4. Powell, H. M., Jamieson, W. A., and Culbertson, C. G., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 80.
5. Schmidt, E. C. H., *Am. J. Path.*, 1948, v24, 97.
6. Warren, J., Russ, S. B., and Jeffries, H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 376.
7. Helwig, F. C., and Schmidt, E. C. H., *Science*, 1945, v102, 31.
8. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
9. McLaren, A., and Sanders, F. K., *Atti Del Vi Cong. Internazionale di Microbiol. Roma*, 1953, v3, 11.
10. Kaliss, N., and Pressman, D., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 16.

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Glucagon Content of Pancreatic Tissue Devoid of Alpha Cells.* (22042)

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In spite of the large amount of work done in the past 30 years to determine the site of origin of glucagon, there is as yet no general agreement as to whether or not glucagon is the alpha cell hormone(1-5). Some have considered that the alpha cell is the source of glucagon because of differences in insulin requirement of alloxan(6) and metahypophyseal diabetes(7) as compared to that of surgical diabetes(8). This opinion is supported by the presence of a hyperglycemic factor in extracts from normal, alloxanized, and alloxanized duct ligated pancreas(3). An alternative explanation for the differences in insulin requirement was that in surgical diabetes intestinal absorption of nutriments is limited(9). This latter view was controverted by other experiments with duct ligated alloxan diabetic dogs(10). A hyperglycemic factor, apparently identical with glucagon, is present in the stomach of the dog. This was considered to confirm the origin of this substance from the alpha cell of the pancreas because of supposed similarity of argentaffin cell of the stomach with the alpha cell(1). Furthermore in one report, gastro-intestinal argentaffin cells are implicated in disturbances in carbohydrate metabolism(11). However, other workers have not found any correlation between tumours of these cells and diabetes(12). Similarly, glucagon could not be extracted from the stomach of the hog despite the presence of argentaffin cells(1). The lack of identity between the alpha cell and the intestinal argentaffin cell, originally demonstrated by Masson in 1924, has recently been confirmed(13, 14). It would therefore be erroneous to consider that the presence of a hyperglycemic factor in tissues containing argentaffin cells supports the conclusion that the alpha cells serve as the source of glucagon. The discovery of chemical agents apparently capable of damaging alpha cells, rather than clarifying this problem, gave further contradictory results(3-5). Some workers reported alleviation of diabetes by cobalt(15,16), and diethyldithio carbamate(16). Others reported no effect of either of these agents or of synthalin A on the severity of alloxan diabetes (13,17,18). In some studies extracts from the pancreas of cobalt treated animals have been reported to contain lesser quantities of glucagon than normal(4,19). On the other hand, one of us(5,20,21), using qualitative methods of glucagon assay could detect no significant differences between normal and cobalt treated pancreas. Others have reported that cobalt treatment increases the glucagon content of the rabbit pancreas, while synthalin A causes its complete disappearance(22). In this connection, it is of interest that the lesions reported by various investigators after cobalt, are not always identical; it seems to vary with the animal species, the dose and the route of

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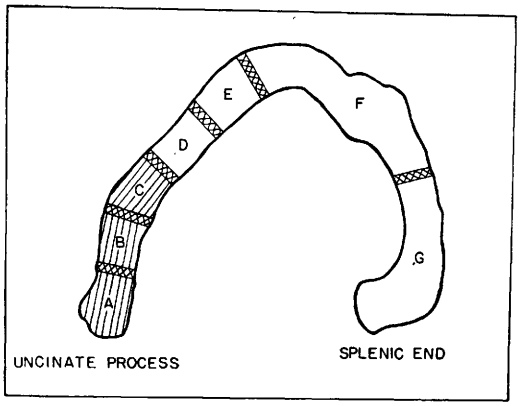

FIG. 1. Tracing of outline of dog pancreas. Longitudinally striated portions A, B and C were free of alpha cells while the remainder contained these cells. Cross hatched areas indicate tissue examined histologically.

administration(4,5,13,23,24).

