 257 ± 47 |

+ATN = Acute tubular necrosis; A2GM: Alpha-2 glycomicroglobulin

++Initial decrease significantly greater (W = 71.5, P = 0.023 by Mann Whitney test) in the group with initial good function than those with ATN. The initial values were obtained from blood samples collected in the first postoperative 12 hours.

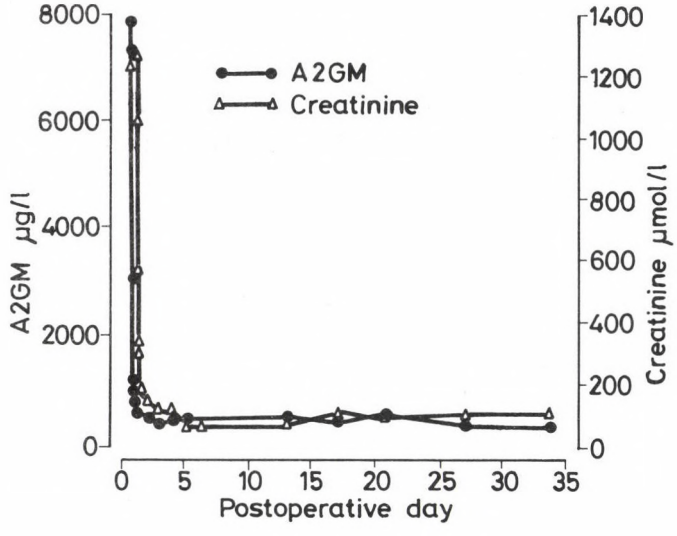


Fig. 1. Post-transplant plasma alpha-2 glycomicroglobulin (A2GM) and creatinine in a patient with uncomplicated clinical course. The patient, S.L., female, aged 13 years, had chronic renal failure due to nephronophthisis. She was maintained on continuous ambulatory peritoneal dialysis until cadaveric transplantation. Note that plasma A2GM and creatinine returned to normal (A2GM 300-600 µg/l, creatinine 80-110 µmol/l) within 5 days of operation

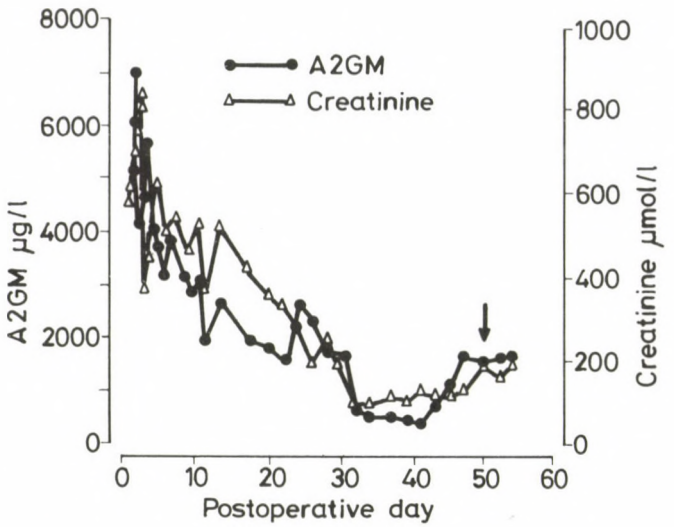


Fig. 2. Post-transplant plasma alpha-2 glycomicroglobulin (A2GM) and creatinine in a patient with complicated clinical course. The patients, A.W., Male, 7 years old, had dysplastic kidneys. Following bilateral nephrectomy and cadaveric kidney transplantation, he was maintained on haemodialysis until day 12; there was acute tubular necrosis and early pyelonephritis on day 4; graft pain (day 8) and acute cellular rejection (day 50, arrowed). Note that the normal levels of A2GM are between 300 and 600 µg/l

Changes in levels of A2GM and creatinine in two patients during the course of the study are shown in Figs 1 (completely uncomplicated clinical course) and 2 (initial ATN and rejection). Following the initial decrease in A2GM in all patients, there was a gradual rise in plasma A2GM (Fig. 2), except in cases where the initial good function was sustained (Fig. 1). This rise was more marked in patients with ATN. In all cases, the plasma levels of A2GM declined with an improvement in renal function.

In this study seven rejections were encountered. Increase in plasma A2GM levels appeared before clinical identification of rejection in five of these episodes. In these cases plasma A2GM rose 48-72 h before significant increases in creatinine were noticed. In the sixth case, when the cellular rejection was mild, there was a significant rise in A2GM about the time of the clinical identification of the rejection; in the last case there was no increase in A2GM at all. In all rejections, prolonged and sustained rise in A2GM appeared to parallel the severity of the rejection crises.

## Discussion

Measurement of LMW proteins in plasma after transplantation may be valuable in the examination of two situations: (i) the immediate postoperative period when anuria or poor function may result from rejection or ATN; (ii) subsequent changes.

The initial decrease in A2GM followed by a gradual rise found in this study is in accord with recent reports on the behaviour of small plasma proteins in kidney transplant recipients /12, 18/. In the initial post-transplantation periods, it has been demonstrated that renal tubular uptake of LMW proteins by a viable transplant kidney occurs prior to perfusion and excretory functions. This agrees with reports that although LMW proteins metabolism by the kidney is primarily by proximal tubular cell reabsorption and catabolism from the luminal side, this is supplemented by a minor, but significant, protein uptake and catabolism from the peritubular capillaries /3, 6, 9, 11/. The progressive decrease in plasma A2GM during initial stages of anuria that followed cadaveric transplantation may therefore suggest that the graft was viable. Although this initial decrease in A2GM was independent of subsequent graft viability and function (Table 1), it was significantly less in patients with initial ATN than those without ATN. Tubules damaged by necrosis would be expected to be less efficient in peritubular uptake and catabolism of A2GM, hence the inferior decrease in plasma A2GM levels in this group of transplants. At this non-diuretic stage, therefore, a decrease in plasma A2GM may indicate functioning tubules; complete absence of this decrease may suggest rejection rather than ATN or lack of perfusion.

Subsequently, the initial fall was followed by a gradual increase in plasma A2GM unless graft function was normal. This could be due to at least two reasons. First, the rapid initial fall of A2GM would presumably trigger off an increase in the synthesis and/or release of the protein as a physiological anti-depletion mechanism. Second, following failure in regain of normal function, the increased synthesis of A2GM may overwhelm the peritubular uptake and metabolism mechanism: A2GM may therefore rise. This rise would however be more rapid in patients with rejection or ATN.

Although the number of rejection episodes studied was not large enough for statistical analysis to be made, some useful trends were observable. In most cases the rise in A2GM during rejection paralleled the rise in creatinine. A2GM was however more sensitive than creatinine in

in detecting rejection by rising 48-72 h before significant increases in creatinine. Five of the seven rejections studied were correctly diagnosed by A2GM, indicating that the protein may have a considerable predictive value in the identification of rejection episodes. Most of the patients were on post-transplantation haemodialysis; for these, plasma creatinine was valueless in mirroring allograft function.

All rejections studied were for the first one to seven weeks and were acute. Later, "chronic" rejections, which may have different characteristics, were not studied. A study of chronic rejection may be useful in further evaluating the clinical usefulness of A2GM in graft function. In conclusion, plasma A2GM monitoring in kidney transplantation has been found to be a useful and fairly sensitive procedure. Its sequential daily determination in plasma may improve the sensitivity of graft function monitoring and has great potential in the early diagnosis and treatment of rejection in most instances. In this respect, the value of A2GM measurement could be in lessening of the severity of acute rejection episodes or immunologic injury to the graft such as scarring and tubular atrophy, which can ensure better long-term function of the graft. The results of this study clearly indicate that there is a basis for further studies on the clinical relevance of A2GM in the detection and evaluation of post-transplantation tubular damage and rejection.

