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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tures. Autotransplantation of previously frozen thyroid has been used to demonstrate survival, since the extensive investigations of Loeb(22) have shown that this type of transfer of normal (unfrozen) organs can be carried out successfully with consistent results. The thyroid gland was further chosen in order to utilize pituitary thyrotropin to increase the growth potential of the transplant and aid in overcoming the injurious effects of transplantation. Silberberg(23) has shown that thyroid autotransplants respond readily to such hormonal stimulus. Successful parathyroid transplants were incidentally encountered when inadvertently frozen and transferred with the thyroid tissue.

Method. 200-400 g guinea pigs were used in these experiments. Autotransplants were made into a subcutaneous pocket in the anterior abdominal wall. In some experiments 1 mg of thyrotropin‡ was administered subcutanously for 3-4 days prior to, or subsequent to transplantation. For storage at −70°C, the thyroid glands were inserted into 20 cc vials which were placed in contact with pieces of solid carbon dioxide in an insulated box. The thyroids were immersed directly into liquid nitrogen to obtain exposure to −190°C. In all of the freezing experiments no attempt was made to accelerate the decrease in temperature of the tissue.

Control Group. In a group of 5 guinea pigs immediate transplants from neck to subcutaneous tissue were carried out. One transplant was removed 24 hours later, another 5 days after transfer, 2 after 13 days, and the fifth after 20 days. Within the first 24 hours necrosis of much of the thyroid had taken place; there remained a few peripheral islands of viable thyroid follicles. By the end of the 5th day fibroblasts had started infiltrating the necrotic tissue, and by the 13th day had completely replaced it. After 20 days there was considerable regeneration of thyroid follicles so that islands of thyroid tissue partially surrounded the organized center. These follicles were lined by flat or cuboidal epithelium and contained pale scant colloid. A parathyroid was found in the 20 day transplant which showed a ring of well preserved epithelial cells surrounding a center of loose connective tissue in which there were a few phagocytes containing a brown granular pigment, presumably hemosiderin. Thyrotropin was administered to a second group of 3 guinea pigs. Two of these received the thyrotropin for 4 days before autotransplantation and the third for a similar period after transplantation. In the former group the transplants were examined after 5 and 11 days. There was less necrosis than in non-injected controls and the areas of preserved thyroid tissue were larger. The follicles were lined by columnar epithelium showing a number of mitoses. Fig. 1 is a section through the 11-day autotransplant. The guinea pig which received thyrotropin after transplantation also showed a well preserved transplant in which there were numerous mitoses even

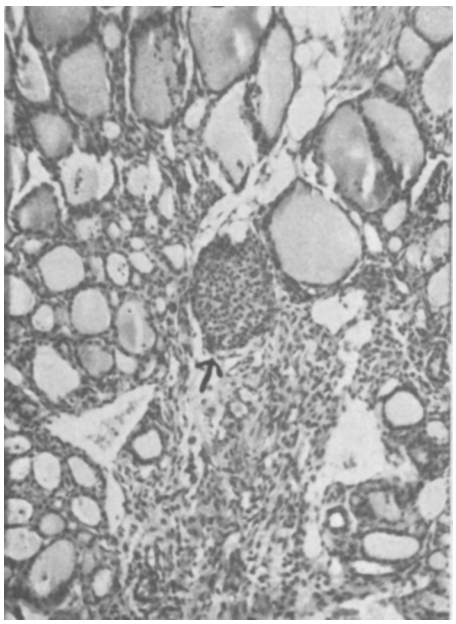

FIG. 1.

11-day autotransplant of thyroid and parathyroid. Guinea pig had received 1 mg of thyrotropin (Armour) daily for 4 days before transplantation. The thyroid follicles are well preserved and lined by cuboidal epithelium. Arrow indicates an area of parathyroid tissue.

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‡ We are indebted to The Armour Laboratories, Chicago, Ill., for supplying the thyrotropin used in these experiments.

as late as 11 days after the last injection.

In order to observe the immediate effects of freezing, one guinea pig received thyrotropin for 3 days, following which its thyroids were removed; one was placed at −70°C for 24 hours, and the second at −190°C for 10 minutes. The glands were then thawed, fixed and sectioned. In both the architecture was well preserved, although the colloid was soft, pale and sparse; there were many pyknotic nuclei. However, the thyroid kept at the lower temperature showed better preserved nuclei and several mitotic figures were found.