As was previously pointed out, it has not been possible by the use of cobalt to prepare pancreas which are completely devoid of alpha cells(4,5). However, it has recently been demonstrated in this laboratory that the dog pancreas can be divided into two portions; one which does not contain alpha cells and which corresponds roughly to the uncinate process, and the balance of the pancreas which contains alpha cells(25). This finding enables a reinvestigation of the problem of the relationship of glucagon to alpha cells, since pancreatic tissue completely devoid of these cells is available for extraction.

Material and methods. The entire pancreas was removed from 6 normal mongrel dogs weighing between 15 and 20 kg which had been killed by exsanguination from the aorta after preliminary nembutal anesthesia. A tracing of the outline of the pancreas was made and the uncinate process was then divided into portions of approximately 1 to 2.5 g each. A complete transverse section of the proximal end of each portion was taken for histological examination and the position of these biopsies was recorded on an outline of the pancreas as indicated by the example in Fig. 1. In addition, transverse sections for morphologic study were obtained from the body and splenic end of the pancreas. The portions of the uncinate process and also the remainder of the pancreas were each immediately placed, separately, in acid alcohol and minced. Extracts prepared by the method of Best *et al.*(26), were dried and kept in the refrigerator until ready for use. The tissue for histological study was fixed in Zenker-Formol solution and sections were subsequently stained with Masson's trichrome, Gomori's chrome alum hematoxylin(27), Gomori's Aldehyde Fuchsin(28) and Mallory-Heidenhain-azan(29). In addition portions of the pancreas from the uncinate process and body

TABLE I. Blood Sugar Response of Fasting Anaesthetized Normal Cats to Intravenous Injection of Dog Pancreatic Tissue. (A) Containing alpha cells; (B) Devoid of alpha cells.

			Blood sugar, mg %					
				Min. after admin. of extract				
Material injected	Dose, g*	Dog	Before inj.	5′	10′	15′	20′	25′
A. Extract of alpha cell containing portion of pancreas	1	L 9	122	172	204	200		164
	"	"	94	131	142	147	133	109
	"	"	111	180	200	202	200	145
	"	L 12	115	169	202	197	187	177
	"	L 13	137	194	200	198	192	140
	"	"	124	137	200	200	182	182
	1/10	L 12	87	117	112	82	69	65
	"	"	102	126	124	110	107	92
	"	"	114	120	136	120	107	100
B. Extract of pancreatic tissue devoid of alpha cells (uncinate process)	1	L 9	112	110	108	100	85	67
	"	"	110	115	106		92	82
	"	"	104	100	92	83	74	66
	"	L 12	112	112	108	97	88	88
	"	L 13	129	127	120	112	101	103
	4	L 12	90	103	102	76	65	53
	"	L 15, 16, 17	114	120	110	94	90	82

* Calculated in term of wt of fresh pancreas.

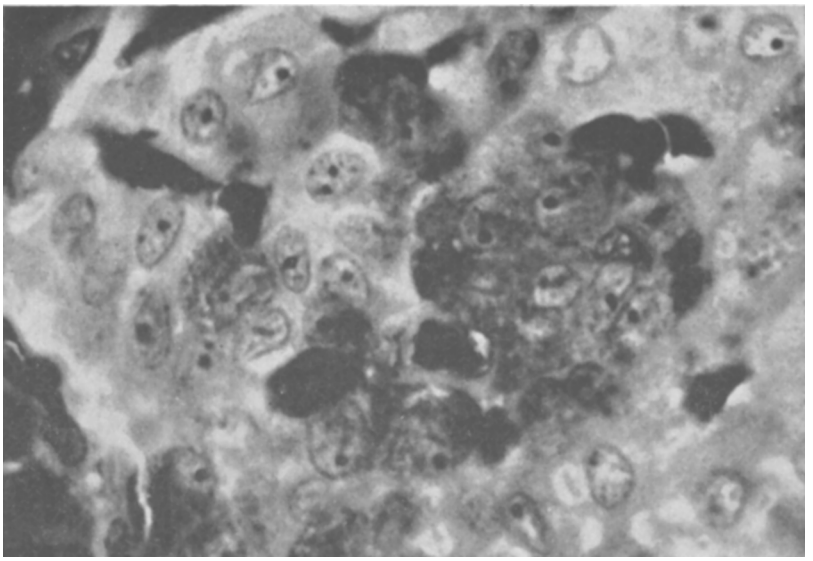

FIG. 2. Pancreatic islet from the alpha cell containing portion of pancreas. Dark finely granulated cells in center of islet are alpha cells and stained black in the original preparation. Zenker-Formol, Masson's Trichrome × 1045.

