ings made from histological sections of necrotic foci of tuberculosis in man and animals. In these drawings the presence of tubercle bacilli in large aggregates is clearly illustrated. Moreover, Middlebrook, Dubos and Pierce(9) have also observed clumped growth of tubercle bacilli in the brain tissue of guinea pigs infected intracerebrally with virulent human strains. It is of interest that the occurrence of this phenomenon has not been more widely discussed. It is possible that modern methods of preparing tissues for histologic examination may not be ideally designed to reveal the nature of the type of growth of *M. tuberculosis in vivo*. On examination of conventionally prepared tissue sections of experimental tuberculous lesions of the lung, virtually all of the microorganisms during the early stages of the infection appear to be intracellular, principally within the monocytes. Under these conditions the tubercle bacilli are present as aggregates but the role of the phagocyte in producing the aggregation cannot be discounted. The principal situation where tubercle bacilli are seen to be extracellular is within areas of caseation where they are also present in clumps. In this situation, also, it is difficult to exclude the possibility that the aggregations were originally formed by the monocytes and were released with disruption of the latter cells.

Thus it cannot be considered as established at present that the clumping of bacilli observed *in vivo* has the same physiologic implications in regard to antimicrobial therapy as the clumping of bacilli *in vitro*.

Steenken and Wolinsky(11) have recently confirmed the observation that subtilin activity *in vitro* is dependent upon the presence of Tween (sorbitan monooleate). They further observed that guinea pigs infected with a virulent human strain, H37Rv, of *M. tuberculosis* were not benefitted by an extensive course of treatment with subtilin.

Summary. It has been observed that the degree of inhibition of tubercle bacilli by subtilin *in vitro* parallels the degree of growth dispersion of the organisms. In a medium permitting diffuse growth, subtilin was highly active. Conversely, in a medium in which growth was clumped, whether because of omission of Tween or from antagonism of Tween by serum, no significant inhibition of growth by subtilin was demonstrable. When serum from rabbits injected with subtilin was tested against tubercle bacilli in a medium containing sufficient Tween, the observed antituberculous activity paralleled the activity against streptococci and staphylococci. If the amount of Tween added to such tests was insufficient to produce diffuse growth of tubercle bacilli, subtilin activity was greatly diminished or absent.

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Relation of Low Temperatures to X-ray Injury of Hematopoietic Tissue of Tadpoles.* (17575)

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Tadpoles of *Rana catesbiana*, from 70-90 mm total length, were subjected to x-ray treatment of definite dosage and killed at the end of specific periods of time during which some were refrigerated and others kept at normal temperature. When tadpoles were refrigerated during irradiation, they were previously kept in the refrigerator for 2 hours. During irradiation, tadpoles were kept in water 7 mm deep, leaving the dorsal surface

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TABLE I.
% of Destruction in 24 Hours.

Exp. No.	Norm. temp., °C	Exp. temp., °C	No. of cases	Norm T. × 500 r norm. T.	Norm. T. × 500 r refrig.	Refrig. × 500 r refrig.	Refrig. × 500 r norm. T.
106	18.8	.0	33	(5) 21.3	(9) 0.6	(8) 0.9	(11) 10.8
99	18.8	.5	39	(16) 18.8	(5) 1.5	(11) 3.2	(7) 14.8
108	16.1	2.7	35	(5) 19.7	(10) 0.8	(11) 0.6	(9) 10.1
109	22.2	5.5	31	(5) 34.9	(9) 0.9	(8) 2.1	(9) 34.4

Figures in parentheses indicate numbers of tadpoles in each division within the experiment. We have extensive controls of unirradiated tadpoles kept at normal temperature and under these different degrees of refrigeration. Especial study was given to those kept at 0°C. Little or no injury resulted from refrigeration.

uncovered. The caudal portions of the mesonephroi were fixed in Bouin's fluid, cut at 5 μ and stained with Wright's or Leishman's blood stain. Counts of normal and dead hematopoietic cells were made with a Whipple grill under oil immersion, the numbers counted in each tadpole ranging from 275-450.

