HISTOCHEMICAL PECULIARITIES OF CARBOHYDRATE METABOLISM IN EXPERIMENTAL HYPOTHYROIDISM

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Despite the huge number of works devoted to investigating the pathologically reduced carbohydrate metabolism in hypothyroidism, many aspects of this problem remain inadequately studied. Among these are the questions pertaining to distribution of the polysaccharides that accumulate in the organs and tissues in association with hypothyroidism. The majority of histochemical investigations that have been carried out on this subject have been devoted to studying the skin in generalized and focal (pretibial) myxedema [1, 6, 9, 19, 17, 23 et al). The authors pointed out the presence of acid mucopolysaccharides in the skin during myxedema, chiefly hyaluronic acid.

The polysaccharide composition of the internal organs in hypothyroidism has been investigated histochemically (and even biochemically) to a lesser degree. The small number of works that exist on this subject contain contradictory data. Thus, Dziewiatkowski [13] found that in experimental hypothyroidism there was a decrease in the incorporation of tagged sulfur into the sulfmucopolysaccharides of the connective tissue, as compared with a control, and regarded a disturbance in the sulfating of acid mucopolysaccharides as a characteristic sign of this pathological condition. In contrast to this, Kowalewski [15] showed an elevation in the incorporation of S^{35} into the mucopolysaccharides of the connective tissue during hypothyroidism in rats.

The work of Speranza and Pennisi [21] also describes an elevation in the blood concentration of carbohydrate compounds containing sulfur during hypothyroidism.

The opinion of scientists also diverges on the question of the accumulation of glycogen in the internal organs during hypothyroidism. Thus, N. A. Isichenko [5] observed an accumulation of glycogen in the liver in association with hypothyroidism caused by methylthiouracil, while O. N. Ganzha [3], under the same conditions, found a slight lowering of its content. A number of investigators [7, 8, 20 et al] describe an elevation in the glycogen concentration within the muscle fibers of the heart associated with hypothyroidism. On the other hand, different authors regard the accumulation of PAS positive material in the muscle fibers of the myocardium during hypothyroidism as "mucinous basophilic degeneration".

In connection with the confusion and ambiguities that exist on the question of polysaccharide accumulation in the internal organs during hypothyroidism, we considered it of interest to carry out a histochemical analysis of the polysaccharides in the internal organs during this pathological condition, using contemporary histochemical methods [2, 16, 18, 22].

EXPERIMENTAL METHOD

Hypothyroidism was caused in 25 male white rats, weighing 150-180 grams, by daily administration of 30 mg of the antithyroid substance, 6-methylthiouracil, in the form of a suspension in 5 ml of water; the material was introduced into the stomach (with the help of a syringe provided with an olive), and the procedure was continued for a period of one month. At the end of that time, the animals were sacrificed by etherization and the internal organs were fixed by the method of Lillie and Shabadash and imbeded in paraffin. In addition to the usual histological methods (staining with hematoxylin- eosin, by van Guisen, for fat with sudan black B, etc.), we applied the following histochemical reactions: staining according to Shabadash, with differentiation of the glycogen by use of

salivary amylase (also used for the determination of neutral mucopolysaccharides and mucoproteids, i.e., like the PAS reaction); staining with toluidine blue, with differentiation through the use of alcohols and testicular and streptococcal hyaluronidase; the reaction of methylene blue binding, with different pH's of the medium; the Hale reaction; the Spicer reaction, with methylation and demethylation for delineating sulfated and nonsulfated acid mucopolysaccharides. The histochemical changes observed were compared with the same features in 10 healthy animals of the same age and weight, kept under identical food and maintenance conditions, and given daily administrations of 5 ml of tap water into the stomach over the course of one month.

EXPERIMENTAL RESULTS

As a result of the investigation, major changes were found in the concentration of glycogen and mucopolysaccharides within the internal organs.

Fig. 1. Unequal coarse accumulation of glycogen in the muscle fibers of the heart during hypothyroidism. Microphoto. The reaction of Shabadash. Ocul. 10, obj. 40.

Thus, an appreciable collection of glycogen was noted in the heart, within the muscle fibers, distributed in an opposite manner to that seen in the control material – not in the form of thin rods under the sarcolemma, but completely filling the girth of the fibers. These coarse accumulations of glycogen were arranged in the form of foci, unequally scattered in cross section of the heart, involving 5-10 somewhat hypertrophied muscle fibers. These foci were predominantly localized on the right side of the heart (Fig. 1). Outside these foci, the structure of the muscle fibers was unchanged, and the glycogen was distributed in the same fashion as in the control material, i.e., as thin rods under the sarcolemma or, occasionally, around the nucleus. This nonuniformity in the distribution of glycogen within the muscle fibers, as seen under a magnifying lens in a section stained by the method of Shabadash, created the impression of a dark-crimson speckling. The stain was completely removed after preliminary incubation of the sections for an hour at 37° with salivary amylase, which confirmed the glycogen character of the inclusions in the muscle fibers.

