

Relation of Glucagon to A Cells of the Pancreas.*† (22339)

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In spite of the amount of work done on origin of glucagon, there is no agreement as to the site of its production(1-5). Some investigators found a significant reduction of glucagon in the pancreas of cobalt-treated guinea pigs and dogs(8,9); others found no diminution in glucagon content of cobalt-treated dogs(6,7); while others stated that the glucagon content of cobalt-treated rabbits was twice that of normal(4). However, these workers(4) found no glucagon in the pancreas of synthalin A treated rabbits. Some but not all controversies on origin of glucagon could be explained by differences in toxic effect on the A cells of different animals(10,11). It has recently been demonstrated by one of us that the uncinate process of the dog pancreas is normally devoid of A cells(12) and that no significant hyperglycemic effect can be obtained with extracts from such an area while there is a marked hyperglycemic effect with extracts from the A cell containing portion of the pancreas(13). Because of the specific morphological differences in the dog pancreas, it was thought that other differences rather than the presence or absence of A cells might explain the regional differences in the glucagon content of the dog pancreas. This hypothesis had to be seriously entertained in view of the conflicting reports on the relation of glucagon to the A cell(1-5). To clarify this relationship, an attempt was made to destroy the A cells of the dog pancreas with cobalt but unfortunately we were unable to do so, although they were easily damaged in the guinea pig(11). Experiments were carried out then with the object of producing complete disappearance of the A cells of the guinea pig with the hope that a pancreas with

these changes would be analogous with respect to its morphology and glucagon content to that of the uncinate process of the dog.

Material and methods. A total of 64 guinea pigs of both sexes were divided as follows: *Group 1:* Five guinea pigs weighing between 600 and 1200 g were used for the study of possible regional differences in the distribution of islet cell types in the guinea pig. The entire pancreas of these animals was embedded and sectioned and in none of the representative sections could we find evidence of any regional differences. *Group 2:* The pancreases of 4 untreated guinea pigs weighing between 900 and 1200 g were pooled and used for glucagon extraction. A small portion of each gland was first taken for microscopic examination. *Group 3:* Four guinea pigs weighing between 400 and 700 g and 6 guinea pigs weighing between 1100 and 1400 g received daily subcutaneous injections of 20 mg of cobalt chloride (B.D.H. Analar) per kg of body weight as a 1% solution for as long as 7 days. The animals of the younger group were all killed at the seventh day. Of the older group, 5 died between the third and fourth days; the survivor was killed on the fifth day. The entire pancreas of all these animals was embedded and studied as in Group 1. The older animals showed the most extensive A cell lesions, but were also more susceptible to the lethal effect of cobalt. The extent of damage to the A cells was variable from one animal to another but of similar degree throughout the pancreas of any one guinea pig. On this basis in subsequent experiments the extent of A cell damage of the portion of pancreas used for glucagon extraction was assumed to be identical to the small one used for histological examination. *Group 4:* Because most animals lost about 10% of their weight during the cobalt treatment 9 guinea pigs were partially starved to make them lose 10% of their weight within 5 days so as to exclude weight

* This work was supported by grants from Banting Research Foundation, Life Insurance Research Fund and National Research Council of Canada.

† Presented at Meeting of Canadian Physiol. Soc., London, Ontario, Oct. 14, 1955.

loss as the cause of the A cell lesion in the cobalt treated guinea pigs. None of these starved animals showed A cell damage. *Group 5:* Thirty-six guinea pigs weighing between 900 and 1200 g were treated with cobalt chloride as in Group 3; fourteen died and were discarded, the remaining 22 were killed between the fifth and seventh day after the beginning of the treatment. A small portion of the pancreas was obtained from each of these animals for microscopic study, the remainder of the organ was used for glucagon extraction. Histologically, the extent of the A cell lesion was graded for each pancreas from 1+ to 4+. Eight animals had 1+; 5 had 2+; one had 3+; and 8 had 4+ lesions of their A cells. Extracts from animals having the same degree of damage were pooled in groups up to 4. All animals had free access to water and Purina rabbit chow supplemented with fresh lettuce and carrots. All guinea pigs were killed at 6 hours after the last injection of cobalt by exsanguination under ether anesthesia. Glucagon was extracted by the method of Best, *et al.* (14), each pancreas was extracted individually and then pooled as described above. The pooled extracts were dried by evaporation in the cold. Every pooled extract was tested in triplicate following the procedure suggested by Staub and Behrens (15). The presence of glucagon was demonstrated by its hyperglycemic effect on fasted, anesthetized cats. The dry extract in an amount equivalent to 2 g of fresh pancreas was dissolved in 2 ml of normal saline (pH 2.5) immediately before injection. The solution was injected intravenously and blood samples were taken prior to and 5, 10, 15, 20 and 25 minutes after the injection. Individual blood sugar determinations were done in duplicate with a modification of the Folin-Wu method. The extract from the animal with a 3+ lesion was tested only once because of insufficient material for more determinations. Tissues for histological examination were fixed in Zenker-Formol, and were processed and stained following the methods previously described (16). Paraffin sections were cut 2.5 μ thick and stained with Gomori's chrome alum hematoxylin stain, the aldehyde fuchsin

stain of Gomori (17) and a recent modification of Masson's trichrome (11).

