teases can induce cell growth in vitro has recently been confirmed experimentally [8, 10].

On the other hand, during blockade of the lysosomes of the macrophages by foreign particles, these cells may lose their ability to form lipid metabolites (of the fatty acid type), albumin–bilirubin complexes, and other substances which, under normal conditions, stimulate hepatocyte proliferation [1].

Inhibition of hepatocyte proliferation during blockade of the Kupffer macrophages of the liver may perhaps be due to a change in the ability of these macrophages to metabolize corticosteroids [9], to a disturbance of the microcirculation in the hepatic sinusoids, and to other causes.

Although the mechanism of the abnormalities of hepatocyte regeneration during blockade of the Kupffer cells are not yet clear, the results described above are certain evidence of the important role of the Kupffer cells in the regulation of reparative regeneration of the liver.

LITERATURE CITED

1. V. A. Konyshev, Patol. Fiziol., No. 4, 86 (1973).
2. N. N. Mayanskaya, V. I. Shcherbakov, L. E. Panin, et al., in: Structure and Functions of the Lysosomes [in Russian], Moscow (1976), pp. 96-98.
3. N. P. Petrovichev, Byull. Éksp. Biol. Med., No. 7, 91 (1975).
4. I. S. Tolmasskii, High-Frequency Magnetic Materials [in Russian], Moscow (1968).
5. L. Desser-Wiest, Cell Tissue Kinet., 8, 1 (1975).
6. I. J. Fabricant, J. Cell Biol., 36, 551 (1968).
7. R. van Furth, Prog. Immunol., 2, 73 (1974).
8. D. A. Hart and J. S. Streilein, Exp. Cell Res., 102, 246 (1976).
9. H. Mekata, Nagoya J. Med. Sci., 34, 89 (1971).
10. Proteases and Biological Control. Conferences on Cell Proliferation, Vol. 2, New York (1975).
11. J. J. Midmann, R. S. Cotran, and H. D. Fahimi, J. Cell Biol., 52, 159 (1973).

EFFECT OF THYROCALCITONIN AND HYPOXIA ON DNA SYNTHESIS IN CONNECTIVE-TISSUE CELLS OF THE REGENERATING SKIN

G. V. Khomullo, T. V. Ivanenko, and V. I. Lotova

UDC 616-003.93:547.963.32:615.357:616-001.8

The effect of thyrocalcitonin (TCT) on proliferative activity of the connective-tissue cells of regenerating skin was studied by [^{3}H]thymidine autoradiography under conditions of a normal and reduced partial pressure of oxygen. Constant saturation of the body with exogenous TCT leads to an increase in the number of cells entering the S period of the mitotic cycle, intensification of DNA synthesis, and considerable dilution of the label during the 24-h period of observation. This may reflect the more rapid passage of the cells through individual stages of the mitotic cycle under conditions of a normal partial pressure of oxygen and hypoxia.

KEY WORDS: regeneration of the skin; thyrocalcitonin; hypoxia, proliferation.

Recent investigations have conclusively shown the activating effect of the thyroid hormone thyrocalcitonin (TCT) on regeneration of bone tissue [4, 5] and skin [1]. This effect can be explained by selective action on the fibroblast population [2, 8].

Department of Biology with General Genetics, Kalinin Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 84, No. 11, pp. 618-621, November, 1977. Original article submitted April 12, 1977.

TABLE 1. Index of Labeled Nuclei of Connective-Tissue Cells (in %; M ± m)

Group	3 h		24 h	
	connective tissue	granulation tissue	connective tissue	granulation tissue
	5 days after operation			
Control TCT	11,70±0,84	9,65±0,95	20,40±1,07	16,80±0,80
	29,50±0,89	22,90±0,76	32,20±0,77	27,40±0,53
P	<0,01	<0,01	<0,01	<0,01
Chronic hypoxia	—	6,85±0,57	—	11,70±0,73
P		<0,20		<0,02
Chronic hypoxia + TCT	19,70±0,87	15,35±0,51	25,30±0,89	18,80±0,67
P	<0,01	<0,02	<0,10	<0,35
	10 days after operation			
Control TCT	13,85±0,76	24,85±1,01	18,10±1,14	25,55±0,76
	20,40±0,91	17,35±0,70	25,00±0,62	20,35±0,78
P	<0,01	<0,01	<0,01	<0,02
Chronic hypoxia	16,70±0,84	13,70±0,69	20,50±0,49	18,65±0,63
P	<0,20	<0,51	<0,30	<0,01
Chronic hypoxia + TCT	21,50±0,68	23,35±0,77	22,85±0,80	25,25±0,67
P	<0,01	<0,55	<0,10	<0,90