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PHYSIOTHERAPY

# THE DESTABILIZATION OF AN ABNORMAL PHYSIOLOGICAL BALANCED SITUATION, CHRONIC MUSCULOSKELETAL PAIN, UTILIZING MAGNETIC BIOLOGICAL DEVICE

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In this study two groups of subjects with indications of chronic musculoskeletal pain are examined statistically after applications of Magnetic Biological Device hereafter referred to as MBD /1, 2/. The first group is a short-term study on 14 indications showing the results after three days of treatments at one per day. The second group is a long-term study on data collected in a survey after the first year of operation in a clinic on 114 indications. The scores indicate that MBD is a desirable alternative to standard therapeutic practices, in the elimination and/or maintenance of chronic musculoskeletal pain due to its superior scoring on the Melzack-McGill Pain Questionnaire, apparent ability to increase the balance of thermal emission patterns, is non-toxic, non-invasive, plus is economically advantageous, due to the limited number of applications required to maintain these conditions.

Keywords: musculoskeletal pain, magnetic biological device

## Introduction

Magnetic Biological Device (MBD) was designed in March of 1982 by David J. Stewart, co-author of this paper. MBD has achieved patents and has been tried in field, clinical, case and control situations.

Accordingly, one broad aspect of this invention resides in providing an electromagnetic device for modifying any growth, repair or maintenance processes in a predetermined local area of a living body that utilizes a signal having a symmetric waveform to excite a magnetic-field-producing coil.

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Another aspect of this invention resides in providing an electromagnetic device for modifying any of the growth, repair or maintenance processes in a predetermined local area of a living body comprising: a coil capable of inducing a magnetic field, wherein the magnetic field has a time-varying spatial-configuration and a time-constant spatial orientation with respect to said coil; means for generating an electrical signal capable of exciting the said coil so as to induce a magnetic field having a time-varying spatial conformation, wherein said signal has a substantially-symmetric waveform; with a frequency below about 100 Hertz; and wherein, when said device is in use, said coil is adapted such that the spatial orientation of said magnetic field with respect to said local area, is capable of being varied in time thereby causing time variations in said magnetic field at any given location in said area which are of about the same range of magnitude as the time variations in the time variations in the spatial-configuration of said magnetic field.

## LONG-TERM STUDY (n=114)

### Methods

The collection of data on changes in reported musculoskeletal pain in relationship to applications of MBD, in the human model commenced in October of 1985. These data consisted primarily of reported pain experience. The subjects consisted of middle to upper income bracket, actively employed or retired, with all being ambulatory.

In mid-1986, Infrared Thermography was added to the data bank to:

a) Assess the possibility of consistent thermographic emmission change in relationship to the various treatment frequencies, the various directions of flux, the various output levels, and the reported pain experience.

b) Due to subjects consistently reported a reduction in pain experience, psychopathic factors had to be eliminated as the contributor.

c) The failure rate, though impressively low, required scrutiny as to:

1) was the application of MBD applied to a wrong physiological area;
2) was there the possibility of a mechanical situation which required surgical correction;
3) was there an addiction present which mimics symptoms;
4) was the subject in a financially 'convenient' situation.

During the month of November 1986, a telephone survey was conducted. Of the 114 musculoskeletal pain subjects contacted, 69 reported total relief, 10 reported excellent response to application with very mild return of pain, 16 reported good response to application with mild return of pain, 11 reported fair response to application with a return of pain that could be tolerated, and eight reported no response to application with five still ex-

periencing the same pain level and three had had surgical correction of mechanical problems, with one having return of pain six weeks after surgery. As it was felt that these two forms of data, thermography and the rough data, would not be enough to satisfy the scientific community of efficacy with the use of MBD in the reduction of musculoskeletal pain, the data was put aside until an additional evaluation tool or one that could statistically verify the collected data became available.

## SHORT-TERM STUDY (n=14)

In January 1989, a sample group of 16 indications of musculoskeletal pain were chosen at random. A time period of six weeks was necessary to achieve this number. The indications were all the result of automobile accidents and/or falls. The sights of indicated pain were cervical radiating to arms or jaw, lumbar radiating to knees and/or ankles, sternum, rib cage, groin radiating to legs, ankles, knees and ankles and wrists. The average pain experience was 5.2 years.

Assessment tools:

a) Melzack-McGill Pain Questionnaire /3, 4/

b) Agema Infrared System 782, Thermal Computer c/w Cannon Color Printer

Therapy tool:

a) Masor, Magnetic Biological Stimulator, Model 6

Materials, equipment and supplies:

a) Agema Infrared System 782

b) Controlled Environment at approximately 21 $^{o}$C

c) Treatment Facility

Procedure

Day 1: a) McGill Pain Questionnaire was administered after a complete medical history was taken. Administration of the questionnaire was as directed by Melzack /3/, in that the subject was allowed to relate freely in relationship to their perceived pain. The various descriptives were discussed, where necessary, until the subject was satisfied that the descriptives were an accurate description of their pain. Administration time for the questionnaire was as necessary, averaging 32.5 min.

b) Thermographic evaluation was conducted in the thermally controlled environment. After the appropriate cooling-down period of 20 min, six to eight views were recorded of each subject on computer disc.

c) Application of MBD: Observing the thermal degree of lateral balance and the vertical spread of one-degree increments, individual schedules were established.

d) Method of application of treatment head: (Standard Pattern). The applicator is moved in a circular pattern of approximately 10 cm diameter at a rate of 30 cm/s, and should take one second to complete one rotation. This pattern is then moved laterally at three cm per revolution to cover an area. The applicator is in contact with the body surface.

e) Spinal Application:

1) Subject is placed face down on the treatment table, fully clothed.

2) MBD is set at negative flux, 2 or 12 Hz (relative to thermal evaluation) at full output for 10 min.

3) Method of application. The standard treatment head pattern is applied beginning at the coccyx and moving up the spinal column to and including the cervical area.

Next the coverage is enlarged until every area of the torso has been exposed. This method of application is repeated until the designated time has elapsed and then repeated with control settings stated in steps 4 through 7.

4) MBD is set at negative flux, 2 or 12 Hz, at 60% output for 5 min.
5) MBD is set at negative flux, 2 or 12 Hz, at 50% output for 5 min.
6) MBD is set at positive flux, 12 Hz, at 60% output for 5 min.
7) MBD is set at positive flux, 12 HZ, at 50% output for 5 min.

f) Sternum and Ribcage Application:
1) Subject is in a sitting position. Subject applies applicator to self using standard pattern and concentrating on tender areas with control settings stated in steps 2 through 5.
2) MBD is set at negative flux, 2 to 12 Hz, at 75% output level for 3 min.
3) MBD is set at negative flux, 2 to 12 Hz, at 55% output level for 3 min.
4) MBD is set at negative flux, 2 to 12 Hz, at 45% output level for 3 min.
5) This application procedure is continued through 35% and 25% output levels and then the same levels are repeated at positive flux, 12 Hz.

g) Ankles, Knee and Wrist Application:
1) Subject is in a sitting position. Subject applies applicator to self.
2) MBD is set at negative flux, 2 to 12 Hz, at full, 50%, 40%, 30%, and 20% output and is applied using standard pattern concentrating on pain site and covering the areas above and below the pain site, where possible for five min.per level.
3) MBD is set at positive flux, 12 Hz, at 50%, 40%, 30%, 20% and full output, five min.each and applied as above.

Day 2: a) McGill Pain Questionnaire administered.
b) Application of MBD, as day 1.

Day 3: a) Thermographic evaluation.
b) Application of MBD, as day 2.
c) McGill Pain Questionnaire administered.

This procedure was complete on 12 of the 16 indications only, as one subject related total pain relief after the first application and requested telephone follow-up due to his demanding work schedule. One subject reported total pain relief after two applications and requested follow-up by telephone. Due to athletic stress, causing re-injury, two indications had to be thrown out of the data.