Results. Morphology. The A cell lesion of the cobalt-treated guinea pig was qualitatively identical in all animals. Cobalt produces a progressive and complete degranulation of the A cell, while its cytoplasm and nucleus remain unaffected. With further treatment, the degranulated A cell undergoes vacuolization of its cytoplasm (Fig. 1,2). It was not possible, however, to see any necrotic cells in the pancreases of cobalt-treated guinea pigs. Our grading of the A cell lesions was based on the proportion of A cells showing complete degranulation or more advanced changes. We considered a 1+ lesion to be present in all those cases in which some cells were observed. When about half of the A cells were so damaged, it was graded as a 2+ lesion. When only a few granulated A cells could be found, the lesion was classified 4+. An attempt was made to interpolate a 3+ lesion, but this could be done on only one occasion.

Glucagon content of the pancreas of untreated guinea pigs: The extracts from the pancreases of these animals invariably showed when tested a marked hyperglycemic effect on the fasted anesthetized cats. Their blood sugar rose from 50 to 153 mg% above the initial level within 15 minutes after the injection of the extracts as indicated in Fig. 3.

Glucagon content of cobalt-treated guinea pigs: When tested, pancreatic extracts from animals having 1+ and 2+ lesions showed a response similar to that of the untreated animals. As noted in Fig. 3, the extracts from a 2+ lesion gave a rise of blood sugar varying from 73 to 104 mg %. In contrast with these results the extract from animals having a 4+ lesion showed on only one occasion a rise of 4 mg % above the initial level. This was followed by a decrease in the blood sugar from 40 to 62 mg % within 25 minutes. The pancreatic extract from the animal having a 3+ lesion showed a rise of blood sugar of 16 mg % above the initial level.

Discussion. An important finding in the present work is the lack of a hyperglycemic effect of the pancreatic extract from animals having 4+ lesions, particularly since the hy-

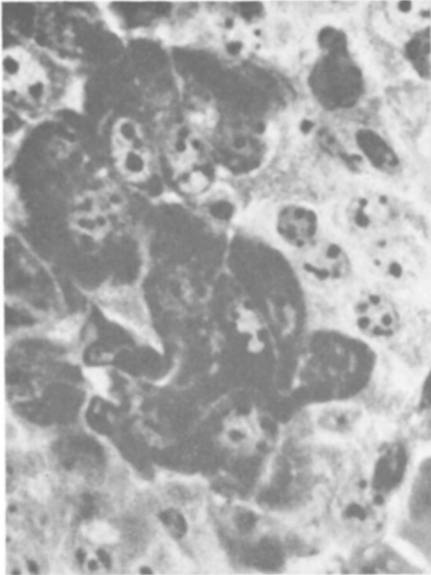


FIG. 1. Pancreatic islet from normal guinea pig showing well granulated A cells in black. Masson's trichrome $\times 900$.

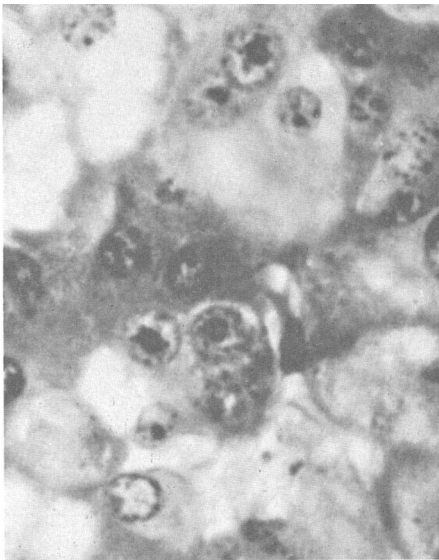


FIG. 2. Pancreatic islet from a cobalt-treated guinea pig. A cells are severely degranulated or vacuolated. They appear clear in the picture. Masson's trichrome $\times 900$.

perglycemic effect of the extract from animals having a 1+ and 2+ lesion is indistinguishable from that of the untreated ones. Our results suggest that the pancreatic extracts from cobalt-treated guinea pigs fail to elicit a hyperglycemic effect, as is the case with ex-

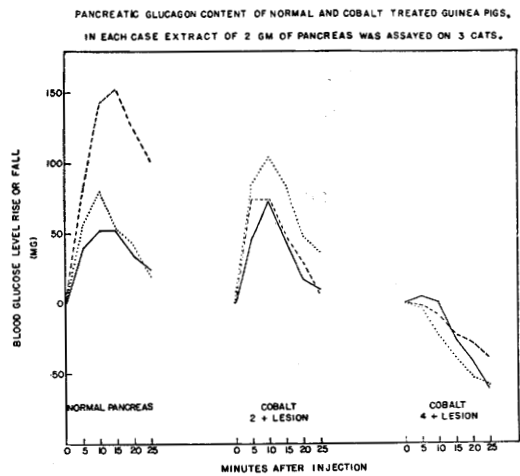


FIG. 3. Typical blood sugar curves in fasting and anesthetized cats after intravenous injection of pancreatic extracts from normal and cobalt-treated guinea pigs.