Experimental Group. Nine lobes of thyroid were autotransplanted after being kept at −70°C for 24 hours; they were examined after periods varying from 5-20 days. All except 2 of these transplants showed only progressive organization of necrotic tissue (Fig. 2). However, in one transplant examined after 13 days there was a well preserved parathyroid gland with a central area of loose connective tissue containing a few phagocytic cells with brown granular pigment (Fig. 3);

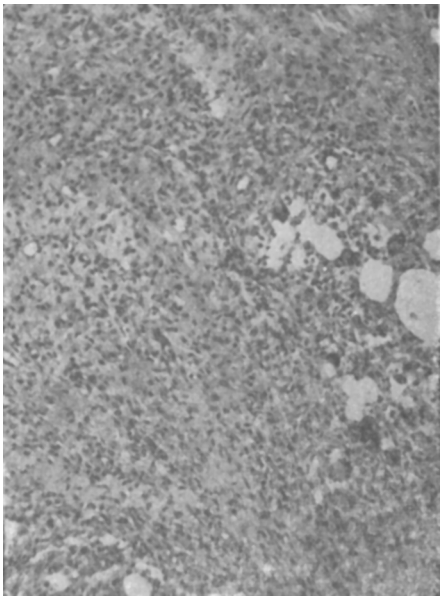

FIG. 2.
14-day unsuccessful thyroid autotransplant following exposure to −70°C for 24 hr. The thyroid tissue has been completely replaced by fibroblasts. The giant cells represent a reaction to necrotic transplanted fat.

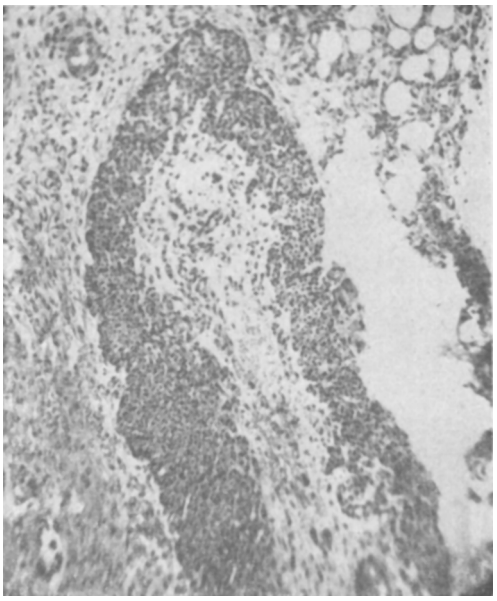

FIG. 3.
13-day parathyroid autotransplant following exposure to −70°C for 24 hr. The parathyroid epithelium forms a ring about a central core of connective tissue.

this was similar to a parathyroid observed in a control autotransplant. Another 13 day transplant contained an area of well preserved thyroid tissue adjacent to organizing necrotic tissue (Fig. 4). The epithelium of the follicles was flat and the colloid soft, pale and sparse; a mitosis was found in one section. In a further experiment one guinea pig received thyrotropin for 4 days prior to removal of a lobe of thyroid which was then subcooled to −70°C for 24 hours and autotransplanted. In 2 other guinea pigs the pituitary hormone was administered for 4 days after subcooling to this temperature and autotransplantation. The first transplant was examined after 11 days, and the latter 2 after 15 days; no preserved thyroid tissue was found in any of these transplants.

Seven guinea pigs received daily injections of thyrotropin for 3 or 4 days, following which a lobe of thyroid was removed, placed at −190°C for 10 minutes and then autotransplanted. Four of these transplants were removed after 3 days when the animals were found dead. The transplants, however, were extremely well preserved; there was no necro-

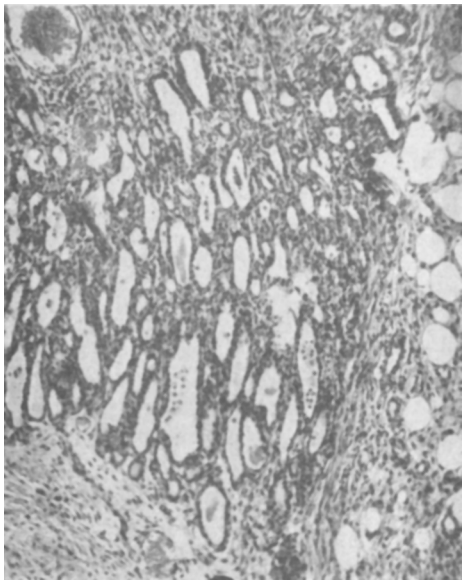

FIG. 4.
13-day successful autotransplant of thyroid following exposure to –70°C for 24 hr. The thyroid follicles are small and contain very little colloid. The epithelial lining is cuboidal in character.

sis, although some of the nuclei were pyknotic; the epithelium lining the follicles varied from a cuboidal to columnar character and several mitoses were found (Fig. 5). Two other guinea pigs were found dead on the fifth day after autotransplantation; although there was some central necrosis in these transplants, there were well preserved peripheral groups of follicles lined by cuboidal epithelium. The fifth transplant was removed after 11 days and showed complete fibroblastic organization.

