Further Studies on Thonzylamine in Treatment of Experimental Tuberculosis. (18876)

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Since many of the symptoms of tuberculosis are believed to result in part from hypersensitivity to the products of the tubercle bacillus(1), we thought it worthwhile to study the effect of an antihistamine on the course of tuberculosis in guinea pigs.

In a preliminary experiment(2) we found that thonzylamine (Neohetramine) reduced the extent of tuberculosis in guinea pigs sacrificed 3 months after infection. In the following paragraphs we report the results of an experiment designed to determine the effect of thonzylamine alone and in combination with non-curative doses of streptomycin on the survival time of tuberculous guinea pigs.

Materials and methods. 160 Tuberculin-negative male guinea pigs, weighing 300-400 g were infected subcutaneously in the region of the groin with *Mycobacterium tuberculosis*, strain H 37 RV, each animal receiving 0.1 mg of bacilli (moist weight) per 500 g body weight. They were then divided into 4 groups as follows: Group 1—60 control guinea pigs received 0.5 ml physiological saline subcutaneously twice daily at 9 A.M. and 5 P.M. Group 2—60 guinea pigs received 6 mg thonzylamine dissolved in 0.5 ml physiological saline twice daily as above. Group 3—20 guinea pigs received 6 mg thonzylamine and 1 mg streptomycin dissolved in 0.5 ml physiological saline twice daily as above. Group 4—20 guinea pigs received 1 mg streptomycin dissolved in physiological saline twice daily as above.

Treatment, started on the day of infection, was continued until the death of the animal. Two months after the experiment was started, the dose of thonzylamine was increased to 9 mg twice daily for all animals receiving that drug.

All guinea pigs were weighed weekly, and tuberculin tests were performed every 7-10 days with varying amounts of O.T., by the Mantoux method. Each animal was autopsied as soon as possible after death, and the extent of tuberculosis in lungs, liver, spleen and lymph glands was scored as previously described(2). Representative sections of these 4 tissues, selected at random, were fixed in Zenker's fluid and examined microscopically. At no time did the examiner know the treatment undergone by the animal being autopsied, or the source of the material under microscopic examination.

Results. Tuberculin tests. At no time during the experiment did the treated animals react differently to intracutaneous O.T. than did the controls.

Weight. Examination of average weight curves disclosed no appreciable difference between the growth rates of the animals in groups 1, 2, and 3, although they suggested animals in group 4 gained more weight in the later stages of the experiment than the others. However, the percent change in weight between the beginning and the end of the ex-

TABLE I. Wt in g.

Group	At beginning of exp. No. animals	At beginning of exp. Avg wt	At end of exp. No. animals	At end of exp. Avg wt	% change
1	(60)	328	(9)	783	+239
2	(60)	326	(14)	782	+240
3	(20)	412	(13)	757	+184
4	(20)	399	(12)	924	+231

TABLE II. Analysis of Survival Data.

Group	1	2	3	4
Mean survival time, days	175.9	189.4	235.4	219.6
$S (y-\bar{y})^2$	216,787	155,433	57,059	110,628
n	56	58	19	20
t	(1 vs 2) 2.5137	(3 vs 4) 1.55	(1 vs 4) 5.9321	

1. Rich, A. R., The Pathogenesis of Tuberculosis, Springfield, Ill., Charles C. Thomas, 1944.

2. Duca, C. J., and Scudi, J. V., *Ann. Allergy*, 1949, v7, 318.

TABLE III. Average Gross Autopsy Scores of Streptomycin Treated, Thonzylamine Plus Streptomycin Treated, Thonzylamine Treated, and Control Tuberculous Guinea Pigs.

Group	Treatment of	No. Guinea pigs	Lungs	Liver	Spleen	Glands	Summary
4	Streptomycin	20	1.1	1	1.5	1.9	5.5
3	Thonzylamine + Streptomycin	19	1	1.1	1.7	1.6	5.4
2	Thonzylamine	58	1.6	1.5	1.9	1.6	6.6
1	Physiological saline	56	1.7	1.8	2.2	2.1	7.8

periment (Table I), indicates that the difference is not significant. On the other hand, the tabulated data indicate that the animals in group 3 clearly gained less than the animals of the other groups. It is possible in this instance that the additional trauma of 2 extra injections daily may be the cause of the decreased weight gain.

Side reactions. At no time did any of the treated guinea pigs display any evidence of local or systemic toxicity due to thonzylamine, either at the 12 or 18 mg daily dose.