TABLE 2. Intensity of Labeling of Nuclei in Fibroblasts of Granulation Tissue (M ± m)

Group	5 days after operation		10 days after operation	
	3 h	24 h	3 h	24 h
Control TCT	32,10±3,10	29,95±2,20	18,80±1,40	18,40±1,80
	49,90±2,30	34,60±2,60	57,95±3,30	30,20±2,90
P	<0,01	<0,01	<0,01	<0,30
Chronic hypoxia	20,45±2,10	20,15±1,60	23,60±1,70	17,25±1,40
P	<0,01	<0,01	<0,01	<0,01
Chronic hypoxia + TCT	37,50±2,30	25,50±2,20	30,15±4,90	20,40±1,70
P	<0,01	<0,01	<0,01	<0,10

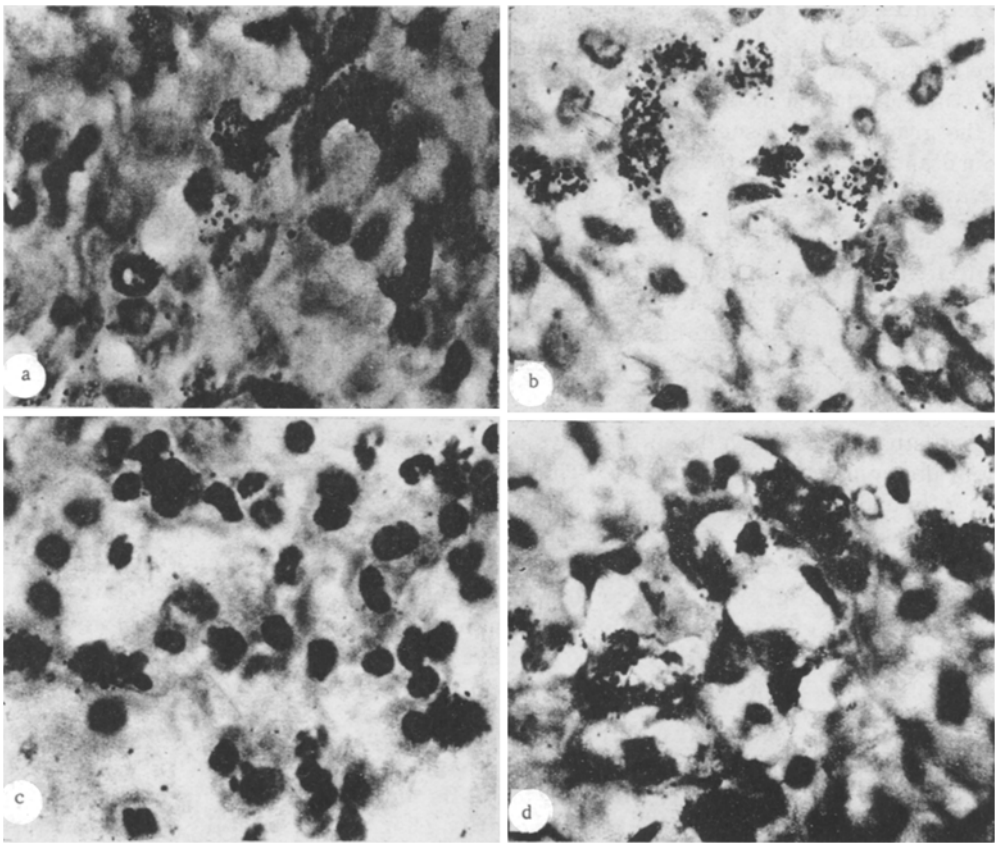

Fig. 1. Incorporation of [^{3}H]thymidine into cell nuclei of granulation tissue 5 days after operation (24 h after injection of isotope): a) control; b) injection of TCT; 3) chronic hypoxia; d) chronic hypoxia and injection of TCT. Hematoxylin, 1100×.

The object of this experimental investigation was to study the effect of TCT, by [^{3}H]thymidine autoradiography, on proliferative activity of the connective-tissue cells of healing wounds under normoxic conditions and in hypoxia, sharply inhibiting the process of regeneration in the skin [3, 6, 7].