During the six week period of data collection, the subjects were instructed that once the procedure was completed, if the pain returned with any degree of intensity, they should return for a further application. One subject returned for one further application. The scores for the 3 days of the short term study are shown on Figs 1-4.

## CORRELATION OF DATA

From the raw data averages (A) and standard deviations (S.D.) were calculated as per Melzack /4/.

| | Day 1 | | Day 2 | | Day 3 | |
|---|---|---|---|---|---|---|
| | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| PRI | | | | | | |
| Sensory | 15 | 7.4 | 5.6 | 3.6 | 2.4 | 2.1 |
| Affective | 2.1 | 2.6 | 14 | 35 | 0 | 0 |
| Total | 24.5 | 13 | 7.9 | 5.7 | 3.2 | 2.8 |
| PPI | 2.5 | 82 | 1.5 | 78 | 86 | 64 |

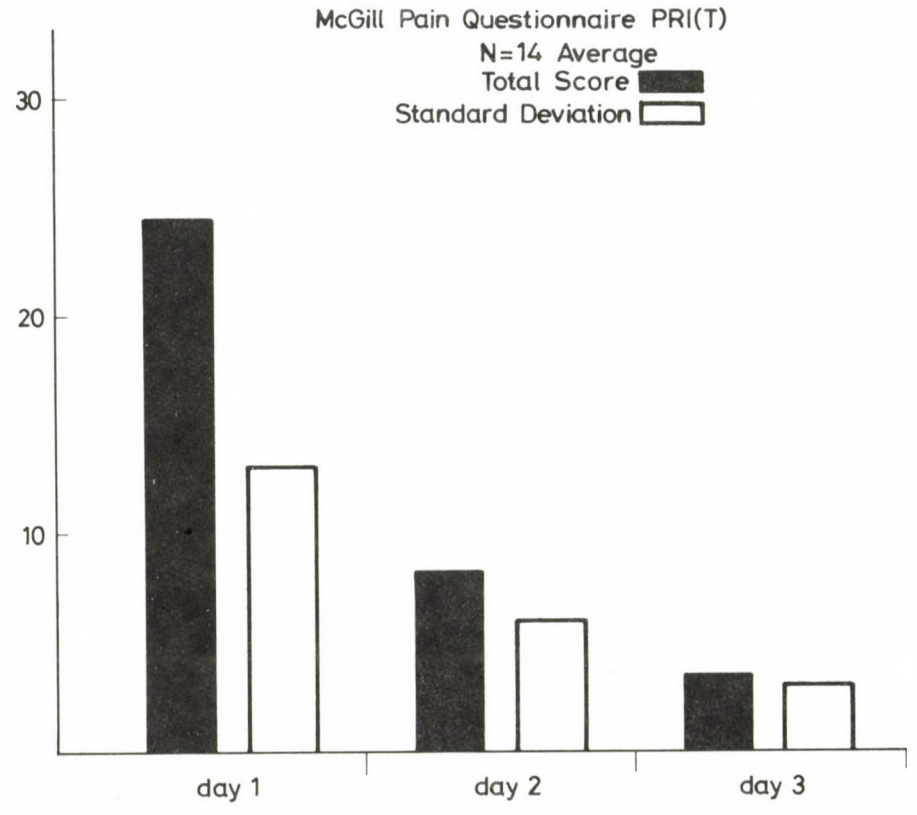


Fig. 1. This Figure represents the PRI(T) scores for the three days of the Short Term Study (Sample Group), plus the Standard Deviations. PRI(T) means the total pain-related index which includes sensory qualities, affective qualities and evaluative words that describe the subjects overall intensity of the total pain experience

Other averages of interest:

a) Age of subjects = 42.13 yrs.
b) Pain experience = 5.21 yrs.
c) Reported pain experience reduction after first application = 63%.
d) Reported pain experience reduction after third application = 83.3%.
e) Number of applications = 2.9.

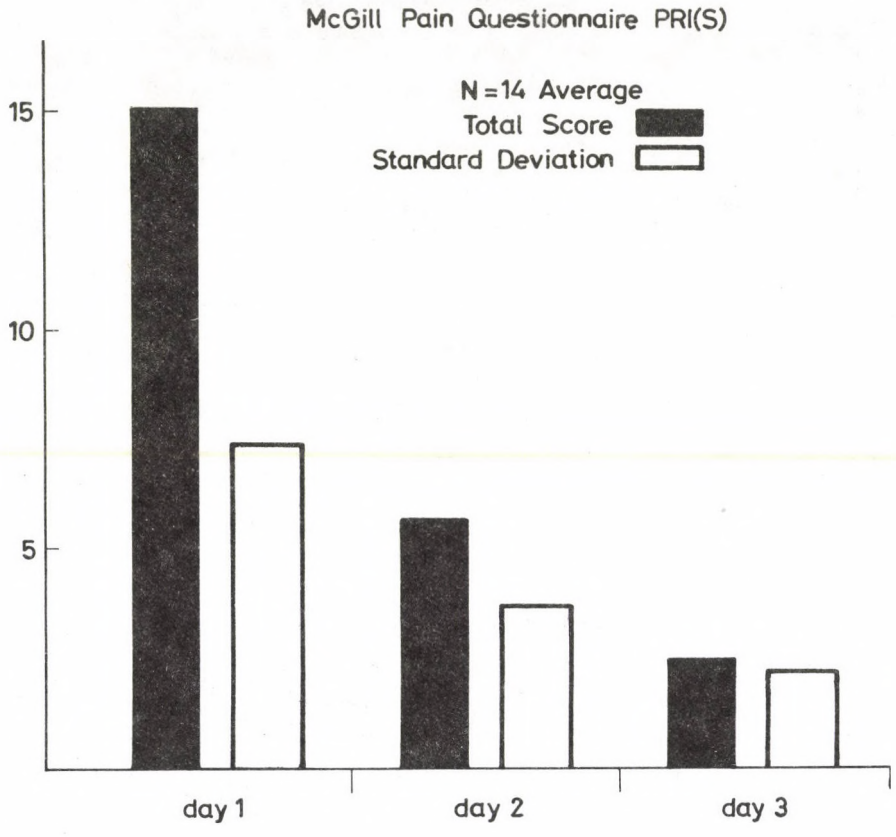


Fig. 2. This Figure represents the PRI(S) scores for the three days of the Short-Term Study (Sample Group), plus the Standard Deviations. PRI(S) means the sensory qualities of the pain experience, in terms of temporal, spatial, pressure, thermal and other properties

