Folate Deficiency During Antituberculous (PAS-INH) Medication

The metabolism of folates is particularly disturbed by folic acid antagonists (aminopterin and methotrexate). A similar disturbance, though of lesser degree, is sometimes seen with the use of many anticonvulsant drugs[1–6] and also barbiturates[4,7–10], ethanol[11,12], pyrimethamine (Daraprim[13], phenylbutazone[14–16] and arsene[17]. In the present study an examination is made of the folate metabolism of patients undergoing antituberculous treatment (PAS-INH).

Material and methods. The control group (Figure 1) consisted of 13 subjects free of gastrointestinal disturbances (7 men, 6 women, mean age 52 years, mean weight 76 kg). The tuberculosis group consisted of 14 patients (8 men, 6 women, mean age 51 years, mean weight 70 kg). According to folate absorption the latter group may be subdivided into 2 parts: patients with a normal absorption curve (Figure 2, 9 patients, PAS 12–14 g pro die and INH 300–400 mg pro die over 4–9 months) and those with a low absorption curve (Figure 3, with individual characteristics in the Table and in bone marrow photograms 4–8, 5 patients, the medication as above).

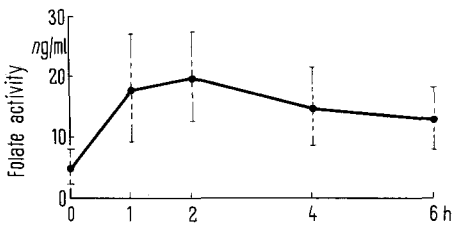

Fig. 1. Folate activity curve (*L. casei*) in serum of 13 control subjects after liver test meal. Mean and standard deviation.

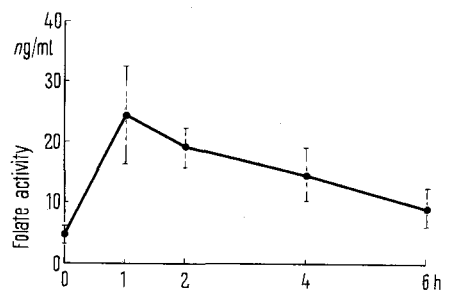

Fig. 2. Folate activity curve (*L. casei*) in serum of 9 patients treated with combined PAS-INH therapy over 4–9 months. Mean and standard deviation.

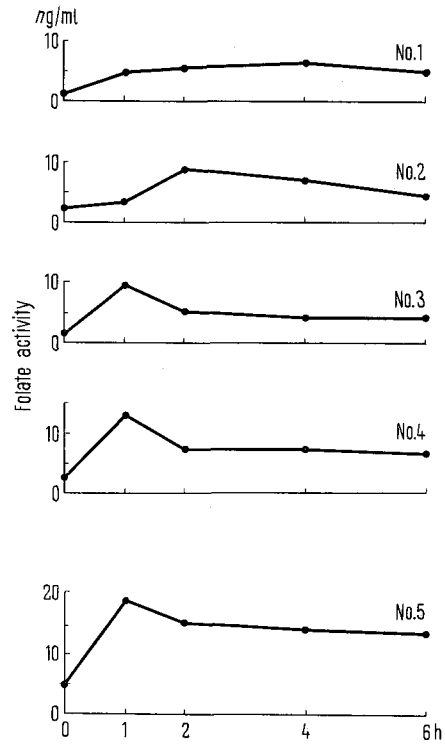

Fig. 3. Low folate activity curves of 5 patients treated with PAS-INH therapy over 5–84 months. The same patients had intermediate megaloblastic changes in the bone marrow as shown in Figures 4–8.

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Individual characteristics of the same patients as in Figure 3. i.m. = intermediate megaloblastic

Patient	Age, years	Weight, kg	PAS-INH, months	Gastric acid	Hb	WBC	Fe	TIBC	Bone marrow
1 ♂	59	57	84	—	12.7	8100	40	188	i.m.
2 ♂	62	57	5	—	12.6	8300	35	340	i.m.
3 ♀	38	51	18	+	13.0	5300	52	315	i.m.
4 ♀	47	87	7	+	13.3	4400	84	270	i.m.
5 ♂	45	76	7	+	14.4	4900	83	268	i.m.

The folate absorption test was performed as follows: After overnight fasting and the taking of fasting blood sample, the test subjects were given natural folates in the form of raw minced calves' liver (8500 *ng* folate activity USP/g wet weight) 1.0 g/kg body weight. Blood samples were taken thereafter according to the schedule seen in Figures 1–3. Before the test all patients had been without antituberculous treatment for 48 h. The serum folate activity was assayed microbiologically with the L. casei method, as described in earlier works[18,19].

The peripheral blood of all subjects was subjected to simple tests (hemoglobin, MCH, WBC). For patients with low folate absorption curves, certain special hematological methods seen in the Table were also used.

