lated both chemically and functionally to produce similar effects. He could not, however, demonstrate any effect with doses similar to ours (10^{-5} or 10^{-6} g/ml). The major difference between these experiments and ours is our use of plasma as suspending medium. The longer duration of our experiments seems a less likely explanation for the divergence.

The gross measurement of sodium concentration in the medium does not permit definitive conclusions regarding the site of action of these adrenal cortical steroids. The results are, however, quite consistent with the view which we have expressed elsewhere that aldosterone hinders sodium efflux[1]. Judging by GLYNN's analysis, this may well represent an action on the transport mechanism itself.

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S. M. FRIEDMAN and
CONSTANCE L. FRIEDMAN

Department of Anatomy, The University of British Columbia, Vancouver (Canada), July 11, 1958.

Zusammenfassung

Natrium- und Kalium-Plasmakonzentrationen und Hamatokritwerte wurden in menschlichem Blut nach Beifügung von kleinen Mengen Aldosteron nach 2-, 4-, 6- oder 24stündiger Kühlung oder nach $^1/_2$-, 1-, 2- oder 3stündiger Erwärmung gemessen. Aldosteron vermindert die Plasma-Natriumzuwachsrate bei Erwärmung und anscheinend auch die Plasma-Natriumverminderungsrate bei Kühlung. Hydrokortison ergab das gleiche Resultat bei Erwärmung, während seine Wirkung bei Kühlung noch nicht abschliessend untersucht wurde.

Some Factors Modifying the Action of Chlorpromazine upon the O_2 Uptake of Brain Homogenates *in vitro*

Studying the direct action of chlorpromazine on isolated tissues[1], we have investigated the *in vitro* effect of Largactil upon the respiration of brain homogenates (albino rats) under various experimental conditions, i.e. in the medium without glucose, in the calcium-free suspension fluid, and on homogenates stimulated with 2,4-dinitrophenol.

The O_2 uptake was estimated by direct Warburg technique, at the temperature of 37°C and in pure oxygen. Medium: phosphate buffered Krebs Ringer solution with or without 0·2% glucose and calcium-free solution (100 ml 0·154 *M* NaCl, 4 ml 0·154 *M* KCl, 1 ml 0·154 *M* $MgSO_4 \cdot 7\,H_2O$, and 30 ml of phosphate buffer) with 0·2% glucose.

Changes in metabolic rate after addition of various concentrations of Largactil are given in Table I.

When Largactil was added to the Krebs Ringer solution without glucose, no significant changes in the O_2 uptake were observed (Table II).

The depressive action of Largactil was diminished in the same way in the calcium-free solution (Table II).

Table I

Oxygen uptake at various concentrations of Largactil in brain homogenates of albino rats. Medium: Buffered Krebs Ringer solution with 0·2% glucose. The inhibition at all concentrations is significant

No. of experiment	Treatment	µl O_2/100 mg wet w./h		Inhibition in %
		M	S.D.	
15	Control	147·04	± 15·15	
	Largactil/ml:			
8	1 µg	128·66	± 8·6	− 12·5
6	5 µg	118·10	± 4·3	− 19·7
13	10 µg	108·29	± 10·1	− 26·4
6	50 µg	108·29	± 16·5	− 26·3
7	100 µg	106·65	± 8·65	− 27·5
5	200 µg	76·34	± 14·5	− 48·2

Table II

Oxygen uptake ot brain homogenates after Largactil in Krebs Ringer solution without glucose$^+$ and in calcium-free solution^{++} with 0·2% glucose. The inhibition is not significant

No. of experiment	Treatment	µl O_2/100 mg wet w./h		Inhibition in %
		M	S.D.	
6	Control	108·28	± 4·64	
5	Largactil (10 µg/ml)	103·50	± 9·34	− 4·42$^+$
5	Control	180·65	± 19·1	
5	Largactil (10 µg/ml)	172·20	± 17·2	− 4·68^{++}

Table III

The inhibition of the O_2 uptake after Largactil in brain homogenates treated with 2,4-dinitrophenol (DNP). Medium: Buffered Krebs Ringer solution with 0·2% glucose. The inhibition is significant

No. of experiment	Treatment	µl O_2/100 mg wet w./h		Inhibition in %
		M	S.D.	
9	DNP (20 µg/ml)	221·94	± 36·64	
9	DNP (20 µg/ml) + Largactil (10 µg/ml)	185·88	± 28·70	− 16·2

In further experiments, Largactil exhibited an antagonizing action upon the increased metabolic activity of brain homogenates stimulated with 2,4-dinitrophenol (Table III).