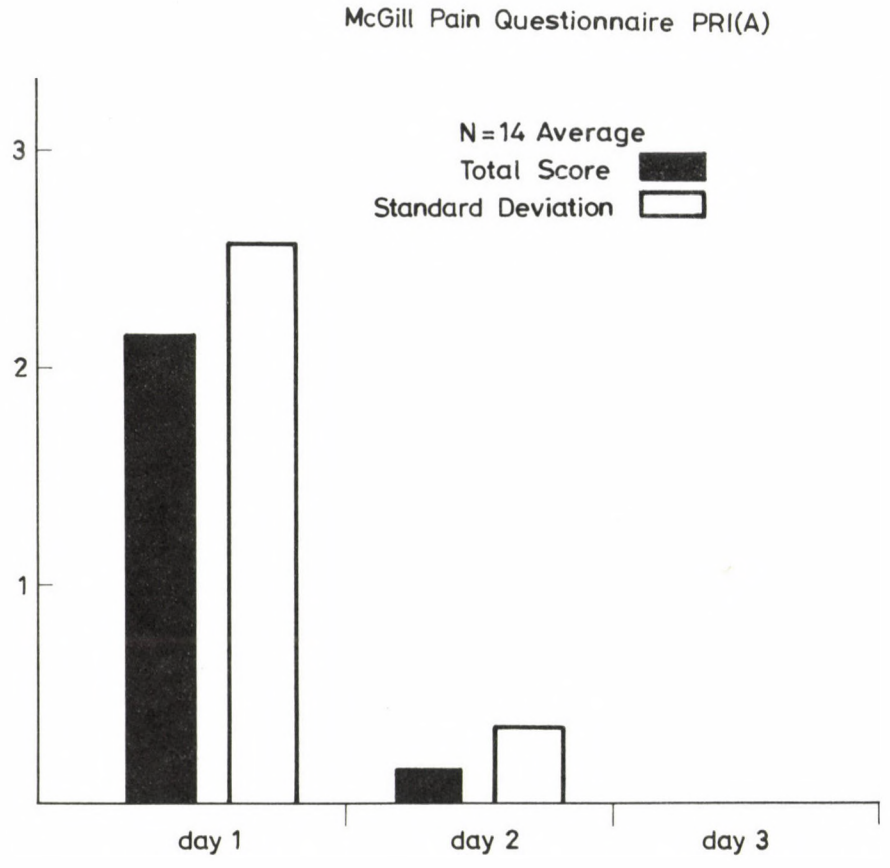


Fig. 3. This Figure represents the PRI(A) scores for the three days of the Short-Term Study (Sample Group), plus the Standard Deviations. PRI(A) means the affective qualities of the pain experience, in terms of tension, fear, and autonomic properties.

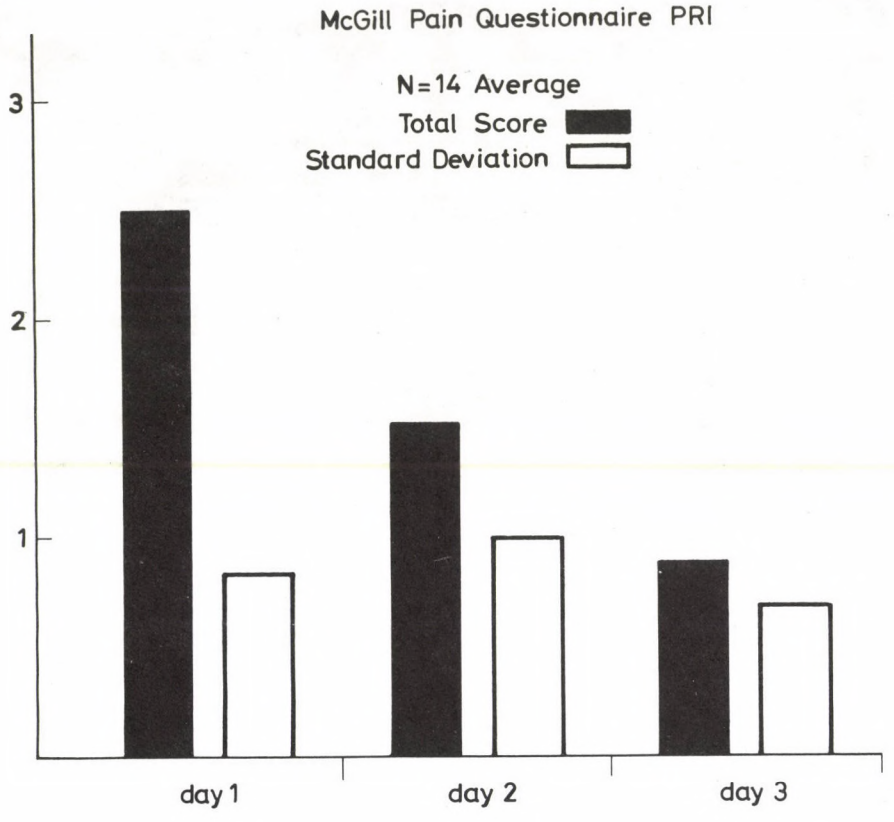


Fig. 4. This Figure represents the PRI scores for the three days of the Short Term Study (Sample Group), plus the Standard Deviations. PPI means the present pain intensity which is scored from 0 to 5, e.g. 0, no pain; 1, mild; 2, discomforting; 3, distressing; 4, horrible; 5, excruciating

## COMPARISON OF SHORT-TERM TO LONG-TERM STUDY

Once the Short-Term Study was completed, and certain values of PRT(T) were established, these scores were then assessed to the Long-Term Study to evaluate long-term effectiveness of the device. It should be realized that these calculations are not fully indicative of the actual PRT(T) scores but will only show a trend, if it exists, in the results over the long term. Scores were assessed equally to all reported data.

If Total Pain Relief is equal to PRI(T) estimated score 0.
If Excellent Improvement is equal to PRI(T) estimated score 1.
If Good Improvement is equal to PRI(T) estimated score 4.
If Fair Improvement is equal to PRI(T) estimated score 8.
If No Change is equal to PRI(T) estimated score 24.5.

Then: N = 114

| | No. of subjects | | | | |
|---|---|---|---|---|---|
| Total Pain Relief | 69 | X | 0 | = | 0 |
| Excellent | 10 | X | 1 | = | 10 |
| Good | 16 | X | 4 | = | 64 |
| Fair | 11 | X | 8 | = | 88 |
| Poor/No Change | 8 | X | 24.5 | = | 196 |
| Total | 114 | | | = | 358 |
| Estimated Average PRI(T) after applications | | | | = | 3.14 |
| Estimated Average improvement: | 358/2793 | | | = | 87% |
| Average number of applications | | | | = | 3.94 |
| Subjects improved per cent | | | | = | 93% |

The estimated data were then compared with the short-term results:

| Description | N=114 | N=14 |
|---|---|---|
| Total Positive Response | 93% | 100% |
| a) Total Pain Relief | 61% | 21% |
| b) Improvement | 32% | 79% |
| Poor/No Change | 7% | 0% |
| Beginning PRI(T) | 24.5 (est.) | 24.5 (S.D.=13) |
| Final PRI(T) | 3.14 (est.) | 3.2 (S.D.=2.8) |
| No. of Applications | 3.9 | 2.9 |
| Total Pain Relief: One Application | 12 | 1 |

The data was further examined and placed in groups of four months after treatment commenced.

| | | |
|---|---|---|
| N = 20 PRI(T) 1 to 4 months | .45 (est.) | with 0 failures |
| N = 29 PRI(T) 5 to 8 months | 2.17 (est.) | with 2 failures |
| N = 41 PRI(T) 9 to 12 months | 4.70 (est.) | with 5 failures |
| N = 24 PRI(T) Ongoing treatment | 3.56 (est.) | with 1 failures |

## Points of interest

In review of the long-term reported pain experience in relationship to the short-term-reported pain experience, these statistics indicate that the positive effects of MBD applications in subjects suffering musculoskeletal pain appears to be ongoing as few subjects had to return for additional applications. As some of the surveyed subjects in the long-term study reported some relief immediately after application, with total relief five to 10 days later, it is reasonable to estimate that some of the 79% showing improvement in the short study will experience total relief within a short period of time or will return for further applications.

