provoking ACTH release[2]; the latter, lethal. Clearly, events within the hypophysis are more appropriately studied by such methods as local implantation of crystalline or highly concentrated solutions of vasopressin[14].

Zusammenfassung. Der antidiuretische Hormon-(ADH, Vasopressin)Gehalt des die Adenohypophyse durchströmenden Blutes wird für die Ruhephase mit 6 mU/ml und nach schwerem Blutverlust mit 2 U/ml berechnet. Die bisher allgemein vertretene Auffassung, nach welcher das ADH in einer für die normale Stimulation der adenohypophysären Sekretion des ACTH ungenügenden Konzentration vorhanden ist, kann nicht aufrechterhalten bleiben.

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Pyruvate Metabolism in Epileptic Tuberculous Patients

Introduction. In a recent paper[1] we have studied the prevention of the convulsant and lethal effects of isoniazid by pyruvic acid. We have shown that isoniazid reduces the levels of blood pyruvate, combining with it to form an hydrazone, and that this reduction was responsible for the acute toxicity of that drug.

The convulsant effect of isoniazid has been known since the first studies on its toxicity[2], as well as the risks involved in the treatment of tuberculous epileptic patients with that drug[3]. The fact that epileptic seizures are aggravated by isoniazid, together with our own results on its convulsant effect, led us to study the pyruvic acid content of the blood of epileptic tuberculous patients and its modification during isoniazid-therapy.

Material and Methods. The results we are about to describe were obtained from epileptic tuberculous patients from the San Sebastian Sanatorium of Rio de Janeiro (Brazil), a control group of non-epileptic tuberculous patients having been examined on the same occasion.

Pyruvic acid was assayed according to FRIEDEMAN and HAUGEN[4]. Blood samples were collected as described by these authors, from the fasting patients and, whenever possible, immediately after the crisis.

Clinical notes on the patients under examination: (a) *Epileptic patients.* GRS, female, 34 years; grand mal epileptic seizures since childhood, presently very frequent. Therapeutic scheme: *p*-aminosalicylic acid (15 g/day), isoniazid (300 mg/day) and streptomycin (1 g/day). – NAS, female, 27 years; frequent epileptic seizures since childhood. Therapeutic scheme: *p*-aminosalicylic acid (15 g/day), isoniazid (300 mg/day), and streptomycin (1 g/day).– DA, female, 22 years; occasional epileptic seizures since childhood. Therapeutic scheme: Isoxyl[5] (3 g/day). (b) *Non-epileptic patients.* DMM, female, 32 years. Therapeutic scheme: *p*-aminosalicylic acid (15 g/day), isoniazid (300 mg/day) and streptomycin (1 g/day).–AS, female, 38 years, and MD, female, 31 years. Therapeutic scheme: Isoxyl (3 g/day).–NC, female, 24 years, and EO, female, 16 years. Therapeutic scheme: thiosemicarbazone (100 mg/day) and *p*-aminosalicylic acid (10 g/day).

The epileptic status of patients GRS and NAS was confirmed by psychiatric examination and EEG tracings, which were nearly normal for patient DA. Treatment with Luminal was suspended during the experiment.

Results and Discussion. Pyruvic acid was assayed in blood samples collected from epileptic and non-epileptic patients during a period of 42 days (September–October 1961), at different intervals, according to the case. The non-epileptic patients of the control group were examined once a week, epileptic patients NAS and DA twice a week and patient GRS, due to the high frequency of seizures, had blood samples drawn every morning and after each crisis.

The results obtained from non-epileptic and from epileptic patients NAS and DA are shown in the Table. All the results quoted in the Table are from fasting subjects, and it can be seen that, for the non-epileptic patients, the concentration of blood pyruvate keeps well within the normal levels[6,7].

As for epileptic patients NAS and DA, there was a sharp increase in blood pyruvate coinciding with each crisis. In the case of patient NAS it can be seen that the concentration of blood pyruvate dropped steadily afterwards, till normal levels were attained. At this point a

[1] R. C. R. BARRETO and D. B. MANO, Biochem. Pharmacol. *8*, 409 (1961).
[2] S. Y. P'AN, L. MARKAROGLU, and J. REILLY, Amer. Rev. Tub. *66*, 100 (1952).
[3] K. I. FETTERHOFF, C. X. HOLMES, and G. E. MARTIN, Amer. Rev. Tub. *66*, 501 (1952).
[4] T. F. FRIEDEMAN and G. E. HAUGEN, J. biol. Chem. *147*, 415 (1943).
[5] 4-4'-diisoamyloxythiocarbanilide (Continental Pharma, Brussels).
[6] T. D. R. HOCKADAY, Biochem. J. *80*, 31 P (1961).
[7] H. KÄSER, Clin. chim. Acta *6*, 337 (1961).

Pyruvate levels in the blood of epileptic and non-epileptic tuberculous patients (C = crisis)

Name	Period of observation (days)																	Notes
	1	2	3	8	11	13	15	17	23	26	28	30	36	39	40	41	42	
NAS	0.31	C	0.89	0.62	0.45	C	0.71	0.51	0.48	C	—	0.75	0.39	C	1.55	—	—	Epileptic
DA	0.35	—	0.45	0.45	0.45	—	0.35	0.45	0.40	—	C	0.71	0.35	—	—	C	0.75	Epileptic
EO	—	—	—	0.53	—	—	—	—	0.37	—	—	—	0.42	—	—	—	—	Non-epileptic
DMM	0.45	—	—	—	—	—	0.45	—	0.51	—	—	0.37	0.35	—	—	—	—	Non-epileptic
AS	0.53	—	—	0.53	—	—	0.45	—	0.37	—	—	0.47	0.42	—	—	—	—	Non-epileptic
MD	0.53	—	—	0.53	—	—	0.45	—	0.45	—	—	0.45	0.35	—	—	—	—	Non-epileptic
NC	0.53	—	—	0.53	—	—	0.53	—	0.38	—	—	0.55	0.37	—	—	—	—	Non-epileptic

new crisis was commenced, completing a cycle of 13 days. Such cycle was not so well delineated for patient DA (which had also a doubtful diagnostic), but it was very clear for patient GRS, as shown in the Figure.

