The Effect of Indole Derivatives on *Mycobacterium tuberculosis*

It was recently shown by ABDEL KADER and ZAKI[1] that tryptophane has a bacteriostatic effect on *Mycobacterium (M.) tuberculosis hominis* both *in vitro* and *in vivo* in guinea pigs. The tuberculostatic effect was attributed as probably due to tryptophane *per se* or to one of its metabolic products. Tryptophane is an indole derivative (α-amino-β-indolylpropionic acid). The presence of the indole ring in the tryptophane molecule might possibly be responsible for its tuberculostatic effect. 5-hydroxyindoleacetic acid has been identified as one of the urinary metabolic products of tryptophane[2]. The sputum of tuberculous patients was shown also to contain indole[3]. This prompted the desire to study the effect of indole and other indole derivatives, which are metabolic products of tryptophane, on the growth of *M. tuberculosis hominis* both *in vitro* and *in vivo*.

For this purpose, indole, 3-indoleacetic acid, and β-[indolyl-(3)]-propionic acid have been used in this investigation. The Proskauer-Beck synthetic liquid medium (Vorwald's modification)[4] has been used to test the effect of these compounds on the growth of *M. tuberculosis hominis*. The synthetic medium consists of magnesium citrate, monopotassium phosphate, magnesium sulphate, glycerol, and asparagine as the only source of nitrogen. The indole compounds were used in different concentrations in the medium either containing asparagine (0·038 *M.*) or without it. Growth was recorded at weekly intervals for a period of 6 weeks. The results are recorded in the Table. After the 6 weeks period of observation, the tightly screwed bottles containing the cultures with indoleacetic acid and indolepropionic acid were autoclaved and extracted with ether. The ether extract was tested for indole by the method of HAPPOLD and HOYLE[5]. Blank experiments were made on similarly autoclaved indoleacetic and indolepropionic acids for comparison. This experiment showed positive results for indole in the cultures, whereas the blanks gave negative results.

It is clear from the Table that indoleacetic acid and indolepropionic acid, in concentrations varying from 0·038 to 0·005 *M*, had an inhibitory effect on the growth of the *M. tuberculosis hominis*. At high concentrations of the two compounds, complete inhibition of growth occurred. Indole in concentrations of 0·038 and 0·020 *M* also inhibited the growth of the mycobacteria. When coupled with asparagine (0·038 *M*), indole affected the growth only to a much less extent.

These findings prompted the desire to study the effect of these indole derivatives on the development of tuberculosis in guinea pigs. For this purpose 21 guinea pigs (weighing between 250 and 300 g, of both sexes) were chosen from the laboratory stock. The technique of FELDMAN and HINSHAW[4] in assessing the effect of indole derivatives on mycobacteria *in vivo* was followed exactly. The guinea pigs selected for investigation were grouped into 7 batches of 3 animals each. All animals were infected with a virulent strain of *M. tuberculosis hominis* (0·001 mg of organism) by intramuscular injection in the inside of the right thigh. The first group of animals (group A) were used as controls. Group B were simultaneously treated with intramuscular injection of 6 mg streptomycin 3 times a week and 5 mg INH. (isonicotinylhydrazide, Rimifon) orally daily. Treatment started immediately after infection and continued for 15 days[6]. Group C and D were given simultaneously indoleacetic acid and indolepropionic acid respectively for 15 days. Doses of 25 mg of these acids were adminisreted subcutaneously. In group E and F, these same compounds in the same doses were administered after 15 days of infection in a manner similar to that in groups C and D.

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The effect of different indole derivatives on the growth of *Mycobacterium tuberculosis hominis* in Proskauer medium for a period of 6 weeks

Compound Added	Concentration (Molar)	Growth during 6 Weeks					
		1	2	3	4	5	6
No Nitrogen		—	—	1+	1+	1+	1+
L(−)Asparagine	0·038	3+	4+	6+	6+	6+	6+
β-[Indolyl-(3)]-propionic acid	0·038	—	—	—	—	—	—
	0·020	—	—	—	—	—	—
	0·010	—	—	1+	1+	1+	1+
	0·005	—	—	1+	1+	1+	1+
3-Indoleacetic acid	0·038	—	—	—	—	—	—
	0·020	—	—	—	—	—	—
	0·010	—	—	1+	1+	1+	1+
	0·005	—	—	1+	1+	1+	1+
Indole	0·038	—	—	1+	1+	1+	1+
	0·020	—	—	1+	1+	1+	1+
L(−)Asparagine	0·038						
+	+						
Indole	0·020	—	—	1+	2+	2+	2+
	0·010	—	—	1+	2+	2+	2+

Degree of growth as judged by the richness of the surface pellicle:
1+ = +, 2+ = ++, 3+ = +++, 4+ = ++++, 5+ = +++++, 6+ = ++++++
Note: The surface pellicle of the tubercle organism in cases of indole derivatives acquires a brownish red colour

The last group G was given, immediately after infection, 5 mg indole subcutaneously every other day for 30 days. The experimental period was two months, after which the animals were sacrificed. During indole administration it was found that a daily 25 mg dose was fatal to the guinea pigs. For this reason the 5 mg dose was chosen to be given every other day to secure no harmful effect. All animals were examined at autopsy and, in all, touch smears from sections of lymph nodes, spleen, liver, and lungs were made, stained, and examined.