Finally an experiment was performed in which two temperatures were employed. The thyroids were first immersed in liquid nitrogen for 10 minutes and then transferred to –70°C where they remained for 24 hours when they were autotransplanted. There were 4 guinea pigs in this group. One guinea pig received thyrotropin for 4 days after autotransplantation, and on the fifth day the transplant showed a well preserved thyroid with follicles lined by cuboidal epithelium; the colloid was sparse and pale staining. The remaining three guinea pigs received thyrotropin before the glands were frozen and autotransplanted. Two of these transplants were examined after 5 days and showed necrosis and fibroblastic organization of the periphery of the transplant, but the third thyroid which was examined after 11 days showed excellent preservation (Fig. 6). The follicles were lined by cuboidal and columnar epithelium; occasional mitoses were found. The colloid was pale and sparse. There was some fibroblastic reaction in the surrounding fat, accompanied by an occasional multinucleated cell, but this reaction was common to many transplants, both successful and unsuccessful, and represents a reaction to transplanted fat frequently injured in the process of inserting the transplant into the subcutaneous pocket.

Discussion. As far as we have been able to determine, these experiments represent the first successful demonstration of the survival of a functional structural unit of an adult homotherm following exposure to extremely low temperatures. Two reports previously cited give evidence of survival of cells other

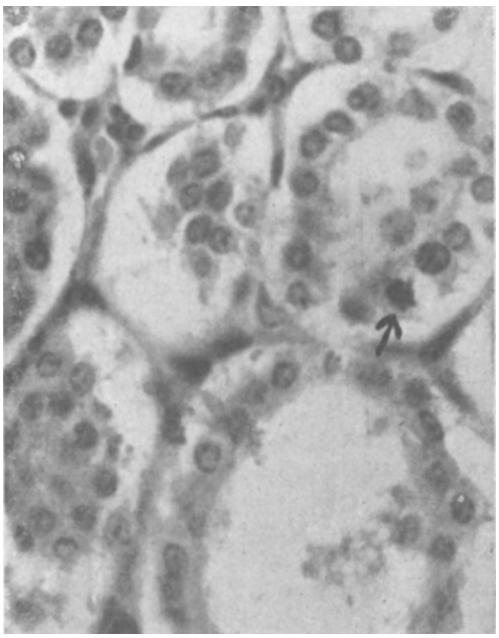

FIG. 5.
3-day autotransplant of thyroid. Treatment previous to transplantation consisted of the injection of 1 mg of thyrotropin daily for 3 days followed by immersion in liquid nitrogen (–190°C) for 10 minutes. The epithelium of the follicles shows an excellent state of preservation and retains a hyperplastic character indicative of a response to thyrotropin. Arrow indicates a mitotic figure.

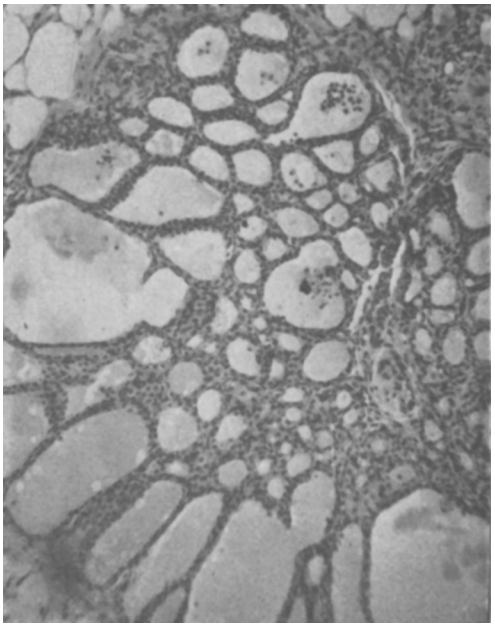

FIG. 6.
11-day autotransplant of thyroid. Treatment previous to transplantation consisted of the injection of 1 mg of thyrotropin daily for 3 days followed by immersion in liquid nitrogen (−190°C) for 10 minutes and storage at −70°C for 24 hr. Note excellent preservation of follicular structure.

than spermatozoa of adult warm blooded animals so treated. In one of these the criterion of viability was movement of the cilia of respiratory epithelium (Breedis and Furth) (7), while in the second there was evidence of mitotic activity in the homologously transplanted epithelial cells of the rat's skin.(8) In the first instance the observation may be criticized on the basis that ciliary movement may have been caused artificially by air or liquid currents; one of these investigators himself subsequently raises objections to movement as a criterion of survival of cells.(18) The criterion of growth as represented by mitotic activity is a much more accurate indicator of viability; such evidence is present in the investigations of Mider and Morton(8) and in our experiments.

