LETTERS TO THE EDITORS

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Penicillin in Leishmania Infections

THE action of penicillin on leishmania infections has not so far been reported in the literature. This note deals with the treatment of four hamsters infected with an Indian strain of the parasite which has previously been employed by me in chemotherapeutic tests¹. The methods used were the same as those already described. The Golden Hamster (*Cricetus auratus*) is the most suitable animal for experimental studies of this infection, since in their case the disease, so far as is known, never shows spontaneous regression and finally causes death. The *in vitro* action of penicillin on tissue forms of the parasite and on flagellate forms in young and old cultures is also described. Thanks are due to the Penicillin Clinical Trials Committee of the Medical Research Council for a supply of material.

The animals weighed 60–80 gm. Two were treated with the calcium salt of penicillin in saline three times daily over a period of eight days with doses of 400 Oxford units at 3–4 hour intervals, partly by the intraperitoneal and partly by the subcutaneous route. Two others received half this amount in the same way, the total dose for each of the two pairs being 9,600 and 4,800 Oxford units respectively, which corresponds to 6–3 million units for a man of normal weight. The drug was tolerated without any untoward symptoms. The spleen infections before treatment varied from 5 to 200 Leishman-Donovan bodies per field, as seen under oil immersion with a $\times 10$ eyepiece, and when smears were examined five days after the end of treatment it was found that the infections had progressed in the same way as those in two control animals. The drug had therefore exercised no influence on the course of the infection and parasitic cell division had taken place normally. In the case of bacteria, Bigger² has suggested that penicillin affects only dividing cells. Garrod³, from his experiments, considers that the evidence on this point is conflicting. It is, however, not certain that the drug can penetrate the splenic cells which normally contain these tissue forms.

In four *in vitro* experiments, infected splenic tissue from hamsters was cultured for six to seven days in media at 25° C. which contained penicillin in various dilutions. A suitable number of control tubes were used at the same time. The results are recorded in Table 1. Accurate counts of flagellates could not readily be made since they tend to become agglutinated in masses under these conditions.

From Table 1 it will be seen that development of flagellates took place in all the culture tubes in which the concentration of penicillin was less than 50 units per c.c., and growth proceeded as in the controls. At that concentration flagellates failed to develop in some tubes and the percentage of failures increased with higher concentrations of drug. It should be noted that the upper concentrations are greater than those likely to be obtained in the blood in clinical practice. After seven days incubation it was found by the agar-cup method that in the highest concentrations about 50 per cent of the penicillin origin-

ally present had disappeared. According to Bigger, the maximum effect of penicillin is exerted near body temperature, but culture of leishmania at 37° C. has not so far been found possible. The results suggest that the drug in the highest concentrations used had exerted some action on the parasite, but our penicillin sample contained only 150 Oxford units per mgm. and the impurities present may have influenced the results.

TABLE 1. HAMSTER SPLEEN INFECTED WITH *L. donovani* CULTURED IN PRESENCE OF PENICILLIN.

Units of penicillin per c.c. of medium	No. of experiment				Totals
	1	2	3	4	
400				5-	5-
200				2+ : 2-	2+ : 7-
50	2+ : 2-	1+ : 4-	5+	7+	15+ : 6-
12.5	3+	6+	6+	7+	19+
3.2		6+	6+		12+
0.8		4+	6+		10+
0.2		5+	6+		11+
Controls	3+	6+	4+	5+	18+
No. of parasites per field in each hamster spleen cultured	1	300	200	40	

+ = active flagellates present.
- = flagellates absent.

Some further experiments were made with flagellate cultures of different ages. Inocula of equal size of young flagellate cultures were sown in media containing penicillin at similar concentrations to the above, as well as in control tubes. After incubation at 25° C. for six to seven days, the contents of the tubes were examined and rough counts made of the number of flagellates present. It was found that the flagellates in tubes containing penicillin were as active as, and their numbers differed but little from, those in the control tubes. These results are summarized in Table 2.

TABLE 2. ACTION OF PENICILLIN ON FLAGELLATE FORMS OF *L. donovani* IN CULTURE MEDIUM.

Units of penicillin per c.c. culture medium	400	200	50	12.5	3.2	Controls
Total no. of tubes used for each conc. of drug	8+ : 1-	13+ : 1-	13+	13+	4+	10+

+ = active flagellates present.
- = flagellates absent.

It will be seen from Table 2 that only in two tubes containing penicillin did flagellates fail to survive six days. We consider that the drug had no action on these forms even at the high concentrations used.

In a final experiment penicillin, in the same medium, was added to six-day old cultures of flagellates to give a concentration of 400 Oxford units per c.c. Four tubes maintained at 25° C. for four days showed no diminution in numbers or activity compared with controls.

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¹ Fulton, J. D., *Ann. Trop. Med. and Parasitol.*, **38**, 147 (1944).

² Bigger, J. W., *Lancet*, **247**, 497 (1944).

³ Garrod, L. P., *Brit. Med. J.*, 107 (Jan. 27, 1945).