on the effects of phlorhizin on the respiratory metabolism of dogs. Only 10 cc samples of blood were used, and since the blood iodine values are all at the normal level the titrations were too small for a high degree of accuracy. However, additions to blood of thyroxin equivalent to 10 mcg of iodine per 100 cc were recovered with an accuracy of over 90%. It is evident that no definite rise in blood iodine occurred in the phlorhizinized animals. This is especially significant if one considers the fact that the basal metabolic rate of intact dogs receiving this amount of phlorhizin increases 70%.[3,4] In cases of thyroid disease presenting such an elevation of metabolism the blood iodine may be increased 20 times or more.

Conclusion. Neither in normal dogs receiving 10 mg doses of thyroxin intravenously, nor in intact dogs receiving phlorhizin, is there any parallelism between the blood iodine level and the basal metabolic rate. The former result conforms with expectations, since the blood is not the site of action of thyroxin. The latter finding is more unexpected, since the increase in metabolism which phlorhizin produces in dogs is very large and is abolished by thyroidectomy.

13968

Effect of Direct Applications of Tyrothricin and Allantoin to Cells *in vitro*.*

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Recent interest in the direct application of sulfonamide crystals to wound surfaces and to body cavities has stimulated research on the effect of such treatment upon cells cultivated *in vitro*.[1,2] In spite of the higher resistance of embryonic cells in tissue cultures to the action of inhibitory agents, workers in this field hold that *in vitro* experiments offer stringent and easily controlled conditions for the analysis of drug toxicity.

In addition to the sulfonamides, a new class of compounds characterized by high bactericidal action is being derived from microörganisms. Of these, tyrothricin and its derivatives, are described as unsafe at present for general use, especially when brought in direct contact with the blood stream.[3,4] Tyrothricin, however, has been found not to interfere with the healing of wounds[3] and therefore may become useful in this direction.

Somewhat better known, allantoin, the chemical associated with the benefits of maggot therapy, has already proved useful at 2% concentrations in stimulating the granulation of chronic suppurative wounds.[5,6] Its action does not appear to be bactericidal and therefore it has been used in association with some disinfectant. Veal[7] has found that allantoin combined with sulfanilamide in a greaseless base was extremely valuable

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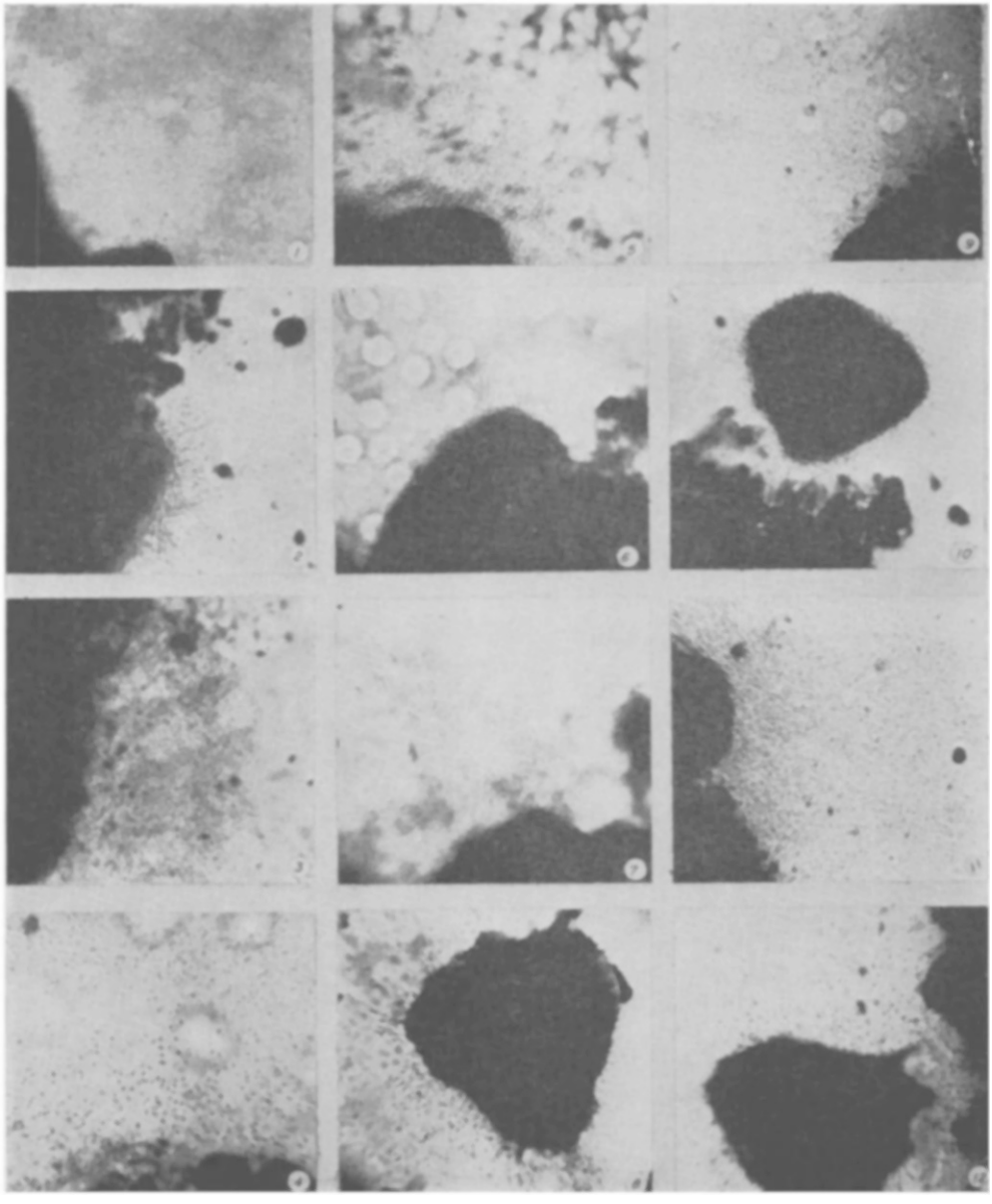