The accumulation of mucopolysaccharides in the stroma of the myocardium, as in the case of glycogen accumulation in the muscle fibers, was focal, corresponding in its localization to the groups of altered muscle fibers. With the corresponding stains, veins of markedly basophilic ground substance were visible between the muscle fibers, which were PAS-positive and resistant to amylase, and manifested a violet β-metachromasia not resistant to subsequent histological treatment with alcohols.

These features were evidence of a simultaneous accumulation of both neutral and acid mucopolysaccharides in these areas, while the β-metachromasia and the susceptibility to alcohols apparently indicated the presence of nonsulfated acid mucopolysaccharides – hyaluronic acid.

In order to verify this hypothesis, control experiments were set up with hyaluronidase. Metachromasia with toluidine blue in the stroma of the myocardium was removed by both testicular and bacterial hyaluronidase, which

might be explained by the presence of either chondroitin-sulfate A or hyaluronic acid. Final resolution of this question was left to the Spicer reaction (methylation – demethylation, delineating sulfated and nonsulfated mucopolysaccharides). In this case, an absolutely clear appearance of metachromasia occurred with demethylation, following its disappearance with preliminary methylation. This was evidence beyond a doubt that the metachromatic substance in the stroma of the hyocardium was hyaluronic acid (Fig. 2).

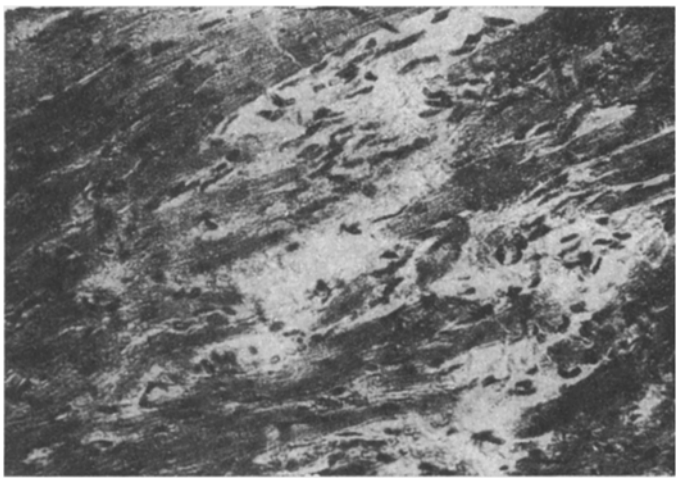

Fig. 2. Accumulation of hyaluronic acid in the stroma of the myocardium, with early sclerosis of the interstitial tissue. Microphoto. Stained with toluidine blue, following the Spicer reaction. Ocul. 7, obj. 20.

Staining for connective tissue fibers (by the method of van Guisen, with azan, and impregnation by the method of Foote) showed dissemination of argyrophilic and collagen fibers in the areas of metachromasia within the myocardium (early focal cardiosclerosis). In the valves of the heart and the aortic intima, marked widening was noted, due to a layer of chromotropic material between the elastic membranes. According to the histochemical data, the composition of this emorphic material included chondroitin-sulfate B and C.

Interesting changes were observed in the kidneys. We observed a brighter red-violet color of the basal membranes in the tubules and of the capillary walls in the glomeruli from the PAS-reaction in the test animals than we did in the control (with staining of the sections in the same vessel), and a resistance to amylase in the former; this was evidence of accumulation of neutral mucopolysaccharides in these tissue elements. Differentiation of them from glycolipids was controlled by resistance to extraction with hot methanol-chlorophorm. In addition to this, when the kidney sections were stained with toluidine blue, we observed manifest, red metachromasia in the interstitial tissue of the medullary layer, chiefly in the region of the renal papillae. Analysis of this metachromasia, using the methods mentioned above (fermentation tests, the Spicer reaction) compelled us to come to an unexpected conclusion: in hypothyroidism, in contradistinction to what takes place under normal conditions [4], chondroitin-sulfate C accumulates in the interstitial tissue of the kidneys. This was also proven by the fact that the basophilic material of the kidneys was resistant to subsequent histological treatment with alcohols, and decolorized methylene blue at pH 3.0.

In experimental hypothyroidism, the liver contained an elevated amount of glycogen, which filled the entire cytoplasm of the hepatic cells, and was present in the form of coarse clumps and granules. In the interstitial tissue of the liver there was an abundant amount of basophilic material, with the same tinctorial and fermentation reactions as the chromotropic material in the stroma of the myocardium. The same was true for the composition of the ground substance in the capsule of the spleen, the interstitial tissue of the lungs, and the submucosa of the stomach and intestine.