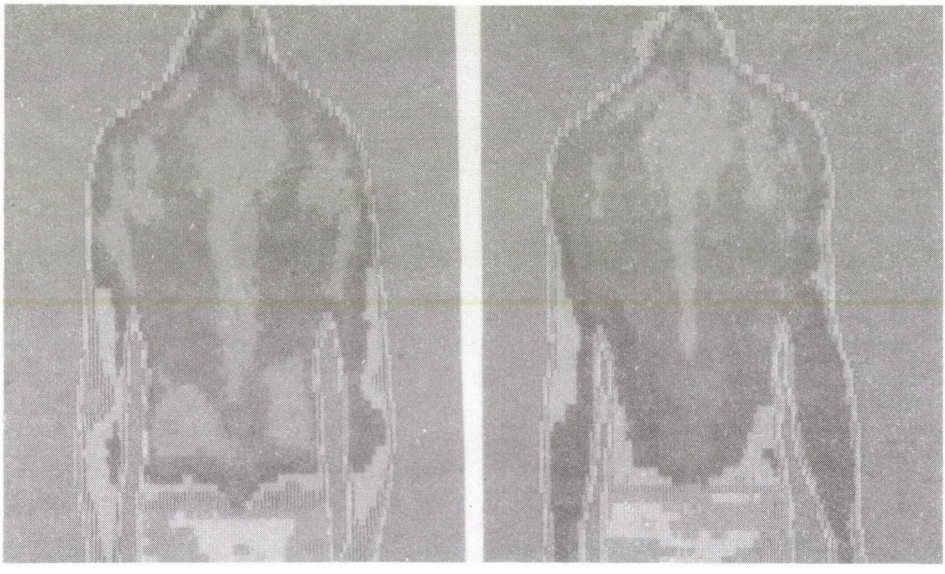

Fig. 5. EXAMPLE 1. In the first printout there is balance from side to side on the back, but little or no density pattern. Also, the arms differ one to the other. The thermal emmission gradient up the back was not apparent. The second printout shows balance is developing from side to side, as are the triangular patterns. The arm temperatures have also changed by plus 2 °C. The thermal gradient up the back is 2.6 °C with an overall reduction in low back temperature of 1 °C. The PRI(T) scores on this subject were: Before First = 13, Before Second = 8, After Third = 3

The values seen in the monthly comparison indicate that either the estimated pain levels increased due to cycling, reinjury or thattreatment schedules were improving. The ongoing treatment subjects, excluding the failure, were being maintained at a tolerable pain level.

During the afore-mentioned telephone survey, a number of questions were asked of the subjects, such as would they use the service again if their pain reverted to its prior level. Of the 114 indications, four subjects stated they would not return, six stated they might return, and all the remaining subjects stated they would use the services again if necessary.

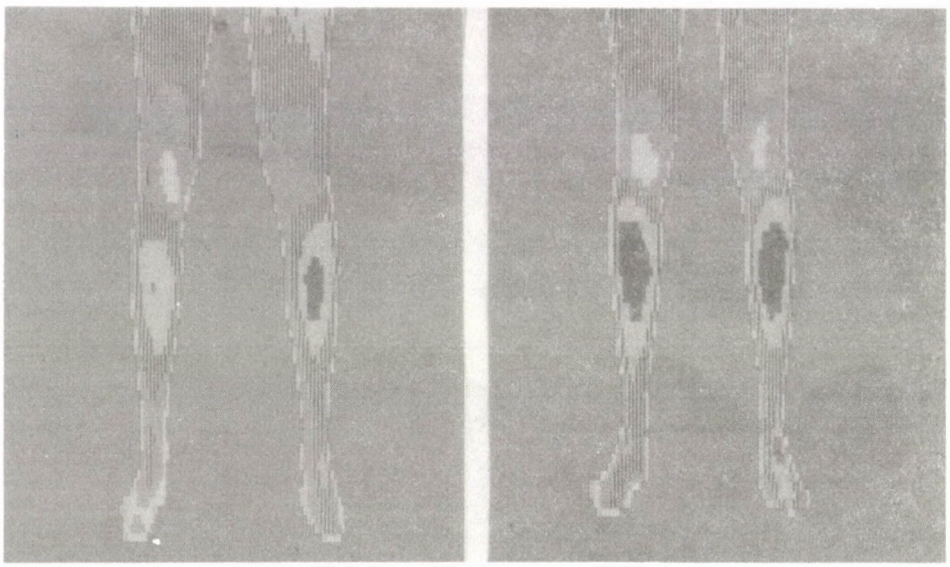

Fig. 6. EXAMPLE 2. In the first printout the dissimilarity of pattern from one leg to the other is very evident. The second printout indicated both legs are balancing and the right foot had changed by plus 1.1 $^{o}$C, while the left knee had changed by 1 $^{o}$C. The PRI(T) scores on this subject were: Before first = 44, Before second = 14, After third = 6

## THE SIGNIFICANCE OF THERMOGRAPHIC EVALUATION IN MONITORING TREATMENT EFFICACY OF MBD

Thermography as a Diagnostic Aid in the Management of Chronic Pain by Pierre L. LeRoy, Walter M. Bruner, Cynthia R. Christian, Roseanne Filasky, and Suzanne LeRoy, states "Thermography aids in following the course of disease and in monitoring treatment efficacy". "Where the body's radiant heat emmission pattern varies from normal symmetry, something is wrong that requires medical interpretation." "Neuropathic disorders are seen to be generally hyperthermic in the acute stage and hypothermic chronically. Unilateral neuropathic disorders of 1$^{o}$C or more are recognizable to the trained neurophysiologist."

The following are examples of the Thermographic Evaluation executed prior to the first and third applications of the MBD. The extent to which the thermography assessments are used by the authors is only to confirm that a change is occurring. If no change occurs, the subject is recommended to return to their assessing physician for further evaluation.

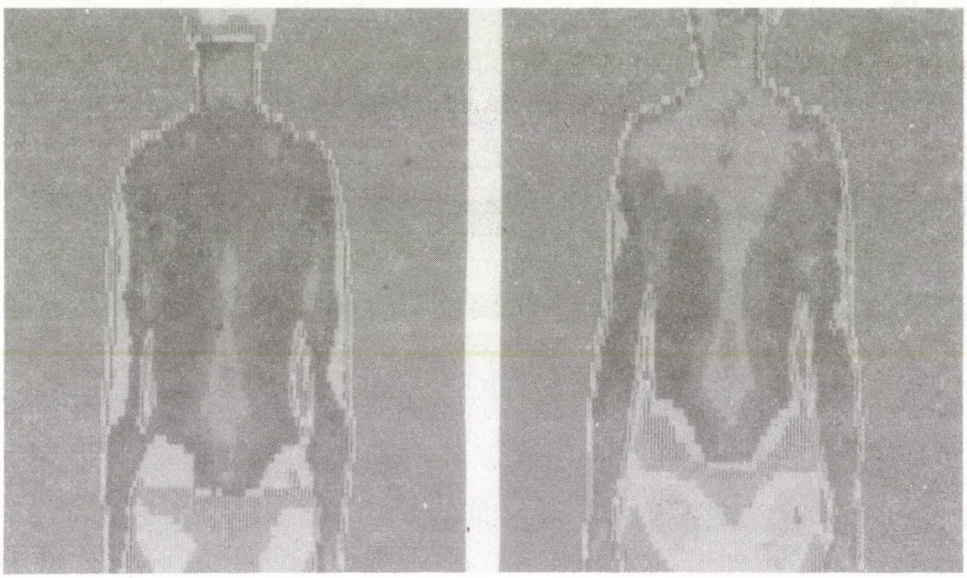

Fig. 7. EXAMPLE 3. In the first printout the pattern was balanced from one side of the back to the other. The thermal gradient up the back was 4 °C, with a further hyperthermic emission of 1 °C over L1. The second printout indicated balance of thermal pattern from side to side, with an increase in temperature of 1 °C over the upper back; a decrease of 1 °C over the buttocks. The thermal gradient up the back had increased to 6 °C. The PRI scores on this subject were: Before first = 23, Before second = 8, After third = 2

Two points were assessed in the evaluation:

a) Are the thermal patterns balanced from one side of the body to the other, plus demonstrating typical triangular patterns of tissue density? Where the anatomical structure have a reference, such as arms and legs, does one differ from the other?

b) Is the thermal emmission gradient up the back relevant to tissue density?

