ON THE MECHANISM OF INCREASING THE ACTIVITY OF LIPOLYTIC ENZYMES UPON THE INJECTION OF LIPIDS INTO TISSUES

(UDC 612.397.3 : 612.397.8)

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Translated from Byulleten' Éksperimental'noi Biologii Meditsiny, Vol. 61, No. 5, pp. 46-51, May, 1966
Original article submitted June 30, 1964

In our previous works, we demonstrated an increase in the lipolytic enzymes—aliesterase and tween-lipase—in the organs and tissues after excess intake of lipids with the food, as well as the appearance of cells containing lipolytic enzymes in the loose connective tissue after local injection of lipids [2, 4]. In view of this, it was of interest to determine the nature of the increase in the activity of lipolytic enzymes under these conditions. We had to determine, in particular, whether this activity is associated only with possible changes in the enzymatic kinetics (elimination of inhibiting factors or appearance of activating factors) or whether it is associated with a true increase in the enzyme synthesis by the corresponding cells, which was induced by substrates – lipids.

Having established a relationship between the induced synthesis of enzymes and the genetic apparatus of the cell, Monod, Jacob, Pardee [7], and others advanced the generally recognized thesis of the role of an unstable mediator – messenger RNA – in the process of protein synthesis. It is known that in the cells of the animal organism, which usually synthesizes the same proteins (i.e., in highly specialized cells, existing under relatively constant conditions), the inhibition of new formation of RNA for several hours and even days has no effect upon protein synthesis. Evidently the messenger and ribosomal RNA already formed is more stable with time in animals than in bacteria; and yet, the process of substrate induction of protein synthesis, including enzymes, is inhibited after blockage of RNA synthesis in the cells of the animal organism just as in bacteria. Thus, a necessary condition for substrate-induced protein synthesis is the synthesis of messenger RNA and DNA.

It is known that the foreign anticancer antibiotic actinomycin D and aurantine, produced in our country at the Laboratory headed by Kh. Kh. Planel'es, entirely block the production of messenger RNA and DNA [1, 6, 9].

We used aurantine in this work to resolve the question of whether we are dealing with activation or induction of the above-mentioned enzymes after the injection of lipids.

EXPERIMENTAL METHOD

The experiments were conducted on 30 white mice weighing 12-13 g, 15 of which received injections under the skin of the back of 0.5 ml of a 10% cholesterol solution in sunflower seed oil, and directly after that, into the hole formed after injection of the lipid, 5 μg of aurantine in 1 ml of physiological saline. The control animals received cholesterol in oil under the same conditions, and instead of aurantine, the same amount of physiological saline. On the following ten days, the mice received daily subcutaneous injections in the femoral region of 5 μg of aurantine in 1 ml of physiological saline (experimental group), and the same amount of physiological saline (control).

After ten days of the experiment, the mice were killed by decapitation. In addition to the animals of this basic group, in a preliminary experiment 5 experimental and 5 control mice, subjected to the same influences, were killed six days after the beginning of the experiment.

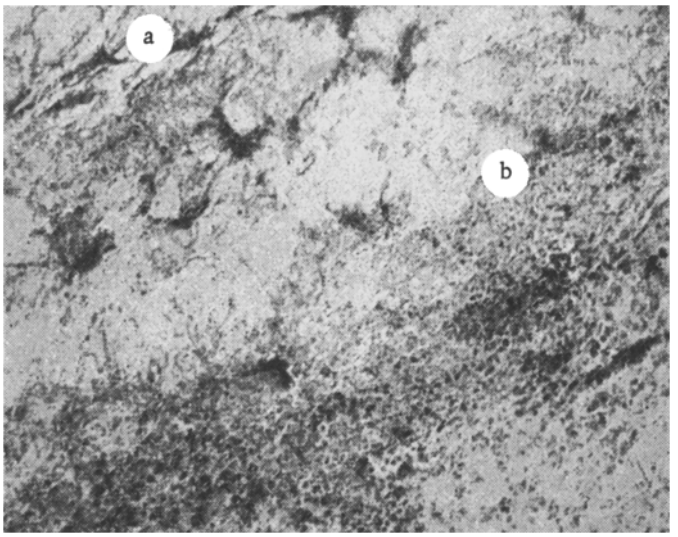

Fig. 1. Weak diffusion of aliesterase activity in connective tissue cells (b) surrounding a lipid conglomerate (a). Experimental animal (blockage of synthesis of messenger RNA). Here and in Figs. 2 and 3: Nachlas-Seligman reaction in the Gomori modification. Differentiation with eserine in a 10^{-5} M concentration. Ob. 8, oc. 7,

The material was fixed in a preliminarily chilled 10% solution of neutral formalin in a refrigerator for 18 h. The histochemical treatment included staining for total lipids with sudan IV, staining for cholesterol and its esters according to Schultz and Vindaus, and polarization microscopy to detect birefringent lipids. Nonspecific esterase was determined according to Nachlas-Seligman with differentiation of cholinesterase by its inhibitor eserine in a 10^{-5} M concentration and aliesterase by its organophosphorus inhibitor E600 in a 1:6000 dilution. Lipase was determined according to the Gomori-Mark reaction with the substrate tween-80.