The direct influence of relatively low doses of Largactil upon the respiration of brain homogenates was confirmed. This agrees with the work of GÄNSHIRT and BRILMAYER[2] who have found a significant decrease of respiration activity in brain homogenates of white mice after Megaphen at concentration of 10^{-6} g/ml. On the other hand, DECSI[3], considering the direct action of chlorpromazine upon the cerebral tissue emphasizes in his recent studies that the inhibition of phosphorylations seems to be prevailing and can be demonstrated even at the dose which does not affect the oxygen uptake. The decrease of the oxygen consumption, according to this further findings, does not

[1] A. ZELENÝ and J. KOZÁK, Pharmazie *13*, 200 (1958). – J. VLK and V. LUKÁČ, J. Physiol. USSR. *44*, 365 (1958).

[2] H. GÄNSHIRT and H. BRILMAYER, Arch. int. Pharmacodyn. *98*, 467 (1954).

[3] L. DECSI, Acta physiol. Hung. *10*, 387 (1956). – L. DECSI and J. HEIDT, Acta physiol. Hung. *13*, 183 (1958).

occur until at high toxic concentrations of Largactil (177 µg/ml).

The next stage in the investigation must be to study these relationships.

J. Kozák, N. Lang, and A. Zelený

Institute of Physiology, Medical Faculty Pilsen (Czechoslovakia), July 27, 1958.

Zusammenfassung

Largactil vermindert auch in niedrigen Konzentrationen (10^{-6} g/ml der Krebs-Ringer-Lösung mit 0,2% Glukose) den Sauerstoffverbrauch von Gehirnhomogenaten weisser Ratten signifikant. Diese depressive Wirkung von Largactil tritt nicht ein, wenn die Krebs-Ringer-Lösung ohne Glukose oder ein kalziumfreies Medium zu den manometrischen Messungen gebraucht werden. Weiterhin hat Largactil eine antagonistische Wirkung gegen 2,4-Dinitrophenol in bezug auf die O_2-Aufnahme von Gehirngewebe.

Tryptophane as a Tuberculostatic Agent

It has recently been shown that certain strains of *Mycobacterium tuberculosis* and *Mycobacterium phlei* are capable of synthesizing nicotinic acid derivative in their body when grown on synthetic liquid media[1]. This confirmed previous findings of Bird[2]. The production of nicotinic acid derivative by the mycobacteria is probably responsible for the relatively high nicotinic acid metabolites found in the blood and urine of tuberculous guinea pigs and humans[3].

The nicotinic acid-synthesizing action of the mycobacteria simulates that of *Escherichia coli*. Ellinger and Abdel Kader[4] proved that *Escherichia coli* are capable of synthesizing nicotinic acid in their bodies when grown on very simple media, and that tryptophane, ornithine, glutamine, and arginine are possible precursors for the nicotinic acid produced in these organisms. These findings prompted the desire to explore for a similar precursor in the mycobacterium organisms.

For this purpose the metabolism of the different amino acids in relation to the nicotinic acid production and growth of the *Mycobacterium tuberculosis* organism were investigated. The liquid medium used for the growth of the organism was the Proskauer and Beck synthetic medium (Vorwald's modification)[5]. This medium consists of magnesium citrate, monopotassium phosphate, magnesium sulphate, asparagine as the only source of nitrogen, and glycerol. The following amino acids were used in equimolecular concentrations in the medium in the place of asparagine (0·038 *M*) using *Mycobacterium tuberculosis hominis* as test organism: L(−) aspartic acid, L(−) ornithine hydrobromide, L(−) arginine hydrochloride, L(−) glutamic acid, L(−) histidine, and L(−) tryptophane. Growth was recorded at weekly intervals and the nicotinic acid content of the whole medium and the cell-free medium was estimated microbiologically (Barton-Wright[6]) using *Lactobacillus arabinosus* 17/5 as test organism. The results are recorded in the Table.

