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EFFECT OF CRYOCONSERVATION AND TRANSPLANTATION FACTORS ON MORPHOLOGY AND FUNCTION OF THE CANINE THYROID GLAND

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During transplantation of any organ, surgeons have to contend with the effects of nonspecific transplantation factors such as denervation, delymphatization, ischemia, operative trauma, and changes in blood supply. During conservation, the organ is additionally exposed to factors accompanying it, and in particular, during cryoconservation: very low temperatures and cryoprotectors.

The aim of this investigation was to study the effect of nonspecific transplantation and low-temperature conservation factors on the ability of the thyroid gland to secrete thyroid hormones and on its morphology.

EXPERIMENTAL METHOD

Experiments were carried out on 30 mongrel dogs weighing from 15 to 25 kg. A model of extracorporeal biological perfusion of the isolated dog thyroid gland was used [6]. In the control group the right lobe of the thyroid gland was removed together with a segment of the carotid artery and perfused after connection to the femoral artery with blood from the same dog, in a thermostatically controlled chamber. By using this approach it was possible to study the effect of nonspecific transplantation factors on the morphology and function of the thyroid gland and to compare it with those of the lobe perfused in situ. In the experimental group, the extirpated lobe of the thyroid gland was subjected to deep freezing before perfusion, which was carried out in the same way, by the method developed at the Institute for Problems in Cryobiology and Cryomedicine, Academy of Sciences of the Uzbek SSR [2]. Saturation of the gland with cryoprotector (a 10% solution of DMSO in Hanks' solution) was carried out by perfusion using a "Peripump" (Hungary) pump; the temperature of the cryoprotector was lowered after 90 min to −2°C. The duration of cryoconservation (−196°C) was between 45 h and 16 days. The organs were thawed in a water bath at 37°C for 5 min. The duration of perfusion in the control and experimental groups was 6 h. Levels of thyroxine and tri-iodothyronine were measured in blood flowing from the lobes perfused in situ and in the constant-temperature chamber by means of commercial kits from "Amersham" and "Corning." At the end of perfusion the thyroid gland tissue was fixed in a 10% solution of neutral formalin and, after histological treatment, sections were stained with hematoxylin and eosin.

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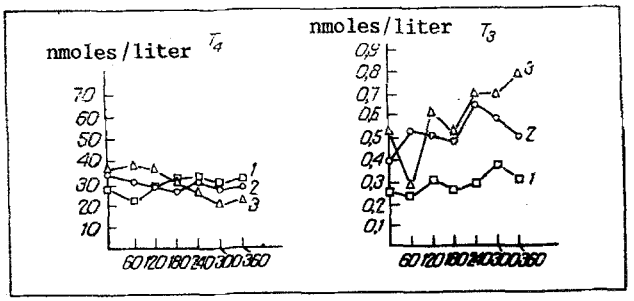

Fig. 1. Thyroid hormone levels in blood flowing from thyroid gland of dogs perfused under different conditions. Abscissa, duration of perfusion (in min); ordinate, hormone level (in nmoles/liter); here and in Fig. 2: 1) in situ; 2) in constant-temperature chamber; 3) in constant-temperature chamber after cryoconservation.

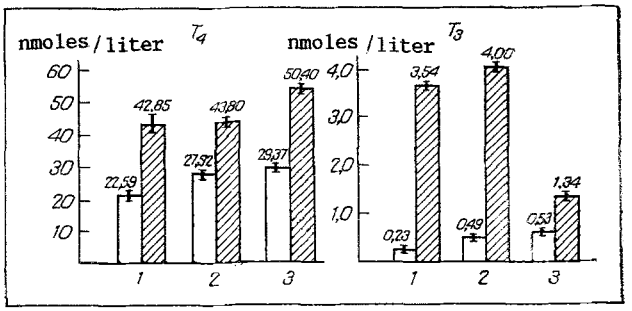

Fig. 2. Effect of TSH on blood thyroid hormone levels under different conditions of perfusion. Unshaded columns – before injection of TSH; shaded columns – after injection of TSH.

EXPERIMENTAL RESULTS

Data on the effect of nonspecific transplantation factors and the conditions of low temperature conservation and thawing on secretion of thyroid hormones are given in Fig. 1. The concentrations of T_3 and T_4 in the two experimental groups were comparable with their values in the control. Some increase was noted in the T_3 concentration in blood flowing from the gland in both experimental groups, and was due to intensification of the conversion of thyroxine into tri-iodithyronine [7]. Potentiation of T_3 secretion cannot be connected with an increase in the blood flow through the gland on account of denervation [1], for it would be logical to expect increased secretion of T_4 also. Absence of elution of hormones from the perfused thyroid gland also was demonstrated previously [6]. The results obtained in vitro on sections of human thyroid gland are evidence of the cryoresistance of the various stages of hormone formation [3-5], which the present experiments confirmed when the integrity of the gland was preserved. Since under conditions of transplantation, when nervous regulation of the thyroid gland was absent and only its humoral regulation remained, it was important to assess the sensitivity of the gland to pituitary thyroid-stimulating hormone (TSH). The results are evidence (Fig. 2) that the thyroid gland, when exposed to nonspecific factors of transplantation and freezing, storage at –196°C, and thawing, remains capable of responding to stimulation by TSH. It must be pointed out that the character of this response was changed: in the cryoconserved thyroid gland, in response to stimulation the increase in the tri-iodothyronine concentration was much smaller than in the control and in the experiments of group 1 (effect of nonspecific factors without cryoconservation), whereas the thyroxine level increased by a greater degree.