## Discussion

The authors' claim of appearing to cause destabilization of an abnormal physiological balanced situation, chronic musculoskeletal pain, with the use of MBD is indicated by the following comparisons with standard therapeutic practices.

a) Analgesics, i.e. morphine, controls reaction to pain through relaxation, apathy and freedom from anxiety. When the effects of the analgesic diminishes after approximately four hours, reaction to the pain returns. The probability of similarity in PRI(T) scores before administration and after excretion is very high. When applying MBD, the reaction to pain after application gradually decreases. In 10% of the subjects, after the first application, there is a decrease, a short period of rebound and then a further gradual decrease. The day following the first application PRI(T) drops by an average of 63%. After three days of application of MBD, one per day, an average decrease in PRI(T) score of 83.3% was indicated in the short study.

b) Chemicals which interrupt the mechanism causing the pain, such as atropine for muscle spasm or ergotamine for migraine, effect temporary or periodic abnormal physiological situations, not those that are balanced as are long-term pain situations.

c) Acetylsalicylic acid affects the threshold of pain perception. MBD applications result in PRI(T) scores consistently lower after application and with yet further decrease over time.

d) Hypnosis averages 40% reduction in perceived pain while MBD averages consistently 83.3%.

e) Distraction, as hypnosis, averages 40% reduction in perceived pain, but the reduction is only indicated while the distraction is ongoing.

f) 'Placebo' and autosuggestion indicates successful results in less than 40% of subjects, where as MBD application scores a success rate, in the short study 100%, and in the long-term study 93%.

g) Transcutaneous Electrical Nerve Stimulation (T.E.N.S.) scores a PRI(T) of 7.8 during application, as per Melzack et al. /4/. While using devices with periodic pulse rates, the nervous system adapts to this type of stimulation over a period of time, lessening efficacy /6/. The effects of MBD appears to be ongoing over time with only 3 to 4 applications necessary, in 61% of the subjects surveyed in the long-term study, to remain pain free.

These discussed comparisons of MBD applications to standard therapeutic practices clearly indicates the effects (efficacy) of MBD, also, to be unique in effect and unquestionably superior to standard therapeutic procedures in the maintenance of chronic musculoskeletal pain. Plus being a non-toxic and non-invasive therapy increases desirability as a therapeutic alternative.

## Conclusion

Past research in this relatively new field seems, generally, to migrate around specific equipment being marketed for bone non-unions. The studies published so far tend to support that equipment. As researchers are seldom electronic design oriented, their research is limited by availability of designs to study. The authors of this paper conclude that this is the reason that MBD and the developed treatment techniques had not been discovered before.

The exact effect of MBD applications on a biological system, beyond destabilization, is not clearly understood. Many studies have been brought forward on the biological effects of pulsed magnetic fields at the cellular level: Ligand binding to the cell membrane /8/, Cyclotron resonance conditions for calcium ion /9/, changes in cell wall permeability /10/ etc. It should be clearly understood that most present research is primarily being conducted as to the frequency effects on cells in the cycle per second and up part of the spectrum using stationary coils and pulsed asymmetrical signals. Presently the MBD uses three primary half-sine symmetrical pulses at 2 Hz, 12 Hz, and 45 Hz. The net effect on tissue is a moving field over an area for less than a second, returning over the same area every second for three seconds and then moving away from that area for six or more seconds. Since the falling edge of the pulse is in a different orientation to the tissue than the leading edge, the 'cancellation effect' is minimized and a net change is left behind, much like a tape head over a moving magnetic medium in a tape recorder. The frequency selected seems to have an effect depending on the acute stage/chronicity of the injured area and could possibly be related to the fluid balance in the damaged site. The time away from the affected area, i.e. six seconds and over, seems to be needed to allow tissue adequate time to respond after each stimulation. The exact effects will become clearer in future research.

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Acta Medica Hungarica, Vol. 46/4, pp. 339—344 (1989)

# BOOK REVIEWS

## IARC Scientific Publications No. 73, Lyon, 1985

### Laboratory decontamination and destruction of carcinogens in laboratory wastes: Some antineoplastic agents

Eds: M. CASTEGNARO, J. ADAMS, M.A. ARMOUR, J. BAREK, J. BENVENUTO, C. CONFALONIERI, U. GOFF, S. LUDEMAN, D. REED, E.B. SANSONE and G. TELLING

The previous Monograph published in this series dealt with the laboratory decontamination of carcinogenic wastes and addressed the problems of worker processing or handling these chemicals during manufacture. This issue is more interesting for those who are continually exposed to these drugs in hospitals or in the pharmacy. The aim of this small book is to increase the safety of hospital work as well as work in those laboratories where the clinical trials are carried out with antineoplastic agents.

Altogether 18 compounds are dealt with (Doxorubicin, Daunorubicin, Methotrexate, Dichlorometithrexate, Cyclophosphamide, Ifosfamide, Vincristine sulfate, Vinblastine sulfate, 6-Thioguanine, 6-Mercatopurine, Cisplatin, Streptozotocin, Chlorozotocin, Lomustine, Carmustine, Semustine, PCNU, Melphalan) in detail, and 12 methods of destruction and decontamination are described.

Appendix A contains the nomenclature and physico-chemical data of 18 antineoplastic drugs, presenting the structural formula, solubility, stability data, too.

Appendix B considers the further reactions of antineoplastic agents relevant to their degradation through biological and chemical methods.

This book is very helpful in the everyday practice of chemotherapy handling and offers safety procedures for those working in hospitals or in research laboratories.

ANNA TOMPA

## IARC Scientific Publications No. 78, Lyon, 1986

### Carcinogenicity of alkylating cytostatic drugs

Eds.: D. SCHMÄHL and J.M. KALDOR

This volume of IARC Monographs considers the secondary carcinogenic effect of long-term chemotherapy. The introductory paper by Professor Schmähl describes the main character and different aspects of the problem. The following papers are related to the biochemical reactions and interactions of chemotherapeutic agents with macromolecules, such as DNA. The antitumour alkylating drugs are cytotoxic, antiinflammatory and immunosuppressive to the host, causing chromosome aberrations and DNA damages also in non-tumour-bearing organs. These malformations can cause non-repaired somatic mutations and develop the biological background of neoplastic transformations. These biological effects may be monitored by epidemiological studies and quantitatively estimated with case-control studies made in patients treated for cancer.

The last chapter of the book deals with the future and report about methods for prevention. The authors (Kaldor and Schmähl) are pessimistic, or even realistic, about the solution of drug-induced cancer as such. They forcast future public health problems concerning these agents, will respect to the magnitude of occupational carcinogens. For example, in Europe and North America there are more than 20.000 new cases of Hodgkin's disease annually and 5% of the treated patients develop acute leukaemia after ten years of treatment. That means approximately 750 new cases of iatrogenic leukaemia per year.

Those who are interested in this problem, will find this volume useful, especially because of its multidisciplinary effort to eliminate this kind of complication caused by chemotherapy.

ANNA TOMPA

IARC Scientific Publications No. 83, Lyon, 1986

Long-term and short-term assays for carcinogens: A critical appraisal

Eds by R. MONTESANO, H. BARTSCH, H. VAINIO, J. WILBOURN and H. YAMASAKI

IARC published a series of critical reports on long-term and short-term studies for detection of chemical carcinogens and mutagens in 1980. The issue under review contains all of the available results from adequately achieved and critically analysed experiments which have been done since 1980. These short-term assays are generally designed to replace the often very expensive and time-consuming animal studies to measure the genotoxic effects of chemical carcinogens or mutagens, and use them in the risk assessment for primary cancer prevention.

Since it is difficult to see how epidemiological studies alone could assess the role of naturally occurring chemicals, dietary components or to elucidate the mechanism of carcinogens formed in the human tissues, an in vitro approach is urgently needed in this field.

This volume contains a series of reports to clarify the aetiopathogenesis of human cancer tested in vitro. In these studies the authors demonstrate the recent developments and provide a better understanding of the endpoints and better validation of the capacity of these short-term assays aimed at detecting carcinogens.