PLATE 1.

Explanation of Figures.

FIG. 1. *Tyrothricin.* Active migration of fibroblasts through a field of powder (left side) 72 hr after direct application to a freshly cut heart fragment. ×30.

FIG. 2. *Tyrothricin.* Growth of fibroblasts in a heart culture after 24 hr incubation. Photograph taken immediately after application of the powder which appears as dense round masses. ×30.

FIG. 3. *Tyrothricin.* Same culture as in Fig. 2 but photographed 48 hr later. ×30.

FIG. 4. *Tyrothricin.* Active migration of leucocytes from a spleen fragment (lower left) treated with powder (lower right) after 48 hr incubation. ×30.

FIG. 5. *Allantoin.* Active growth of fibroblasts in a field of crystals 72 hr after application to a freshly cut heart explant. ×30.

FIG. 6. *Allantoin.* Growth of fibroblasts in a heart culture after 24 hr incubation. Photograph taken immediately after application of crystals (right side). ×30.

FIG. 7. *Allantoin.* Same culture as in Fig. 6 but 48 hr later. Migration of fibroblasts is seen to have continued following treatment. ×30.

FIG. 8. *Allantoin.* Migration of leucocytes from a spleen fragment in the presence of crystals. 24-hr culture. ×30.

FIG. 9. *Tyrothricin-Allantoin.* Active growth of fibroblasts 72 hr after treatment of a freshly prepared explant of chick heart. ×30.

FIG. 10. *Tyrothricin-Allantoin.* Growth of fibroblasts in a heart culture after 24 hr incubation. Photograph taken immediately after treatment with dry mixture of tyrothricin and allantoin (lower left area). ×30.

FIG. 11. *Tyrothricin-Allantoin.* Same culture as in Fig. 10 but 48 hr later showing continued active growth of fibroblasts. ×30.

FIG. 12. *Tyrothricin-Allantoin.* Migration of leucocytes from a spleen fragment in the presence of powder mixture (right) after 24 hr incubation. ×30.

TABLE I.

Effect of Tyrothricin and Allantoin Powder Applied Individually or in Combination to Fibroblasts and Leucocytes Grown Cultivated *in vitro*.

Compound used	Tissue	Individual results					
Tyrothricin	Heart fresh explant	+++	++	+++	++++		
	" 24-hr growth	++++	++++	++++	++	+	++
		++++	++++	++++	++	+	++
	Subcultured		+++		+++		+++
	Spleen	+++		++	++++	+++	++
		+++		++	++++	+++	++
Allantoin	Heart fresh explant	++++	++++	+	+++		
		++++	++++	+	+++		
	" 24-hr growth	+++	+++				
	Spleen	++	++	++	++	+++	++
Equal parts tyrothricin and allantoin	Heart fresh explant	++++	+++	+++	++++		
	" 24-hr growth	++++	+++	++++	++++		
	Spleen	++	++	+	+++	+++	+++

in the preparation of extensive burns for skin grafts.

