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ANTITUBERCULOUS EFFECTS OF CERTAIN INDOLE DERIVATIVES

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Certain heterocyclic compounds were proved to have antituberculous action[1)~6)10)11)]. Some of them were found to be increased in the blood and urine of tuberculous animals and patients[7)~9)]. This study was conducted to study the tuberculostatic actions of 5-OH-tryptophan, 5-OH-typtamine (Serotonin), tryptamine HCl, 3-OH-tryptamine, nicotinic acid, nicotinamide and nicotinuric acid (Sigma Chemical Co.).

Methods of Experiments

Two types of investigations were carried out using *Mycobacterium tuberculosis*, H_{37} Rv. (A) In vitro studies were made to test the antituberculous effect of some indole derivatives on Mycobacterium tuberculosis. The compounds tested were 5-OH-tryptophan, 5-OH-tryptamine (Serotonin), tryptamine-HCl, 3-OH-tryptamine, nicotinic acid, nicotinamide and nicotinuric acid. Abdel Kader and Suleiman Modification[10)] of Vorward's medium was used for investigation. It is composed of 5 gm monopotassium phosphate, 5 gm Aspargine, 2.5 gm Magnesium citrate, 0.6 gm Magnesium sulphate, and 20 ml glycerol. All dissolved in distilled water in the order given and completed to 1,000 ml (pH 7 and solidification by 1.8 gm agar).

After sterilization, the inoculation with mycobacteria was made and the growth was recorded weekly for six weeks.

The results of the in vitro studies were further extended to the in vivo investigations to test the therapeutic affects of serotonin as antituberculous agent in animals. (B) In vivo Experiments: Therapeutic effect of serotonin given 0.15 mg/ml intraperitoneally daily was examined in guinea-pigs infected with Mycobacterium tuberculosis. This dose was equal to that previously used with histamine.[11)] Four groups of animals, 8 male guinea pigs each, were selected from laboratory stock, and their body-weights ranged from 250 to 300 g. They were kept on a stock diet composed of rye and green clover during the experimental period. The animals were housed separately. All animals were infected with 0.001 mg of *Mycobacterium tuberculosis* H_{37} Rv by the intramuscular injection in the right thigh.

The first group (I) infected and untreated was used as the control, the second group (II) was simultaneously treated with intramuscular injection of streptomycin, 3 times a week, and 5 mg isonicotinyl hydrazide daily. Treatment was continued for 15 days.

The third group (III) was given a daily intraperitoneal dose of 0.15 mg serotonin as 1 ml sterile aqueous solution. The fourth group (IV) was treated with the same daily dose of serotonin starting from 15 days after the tuberculous infection. The serotonin therapy in the groups III and IV was continued for 15 days.

Results

In vitro Experiments:

The tryptophan metabolites tested showed different degree of tuberculostatic effects. The highly potent compounds were the hydroxylated derivatives (5-hydroxytryptophan, 5-OH-tryptamine HCl and nicotinuric acid).

The tryptamine HCl had moderate effect. A very slight antituberculous effect was shown by nicotinic acid,

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Table 1. In Vitro Effect of Some Tryptophan Metabolites (0.02 M) on Growth of Mycobacterium Tuberculosis.

	1	2	3	4	5	6	Notes
Control	2+	3+	3+	4+	4+	4+	Black colour masked any microscopic picture so Zeihl Nelson's films were done to prove the absence of any growth.
1. 5-OH-tryptamine (Serotonin)	−	−	−	−	−	−	
2. 5-OHL-tryptophan	−	−	−	−	−	−	
3. 3-OH-L-tryptamine	−	−	−	−	−	−	
4. Nicotinuric acid	−	−	−	−	−	−	
5. Tryptamine HCl	+	+	+	+	+	+	
6. Nicotinic acid	+	2+	3+	3+	3+	3+	
7. Nicotinamide	+	2+	3+	4+	4+	4+	

