should have involved bones as well as soft tissues(4). Unfortunately, these data are capable of other interpretations. Attempts to demonstrate increased potassium content in rat liver have not been successful.

*Summary.* Measurements were made of muscle intracellular water, potassium and sodium concentrations in 2 groups of rats. The right hind limb of the experimental animals was immersed in a bath of acetone and ice, the right hind limb of the control animals in a bath of tepid water. Exposure of the leg to external cooling was associated with an increase in intracellular water in the muscles of that leg and a decrease in potassium concentration per liter of intracellular water. There was no difference in sodium concentration in the muscles from the chilled extremity from that of the control muscles.

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## Chemotherapeutic Studies on Cutaneous Leishmaniosis. (26475)

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The majority of experimental studies on antileishmanial drugs were conducted on visceral leishmaniosis. The method most frequently used for testing chemotherapeutic agents is that recommended by van Dyke and Gellhorn(1) using hamsters infected intraperitoneally with *L. donovani.* Stauber *et al.*(2,3) recently described a method which permits rapid completion of the test by counting the number of organisms in liver impression smears 8 days after a heavy intracardial inoculation with *L. donovani.*

Being interested in the therapy of the cutaneous forms we utilized an infection to which the mouse is quite susceptible, obtained with a strain of *L. brasiliensis* from a clinical condition—leishmaniosis tegumentaria diffusa—described by Convit(4). In man, this infection attacks the entire skin surface and resembles lepromatous leprosy. The disease does not affect the viscera and mucous membranes, though it sometimes induces a slight infiltration around the lower nasal septum. The cutaneous lesion is formed "by a specific granuloma composed of large macrophages, which are practically full of parasites in their highly vaculated protoplasm"(5). Mayer, Convit and Pifano(6) have experimentally investigated the infection and induced lesions on the caudal zone of the back in mice, first in ulcerative, later in nodular form, with an impressive abundance of parasites. Pifano(7) in a geographical distribution study concludes that the American tegumentary leishmaniosis is formed by various sub-entities (espundia, uta, *úlcera de los chicleros*), one of them being represented by the leproid form considered here. Interestingly enough, the intradermal Montenegro test with phenolated suspensions of *L. brasiliensis*(8) remained negative only in the latter clinical manifestation (5,6).

The possibility to follow accurately the effect of the treatment by gross and microscopic observation of the mouse infected with

this strain, gives to each individual experiment the value of a case history. We present here our findings concerning the action of drugs which have been found effective in *L. donovani* infections, namely tri- and pentavalent antimonials, Hydroxy-stilbamidine, the antibiotics Fungizone and Fumagillin.*

*Materials and methods.* A strain isolated from a chronic clinical case, carried through mice for a number of years by Prof. Pifano, has been used for investigation. In the early part of our experiments, ca. 0.1 cc of exudate taken from a heavily infected lesion was injected with a Pasteur pipette subcutaneously into the caudal zone of mice; later, finely cut saline suspensions of nodules were injected with syringe. In this way, up to 60 mice could be infected with a nodule suspension, dense in parasites, obtained from one donor. The take of the infection varied from 51 to 100% in different experiments. The infection developed after 1 to 3 months in 52%, 3 to 5 months in 32%, 6 to 9 months in 13% and 10 months or more in 3% of 159 mice. In 3 mice the infection was first visible 320 to 350 days after inoculation.

For treatment, animals with well developed lesions, rich in parasites, were selected: they were observed macroscopically and by preparing stained smears of the material aspired from the lesion.