With hypothyroidism, we noted a marked increase in the number of fat cells within all the organs and tissues, distributed in the myocardium, for example, next to or within the areas of metachromasia. The increase in the number of fat cells was especially great in the perivascular connective tissue of the lungs, in the submucosa, muscle

layers and serosa of the gastro-intestinal tract, and in the areolar tissue surrounding the lymph nodes (Fig. 3). The fat cells were coarse, and filled to overflowing with metachromatic granules, often with discharge of these granules. A comparison of the number of fat cells in the gastric submucosa of 10 healthy rats and 25 rats with experimental hypothyroidism showed that while in the normal animals, in a section 5 micra in width (based on inspection of 50 sections, we observed 12.24 ± 0.225 cells, in the hypothyroid animals this number rose to 27.01 ± 1.327.

The basophilic material in the fat cells decolorized methylene blue at pH 2, which indicated the presence of heparin. Besides heparin, they also contained hyaluronic acid, which was shown by attenuation of the metachromasia after methylation – demethylation by the method of Spicer. As a rule, the fat cells were PAS positive in hypothyroidism. In the opinion of Lillie, this also indicates that they contain the monosulfate of heparin, possessing 1,2-glycol groups, and thus reacting with the Schiff reagent after oxidation with periodic acid. In the opinion of a number of authors [10, 12 et al], the accumulation of fat cells in the connective tissue during hypothyroidism is connected with the increased output of thyrotropic hormone from the hypophysis, causing activation of the cellular elements in the loose connective tissue.

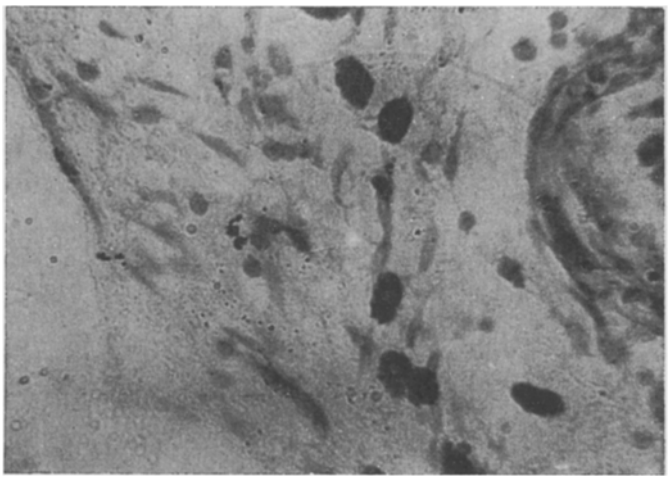

Fig. 3. Increase in the number of fat cells within the loose connective tissue. Microphoto. PAS-reaction, counterstained with methylene blue. Ocul. 10, obj. 40.

In concluding, we should mention the mucoproteid composition of the blood and lymph in hypothyroidism. In observing preparations of various organs, treated with the Periodic Acid – Schiff reagent, it was possible to note unusually intense staining of the blood plasma within the vessels, which was resistant to amylase and to extraction with hot methanol-chlorophorm; we also observed an intense staining of the arterial walls, up to a violet-black color, in the areas of plasmorrhagia often encounted in hypothyroidism. In the sinuses of the spleen and lymph nodes, and in the follicles of the intestine and lungs, we observed accumulation of fluid which intensely stained a violet color when treated with periodate and Schiff's reagent, and was resistant to treatment with amylase.

Inasmuch as at this time we are still unable histochemically to differentiate neutral mucopolysaccharides from mucoproteids [18], but their distribution in the blood and lymph is characteristic specifically for the latter group of mucoids, we have considered it feasible to regard the obtained changes as an accumulation of mucoproteids. These data correspond to the evidence in the literature [14], showing an elevation of the mucoproteid content in the blood associated with hypothyroidism.

SUMMARY

Histochemical analysis of polysaccharides was done in internal organs in experimental hypothyroidism. A considerable rise in glycogen content was noted in the parenchyma of the liver and heart; glycogen distribution in the cardiac muscle fibers was uneven, in the form of separate large foci, surrounded by mucoid edema of the stroma. In the interstitial tissue of internal organs accumulation of acid mucopolysaccharides is observed, the composition of which varies in different organs (in the heart – preponderantly hyaluronic acid, in the kidneys – chondroitinsulfate C, etc). Mucoproteid accumulation is noted in the blood plasma and lymph in experimental hypothyroidism cases.

* This number represents the mean error for the determined figure, obtained according to the formula $m = \sigma/\sqrt{n}$, where σ represents the mean quadratic deviation, and n is the number of observations.

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