The *postmortem* examination of the control animals showed the spread of infection, manifested by enlargement and caseation of the regional lymph glands at the site of inoculation with generalization, especially in the liver and spleen which were markedly enlarged and full of macroscopic miliary nodules all over. Films made and stained with Ziehl-Neelsen and examined microscopically revealed the presence of the tubercle organism. On the other hand, the animals treated with INH and streptomycin showed no macroscopic lesions; the spleens, livers, and lymph nodes were normal. Microscopic examination proved negative for T. B. The animals treated with indole were similar to those treated with INH and streptomycin showing no lesions. The regional lymph nodes were markedly fibrosed, and microscopic examination of the films showed negative results for T. B.

Indoleacetic acid and indolepropionic acid showed also a certain activity in limitting the spread of tuberculous infection. The lesions in the spleen and liver were much less than in the control animals. The regional lymph nodes, on the other hand, were similar to those of the control animals. Indoleacetic acid was slightly more effective than the indolepropionic acid.

The most interesting finding here is the tuberculostatic effect of indole in therapeutic dose (5 mg per animal) comparable with that of the INH. Indole itself shows this tuberculostatic effect *in vitro* and interferes also with the utilisation of asparagine for the growth of the tubercle organism. The tuberculostatic effect of indoleacetic acid and indolepropionic acid *in vitro* may be due to the oxidation of the side chain by the microorganism which leads to indole production, and this in turn causes the apparent inhibition. The same mechanism of producing indole might take place in the tissue cells of the guinea pig. This might explain the slight tuberculostatic effect of these compounds. It seems possible also that the lengthening of the side chain in the 3-position of the indole ring lessens the tuberculostatic effect of the indole derivative. This is shown from the higher activity of indoleacetic acid than the indolepropionic acid. Of course this suggestion requires confirmation by examining different indole derivatives with longer side chain in the 3-position of the indole ring. The main drawback of indole is its toxicity. ETS and FEINBERG[7] obtained paralysis of the dog jejunoileum by injecting intravenously 25 or 60 mg indole per kg body weight. It is considered that indole has a depressant effect due to a direct action on the smooth muscle fibres[8]. In fact, indole in 100 mg/kg body weight was fatal to the guinea pig.

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Résumé

L'étude des effets de l'indole sur l'organisme *M. tuberculosis hominis* révéla qu'il est bactériostatique *in vitro* et *in vivo* chez les cobayes infectés par lui (5 mg par jour pendant 15 jours). Les acides indol-acétiques et indol-propioniques sont aussi légèrement tuberculostatiques.

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Cholesterin und Fettsäuren als Ersatz von Serum in der Kultur von *Trichomonas foetus*

Für Trichomonaden wurden halbsynthetische Medien entwickelt, die Serum, Trypticase und Agar als nicht definierte Bestandteile benötigen[1,2]. Neben diesen enthalten sie eine Vielzahl von organischen und anorganischen Stoffen, deren Bedeutung im einzelnen nicht völlig abgeklärt ist. Unter diesen Bedingungen scheint dem Serum der Charakter eines essentiellen Wuchsfaktors zuzukommen.

Nach Untersuchungen von SPRINCE[3] sind zwei Fraktionen von Rinderserum wichtig, eine wasserlösliche und eine ätherlösliche. Von den in der ätherlöslichen Fraktion vorhandenen Stoffen gilt Cholesterin als wesentlich[2,4,5]. Die quantitativen Verhältnisse in Beziehung zur wasserlöslichen Fraktion und die Bedeutung anderer lipoidlöslicher Stoffe sind vorläufig nicht genügend abgeklärt[6]. Aus diesem Grunde wurde versucht, zunächst die ätherlösliche Fraktion aus Serum weiter zu charakterisieren. Es wurden vor allem Cholesterin und Cholesterin-Fettsäure-Mischungen geprüft.

Das Ergebnis der Versuche mit *T. foetus* ist folgendes: Auf einem der genannten leicht modifizierten halbsynthetischen Nährböden vermögen Trichomonaden nur in Gegenwart von mehr als 0,3% der wasserlöslichen Fraktion des Serums allein zu wachsen. Bei niedrigeren Konzentrationen ist jedoch mit Zusatz von Cholesterin ($10^{-5,5}$) ein gleich starkes Trichomonadenwachstum wie mit etwa 1% unfraktioniertem Serum möglich. Die verdünnte wasserlösliche Serumfraktion wird somit durch Cholesterin komplettiert. Cholesterin allein in entsprechenden Konzentrationen besitzt keinen Effekt. Die Frage, welcher Art der wasserlösliche Faktor ist, der Cholesterin als Supplement bedarf, um Trichomonadenwachstum zu gestatten, wurde hier nicht näher verfolgt.

In Gegenwart einer für die Vermehrung der Trichomonaden allein ungenügenden Menge der wasserlöslichen Fraktion (0,3%) ergibt der Zusatz von Fettsäuren teilweise eine beschränkte Vermehrung (vgl. Tabelle), die im Maximum 1/5 der in Gegenwart von Cholesterin (10^{-5}) auftretenden Zellzahl erreicht. Hierbei fördern Stearin-, Elaidin- und Ölsäure das Wachstum in Konzentrationen von 10^{-5} und weniger, wogegen höher ungesättigte Fettsäuren mit derselben Anzahl C-Atomen (18), nämlich Linol- und Linolensäure, in keiner der geprüften Konzentrationen fördernd wirken. Höhere Konzentrationen der

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