It was the object of the present study to submit tyrothricin, allantoin and a combination of equal parts of both compounds to the test of direct application to cells grown in tissue culture.

Experimental. The technic employed was identical with that described by Pomerat, Capouya and Greenleaf[2] for a similar study of sulfonamide compounds. Hanging-drop preparations were made using equal parts of rooster plasma and a 20% extract in Tyrode's solution, of chick embryos incubated for 7 to 10 days. Three types of cultures used were: (1) freshly explanted fragments from the ventricle of chick embryos incubated for 8-10 days, (2) heart explants prepared as in (1) but grown 24 hr before treatment, and (3) spleen fragments of 1-2 day old chick from which leucocytic migration could be observed. The compounds to be tested were placed on one side of the explant in a quantity sufficient to exceed saturation at 38°C for the entire period of observation. One untreated control culture was set up for each group of 5 experimental preparations.

Photomicrographs were taken at the time of treatment and at various intervals up to 72 hr for the measurement of fibroblast growth and to 24 hr for the migration of leucocytes. The object of the study was to test growth *versus* non-growth in the presence of the undissolved compounds. No exact quantitative measurement was made of the rate of growth but this was roughly estimated in terms of control cultures and is reported by means of plus signs, the largest rate being given as ++++.

Results. Table I and Plate 1 illustrate the results obtained for the 3 types of tissue preparations in relation to the compounds studied.

(A) *Tyrothricin.* When this is added to the culture medium it tends to spread moderately and to form a film with a very thin edge. Cells growing on the surface of a clot which is covered by a very thick layer of

powder appear to be mechanically obstructed and show almost complete inhibition. Fibroblasts and leucocytes which are in the immediate vicinity, but which are not heavily covered, are not damaged and undergo proliferation typical of control cultures. This was found true for freshly cut fragments of chick ventricle and spleen, as well as for heart cultures which had proved a capacity for active growth following 24-hr incubation.

(B) *Allantoin.* Heart tissue dusted with allantoin immediately after fragments were set in clotted medium or after fibroblasts had been growing for 24 hr were not inhibited, but growth did not progress beyond that of the controls. Allantoin did not prevent chance contamination of some cultures. These, however, are not included in the table since the data were restricted to cultures which remained sterile throughout the experiment.

The migration of leucocytes from spleen fragments was not as active in allantoin treated cultures as in the controls, but complete inhibition was not observed in any of the cultures so treated which were studied.

(C) *Mixture of tyrothricin and allantoin in equal proportions.* While the total number of test cultures is small and the method of evaluating the amount of growth is not highly quantitative, some impressions are justified. Fibroblasts appeared to grow somewhat more actively than in either of the components of the mixture when used alone. This growth, however, did not exceed perceptibly that of the controls, so that cell stimulation is not a justifiable interpretation. Leucocyte migration from spleen was as active as in the presence of allantoin alone, but it was somewhat less active than in the presence of tyrothricin alone. Leucocytic activity, however, was far less than that of control cultures.

Discussion. In spite of its well known hemolytic effect,[3] tyrothricin does not appear to inhibit materially either mitosis or migration of fibroblasts, or the activities of leucocytes following its direct applications to tissue culture media.

Three cultures treated with tyrothricin powder after growth for 24 hr and then incubated for 48 hr were subcultured and exhibited growth in the second passage equal to that obtained in controls.

Heart fibroblasts treated with allantoin have been studied in tissue cultures by Shipp and Hetherington.[8] These authors report the addition of allantoin to Tyrode's solution before the extraction of the embryos in quantities sufficient for a final dilution of 0.5%. This method differs from that of the present study wherein allantoin crystals were dusted directly onto the clotted culture medium. The author's methods were not sufficiently quantitative to corroborate Shipp and Hetherington's statement that allantoin is slightly stimulating to fibroblasts grown *in vitro,* but they do indicate that crystals of this compound are relatively non-damaging to cells in tissue cultures.

These results resemble those obtained for similar experiments using sulfonamide compounds[1,2] in which the less soluble members of the group exhibited extraordinarily low toxicity when dusted directly onto cells cultured *in vitro.*

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