9. V. A. Shakhlamov, Capillaries [in Russian], Moscow (1971).
10. C. K. Drinker, The Clinical Physiology of the Lungs, Springfield (1954).
11. M. J. Oyarzum and J. A. Clements, Am. Rev. Resp. Dis., 3, No. 5, 879 (1978).

INCORPORATION OF ^{3}H-THYMIDINE BY INTERSTITIAL CELLS OF THE RAT MYOCARDIUM AFTER A SINGLE INJECTION OF THE LABEL

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An important role in cardiac function is played by interaction between cardiomyocytes and connective tissue, for the latter not only forms a supportive carcass, but also mediates the metabolic processes of the cardiomyocytes. It is accordingly interesting to study the character of renewal of connective-tissue cells in the heart. We know that if a single injection of ^{3}H-thymidine is given to an animal, the precursors of different types of connective-tissue cells require 2-4 days in order to divide, migrate into the organ, and specialize [4, 5]. The kinetics of connective-tissue cells incorporating ^{3}H-thymidine was found to be similar for different organs. Maximal incorporation of label is observed on the 4th day after a single injection, and the level of labeling thereafter falls for 1-2 weeks [7, 15]. However, despite the similar time course of labeled connective-tissue cells, the highest percentage of them in different organs and under different conditions of testing, differed considerably, possible evidence of a difference in the degree of renewal of the connective-tissue, and a sign of organ specificity. The heart has not been investigated deliberately from this point of view, and the investigation described below as accordingly carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Wistar rats weighing 60-115 g. The animals were given a single intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 1 TBq/mmole). Thoracotomy was performed on the animals under pentobarbital anesthesia (0.05 mg/g) 1, 2, 3, 4, 5, 6, 7, 14, and 21 days later and the heart was perfused with Hanks' solution and then with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 10-15 min. The heart was then removed. A lamina 1 mm thick was excised from the middle third of the left ventricle, perpendicularly to its axis. The laminae were fixed in 1% osmium tetroxide solution in phosphate buffer, and them embedded in Epon by the standard method. Three blocks 0.5 mm wide were excised from the middle third of the lamina and semithin sections cut from them through the whole thickness of the wall of the left ventricle. The sections were covered with type M emulsion and exposed at 4°C for 2 weeks. After development, the sections were stained with methylene blue. Cells were considered to be labeled if there were 7 grains of silver or more above the nucleus. The background level was 1-4 grains per cell. The percentage of labeled cells on the whole surface of the section and separately for each layer of the myocardium was counted. The number of cells counted each time was 2500-6000. The criterion for isolation of a layer was the direction of the muscle fibers and the connective-tissue bands containing large blood vessels. According to these features three layers were distinguished: subendocardial, subepicardial, and middle. The numerical results were subjected to statistical analysis [8].

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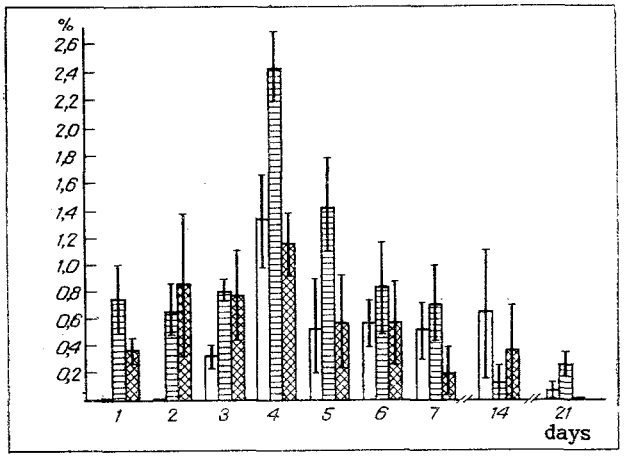

Fig. 1. Time course of incorporation of label by interstitial cells in different layers of myocardium after a single injection of ^{3}H-thymidine. Unshaded columns – subendocardial layer of heart wall, horizontally shaded – middle layer of myocardium, cross-hatched – subepicardial layer of myocardium (M ± m).

EXPERIMENTAL RESULTS

The fraction of labeled cells in sections obtained from different animals at the same time of investigation after a single injection of the label was statistically homogeneous. The label was incorporated after the 1st day (0.40 ± 0.08%) and the greatest number of labeled nuclei was found on the 4th day (1.66 ± 0.1%), and this was followed by a decrease in the content of label until the 7th day, almost to its initial level (0.51 ± 0.1%). Later, on the 14th and 21st days, very few labeled cells were found (0.30 ± 0.09 and 0.01 ± 0.14%); most frequently these were cells which incorporated the label intensively. Thus the time course of ^{3}H-thymidine incorporation into the nuclei of the connective tissue cells did not differ from that described in the literature for other organs [5, 10]. The fact will be noted that the intensity of labeling of the connective-tissue cells of the intact myocardium was significantly lower than that stated for the subcutaneous connective tissue. Lange and Khrushchov found about 20% of labeled cells in normal subcutaneous connective tissue on the 4th day after a single injection of ^{3}H-thymidine, the initial level being about 2%. A low level of labeling index of the interstitial tissues of the myocardium also was observed by previous investigators [13]. It can be assumed that the organization of renewal of the myocardial interstitial cells obeys the same rules as that of other organs, but the intensity of renewal is lower.