Early in the 1980s there were high expectations for the use of these methods alternatively or in addition to long-term carcinogenicity testing. Now the short-term assays are playing an essential complementary role in the extrapolation of these studies to human, using human cells for testing. Use of these tests has enabled us to understand some of the crucial metabolic pathways in chemical carcinogenesis and the chemical and cell interactions.

The first two reports (G. Della Porta and P. Bannasch) deal with evaluation of bioassays for carcinogenicity in animals as well as with early preneoplastic lesions. In the third report the topic is the assay for initiating and promoting activities during chemical carcinogenesis, using a mouse skin model, rodent liver, urinary bladder, etc.

In the second part of the volume in vitro methods are used to detrect the DNA damage and repair or different end-points in genetic changes, using bacterial tests, mammalian cells or host-mediated assays. The assay for germ-cell mutations in mammals, mammalian cell transforma-

tion in culture or in vitro assays for tumour-promoters are also critically reviewed. The studies made on fungi or in drosophila melanogaster are also reviewed.

The new and very promising field of the biological monitoring of carcinogenic potential of certain environmental chemicals are the short-term assays for the analysis of body fluids and excreta.

At last, we can find the personal overview of the development of short-term tests by B.A. Bridges. Presenting personal opinion about this rapidly changing field reflects a very impressive courage of the author We should agree with him that these two branches of carcinogenesis research are not confrontable but complementary to each other. On the future, these methods are usable to correlate their results statistically in evaluation of different environmental chemical carcinogens and/or mutagens.

ANNA TOMPA

## IARC Scientific Publications No. 85, Lyon, 1988

### Environmental Carcinogens. Methods of Analysis and Exposure Measurement: Vol. 10. Benzene and Alkylate Benzenes

Eds: L. FISHBEIN and I.K. O'NEILL

Benzene was dealt with in three earlier issues of IARC, Monographs Supplement No. 7 and in Vol. 7 and 29. This volume contains not only benzene-related information but also presents data for xylenes and toluene. These aromatic hydrocarbons are produced for a wide variety of applications, primarily as solvents. Since benzene is a human carcinogen, xylene and toluene are replacing it in the chemical industry and in laboratories.

Many case reports and epidemiological studies have dealt with the association of exposure to benzene and leukaemia in humans, although these epidemiological studies are unable to evaluate the risk for leukaemia among exposed people. The haemopoietic tissue is very sensitive to benzene, which causes "benzene haemopathies". 10 ppm benzene inhalation increased the chromosomal damages (mainly chromosome breaks) by 20, 25 and 30%. IARC in 1982 established that benzene is a human carcinogen, however, there is only limited evidence in experimental animals to its carcinogenicity.

Xylene and toluene have no human carcinogenic activity according to the available data, but benzene impurities in these solvents should be taken into account. These aromatic hydrocarbons require metabolic activation to be active to biological systems, explaining the combined effect of the rate of metabolic activation with the clastogenic effect of phenol. Benzene, toluene and xylenes are highly volatile and are absorbed through the respiratory route or through the skin. This manual contains the precise data of the toxicokinetics of these solvents and those factors which affect pharmacokinetic processes in the living body. The interaction with ethanol-induced alterations of benzene, toluene and xylenes is also discussed.

In this book we can find all of the present-day data in this field from the places of exposure to biological monitoring. There is some useful study in the sampling and analysis of industrial air to identify specific pollutant sources showing the way to the moritoring strategy.

Determination of the levels of exposure to solvent vapours in the industrial atmosphere has long been a difficult problem, with sample collection and concentration. The colorimetric methods as well as the direct spectrophotometric methods are subject to interferences from a broad spectrum of compounds. Unfortunately, some of the older exposure measurements did not eliminate this interference problem.

The analysis of volatile compounds such as benzene, toluene or xylene is prescribed for waste sites by gas chromatography with photoionization detection or with mass spectrometry.

In the last Chapter, 11 methods are detailed for determination of these solvents or their metabolites by gas chromatography.

ANNA TOMPA

IARC Scientific Publications No. 89, Lyon, 1988

Methods for detecting DNA damaging agents in humans:

Applications in cancer epidemiology and prevention

Eds by H. BARTSCH, K. HEMMINKI and I.K. O'NEILL

This volume comprises the proceedings of symposium on Detection of DNA-damaging Agents in Man, held in Espoo, Finland between 2-4 September 1987.

The participants of the symposium discussed the possibilities that now exist for measurements and the methods quantifying the interaction of carcinogens with the critical target organs or molecules. Most of the promising dosimetric methods were presented and their validity to improve the methods in cancer prevention were discussed. Each of the papers deals with the viewpoints of public health to protect people against harmful exposure, therefore, they reviewed the progress in the practical applications of DNA research. They emphasized the new trend in cancer research in which there the DNA interaction with chemicals predominated. DNA damage may activate oncogenes or the lesion in DNA may trigger an uncontrolled cellular growth. These changes can be detected by chromosomal analysis, by sister-chromatid exchanges, or with methods to detect point-mutations. These are the basic methodology in the human population, monitoring studies which provide better epidemiological data and more opportunity to make an inventory of the present status of our knowledge.

Several industrial countries have adopted premarketing requirements for new chemicals and include testing for genotoxic activity and carcinogenicity.

This book review the available methods in this field and give ideas for evaluation of chemicals and committing policies and notifications to avoid genotoxic exposure.

ANNA TOMPA

IARC Scientific Publications No. 92, Lyon, 1988

Cell differentiation, genes and cancer

Eds: T. KAKUNAGA, T. SUGIMURA, L. TOMATIS and H. YAMASAKI

The role of aberrant differentiation in cancer development has been reviewed in the literature several times and the topic became current again by the discovery of tumour markers related to the differentiation of cancer cells. There is a hypothesis suggesting that aberrant differentiation could be the molecular basis of early stage of neoplastic processes or cell transformation. Really, new developments, particularly new methods, in cell biology have promoted the progress in this area.

The aberrant differentiation in mouse-skin model is described by the famous expert of this field, S.H. Yuspa, and his collaborates. The gene regulation and tumorigenic expression in the somatic cell hybrids is discussed by R.J. Stanbridge, and the c-myc expression in mouse plasmocytoma line in summarized by T. Oikawa. The role of the intracellular factors in the erythroleukaemia cells is presented by T. Watanabe.

There is a very fascinating field of cell regulation controlled through cell-to-cell communication and through signal transduction. The problem is covered by Yamasaki et al. They conclude that the development of gap junction between transformed and non-transformed cells may serve as a route for intercellular exchange of normal signals of differentiation. Unfortunately, these junctions are not willing to develop spontaneously between growing tumours and host tissue. There is a definite change in genes during carcinogenesis and these transforming genes or oncogenes have an essential role in certain tumours' malignant expression. The oncogenic viruses and these changes are also related to the complex mechanism of development of Burkitt's lymphomas and in avian leukaemias as well as in human T-cell lymphomas. These problems are also described in the last chapter of this volume, written by L. Tomatis, A. Calendar, C.C. Harris and many other famous experts of this field.

The content of this book is an up-to-date summary of oncogenes and cellular gene-regulating differentiation related to carcinogenesis.

ANNA TOMPA

IARC Scientific Publications No. 81, Lyon, 1987

Methods of Analysis and Exposure Measurement

Passive Smoking

Eds: I.K. O'NEILL, K.D. BRUNNERMANN, B. DODET and D. HOFFMANN

Volume 9 in: Environmental Carcinogens. 372 pages

Passive smoking is the subject of many recently published studies. Many of the studies carried out to date are difficult to interpret and compare. The present volume of the excellent IARC Manual Series presents methods of sampling and analysis for a number of tobacco-derived substances.