There are contradictory reports in the literature as to whether rapid or slow freezing is less injurious to cells. The primary factor in producing death in frozen tissue is apparently the formation of ice crystals.(24,25) Luyet and Gehenio(24) have divided the organisms which resist cold into two groups, namely (1) those that resist cold only in a dry state, and (2) those that resist cold with their full water content. Of the latter, some organisms survive only if special precautions for rapid cooling and rewarming are taken in order to prevent the formation of ice crystals. The critical range of temperature in subcooling tissues would therefore be that span in which ice crystals may form. If this range is passed through rapidly, a vitreous state (Luyet and Gehenio) results which is apparently not injurious to cells in most instances, and in which state they probably may be preserved indefinitely. On the other hand, several investigators report better results in tumors(7,8,18) and in homologous transplants of rat's skin(8) with slow freezing. Breedis (18) explains this on the basis that the initial freezing of water occurs in the extracellular fluids which then results in the concentration of osmotically active material outside of the cell. It is believed that this results in partial dehydration of the protoplasm which would then become a less favorable site for ice crystal formation. Since in the present experiments considerably better results were obtained with rapid immersion in liquid nitrogen it would appear that these experiments tend to substantiate the observations of Luyet and Gehenio. However, it is possible that different tissues respond differently after exposure to low temperatures.

We have pointed out in the introduction that the successful transfer of frozen tumor material is believed by some investigators to indicate the transfer of a virus, since they assume that normal and malignant cells cannot survive exposure to freezing and below freezing temperatures. The present studies show that this is not the case. Nor does the successful transfer of frozen, dried tumor material necessarily prove the presence of a virus. The investigations of Luyet and Gehenio(24) indicate that dehydration may facilitate rather than impede survival of tissues at extremely low temperatures.

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Summary. Thyroid gland of guinea pig was subjected to low temperatures (−70°, or −190°C) and subsequently autotransplanted. In some instances pituitary thyrotropin was administered before or after transplantation in order to increase the growth potential of the transplant and overcome the injurious effects incident to transferring the tissue. There was one successful transplantation of thyroid gland in 12 attempts after exposure at −70°C and one parathyroid transplant. Eight viable thyroid grafts in 12 attempts were obtained after immersion at −190°C. The significance of these findings with respect to rapidity of freezing is discussed in their relation to the mechanism of survival of mammalian cells and tissues exposed to extremely low temperature. The relation of these results to evidence purported to indicate a viral transmission of transplanted frozen-dehydrated tumor material is also pointed out.

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Comparative Study of Agglutinins and Complement-Fixing Antibodies for Brucella in Blood and Cerebrospinal Fluid. (17577)

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Although brucellosis commonly causes symptoms referable to the nervous system, objective evidence of the results of invasion of the central nervous system is frequently lacking. Over a period of 12 years at the University of Minnesota and cooperating hospitals, a total of 161 cases of active brucellosis, 86 of which were culturally proved, have been studied, and the diagnosis of encephalomeningitis was made in four cases.(1) A fifth case exhibited a severe cerebral vascular accident secondary to subacute bacterial endocarditis, which was due to *Br. abortus.*(2) Except for the cases cited, there was no evidence of organic cerebral changes in the remaining cases, although psychic disturbances with emotional instability and mental depression were commonly encountered. Apter and his associates(3) were impressed by the "disturbances in the adaptive capacities" of patients having chronic brucellosis and carried out a battery of neuropsychologic studies, as outlined bp Halstead,(4) on a series of 10 patients. As a result of their investigations, it was concluded that organic damage to the frontal lobes had occurred in 7 of the 10 patients. While such a study suggests a reasonably high incidence of brain damage in patients with chronic brucellosis, there remains an urgent need for acquiring more information on the frequency with which the central nervous system is invaded by *Brucella.* Since serologic techniques are applied successfully to an examination of the cerebrospinal fluid in individuals with suspected syphilis, there was a possibility that serologic methods utilized for the diagnosis of brucellosis might be of some aid in resolving the problem of neurobrucellosis. Simultaneous tests on the blood and cerebrospinal fluid of patients with brucellosis were performed, utilizing procedures for detecting the presence of agglutinins and complement-fixing antibodies.

Methods. Specimens of venous blood and

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