*Results. Tartar emetic* in daily subcutaneous doses of 15 mg/kg, given 52 to 82 times, induced no changes in 5 mice. Similarly, 2 × 50 mg/kg i.p. remained ineffective in a mouse which died after 10 days. *Antimony trisulfide,* given daily in 150 and 500 mg/kg oral doses induced after the first treatments morphological variations of the leishmania, which appeared larger and granulated in both cases. Besides, significant transitory decrease of the parasites was encountered in the mouse treated with the 150 mg/kg dose; however, during the treatment period the parasite number increased and stayed at pretreatment value after 33 administrations. *Fuadin* induced in 2 mice, after the second subcutaneous treatment with 50 mg/kg, a temporary reduction in number of leishmania with morphological changes consisting in disappearance of nucleous and/or blepharoplast and presence of cytoplasmatic granules in preparations stained with Wright-Giemsa. Yet, by continuing treatment, with 100 mg/kg daily, the organisms reacquired their normal shape and the lesion was larger than initially after the 32nd treatment. *Hydroxy-stilbamidine* was given 14 times in daily doses of 12.5 mg/kg (i.p.) to 4 mice. In 3 animals no effect rerulted (in fact, in 2 of them the lesions were larger after treatment), while in 1 out of 4 the lesion decreased and no parasites were encountered after 4 months. *Fungizone,* 20 mg/kg daily (i.p.), given for 14 days, showed no activity in 4 mice, nor did 2 mice injected intravenously with 17 mg/kg × 3 respond to treatment. *Fumagillin,* injected intraperitoneally 14 times, in daily doses of 300 mg/kg, induced no effect in 3 out of 4 animals. In a fourth, the caudal lesion disappeared after 5 months, to reappear 8 weeks later. *Sodium-stibogluconate (Pentostam),* given intraperitoneally in 19 daily doses of 75 mg/kg did not influence the lesions of 3 mice, which became even more extensive during this treatment period. After an additional treatment course of 5 × 100 mg/kg the nodules started to decrease, but fully redeveloped within 3 months. *Antimoniate of N-methyl-glucamine (Glucantime)* in 4 mice treated subcutaneously, twice daily with 500 mg/kg, a total of 52 times, reduced the diameter of the nodules from 0.3-0.6 cm to 0.1 cm. In 2 of the mice the nodules increased again 3 months after cessation of treatment, and a secondary lesion of the foreleg developed in one of them. Four mice were treated 13 times with 1000 mg/kg daily: the larger ulcerated lesions of 2 mice remained unchanged, while in 2, with similar nodules, the lesion continued to increase (from 0.1 to 0.6 cm diameter). Six treatments with 1000 mg/kg daily showed no effect in 3 out of 4 mice, while the lesion disappeared temporarily in one and relapsed after 3 months. One of 2 mice treated 22 times with 500 mg/kg daily did not respond to treatment; in the other

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the initial lesion was not visible 2 weeks after the last subcutaneous injection, but a secondary lesion rich in leishmaniae appeared in the right hind leg 10 weeks later. In mouse No. 52, treated twice with 1000 mg/kg, the parasite count lowered within 6 days and the lesion disappeared within 4 weeks, but a secondary lesion in the left foreleg appeared 2 months later. The only animal completely cured was another treated with the same dosage: the initial lesion, containing 1000 parasites/field in the exudate smear (400×), disappeared completely 10 days after the second injection.

*Conclusions.* It would appear from the experimental findings that Fungizone, Fumagillin and Hydroxy-stilbamidine, effective against *L. donovani*, possess no action against the strain of *L. brasiliensis* obtained from a patient affected with leishmaniosis tegumentaria diffusa, employed for the cutaneous infection of mice. *In vitro,* Fungizone showed a high leishmanicidal action (0.01 μg/cc) on a strain of *L. brasiliensis* studied by Furtado *et al.*(9). Prolonged treatment with pentavalent antimonials, Pentostam and Glucantime, induced temporary decrease of the parasite number in the exudate, occasionally a transitory regression of the lesion, but definitely no curative effect. The lesion in most cases reappeared on the initial (caudal) site of the inoculation and exceptionally in another zone, in the legs. The temporary action observed was very clear cut and proves the value of the method for a search of drugs acting more specifically on this infection, or possessing, in a more general sense, a different strain specificity than the known agents. In some cases morphological variations were noted in the early period of treatment (Fig. 1). The significance of these changes is under investigation. There is a parallelism between clinical and experimental results, since according to the experience of the clinicians antimonials give only a temporary regression or diminution of symptoms. In conclusion, one can state that the effect of the presently known antimonials with antileishmanial action in this particular form of cutaneous infection is limited to a temporary effect on the lesion, or a transitory clearing without curative action.