RESULTS

In the autopsy of the animals of the basic group, attention was paid to the greater size of the lipid cavity in the experimental animals than in the controls. The volume of the cavity containing cholesterol in oil was an average of 1.1 ml in the experimental mice and about 0.7 ml in the controls. In the experimental animals, when the cavity was cut open, the oil was of the same consistency as when it was injected (it had only become somewhat turbid), while in the control the liquid was denser, with a pearly white deposit of cholesterol in the wall of the cavity and its lumen.

In the animals of both groups, the wall of the cavity was soft, almost transparent, while in the experimental mice it was flaccid and easily torn. In a histological investigation of the experimental animals, the capsule of the granuloma was indistinct and represented macrophages scattered in the edematous stroma, individual adipose cells, as well as polymorphonuclear leukocytes, frequently with signs of decomposition, predominating in the histological picture. The cytoplasm of the macrophages was frequently vacuolized, apparently vacant, without appreciable basophilia. Practically no fibroblasts were detected in the capsule of the adipose vesicle. In the lumen of the cavity, numerous small and larger drops of fat (frequently surrounded along the periphery by individual small macrophage cells) and leukocytic detritus were visible. Cholesterol crystals in the lumen of the cavity were few; they were not found in the wall.

In an investigation of esterase, together with the noticeable enzyme activity in the muscle fibers adjoining the lipid granuloma (neuromuscular spindles, rich in acetylcholinesterase, were brightly stained there) and the active reaction for lipolytic enzymes in the skin and subcutaneous adipose tissue, an almost total absence of the corresponding reactions in the elements forming the capsule of the lipid granuloma could be noted. Only in individual portions, adjoining the lipid conglomerate, were there groups of macrophage cells, weakly positively stained in the detection of esterase (Fig. 1).

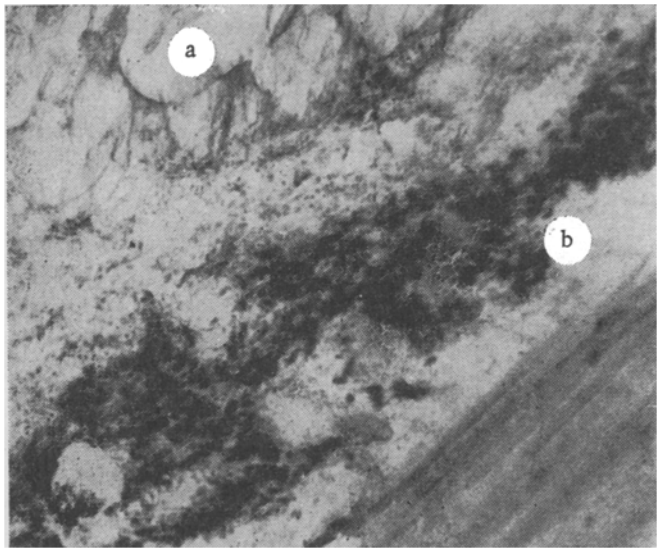

Fig. 2. High activity of aliesterase (dark lumps and spots) in the cellular zone (b) surrounding the injected lipid (a). Control animal.

The activity of tween-lipase – lumps of a deposit of lead sulfide in the cells surrounding the lipid conglomerate – was somewhat more distinctly represented in these animals than the esterase activity. The small number of these lumps was striking.

In the mice of the control group, a pronounced capsule of the lipid sac, where together with numerous polymorphonuclear leukocytes, well-expressed macrophages and gigantic cells of foreign bodies, forming an inner granuloma belt, was detected at the same period as the experiment. In the granuloma belt, numerous precipitated (probably as a result of the more rapid cleavage and assimilation of the solvent oil) cholesterol crystals were visible, usually included in the cytoplasm of the gigantic cells. These cells possessed a maximum activity both of esterase and of lipase (Figs. 2 and 3). High lipolytic activity was also exhibited by the macrophage cells, represented in the middle granuloma belt. In the central zone there was a substantial number of adipose cells. Finally, in the outer zone, where proliferation of the fibroblasts was observed, high activity of nonspecific esterase was noted. However, when the organophosphorus inhibitor E600 was used for differentiation (in a 1:6000 dilution), it was found that the enzyme is not lipolytic – aliesterase, but proteolytic, the so-called esterase C (this enzyme is close to cathepsin [9]).