Results. The results of the tests are illustrated in Figures 1–8 and in the Table. There is evidence that in this unselected material 5 out of 14 tuberculous patients (36%) had folate absorption curves below the normal variation (Figure 3). In these patients intermediate megaloblasts were found in bone marrow samples, indeed more clearly than is usual in cases of folate deficiency (Figures 4–8), as well as low folate levels (1.2–4.5, mean 2.4 *ng*/ml), and in 3 out of the 5 low serum iron levels, too (Table). No definite anaemia could be verified in the peripheral blood. The PAS-INH treatment of these patients had lasted 5-84 months (Table). The other tuberculous patients showed absorption curves comparable with normal ones (Figure 2). Their PAS-INH treatment had lasted 4-9 months, mean 6 months.

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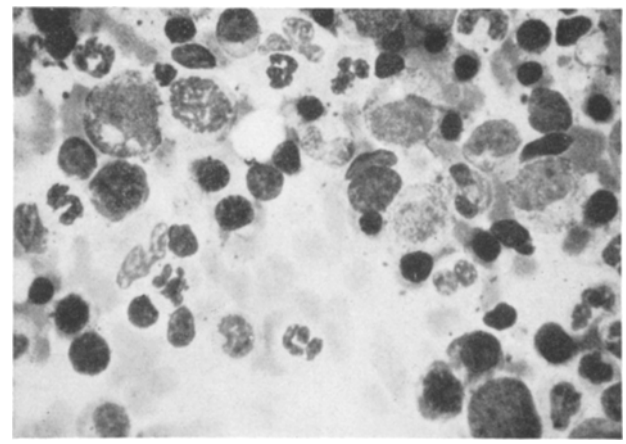