In recent years attention has been paid by research workers to the study of different layers of the myocardium. Different layers have been shown to have different metabolic characteristics [11]. Different layers of the heart wall probably play different roles in the mechanism of systole, and as a result, cardiomyocytes in different layers differ from one another in size and ratio with capillaries, and also in the quantity of work done [1, 2, 9, 12].

All the differences mentioned above presuppose a different character of organization of the responsible interstitial cells. Under normal conditions the architectonics of the connective-tissue carcass of the heart is similar in all its parts [3], but the quantitative and qualitative (type of fibers) distribution of connective tissue in different parts of the heart differs significantly [1, 9]. It is accordingly interesting to study the characteristics of labeling in connective-tissue cells in different layers of the myocardium. At the maximum of labeling (4th day) the subendocardial and subpericardial layers had almost identical labeling indices (1.36 ± 0.36 and 1.16 ± 0.24%), the corresponding values for the middle layer being 2.44 ± 0.24% of cells ($p < 0.05$). Analysis of the kinetics of incorporation of label by cells in different layers revealed the same pattern as for the myocardium as a whole. However, there were also certain differences (Fig. 1). Labeled cells appeared in the subendocardial layer only on the 3rd day after injection of ^{3}H-thymidine, whereas in the other cases, they appeared with effect from the 1st day. At subsequent times of the

investigation, on the 14th and 21st days, the labeling index varied considerably both in different animals and in different layers, the aggregated value of this parameter being significantly low. Meanwhile the intensity of labeling in the nuclei as a rule was high, i.e., there were most probably cells which had passed through not more than one or two divisions or cells which had not divided even once after incorporation of the label [6]. These cells were few in number, they were found not in every preparation, and not in every animal. A phenomenon of this kind can be regarded as the result of the existence of two populations of fibroblasts, differing in proliferative activity and characterized by faster renewal in one subpopulation and slower renewal in the other. These data are in agreement with the view published in the literature in histogenesis of connective-tissue cells [5, 7, 10, 14].

The following conclusions can thus be drawn from this investigation. The intensity of renewal of connective-tissue cels is lower in the intact myocardium than in other organs. The time course of labeled cells at different times after a single injection of ^{3}H-thymidine, in the myocardium coincides with data in the literature for other organs, i.e., renewal of connective-tissue cells exists on account of precursor cells migrating into the organ. The intensity of incorporation of the label in different layers of the heart wall differs. It is highest in the middle layer of the myocardium. The character of the time course of labeled cells in different layers suggests asynchronous renewal of connective-tissue cells in these layers, possibly due to the different functional loads carried by the layers during contraction.

LITERATURE CITED

1. G. G. Avtandilov and G. A. Gevondyan, Arkh. Anat., No. 7, 33 (1980).
2. L. I. Gabain and É. A. Adyshirin-Zade, Arkh. Anat., No. 12, 37 (1986).
3. L. D. Krymskii, Arkh. Anat., No. 2, 53 (1956).
4. M. A. Lange and N. G. Khrushchov, Arkh. Anat., No. 6, 93 (1977).
5. M. Lange and N. G. Khrushchov, Zh. Obshch. Biol., No. 5, 752 (1973).
6. G. E. Onishchenko, Yu. S. Chentsov, and A. B. Iordanskii, Tsitologiya, No. 2, 233 (1972).
7. G. P. Satdykova, "Fibroblast-like cells in a focus of aseptic inflammation," Author's abstract of dissertation for the degree of Candidate of Biological Sciences [in Russian], Moscow (1974).
8. R. B. Strelkov, An Express Method of Statistical Analysis of Experimental and Clinical Data [in Russian], Moscow (1986).
9. V. A. Fedoseev and T. V. Pistsova, Byull. Éksp. Biol. Med., No. 9, 349 (1985).
10. N. G. Khrushchov, Histogenesis of Connective Tissue [in Russian], Moscow (1976).
11. M. Aomin, M. Arita, S. Imanishi, et al., Jpn. J. Physiol., 32, 895 (1982).
12. Y. Boucher, S. Roberge, and P.-E. Roy, Microvasc. Res., 29, 305 (1985).
13. F. Cluzeaut and B. Maurer-Schultze, Cell Tissue Kinet., 19, 267 (1986).
14. B. E. Hull, S. E. Sher, S. Rosen, et al., J. Invest. Dermatol., 81, 436 (1983).
15. Z. F. G. Jaworski, B. Duck, and G. Sekaly, J. Anat. (London), 133, 397 (1981).