Survival time. As in any long term experiment with guinea pigs, a small number of the experimental animals died from causes other than tuberculosis. These causes of death, usually bronchopneumonia or evident trauma, are easily differentiated from tuberculosis either at gross autopsy or during microscopic examination of the tissues. The few animals which clearly died from extraneous causes were routinely eliminated from further consideration. The percentages of surviving animals plotted against time indicated that the animals receiving the combined treatment (Group 3) survived the longest mean time, the streptomycin treated animals the next longest, the thonzylamine group the third longest, and the control groups the shortest. However, statistical analysis of survival time indicates (Table II) that the differences in survival times are significant only when the thonzylamine group is compared with the controls ($p = .02$) and when the streptomycin treated animals are compared with the controls ($p = .001$). The difference in survival times between those receiving the combined treatment and those receiving the streptomycin treatment is not significant statistically ($p = .1$).

Extent of tuberculosis. Considering only those animals in which tuberculosis was the cause of death, average gross autopsy scores were determined (Table III). The animals in group 4 were given streptomycin in doses too small to effect a cure, but in agreement with others(3), a distinct beneficial effect was obtained. The results were not improved by the addition of the antihistamine (Group 3). The animals receiving thonzylamine alone (Group 2) appeared, at autopsy, to exhibit less tuberculosis than the controls (Group 1). The difference is small, but in view of the random method of evaluation and the large numbers of animals, it is felt that the difference is real.

In the present experiment the thonzylamine decreased the extent of the disease, but not as significantly as it did in our earlier experiment(2). An explanation for the difference lies, we believe, in differences between the two sets of experiments. In the earlier experiment, animals were autopsied 3 months after infection, whereas, in the present experiment, death due to tuberculosis was taken as the end point. Since thonzylamine possesses no significant antimycobacterial activity, it can act only to diminish the allergic reaction of the host to tubercle bacilli. Once spread, however, the organisms would proliferate normally and would cause lesions. Thus, the effectiveness of the antihistamine would diminish as the disease progressed, and the difference in the extent of the disease in the control and the thonzylamine treated animals would tend to diminish with time. If this is so, one would expect a greater influence of the antihistamine drug to appear in the earlier short-range experiments than appears in the present long-range experiments.

3. Karlson, A. G., and Feldman, W. H., *Am. Rev. Tub.*, 1948, v58, 129.

Microscopic examination. All animals of Groups 3 and 4 were examined, but in the larger groups, 25 sets of sections were selected at random from each group for examination. Study of Ziehl-Neelsen and hematoxylin-eosin stained sections disclosed no difference between the controls (Group 1) and thonzylamine treated animals (Group 2) with respect to type of lesions or to numbers of bacilli present. All animals in these two groups showed predominantly fibrotic lesions with moderate caseation which contained small numbers of acid fast bacilli. The streptomycin treated guinea pigs (Group 4), and those receiving both drugs (Group 3) showed more fibrosis, fewer caseating lesions, and bacilli were difficult to find. The lesions in the latter two groups were similar to the lesions described by Karlson and Feldman(3) in tuberculous guinea pigs given the same dose of streptomycin.

Discussion. Thonzylamine, an effective antihistaminic agent which is capable of suppressing allergic reactions, was used in this and in our preceding study in an effort to determine whether or not it would aid in the chemotherapy of infectious diseases. It is evident from the above experiment that thonzylamine causes a slight but statistically significant increase in the survival time of experimentally infected tuberculous guinea pigs, and this appears to be reflected in a lessened spread of the disease. With curative doses of streptomycin (unreported experiments) the disease responded promptly and no effect attributable to thonzylamine could be discerned. With sub-curative doses of the antibiotic, our findings were in agreement with those reported by Karlson and Feldman(3) but, here again, thonzylamine did not influence nor aid streptomycin therapy. It must be concluded that thonzylamine is not a practical aid in the therapy of experimental tuberculosis in guinea pigs, but the slight beneficial result still leaves open the possibility that antihistaminic drugs may be useful in combatting the allergic component of infectious diseases.

Conclusions. Thonzylamine caused a slight reduction in the extent of tuberculosis in experimentally infected guinea pigs and caused a slight but significant increase in the length of survival. It did not supplement the effects of streptomycin.

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Evidence that Hyaluronidase is Not the Factor in Testicular Extract Causing Increased Vascular Permeability.* (18877)

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A factor capable of increasing vascular permeability was demonstrated to be present in bull testis extracts by Duran-Reynals(1). This finding has been amply confirmed(2-5). Duran-Reynals and others have assumed that hyaluronidase is responsible for both spreading and increase in vascular permeability.

We have investigated hyaluronidase preparations, more highly purified than those previously available, for vascular permeability-increasing activity. Two approaches were used: (1) a study of the local extravasation of an intravascular colloidal dye following

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1. Duran-Reynals, F., *Yale J. Biol. and Med.*, 1939, v11, 601.

2. Menkin, V., *Am. J. Physiol.*, 1940, v129, 691.

3. Aylward, F. X., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 342.

4. Elster, S. K., Freeman, M. E., and Dorfman, A., *Am. J. Physiol.*, 1949, v156, 429.

5. Benditt, E. P., Schiller, S., Wong, H., and Dorfman, A., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 782.