Fig. 6

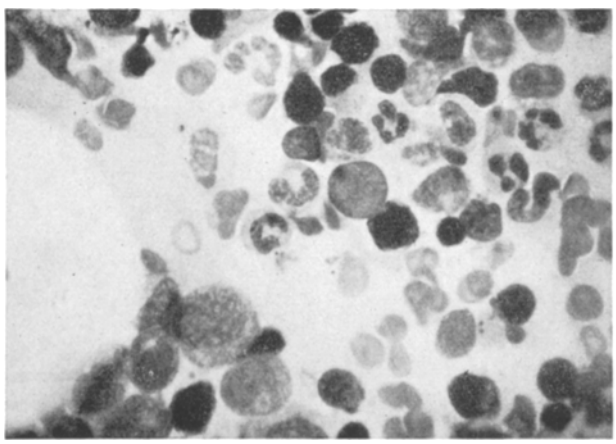

Fig. 4

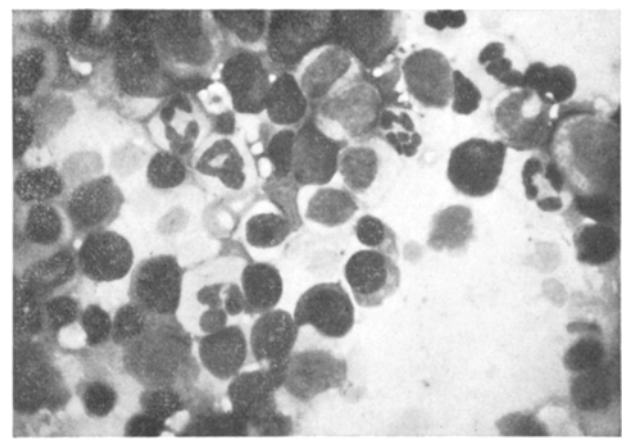

Fig. 7

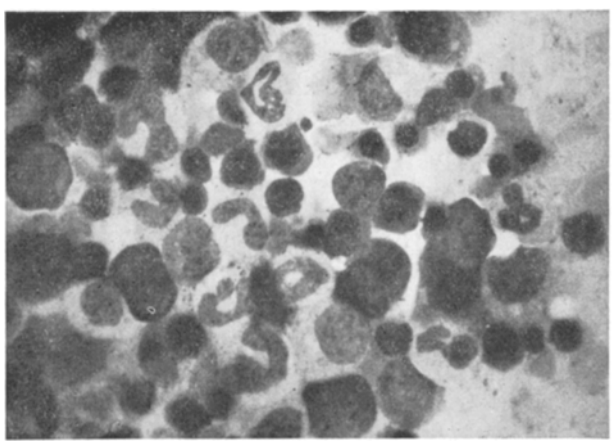

Fig. 5

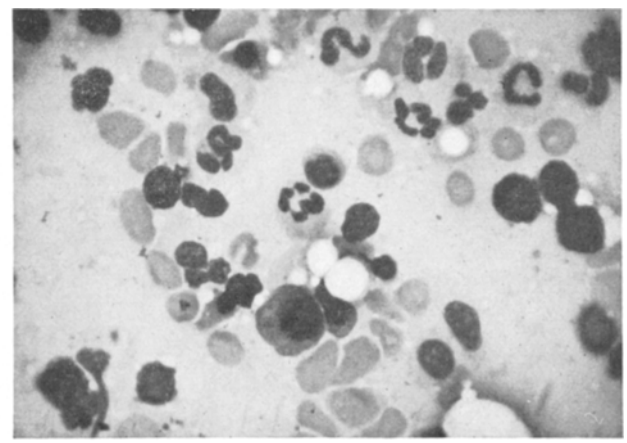

Fig. 8

Discussion. It is very evident, in our opinion, that the intermediate megaloblastic changes in the bone marrows of the patients in question are due to folate deficiency. Because the patients have received combined PAS-INH treatment it is naturally impossible to decide which of the drugs might be responsible, or if they might act in a sort of synergism. In a recent paper an undesirable effect of PAS-therapy has been described, in which it specifically counteracts the absorption of vitamin B_{12}[20]. If this is so, it might perhaps be advisable to investigate the untoward effects of PAS which possibly influence the fate of folates, too. According to these preliminary observations of ours there are at least 2 possibilities: antituberculous drugs may have adverse effects on the digestion of naturally occurring folate conjugates (conjugase defect or inhibition?) and/or on their absorption (biochemical defect(s) in the mucosal cells?). A wider question is also raised: how this antituberculous treatment interferes with the availability and metabolism of trace nutrients as investigated on a wider scale.

Zusammenfassung. Absorption natürlicher Folsäurekonjugate (in der Form vom Leberbrei) wurde bei 13 Kontrollen und 14 tuberkulösen Patienten nach monatelanger PAS-INH-Therapie untersucht. Die Folsäureaktivität im Serum wurde mit *L. casei* analysiert. Fünf von 14 Patienten hatten pathologisch niedrige Absorptionkurven und intermediär megaloblastische Veränderungen im Knochenmark, offenbar ein Zeichen für Folsäuremangel.

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Depletion and Resynthesis of Pituitary FSH: Time Course of Events Following Treatment with Hypothalamic FSH-Releasing Factor

Studies from a variety of laboratories seem to denote the presence, in the stalk-median eminence (SME) area of the hypothalamus, of a neurohormonal factor capable of stimulating the release of pituitary follicle stimulating hormone (FSH)[1-3].

Recently, DAVID et al.[4] have developed a procedure permitting the quantitative evaluation of the FSH-releasing factor (FSH-RF), utilizing the sexually mature male rat. This method, which is based on the ability of SME extracts to reduce (deplete) pituitary FSH stores in vivo, has been modified in this laboratory. Furthermore, in the course of determining the proper time to kill the animals (i.e. the period of maximum pituitary FSH depletion), following the SME injections, it was discovered that after such maximum depletion, the resynthesis of hypophysial FSH ensued. This report will therefore provide preliminary information on the sequential changes occurring in pituitary FSH subsequent to treatment with SME extracts.

Intact, mature male rats (Sprague-Dawley, 196 $\pm$ 5 g) were used as the recipients (5 rats/group) of the crude, acidic SME extracts which were derived from 60-day-old, intact, normally cycling Sprague-Dawley females (killed in diestrous/metestrous stage of cycle). The material was injected into the jugular vein at a dose of 2 SME/ml/recipient rat. The animals were decapitated at varying intervals following treatment; their pituitaries were then removed, weighed, appropriately pooled, homogenized and diluted with saline for employment in the FSH assay of STEELMAN and POHLEY[5]. A 2 + 1 design was utilized (5 animals/dose), the reference standard being NIH-FSH-S2. The results were converted for expression in terms of NIH-FSH-S1.

The results (see Table) demonstrate that within 15 min following the jugular vein injection of the SME extract, pituitary FSH depletion ensues. It is not until 45 min post-treatment that maximum depletion takes place. Resynthesis of FSH then occurs, with normal pituitary FSH values being restored approximately 4 h after treatment. The rate at which pituitary FSH repletion takes place appears rather rapid – requiring approximately 3 h from the point of maximum depletion to the re-establishment of near normal storage levels (rate of resynthesis during this latter time period = 15 μg FSH/h/mg pituitary).

It is important to direct attention to the recent study of MÜLLER and PECILE[6], who demonstrated, in a similar manner, the time course of events in pituitary growth hormone (GH) following an initial treatment with a rat SME extract containing GH-releasing factor.

Depletion and repletion of rat pituitary FSH following jugular vein injection of SME extract

Treatment	Time interval, treatment to sacrifice	μg FSH/mg pituitary	% FSH depletion	λ^b
Uninjected	–	98 (47.1–203.8)[a]	0	0.264
Saline	45 min	95 (54.0–167.2)	0 (initial control value)	0.204
2 SME/rat	15 min	65 (36.3–116.4)	32	0.211
2 SME/rat	30 min	53 (25.2–111.3)	44	0.270
2 SME/rat	45 min	41 (22.9– 73.0)	54	0.210
2 SME/rat	1 h	54 (25.7–113.4)	43	0.269
2 SME/rat	2 h	75 (41.7–135.0)	21	0.213
2 SME/rat	4 h	90 (76.7–156.6)	5	0.200

[a] Mean (95% confidence limits). [b] Index of precision.

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