In the Figure we plotted the daily concentrations of pyruvate, assayed in blood samples collected from the fasting patient (empty marks), besides those drawn just after each crisis, regardless of fast (black marks). As it can be seen, during a first period of observation of 25 days the patient had six seizures (A, B, C, D, E, and F). The very high concentrations of blood pyruvate found in B and F were assumed to be due to the fact that the samples were drawn in the afternoon, after the heavy midday meal. During this period the patient was under INH-therapy.

The results shown in the Figure suggest the existence of a 'pyruvate cycle' in epileptic patient GRS, similar to that found for patient NAS (Table). Attention must be called to the fact that, according to the results obtained before crisis D, E, and F, the increase in blood pyruvate may procede the seizures. As before, convulsions seem to be started by the fall of blood pyruvate down to the levels found for normal patients (Table).

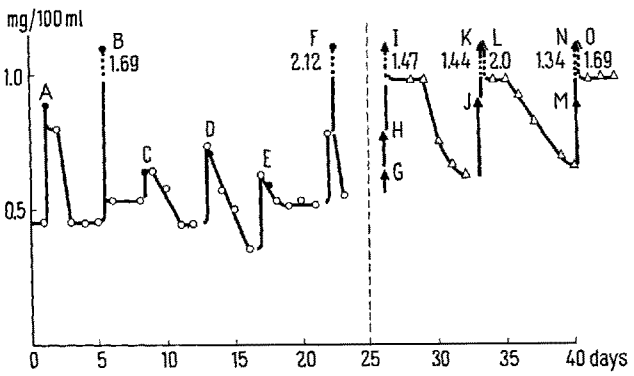

Fig. 1. Pyruvate cycle in the blood of an epileptic tuberculous patient (GRS) during INH-therapy (o–o) and after INH was suspended (Δ–Δ). The empty marks are for fasting values and the black ones for the values found after the crisis, regardless of fast.

The peculiar toxicity of isoniazid for epileptic tuberculous patients would be explained by the augmented consumption of blood pyruvate during the formation of the isonicotinyl-hydrazone of pyruvic acid[1] and consequent shortening of the 'pyruvate cycle'. This was confirmed by interrupting INH-therapy, as shown in the second part of the Figure. The 'pyruvate cycle', which was of 3 to 5 days during INH-therapy, was augmented to 7 days, with a correspondent spacing of the crisis.

Much higher levels were found for blood pyruvate after INH was suspended. Besides that, special attention must be paid to the fact that seizures, if more spaced, were now recurrent (GHI, JKL, and MNO), blood pyruvate increasing with each crisis. We tried to avoid that by keeping supposedly safe high levels of blood pyruvate. The number of crisis was reduced to a single one weekly when sodium pyruvate was orally administered in two daily 200 mg doses. However, this was the sole noticeable result, even when the daily dose was augmented to 600 mg[8].

Résumé. Les auteurs ont étudié la variation de la concentration de l'acide pyruvique dans le sang de 3 tuberculeux épileptiques. Ils ont vérifié l'existence d'un cycle dont le maximum coïncide avec la crise épileptique et dont le minimum la précède et semble la causer. L'effet nuisible de l'isoniazide pour les épileptiques serait dû à la consommation accélerée de l'acide pyruvique, utilisé pour la formation de l'hydrazone respective.

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On Driving and Synchronization of the Rate of Clonic Discharges of the Two Autonomous Hippocampal Epileptogenic Foci by Thalamic Stimulation

It has been demonstrated in a previous paper[1] that clonic discharges, evoked by a discrete, separate electrical stimulation of two bilateral, widely separated and mutually unrelated areas of the cerebral isocortex, could be controlled, synchronized and prolonged beyond their intrinsic durations by single shock stimuli applied to the intralaminar thalamic nuclei.

The present experiments have been designed to see if the activity of the two bilateral, autonomous, hippocampal epileptogenic foci, having various rates of discharge, could equally be controlled and synchronized by appropriate electrical stimulation of midline thalamic nuclei.

Cats were used for this investigation. Under Nembutal anaesthesia, having taken all the necessary aseptic precautions and by means of the standard stereotaxic technique, a minute amount of alumina cream was introduced, first into the hippocampus proper of one side and, several days later, into the homologous structure of the other side of the brain. A typical 'injury discharge' indicated the penetration of the combined needle-electrode shift into the desired depth of the brain. After the substance has been instilled, the needle was removed and the operative wound closed properly. Twenty days following the application of alumina cream, the animals were reoperated. Needle electrodes were introduced into the hippocampus of both sides and into the intralaminar thalamic nuclei. After complete recovery from anaesthesia, the electrical activity was recorded using an Alvar-Reega EEG apparatus. Grass stimulator, Model 4 S, with stimulus isolation unit, was employed for stimulating the thalamus. The single unidirectional, 0.2 msec pulses were delivered to the thalamus at the rate of 0.5–3/sec. After the experiments have been completed, the position of the electrodes was

[1] Lj. Mihailović, Exper. *15*, 119 (1959).