The book consists of two parts. The first part is divided into 12 chapters. The book starts with an Overview (Chapter 1-6), which is followed by an other series of 6 chapters named General Analytical Considera-

tions. The second part contains the methods of sampling and analysis in smoke and air. Informations are available on the determination of carbon monoxide, oxides of nitrogen, volatile aldehydes, volatile N-nitrosamines, nicotine and minor tobacco alkaloids, polycyclic aromatic hydrocarbons, aromatic amines, phenols, cotinine, thiocyanate, hydroxyproline. There is a very interesting Appendix at the end of the monograph, that is a Questionnaire used in the International Study on Exposure to Other People's Smoke and Urinary Cotinine Levels in Non-Smokers.

The book is important for those, who are interested in studies of environmental damages and who would like to answer the question, to smoke or not to smoke. From the book nobody will be able to know the correct answer, the careful study of the methodology summarized in the publication will, however, help a lot to find a scientifically proved solution about this important problem of our age: are the passive smoking dangerous or not.

There is one thing which is lacking and which would be important to the users, that is a comprehensive subject index.

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Vancouver, British Columbia, Canada
July 15-18, 1990

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THIRTY-SECOND ANNUAL MEETING

**International College**
**of Angiology**

TORONTO PRINCE HOTEL
TORONTO, ONT., CANADA
JUNE 24 - 29, 1990

All communications relating to this Meeting should be addressed to:
Denise M. Rossignol, Executive Director
Continuing Medical Education Programs
INTERNATIONAL COLLEGE OF ANGIOLOGY
1044 Northern Boulevard, Ste. 103
Roslyn, NY 11576 U.S.A.

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**The 34th NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF RADIOLOGY AND NUCLEAR MEDICINES (SIRMN 1990)**
**will be held, together with the 3rd Edition of the Mediterranean Meeting of Radiology, in Turin from May 30 to June 3, 1990**

SEGRETERIA SCIENTIFICA
Istituto di Radiologia dell'Università
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# THE WORLD CONGRESSES OF GASTROENTEROLOGY

**9th Congress of Gastroenterology**
**7th Congress of Digestive Endoscopy**
**4th Congress of Colo-proctology**

**SYDNEY - AUSTRALIA**
**26 - 31 AUGUST, 1990**

**Official languages**

English is the official Congress language.

Some Congress halls will be provided with simultaneous translation into English, French, Spanish and Japanese.

**Congress secretariat**

The World Congresses of Gastroenterology
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## 22nd ANNUAL MEETING OF THE EUROPEAN PANCREATIC CLUB

**BASEL, SWITZERLAND**
**OCTOBER 15 - 17, 1990**

### Information on the Meeting

The 22nd Annual Meeting of the European Pancreatic Club (EPC) will be held in Basel, Switzerland from October 15 to October 17, 1990.

Traditionally this Symposium has focused on various aspects of pancreatic research and disease. For the Basel Meeting, we would also like to attract medical and research people working with the endocrine part of the pancreas. Especially we plan to have sessions on interactions between the islets and the exocrine pancreas, on nutritional diabetes and pancreatitis, and on other related topics in addition to traditional subjects. This Symposium should provide a review of latest research data of basic and clinical aspects of exocrine and/or endocrine pancreatic functions. It should also provide a forum for discussion together with an extension of friendship among physicians and scientists from various countries. We do hope to attract many young researchers to the Basel Meeting to stimulate future pancreatic research.

Basel, the City on the Rhine, is the second largest town in Switzerland with a population of 200 000. It offers excellent facilities for scientific meetings in combination with excellent possibilities for distraction and recreation.

The Meeting will take place at the "Zentrum für Lehre und Forschung" which belongs to the Medical Faculty of the University Hospital of Basel. Several hotels will host all the participants at reasonable prices.

Basel can easily be reached by car, train or airplane. It has three railway stations in the city: the Swiss, French, and German. It is also an international motorway junction. The international airport Basel-Mulhouse is actually situated on French soil, but is only 10 minutes from the city.

We are looking forward to welcoming you to the 22nd Annual Meeting of the EPC.

The Local Organizing Committee

Klaus Gyr
Christoph Beglinger
Carita Frei
Jacqueline Gass

If you are interested in further information
please contact:

Carita Frei, Secretary
Division of Gastroenterology
University Hospital
CH-4031 Basel, Switzerland
Phone +/41/(0)61/25 25 25,
extension 5174
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**RADIOLOGY POSTGRADUATE EDUCATION:**

OB/GYN AND ABDOMINAL SONOGRAPHY: UPDATE 1990
March 16-18, 1990 San Francisco, CA

33rd ANNUAL DIAGNOSTIC RADIOLOGY - Postgraduate Course -
March 19-23, 1990 San Francisco, CA

DIAGNOSTIC IMAGING: 1990
March 26-30, 1990 Kauai, HI

MR IMAGING UPDATE: CORRELATION WITH ULTRASOUND AND CT
COURSE AND SYMPOSIUM - 1990
April 2-6, 1990 Bora Bora, Tahiti

CT, MR, IN BODY IMAGING: A Comprehensive Course
May 22-25, 1990 Monterey, CA

**DIAGNOSTIC CYTOPATHOLOGY FOR PATHOLOGISTS**

**1990 POSTGRADUATE INSTITUTE**

The Johns Hopkins University School of Medicine offers the

**31ST ANNUAL POSTGRADUATE INSTITUTE FOR PATHOLOGISTS IN CLINICAL CYTOPATHOLOGY.**

This Institute, Course A and Course B, is an intensive program in all aspects of **Clinical Cytopathology**, with time devoted to newer developments and techniques, special problems, research, and recent applications including **immunodiagnosis and needle aspiration**. It is **solely for pathologists** who are Certified (or qualified for certification) by the American Board of Pathology (or its international equivalent). It is designed as a subspecialty residency in **Clinical Cytopathology** which is then highly compressed for the busy Pathologist into **152** AMA Category I credit hours in two courses, **both** of which **must** be taken:

- February through April 1990, Home Study Course A is provided each registrant for personal reading and microscopic study in their own laboratory in preparation for Course B; and
- April 23 to May 4, 1990, In-Residence Course B is an extremely concentrated lecture series with intensive laboratory studies and vital **clinical experience** at the Johns Hopkins Medical Institutions, Baltimore, MD, U.S.A.

Topics are covered in lectures, explored in small informal conferences, and discussed over the microscope with the Faculty. Abundant self-instructional material is available to maximize learning at each individual's pace. The Institute begins in **February 1990.** The Home Study Course A must be successfully completed before starting Course B on April 23rd in Baltimore. Upon completed preregistration the loan set of slides with texts (Course A) will be sent to each participant within the United States and Canada for home-study during February through April. Participants **outside** of the United States and Canada **must** make **prior** special arrangements to study Course A in adequate time before Course B.

Application and completed pre-registration is advised at the **earliest** date possible, (before the end of the year) to assure an opening. Completed pre-registration, however, **must** be accomplished **before March 23, 1990,** unless by **special arrangement.**

For details, contact: John K. Frost, M.D., or Ms. Betty Ann Remley, 111 Pathology Building, The Johns Hopkins Hospital, Baltimore, MD 21205, U.S.A.

**The entire Course is given in English.**

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
World Health Organization
Lyon - France

## FELLOWSHIPS FOR RESEARCH TRAINING IN CANCER 1990-1991

**Applications for training fellowships in 1990-1991 are invited from junior scientists wishing to be trained in those aspects of cancer research related to the Agency's own programme: epidemiology, biostatistics, environmental and viral carcinogenesis and mechanisms of carcinogenesis.**

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.

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## VISITING SCIENTIST AWARD
## 1990-1991

**This award is intended for established cancer research workers, with a minimum of five years postdoctoral experience, who wish to spend 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 remuneration and the cost of travel will be met.

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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

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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.

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

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