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[6] 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).

Effect of Different Amino Acids (0·038 *M*) on Growth and Nicotinic Acid Production of *Mycobacterium tuberculosis hominis* in Proskauer Medium

Compound added	Growth during 6 Weeks						Nicotinic acid µg/ml	
	1	2	3	4	5	6	Whole medium	Cell-free medium
No nitrogen	—	1+	1+	1+	1+	1+	0·4	0·25
L(−) Asparagine	—	2+	3+	4+	6+	6+	4·4	4·30
L(−) Aspartic acid	—	2+	2+	3+	4+	4+	1·5	1·30
L(−) Glutamic acid	—	2+	2+	3+	4+	4+	2·5	2·20
L(−) Ornithine hydrobromide	—	2+	3+	4+	5+	5+	4·6	4·20
L(−) Arginine hydrochloride	—	2+	2+	3+	5+	5+	4·3	4·00
L(−) Histidine	—	±	±	1+	1+	1+	0·10	0·10
L(−) Tryptophane	—	±	±	±	1+	1+	0·4	0·4

Degree of growth as judged by the richness of the surface pellicle: 1+ = +; 2+ = ++; 3+ = +++; 4+ = ++++; 5+ = +++++; 6+ = ++++++.

tophane or one of its metabolic products which is responsible for the tuberculostatic effect. The presence of tryptophane in excessive amounts will lead to the accumulation of the metabolic products kynurenine, kynurenic acid, quinolinic acid, and nicotinic acid. One of the last mentioned metabolites, besides tryptophane might possess this T. B. growth-inhibiting action. The presence of tryptophane (an essential amino acid) in excessive amounts might raise the resistance of the animal against the invading organism. However it must be noted that tryptophane itself proved to be tuberculostatic *in vitro*. *In vitro* experiments showed that neither nicotinic acid nor its amide (in comparatively big concentrations up to 10 mg %) had any inhibiting effect on the growth of the T. B. organism[7].

Naturally, these preliminary findings should be repeated on a larger number of animals for further confirmation. The results are rather encouraging for the exploration of other tuberculostatic drugs containing these basic heterocyclic rings. This will be the theme of our future work in this field.

M. M. ABDEL KADER and O. ZAKI

Department of Biochemistry, Faculty of Medicine, Cairo University and Department of Bacteriology, Faculty of Veterinary Medicine, Cairo University (Egypt), July 12, 1958.

Résumé

L'étude d'un précurseur probable dans la production de l'acide nicotinique par le *Mycobacterium tuberculosis hominis*, révéla que les acides aminiques hétérocycliques, qui sont indispensables à plusieurs organismes, comme le tryptophan et l'histidine, sont bactériostatiques à l'organisme. Cet organisme se développait dans une solution synthétique de Proskauer & Beck (VORWALD's modification). Les acides aminiques tels que l'ornithine et l'arginine aidèrent à sa croissance et favorisèrent la production d'acide nicotinique, ressemblant sur ce point à l'asparagine.

Des études préliminaires faites sur des cobayes infectés par l'organisme tuberculeux confirmèrent l'effet bactériostatique du tryptophane *in vitro*. Du tryptophane fut administré, de façon sous-cutanée, à raison de 125 mg par jour pour 10 jours consécutifs, immédiatement après l'infection, à un groupe d'animaux. Un second groupe subit le même traitement, mais à deux semaines du jour de l'infection. Un troisième groupe fut infecté, mais laissé sans traitement. Tous ces animaux furent observés pendant 9 semaines. Après examen bactériologique, microscopipue et macroscopique, l'effet bactériostatique du tryptophane *in vivo* put être démontré.

[7] M. M. ABDEL KADER and O. ZAKI (1958), Unpublished data.

Zur Frage der Verteilung von Radiolutetium (^{177}Lu) im Organismus der Maus bei unterschiedlicher Applikationsart

Über das Verhalten von Radiolutetium im Körper ist bisher in der einschlägigen Literatur wenig berichtet worden, wenn man von einigen nur begrenzt zugänglichen Mitteilungen absieht. KYKER *et al.*[1] teilten Versuche über eine mögliche Selektivbestrahlung von Lymphknoten nach interstitieller Applikation von ^{177}Lu mit. Auch DURBIN *et al.*[2] erwähnen einige Ergebnisse mit Lutetium im Rahmen ihrer vergleichenden Untersuchungen über das Stoffwechselverhalten Seltener Erden.

Im folgenden sollen daher unsere bisherigen Befunde über Verteilung und Ausscheidung von ^{177}Lu bei Mäusen mitgeteilt werden.

Methodik. Die Versuche werden mit 64 weissen Mäusen beiderlei Geschlechts eines institutseigenen Inzuchtstammes mit einem Gewicht von 13–18 g durchgeführt. Die Tiere erhielten 4 µC ^{177}Lutetium als Chlorid in 0,2 ml physiologischer NaCl-Lösung, pH 3,0, entweder intraperitoneal oder subkutan injiziert. Dies entspricht bei der spezifischen Aktivität des verwendeten Lutetiums einer Gewichtsdosis von $\approx 10^{-6}$ g.

Zu verschiedenen Zeiten nach der Injektion (zwischen 1 h und 30 Tagen) wurden die Versuchstiere getötet und folgende Organe bzw. Körperflüssigkeiten auf ihren Gehalt an Radioaktivität untersucht: Lungen, Leber, Milz, Pankreas, Nieren, Femur, Blut und – soweit gewinnbar – Blasenharn. Die Organe wurden hierzu zerkleinert und gleichmässig auf Meßschälchen verteilt. Nach erfolgter Trocknung wurde die Aktivität mit Hilfe eines Szintillationszählers gemessen und nach einer Korrektur entsprechend der Zerfallrate auf die injizierte Dosis bezogen. Die Angaben erfolgen in % der injizierten Dosis pro 100 mg Gewebe (Frischgewicht). Ausserdem wurde die Ausscheidung in Harn und Kot bestimmt.

Ergebnisse:

1. Subkutane Applikation. Die Resorption erfolgte nur sehr geringgradig und langsam, nach 30 Tagen fanden sich noch etwa 77% an der Injektionsstelle wieder. Die Werte für die verschiedenen Organe sind in der Tabelle I wiedergegeben. Auffallend ist vor allem, dass im Gegensatz zu anderen Seltenen Erden (zum Beispiel Cer, Lanthan)[3] die

[1] G. C. KYKER, W. M. CHRISTOPHERSON, H. F. BERG und M. BRUCER, Cancer *9*, 489 (1956).

[2] P. W. DURBIN, M. H. WILLIAMS, M. GEE, R. H. NEWMAN und J. G. HAMILTON, Proc. Soc. exp. Biol. Med. *91*, 78 (1956).

[3] E. SPODE und F. GENSICKE, Naturwiss. *45*, 117, 135 (1958).