The structure of the dogs' thyroid gland after perfusion in situ (control) consisted of follicles of different sizes with dense oxyphilic colloid, partially separating from the walls of the follicle, and with infrequent resorption vacuoles. The thyroid epithelium was

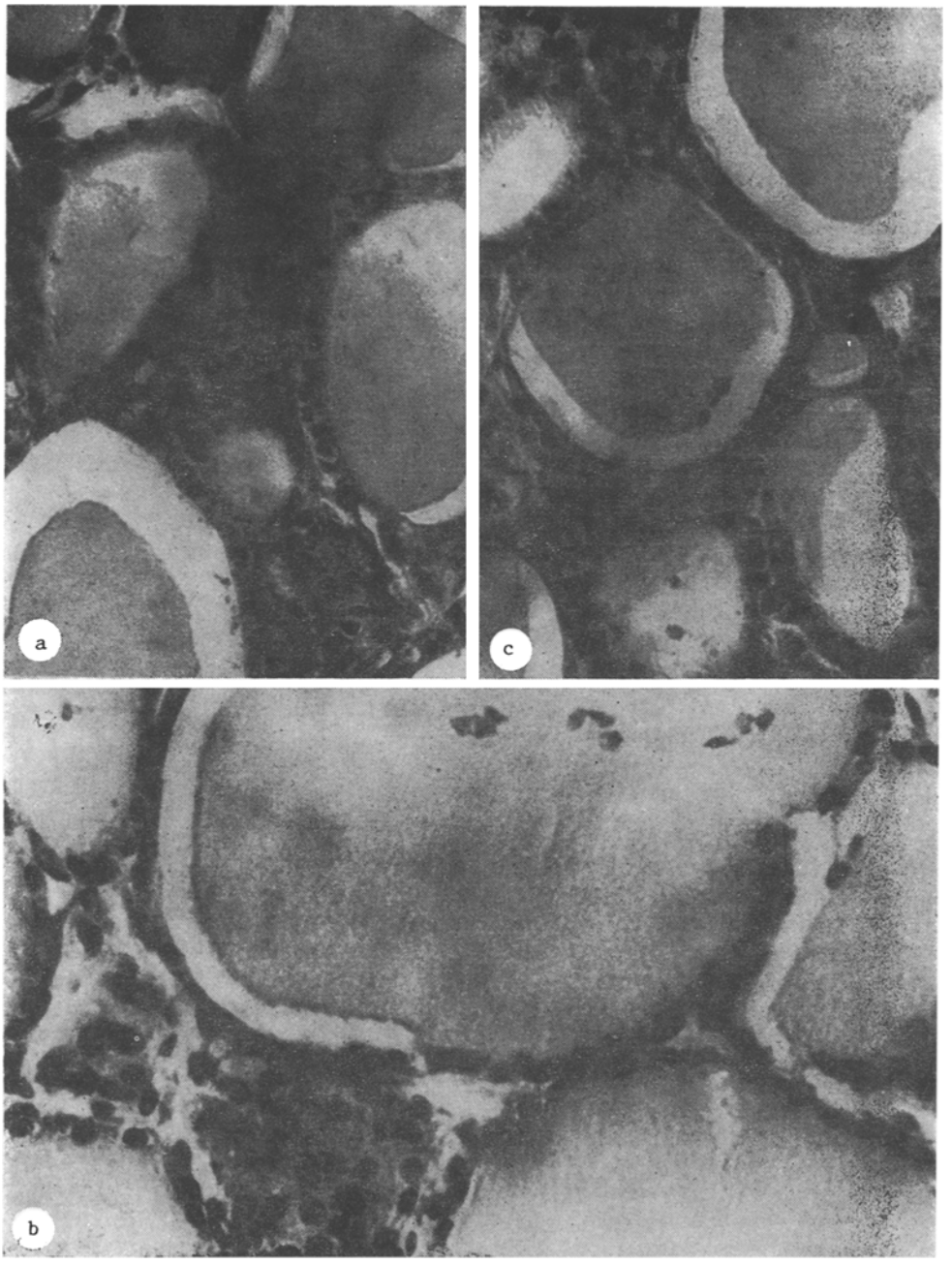

Fig. 3. Morphological picture of dog thyroid gland: a) intact thyroid gland perfused in situ. Here and in Fig. 3b, c) stained with hematoxylin and eosin. 200 ×. b) Structure of canine thyroid gland after perfusion for 6 h in constant-temperature chamber; c) structure of canine thyroid gland perfused in constant-temperature chamber after cryoconservation.

flattened or cubical, the nuclei of the thyrocytes dense and of average size, and in the cubical thyrocytes the nuclei were situated basally. The interfollicular epithelium was found in the intervals between follicles, and regions of proliferation with the formation of young follicles were present (Fig. 3a).

The structure of the thyroid gland after perfusion in the constant-temperature chamber differed only a little from the control. No marked signs of tissue ischemia were found (Fig. 3b). The morphological structure of the thyroid gland perfused in a constant-temperature chamber after cryoconservation and deconservation revealed all the characteristic features of active and viable tissue. Most follicles were lined with cubical epithelium, with

large basal nuclei and with oxyphilic colloid, containing resorption vacuoles of different sizes, and often not filling the whole cavity of the follicles; some empty follicles were seen. No evidence of diapedetic hemorrhage could be seen near the small veins (Fig. 3c).

The results of the biochemical and morphological investigations of the thyroid gland thus demonstrate preservation of the basic functional and morphological characteristics after exposure of the gland to nonspecific factors of transplantation and very low temperatures.

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