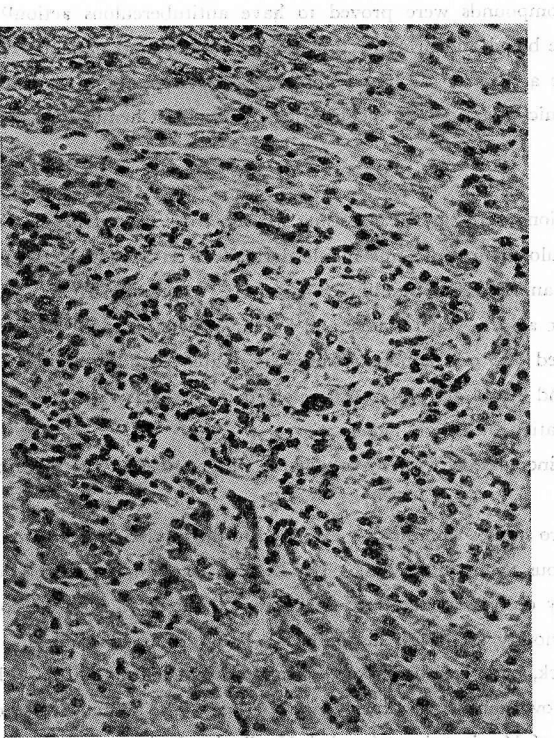

Fig. 1

while nicotinamide did not prove any effect.

In the in vivo experiments and post-mortem examination, the control animals, group I, showed evidence of infection manifested by enlargement and caseation with generalisation especially of spleen and liver. The lungs showed discrete tubercles, discrete alveolar obstruction and increased vascularity. Ziehl-Nelson's staining revealed the presence of tubercle bacilli.

In animals of group II (Streptomycin+INH) did not show any sign of generalization and the regional lymph nodes were slightly enlarged, fibrosed and showed no caseation. Microscopic examination revealed normal spleen and liver, but the lungs showed multiple tubercles with no giant cells and consolidation of 2/3 of the lung tissue. There was also increased vascularity of the lung tissue.

The group III treated with serotonin simultaneously with infection showed minimal signs of tuberculosis. The liver showed no signs of active tuberculosis except minimal fibrosis around the portal tract. Spleen presented fibrotic follicles with occasional lymphocytic infiltration. The lung also showed focal lymphocytic infiltration.

The group IV treated with serotonin 15 days after infection showed the following pathological pictures. The

Table 2. In Vivo Antituberculous Effect of Serotonin (Pathological and Bacteriological)

	Tuberculous activity as shown by microscopical examination of different organs					Bacteriological Examination	
	Lymph nodes	Liver	Spleen	Lung	Total activity	Liver	Spleen
Group I: Infected untreated (control)	4+	4+	4+	+	4+	2+	2+
Group II: streptomycin+INH treated group	±	−	−	2+	2+	−	−
Group III: Serotonin group, treated immediately after infection	±	−	−	−	±	−	−
Group IV: Serotonin group, treated 15 days after infection	±	+	+	±	+	−	−

liver showed few macrophagic accumulation surrounded by minimal amount of fibrosis and occasional portal occlusion. The spleen showed few tubercles composed mainly of macrophages. The macrophages were not fixed but mobile (Fig. 1). The macrophages did not form epithelioid cells. The lung showed focal lymphocytic infiltration in peripheral area.

Discussion

In the in vitro experiments, the nicotinuric acid and the 5-hydroxylated derivatives of tryptophan seemed to be most potent. The blackish discolouration might be due to polymerization and formation of the blackish stain melanin. That the nicotinamide and nicotinic acid were least effective as antituberculous drugs, might be well explained by the previous finding[3] that nicotinamide might be metabolic product of the tubercle bacillus.

In the in vivo experiments, the infected, untreated animals showed the typical picture of tuberculosis[16]. The animals treated with streptomycin+INH showed normal spleen and liver. Yet there was exacerbation of the tuberculous process in the lung tissue manifested by the presence of multiple tubercles and consolidation of two-thirds of ung tissue. This exacerbation in the lung was seen 6 weeks after stopping the treatment, and it was not found in the control untreated tuberculous animals (group I). These facts indicate diminished tissue resistance. This exacerbation might probably be similar to the remission which occurs in human tuberculosis, whenever the antituberculous treatment with the current drugs is not continued for at least one year[15]. In this group of animals the traditional antituberculous drugs were able to protect both spleen and liver from the disease without any remission after stopping them.

Comparing the action of serotonin in groups III and IV, serotonin was more effective when given immediately with infection (Table 2). This apparently might not match the previous hypothesis[10][11] that histamine might be an early endogenous antituberculous agent, while serotonin is an endogenous antituberculous factor which is liberated by the body in chronic tuberculous conditions. Such discrepancy might be explained that our results were gained by exogenous amines administered, thus serotonin was more effective when given early. Also one might consider the established facts that both biosynthesis and catabolic mechanisms for monamines are very much interrelated[12].

Further works are needed to clarify the real endogenous mechanism.

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