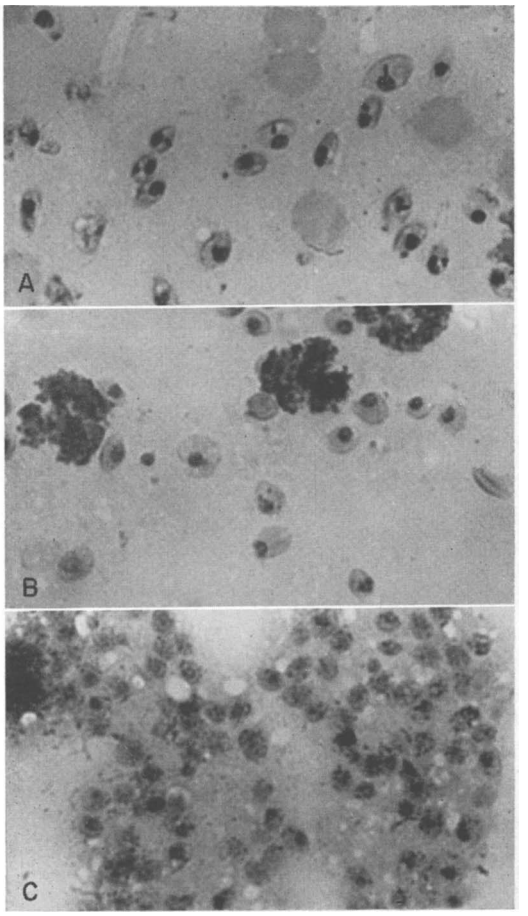


FIG. 1. Smears from local lesions of mice. In *A*, normal appearance of leishmania. In *B*, disappearance of blepharoplast and in *C*, cytoplasmatic granules after 2 treatments with 50 mg/kg (s.c.) Fuadin. In both cases these changes were temporary; the parasite reacquired its usual form during successive treatment with an increased dosage (100 mg/kg × 29).

*Summary.* The cutaneous infection of mouse obtained with a strain of *L. brasiliensis* causing in man the leproid form represents a suitable test for chemotherapeutic investigation. The infection has not been influenced by the antibiotics Fungizone and Fumagillin recently reported to possess antileishmanial action against *L. donovani*(10). A limited effect followed administration of pentavalent antimonials, consisting in a drop of the parasite number in the exudate and a temporary regression of the lesion, without indication

for curative action. Thus, the drug sensitivity of the strain is totally different from the ones studied so far, which offers the possibility to reveal agents with a different strain specificity than the known ones.

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## Deletion of Serine Dehydrase and Cystathionine Synthetase Activities During Azo-Dye Carcinogenesis. (26476)

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The Novikoff hepatoma, a tumor induced in rat liver using 4-dimethylaminazobenzene (DAB)(1), was reported to be essentially devoid of serine dehydrase activity(2). Recently, Selim and Greenberg(3) reported that a purified protein from rat liver exhibited both serine dehydrase and cystathionine synthetase activities. The authors suggested that their data indicated both activities were exhibited by a single enzyme(3). Assuming this suggestion to be correct, and further assuming that the enzymatic constitution of transplanted lesions of hepatic origin represented multiple deletions during hepatocarcinogenesis(4,5), it was reasonable to expect that cystathionine synthetase would also be essentially absent in the Novikoff hepatoma. It was the purpose of this investigation to determine (a) whether both serine dehydrase and cystathionine synthetase activities were absent in several transplanted hepatomas and (b) whether both enzyme activities were altered during hepatocarcinogenesis with 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB).

*Materials and methods.* The manner in which transplanted hepatomas were carried, precancerous liver tissues were obtained, and primary lesions were induced with 3'-Me-DAB have been previously described(6-8). Pyruvic acid formation, determined by the method of Friedemann and Haugen(9), was used as the criterion for assaying serine dehydrase activity of tissue homogenates, while serine utilization, determined by the periodate oxidation method of Frisell *et al.*(10), was used to assay cystathionine synthetase activity. The chromogen formed from chromotropic acid and formaldehyde was measured at 570 mμ and compared to an appropriate standard curve constructed with L-serine. All optical density measurements were made using a Bausch and Lomb Spectronic 20 Colorimeter.

Direct estimation of cystathionine was achieved by one dimensional paper chromatography(11) using n-butanol, acetic acid and water (60:15:25) as the solvent system. Known compounds and supernatants obtained by centrifugation of reaction mixtures treated with 10% trichloroacetic acid (TCA) were spotted on 47 cm × 57 cm sheets of Whatman No. 3 paper and subjected to descending solvent flow. Following partition,

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