EXPERIMENTAL METHOD

Four series of experiments were carried out on 80 male guinea pigs: I) control, II) injection of TCT, III) chronic hypoxia, IV) chronic hypoxia plus injection of TCT. Full-thickness skin wounds with an area of 225 mm^2 were inflicted on all the animals on the left lateral surface of the body. TCT (USSR product) was injected intraperitoneally in a dose of 5 units per animal daily throughout the period of investigation (15 days). The state of chronic hypoxia was produced in a pressure chamber. The parameters of hypoxia were: pressure 250 mm Hg, exposure 8-10 h/day. During healing of the wound the area of the open wound surface was measured daily, biopsy material was taken from the wound edges 5, 10, and 15 days after the operation, and sections were stained with hematoxylin–eosin. The number of fibroblasts in the surface layers of the granulation tissue was counted per field of vision of the microscope (magnification 900 times). [^{3}H]Thymidine with a specific activity of 1.4 Ci/mmole was injected intraperitoneally in a dose of 1 μCi/g body weight 3 and 24 h before decapitation. The animals were killed 5 and 10 days after wounding. Autoradiographs were prepared with type M emulsion. The index of labeled nuclei (ILN) was calculated in percent for cells of the central and peripheral parts of the wound. The intensity of labeling (IL) above the nuclei of the fibroblasts was determined by counting the number of grains of silver visible.

EXPERIMENTAL RESULTS

After injection of TCT the duration of the individual stages of reparative regeneration of the skin was reduced. The sharp acceleration of regenerative changes as a result of activation of cells of the macrophage system led to the earlier formation of granulation tissue. This tissue was characterized by its considerable vascularization, its abundance, and the polymorphism of its cells and fibrous structures. The intensive fibroblast reaction was manifested as an increase in the number of fibroblasts (18.24 $\pm$ 0.63 and 53.92 $\pm$ 1.33 per fields of vision 5 and 10 days after the operation compared with 9.04 $\pm$ 0.50 and 19.14 $\pm$ 0.61 per field of vision in the control), the many processes given off by the cells, and the presence of a large, well-stained nucleus with two nucleoli. Differentiation of the tissue cells took place more rapidly, thereby facilitating the earlier differentiation of the granulation tissue. TCT favored growth of the epithelium on account of the marked contraction of the wound associated with the rapidly formed layer of horizontal fibroblasts. Final healing of the defects took place after 11 days, i.e., 2-3 days sooner than in the control.

A study of the autoradiographs of the animals receiving TCT showed that after 5 days the highest intensity of incorporation of label was into fibroblasts of the peripheral part of the wound (Table 1). IL reached a maximum after 24 h when it was twice the control level. The percentage of DNA-synthesizing cells in the granulation tissue also was much higher than in the control. Besides nuclei of the fibroblasts, nuclei of the adventitial and endothelial cells and, less frequently, of histiocytes, polyblasts, and fat cells, also were radioactive (Fig. 1a, b).

The subsequent study of DNA synthesis, 10 days after the operation, revealed a marked decrease in proliferative activity of the connective-tissue cells. This dynamics of ILN was the result of the more rapid differentiation of the cells and maturation of the granulation tissue under the influence of TCT. The intensity of incorporation of the isotope into cells of the peripheral part of the wound was unchanged in the control animals during this same period of the investigation, whereas in the granulation tissue, on the other hand, it reached maximal values. The number of grains of silver above the fibroblasts of the granulation tissue of the rats receiving TCT was considerably greater than the corresponding number of the control (Table 2). During the 24-h period the intensity of labeling fell sharply (by 30% 5 days and by 52% 10 days after the operation), as a result of its dilution during subsequent divisions, whereas in the control it changed only very slightly.

Prolonged exposure to the extremal factor inhibited the development of regeneration and the formation of granulation tissue, which was distinguished by plasmorrhagia, multiple hemorrhages, and destructive and degenerative changes of increasing severity affecting the cells and the newly formed vessels and fibrous structures. Weak contraction of the wound edges and degenerative changes in the cells of the regenerating epithelium led to delayed epithelization of the experimental wounds (after 15 days the area of the wound in the rats of this series was 48.6 $\pm$ 2.0 mm^2).

Under the influence of chronic hypoxia, marked inhibition of formation of the regenerating structures was accompanied by weakening of the ability of the cells to take up the isotope. Under hypoxic conditions no con-

nective tissue was formed 5 days after the operation. The granulation tissue developing in the peripheral parts of the wound contained a few radioactive nuclei (Fig. 1). Among the cells incorporating the isotope, most were undifferentiated adventitial cells, and labeled fibroblasts were infrequently seen.