Of the amino acids examined, ornithine and arginine supported growth and produced nicotinic acid, both in the cell-free medium and whole medium, just as asparagine. The growth and nicotinic acid-production was partially inhibited by the heterocyclic amino acid histidine. It was also very interesting to find that tryptophane, a heterocyclic amino acid, completely inhibited the growth and nicotinic acid-production of *Mycobacterium tuberculosis hominis*. In higher concentrations (0·038 *M*), the action of tryptophane was bactericidal, while at lower concentration (0·019 *M*) it was bacteriostatic. The bacteriostatic effect was also demonstrated on *Mycobacterium tuberculosis bovis, avis*, and *Mycobacterium phlei*.

The most interesting finding is that the tuberculostatic effect of tryptophane is rather marked and that the tryptophane-nicotinic acid relationship of *Escherichia coli* does not hold true for the mycobacterium organisms.

The *tuberculostatic action of tryptophane* remained to be studied *in vivo*. For this purpose 9 guinea pigs (weighing between 250–300 g and of male sex) were chosen from the laboratory stock. The technique of Feldman and Hinshaw[5] in assessing the tuberculostatic effect of tryptophane was strictly followed. The guinea pigs selected for the experiment were subdivided into three groups of three animals each. All animals were infected with a virulent strain of *Mycobacterium tuberculosis hominis* (10 mg wet organism) by subcutaneous injection in the inside of the right thigh. The first group of animals were used as controls. The second group was infected and immediately given tryptophane (125 mg/day) for 10 consecutive days. In the third group, the tryptophane was administered in the third week after infection in a manner similar to that in the second group. Sterile tryptophane solutions (12·5 mg/ml in physiological saline) were administered subcutaneously; the experimental period was 9 weeks, after which the animals were sacrificed.

On post-mortem examination, the control animals showed evidence of infection manifested by enlargement and caseation of the regional lymph glands at the site of inoculation, with generalisation especially in the spleen and kidneys. Films made and stained with Ziehl-Neelsen and examined microscopically revealed the presence of the T. B. organisms.

On the other hand, when the animals of the other two treated groups were examined, it was found that there was no evidence of generalisation and, although the regional lymph glands were enlarged, they showed no caseation and were markedly fibrosed. Microscopic examination of the films was negative for T. B. organisms.

This animal experiment proved that tryptophane, in the concentrations used, was tuberculostatic.

It seems probable that amino acids with a heterocyclic ring possess an inhibitory action on the growth of the T. B. organism. This is shown by the effect of tryptophane and histidine *in vitro* and the effect of tryptophane *in vivo*. This supposition requires further support by examining the effect of other amino acids with heterocyclic rings.

The bacteriostatic effect of tryptophane might be explained in many ways. In the guinea pig, it is either tryp-

[1] M. S. El-Ridi, M. M. Abdel Kader, Habib Angel, and O. Zaki, Proc. pharm. Soc. Egypt *34*, 49 (1957).

[2] O. D. Bird, Nature *159*, 33 (1947).

[3] M. M. Abdel Kader, Habib Angel, and M. Saadany, J. R. egypt. med. Assoc. *34*, 108 (1951). – M. S. El-Ridi, M. M. Abdel Kader, Habib Angel, and A. Abdel Aziz, J. R. egypt. med. Assoc. *36*, 435 (1953). – M. S. El-Ridi, M. M. Abdel Kader, Habib Angel, and M. Saadany, Hoppe-Seyler's Z. *310*, 275 (1958).

[4] P. Ellinger, M. M. Abdel Kader, and A. Emmanuelowa, Brit. J. exp. Path. *28*, 261 (1947). – P. Ellinger and M. M. Abdel Kader, Proc. biochem. Soc. *41*, IX (1947); Nature *160*, 675 (1947); Proc. biochem. Soc. *43*, IX (1948); Biochem. J. *44*, 285 (1948); Nature *163*, 799 (1949); Biochem. J. *44*, 627 (1949).

[5] W. H. Feldman and H. C. Hinshaw, Amer. Rev. Tuberc. *51*, 582 (1945).

[6] E. C. Barton-Wright, Biochem. J. *38*, 314 (1944).