There were 4 types of treatment:
(1) Normal temperature before and during x-ray → Normal temperature.
(2) Normal temperature before and during x-ray → Refrigeration.
(3) Refrigeration before and during x-ray → Refrigeration.
(4) Refrigeration before and during x-ray → Normal temperature.

"Normal" temperature is that to which tadpoles were subjected in bowls kept in shade out-of-doors during August, September and October. The average temperatures for the dates of the experiments are given in the second column of the table. X-ray treatment was given with 250 KV, 15 m.a., .21 inherent Cu + .5 mm. Parab. Cu + 1 mm Al, h.v.l. 1.85 mm, Cu 35 cm distance F.O.D. Roentgens measured in air. Tadpoles placed in water leaving dorsal half exposed.

The results are shown in Table I.

A. X-ray irradiation of a dosage very destructive under normal temperatures can be prevented from producing appreciable visible effects on the hematopoietic cells if the tadpoles are maintained at 0° to 5.5°C.

B. Refrigeration during irradiation certainly does not prevent injury of hematopoietic tissue in tadpoles subsequently kept at normal temperatures. To what degree, if any, it may lessen the proportion of destruction is a subject for further study. In an experiment using 58 tadpoles, and extending from 3 to 4 days, we had similar results. Tadpoles irradiated and refrigerated for 2 days, then removed to normal temperature for 1 or for 2 days, showed very heavy cell destruction, while others treated identically and kept refrigerated at 2°C until killed at the end of 4 days, showed extremely little cell destruction. This will be fully discussed in our complete report.

Discussion. Our findings regarding the arrest of x-ray destruction of hematopoietic cells by refrigeration run parallel to the work of Patt,(1) Swift, Tyree and Clayton who showed that frogs that have been heavily x-rayed can be maintained alive for several months so long as they are kept refrigerated at 6°C. When transferred to normal temperature they quickly die.

These results might be due either to an arrest of metabolic activity or of mitosis. The work of Gluckmann and Spear(2) upon the arrest of mitotic activity in the germinal zone of the retina of tadpoles kept at low temperatures after gamma ray treatment would appear to favor the latter alternative. We have begun several lines of experimentation to solve this problem but the work is not far enough advanced to justify conclusions. The problem is quite complicated, indeed both factors might be involved. Tadpoles offer great advantages in experimental technics and in cytological studies of results.

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Summary. So long as tadpoles are maintained at temperatures from 0° to 5.5°C the destructive effects of x-ray exposure are very slight in the hematopoietic cells. The temperature of tadpoles at the time of x-ray treatment makes little if any difference in the destructive processes that develop later.

These destructive effects depend primarily upon the temperature to which the tadpoles are subjected after irradiation.

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Survival of Guinea Pig Thyroid and Parathyroid Autotransplants Previously Subjected to Extremely Low Temperatures.* (17576)

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Numerous investigations have been carried out on the effects of freezing and below freezing temperatures on infectious organisms and on the cells and tissues of plants, lower animals and cold blooded vertebrates, but relatively few studies have been reported in homotherms. In the latter group there have been reports of the survival of spermatozoa,(1-5) embryonal tissues,(6) the epithelium of chicken trachea(7) and the squamous epithelial cells of homologous skin grafts in the rat.(8) There are also numerous reports of the storage of tumor tissues at low temperature for periods up to two years with subsequent successful animal transfer.(7-21) Recently Gye and also Mann have stated that these results in tumors indicate the transfer of a tumor producing virus. Mann and Dunn(21) believe they have further support of this thesis in that they have been able to propagate mouse carcinoma by dried, frozen tumor tissue. However, the acceptance of such an interpretation rests, at least partly, on the proof that normal cells cannot survive similar treatment.

The present report presents data which indicate that normal mammalian tissues can survive exposure to extremely low tempera-

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