In the later stages of repair (10 days) the percentage of labeled nuclei of the connective-tissue cells increased a little compared with the previous time, but it remained significantly below the control value. The principal cells incorporating the label were endothelial cells; as before the number of labeled fibroblasts was small.

The ILN was considerably lower than in the control; the absence of change in these indices during the 24-h period of observation 5 days after the operation is evidence that further dilution of the label had ceased and it evidently means that under the influence of hypoxia, passage of the cells through all or some of the phases of the mitotic cycle was sharply delayed. Subsequent determination of ILN (10 days) revealed a very small increase 3 h after injection of the isotope and some decrease 24 h after injection, probably indicating the development of adaptation to the action of hypoxia.

Injection of TCT against the background of chronic hypoxia largely restored the normal course of post-traumatic regeneration, the positive effect of the hormone was most clearly manifested in the growth and development of the connective-tissue basis of the skin. The granulation tissue was well vascularized, it contained no hemorrhages, and the vessels themselves were moderately filled with blood but were not as dilated as in the hypoxic animals. The granulations consisted of a variety of cells of hematogenous and tissue origin, a mass of ground substance, and collagen and elastic fibers. Under these conditions there was an increase in the number of fibroblasts in the granulation tissue (12.16 ± 0.48 and 15.32 ± 0.41 per field of vision after 5 and 10 days compared with 5.20 ± 0.44 and 10.82 ± 0.62 per field of vision in hypoxia. Destructive and degenerative changes were reduced in the cells of the regenerating epithelium, so that healing of the wounds could take place more rapidly. Towards the end of the investigation (15 days) only a small (25.6 ± 1.9 mm^2) area of the wound surface still remained under the scab.

Injection of TCT considerably increased ILN in the granulation tissue of rats exposed to hypoxia at all stages of healing. Characteristically, the percentage of radioactive nuclei not only was greater than in the animals of the hypoxia series, but it was also higher than in the control. The dynamics of IL for the connective-tissue cells mainly followed the rule discovered for the control rats. The cells of the fibroblastic series were the most numerous of the labeled cells (Fig. 1d).

The period of maximal values of IL in the animals of this series occurred 5 days after the operation. The marked decrease in concentration of grains of silver above the labeled nuclei during a 24-h period of investigation both 5 and 10 days after the operation must be stressed.

It can be concluded from these findings that TCT stimulates the processes of proliferation of connective-tissue cells during the healing of skin wounds under the conditions of both a normal and a reduced partial pressure of oxygen. The validity of this conclusion is confirmed by the following facts: a considerable increase in the number of cells entering the S period of the mitotic cycle during constant saturation of the body with exogenous TCT, intensification of DNA synthesis in the fibroblast nuclei, and considerable dilution of the label during the 24-h period of observation, which reflects the more rapid passage of the cell through individual stages of the reproductive cycle.

LITERATURE CITED

1. T. V. Ivanenko and V. I. Lotova, in: Thyrocalcitonin and Reparative Regeneration of Tissues under Experimental and Clinical Conditions [in Russian], Moscow (1974), pp. 50-55.
2. T. V. Ivanenko, in: Thyrocalcitonin and Reparative Regeneration of Tissues under Experimental and Clinical Conditions [in Russian], Moscow (1974), pp. 56-61.
3. V. I. Lotova, "Special Features of regeneration in the skin during hypoxia and supplementary administration of STH," Candidate's Dissertation, Kalinin (1974).
4. G. V. Khomullo, A. I. Briskin, I. Kh. Khusid, et al., Abstracts of Proceedings of the Second Zonal Inter-Institute Scientific Conference on Regeneration and Transplantation of Mammalian Organs and Tissues [in Russian], Erevan (1973), pp. 108-109.
5. G. V. Khomullo, in: Thyrocalcitonin and Reparative Regeneration of Tissues under Experimental and Clinical Conditions [in Russian], Moscow (1974), pp. 11-16.
6. G. V. Khomullo and V. I. Lotova, Byull. Éksp. Biol. Med., No. 1, 64 (1975).
7. G. V. Khomullo, T. V. Ivanenko, and A. N. Chernyaev, Kosmich. Biol., No. 2, 68 (1976).
8. A. Lupulescu, J. Morphol., 142, 447 (1974).