Differentiation of nonspecific esterase in the macrophage elements and gigantic cells indicated (according to the results of the reaction with E600 and eserine) that this enzyme is lipolytic – aliesterase – in the mice of the experimental group and in the controls. In the animals of the control group, a substantial increase in the activity of another lipolytic enzyme was also noted – tween-lipase, which was expressed in the appearance of numerous large, brightly stained, fused lumps of lead sulfide, both in the lumen of the cavity and in its wall.

The data of a count of the macrophage cells containing aliesterase in the cytoplasm (a count of 500 macrophage cells in each experimental and control animal) were the following: control 399.0 ± 13.26, experimental 13.3 ± 1.18. These differences are statistically significant ($P < 0.005$). On account of the coarse deposits of the final reaction product, which do not coincide with the boundaries of the cells, in the determination of lipase this reaction is not subjected to a quantitative consideration. However, a visual comparison revealed a sharp intensification of this reaction also among the animals of the control group.

Analogous results were also obtained in a histochemical study of the material of a preliminary experiment (mice, killed after six days of the experiment): of five control animals, activation of the lipolytic enzymes in the macrophage cells of the capsule of the oil sac was noted in four; in the experimental group, in not one mouse was active aliesterase detected in the macrophages of the cholesterol granuloma.

Thus, blockage of the synthesis of messenger RNA on DNA, induced by aurantine, substantially reduced the activation of lipolytic enzymes in the connective tissue cells in response to the injection of lipids into the latter.

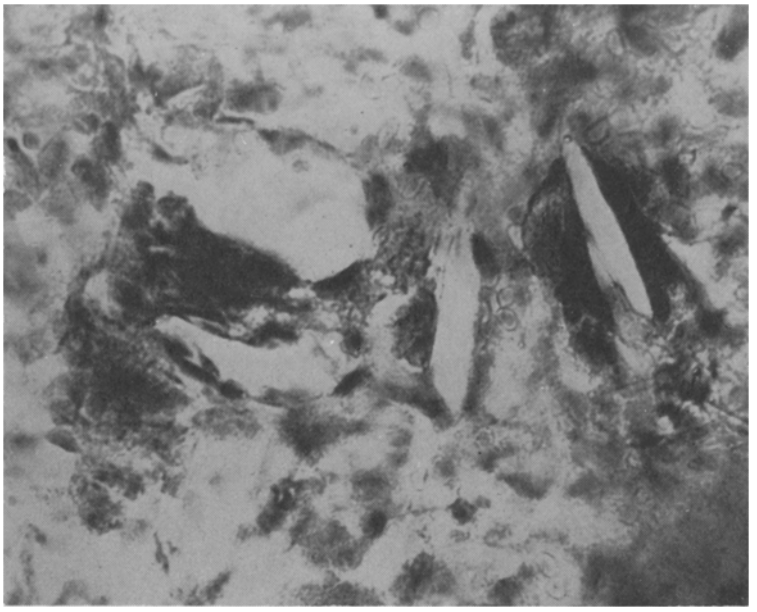

Fig. 3. High activity of aliesterase in gigantic cells of foreign bodies, phagocytizing cholesterol crystals (they look like needle-shaped cavities). Control animal. Ob. 40, oc. 15.

This is evidence that the mechanism of the intensification of the activity of lipolytic enzymes in response to the injection of lipids is associated not only with a possible change in the enzyme activity, but also with substrate-induced synthesis of enzyme molecules.

The fact that the lipolytic enzymes belong to the group of inducible enzymes is also emphasized by the substantial effect of endocrine and neurogenic influences upon their activity [3], which recently has also been demonstrated for the phenomenon of induction of other enzymes. Thus, Huebner [5] indicates an influence of corticosterone upon the substrate induction of tyrosine transaminase, while Kenney and Kull [8] indicate an increase in the synthesis of glutamine-alanine transaminase under the influence of hydrocortisone.

The data cited in the inducibility of lipolytic enzymes once more demonstrate their high adaptive properties. This indicates an ability of this enzyme system to adapt to the requirements of the organism through a substantial and flexible variation of synthesis.

SUMMARY

Blocking of the synthesis of information RNA on DNA with the antibiotic aurantine considerably reduces the activation of the lipolytic enzymes in the connective tissue cells in response to injection of lipids. This is evidence that a substrate-induced synthesis of enzymes takes part in the mechanism of intensification of the activity of lipolytic enzymes in response to injection of lipids.

Data on the inducibility of lipolytic enzymes demonstrate their high adaptational capacity, i.e., the ability of a given enzymatic system to adapt itself to the body requirements by means of extensive and flexible variations in synthesis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