tracts from the A cell-free portion of the dog pancreas when the number of granulated A cells present is reduced to a certain minimum which apparently is achieved only in our 4+ lesion. Because 4+ lesions occurred in only 40% of cobalt-treated animals, it is possible that the 60% reduction of glucagon content obtained by Vuylsteke *et al.*(8) in their pooled extracts from pancreases of cobalt-treated guinea pigs was due to the fact that these authors did not grade the extent of their lesion. From our experience with cobalt in various species(11) from the work with the uncinat process of the dog(12,13) and since none of the substances toxic for the A cell have resulted in pancreas completely devoid of them(1,3,4), it seems that the conflicting results on the relation of glucagon to the A cell may be due to the great hyperglycemic activity of minute amounts of glucagon remaining in the pancreas when a few granulated A cells are present. We are, however, unable to explain on the basis of our results the work of Fodden and Read(4) who found that extracts from cobalt-treated rabbits had double the normal amount of glucagon while that of synthalin-treated ones had none despite the fact that A cells were severely damaged in both groups. It is unfortunate that these investigators did not make a detailed comparison of the A cell lesion as it occurred in

the rabbits treated with these two substances. The results of our work are interpreted as strongly suggestive that glucagon originates from the A cells. Quantitative determination of the glucagon content of pancreas treated with drugs toxic for the A cell together with detailed morphological study of the type of A cell lesion, will aid in the elucidation of this problem.

Summary. Pancreatic extracts of cobalt-treated guinea pigs with a 4+ lesion fail to produce a significant hyperglycemia when assayed in cats; however, if well-granulated A cells remained in amounts roughly estimated above 25% of the normal, the extracts from these pancreases invariably elicited a hyperglycemia indistinguishable from that of the normal. These results are interpreted as strongly suggestive that glucagon originates from the A cells.

1. Volk, B. W., Lazarus, S. S., and Goldner, M. G., *A. M. A. Arch. Int. Med.*, 1954, v93, 87.
2. Cavallero, C., *Rev. Canad. biol.*, 1953, v12, 509.
3. De Duve, C., *Lancet*, 1953, v2, 99.

4. Fodden, J. H., and Read, W. O., *Endocrinology*, 1954, v54, 303.
5. Foa, P. P., *Advances in Int. Med.*, 1954, v6, 29.
6. Lazarus, S. S., Volk, B. W., and Goldner, M. G., *Metabolism*, 1954, v3, 449.
7. Goldner, M. G., Volk, B. W., and Lazarus, S. S., *J. Clin. Endocrinol.*, 1954, v14, 184.
8. Vuylsteke, C. A., Cornelis, C., and De Duve, C., *Arch. Internat., physiol.*, 1952, v60, 128.
9. Dorner, M., and Stahl, J., *Comp. rend. Soc. biol.*, 1954, v148, 590.
10. Creutzfeldt, W., and Schmidt, W., *Arch. exp. Path. u. Pharmacol.*, 1954, v222, 487.
11. Bencosme, S. A., *Rev. Canad. biol.*, 1955, v14, 175.
12. Bencosme, S. A., and Liepa, E., *Endocrinology*, 1955, v57, 588.
13. Bencosme, S. A., Liepa, E., and Lazarus, S. S., *Proc. Soc. Exp. Biol. and Med.*, 1955, v90, 387.
14. Best, C. H., Haist, R. E., and Ridout, J. H., *J. Physiol.*, 1939, v97, 107.
15. Staub, A., and Behrens, O. K., *J. Clin. Invest.*, 1954, v33, 1629.
16. Bencosme, S. A., *Arch. Path.*, 1952, v52, 87.
17. Gomori, G., *Am. J. Clin. Path.*, 1950, v20, 665.

Received February 15, 1956. P.S.E.B.M., 1956, v91.

Effect of Splenectomy on Antibody Formation in Cortisone Treated Rats. (22340)

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The hemolysin formation in rats after a single intraperitoneal injection of sheep erythrocytes was the object of a previous study (1). It was suggested that at a large dose of the antigen (about 2 ml of a 10% suspension) a considerable part of the sheep cells enters the blood stream and is taken up by the spleen. The spleen may then be responsible for a large fraction of the circulating antibodies measured. However, at an antigen dose 30-40 times smaller, splenectomy preceding (2,3) or subsequent to (unpublished experiment) the intraperitoneal antigen injection did not appreciably alter the hemolysin response. In another series of experiments some factors which condition the antibody-

reducing effect of cortisone were studied (4). It was shown that the hemolysin response after a large intraperitoneal dose of sheep cells was less affected by cortisone than that after a small one. One possible explanation involved the assumptions: 1) that the spleen produces a dominating part of the hemolysins with a large antigen dose but not with a small one, and 2) that splenic antibody production is more resistant to cortisone than is extra-splenic antibody formation. The effect of cortisone should then be less with a large than with a low antigen dose.

The following experiment was an attempt to verify the two assumptions.

Material and methods. Animals. White