A MODIFICATION OF GOMORI'S STAIN

The Demonstration of Beta Granules in the Islets of Langerhans

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In 1911 Bensley[1] employed special stains to bring out the morphology of the alpha, beta and gamma cells of the pancreatic islets. They work well on guinea pig pancreas. With other species the results are sometimes uncertain. The technic is also difficult. In 1939 Gomori[2,3] introduced a new stain. The technic is much simpler and fails less frequently.

In our experience, while Gomori's stain usually works well on tissues fixed in Bouin's fluid or in formalin, it frequently fails to bring out the beta granules. The failures have been so frequent that one cannot be sure that the beta granules are really absent when the stain fails to show them. Only positive results are dependable. The stain works poorly on tissues preserved in paraffin blocks and usually fails on tissues preserved for some time in formalin. I have attempted to determine the cause of the variations and, if possible, to alter or correct them. I think I have succeeded in simplifying the technic and in increasing the dependability of the stain.

EXPERIMENTAL WORK

On several occasions I observed that when rat tissue failed to take the stain properly, the beta cell granules would stain a faint pink instead of blue. This suggested a change in the *p*H. Gomori recommended Bouin's fixative. This is acid (*p*H 2.1) and seems to inhibit the staining of the beta granules especially on old formalin-fixed tissue. I have also found this fixative unsatisfactory because it makes the tissue very hard to cut, and with a very small piece of tissue, especially with biopsies of the pancreas, it becomes so fragmented that it is difficult to find the islets.

I employed formalin and found that formalin-fixed tissues were easy to cut; thin, even sections could be obtained. Two sets of fixatives were prepared, varying the *p*H. One contained acetic acid, the other ammonium hydroxide. It was found that when the material was fixed with the acetic acid-formalin all the granules, both alpha and beta, dissolved out, while the tissue fixed with the ammonium hydroxide formalin stained well. A number of alkaline salts and alkalis were then tried in the fixative instead of ammonium hydroxide. Ammonium hydroxide and potassium dichromate were found to yield superior results. An objection to ammonium hydroxide was its tendency to cause disintegration if the tissues were left too long in the strongly alkaline solution.

I found with both human and animal tissues that excellent staining of the islets could be secured with the following technic. The tissues were fixed in 4 per cent formalin, cut and then mordanted either in a concentrated ammonium hydoxide 8 per cent-4 per cent formalin solution; or a 16 per cent potassium chromate-4 per cent formalin solution. The use of the 16 per cent potassium chromate-4 per cent formalin mixtures as a mordant yielded excellent results in dealing with human, rodent and guinea pig material. One of the advantages is ease in cutting the fixed tissue on the microtome.

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With the above technic, very old human tissues preserved in paraffin blocks stained clearly; some specimens were 22 years old. In several cases, tissues preserved in formalin for three years also stained well. The amount of beta granulation is easily determined. The acinar tissue stains a pale red to pink; the zymogen granules stain a very bright red. The beta cells show a dark blue granulation; the alpha cells a bright red granulation.

STAINING TECHNIC

Tissues which have been fixed in formalin for 24 hours or more are embedded in paraffin, sectioned at 5-8 micra, and affixed to slides in the usual fashion. The tissues on the slides are then mordanted overnight in a 16 per cent potassium chromate-4 per cent formalin solution. The following morning they are washed in tap water for a few minutes and then immersed in solution containing equal parts of a 0.3 per cent potassium permanganate and a 0.3 per cent sulphuric acid solution for five minutes. The potassium permanganate and sulphuric acid are mixed just before using.

The tissues are then put in a 2.5 per cent sodium bisulfite solution for five minutes, washed in tap water for ten minutes, and stained in chromium hematoxylin for 4-8 minutes. Staining in the chromium hematoxylin is done in the tissue oven at an elevated temperature (45°-55°C.). The tissues are then decolorized in 1 per cent acid alcohol for one minute, washed for one hour in tap water, and stained in 5 percent phloxin for 10 to 15 minutes. They are then rinsed in tap water, placed in 5 per cent phosphotungstic acid solution for one minute, and washed in tap water for ten minutes. The tissues are then carried through 95 per cent alcohol, two changes of absolute alcohol, three changes of xylol and coversliped with clarite or balsam.

The chromium hematoxylin stain is prepared as follows: Equal amounts of a 1 per cent aqueous hematoxylin solution and 3 per cent chrome alum solution are mixed. To 100 cc. of this solution are added 2 cc. of 5 per cent potassium bichromate and 2 cc. of 2.5 per cent sulphuric acid solution respectively. The stain should be allowed to ripen for a few days before using. It will give excellent results for at least one month and needs no special storing.

SUMMARY

A modification of Gomori's technic for the selective staining of islet cells has been described. It is a dependable stain for both human and animal pancreas and gives constant results. It is especially useful for the study of small biopsies of the pancreas.

REFERENCES

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[2] Gomori, G.: A differential stain for cell types in the pancreatic islets. Am. J. Path. *15*:497-500, 1939.

[3] Gomori, G.: Observations with differential stains on human islets of langerhans. Am. J. Path. *17*:395-406, 1941.

The Detection of Degenerative Diseases

In private practice the physicians can do far more in the prevention, retardation, and alleviation of disability that is due to chronic progressive disease in the aging and the aged than is being accomplished at present.[1] Aside from the instruction, guidance, and stimulation of motivation for effort toward the construction of optimum health by personal, tutor-like instruction,[2] the most important potentiality for service is the earliest possible detection of degenerative disease and the institution of measures to correct the known and/or suspected etiological factors. As previously pointed out, these disorders begin so silently and insidiously that they must be searched for if they are to be discovered in time to be amenable to therapy. To wait until obvious symptoms and signs appear is to wait too long. Irresversible damage is the price of procratination and lack of thoroughness in clinical study.

—From *"Chronic Illness and Senescence" by Edward J. Stieglitz, M.D.* in *The Journal* of the American Medical Association, October 4, 1952

[1] Geriatric Medicine The Care of the Aging and the Aged, edited by E. J. Stieglitz, ed. 2, Philadelphia, W. B| Saunders Company, 1948, chap. 6.

[2] Stieglitz, E. J.: Aging as an Industrial Health Problem, J. A. M. A. *116*:1383 (March 29) 1941; The Potentialities of Preventive Geriatrics (Delta Omega lecture), New England J. Med. *225*:247 (Aug. 14) 1941; Preventive Geriatric Medicine, Journal-Lancet 65:60 (Feb.) 1945. Footnote 1.