of 3 of the dogs were placed in 10% formol and impregnated with silver according to Fontana's method. For estimating the glucagon content the dried extracts were dissolved in physiologic saline (0.9% sod. chloride) acidified to pH 2.4 with HCl. One cc of solvent was used for each g of pancreas. The procedure suggested by Staub and Behrens(30) was followed. After an overnight fast, normal cats weighing between 2½ and 3½ kilo received 75 mg/kilo of sodium amytal. The inguinal veins were exposed bilaterally and duplicate blood samples were obtained just prior to, and at 5, 10, 15, 20, and 25 minutes after the injection of the test material into the contralateral vein. The blood glucose was determined by a micromodification of the Folin-Wu method(31). Extracts of 1 g of pancreatic tissue from each of the various portions were tested as per Table I. In addition 1/10 g portions of the body of the pancreas (Dog L 12) were tested in triplicate. Also, extract from 4 g of the uncinate process of Dog L 12 and a 4 g portion prepared by pooling the extracts from the uncinate process of dogs L 15, 16, 17, were used.

Results. Examination of the histological sections showed that most of the uncinate process, which amounts to approximately 15 to 20% of the total weight of the pancreas, is devoid of alpha cells. The islets of this portion are smaller than those of the rest of the pancreas and are roughly estimated to be, at most, only 1/10 of the volume of the latter. In addition, the islets of the uncinate process contain certain cells which stain red with the Masson trichrome in contrast to the alpha cells which stain black when a well ripened haematoxylin is used (Fig. 2 and 3). Argentaffin cells were demonstrated by the Fontana silver method in both portions of the pancreas, and seemed to be related to the medium sized ducts. No difference was observed in their number in either area.

The changes in blood sugar levels of the fasting anesthetized cats after the injection of each extract are represented in Table I. As was to be expected, the alpha cell containing portion of pancreas uniformly elicited an initial marked hyperglycemic response. The peak value, with 1 cc of extract, was reached at 10 to 15 minutes after injection and ranged from 53 to 91 mg% above the initial level. The blood sugar did not return to the fasting value by 25 minutes. On the other hand, one cc of extract from the portion of pancreas devoid of alpha cells did not cause an initial rise in the blood glucose concentration, except in

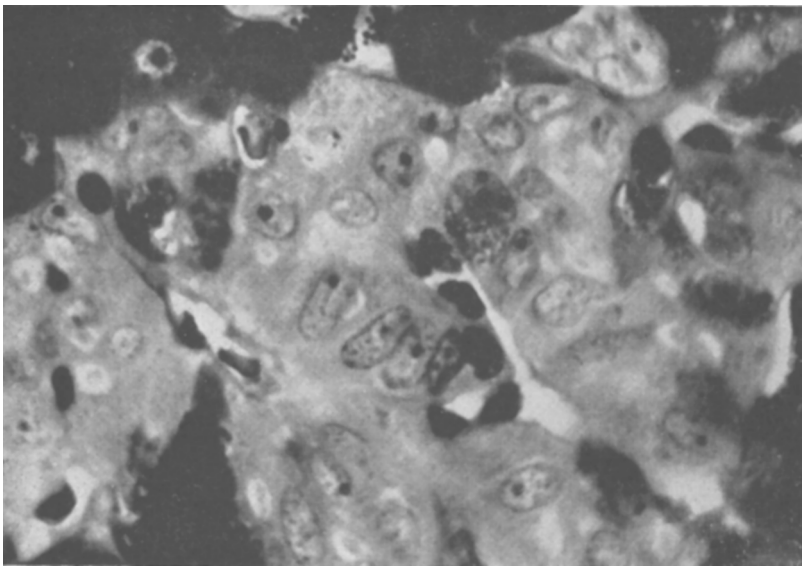

FIG. 3. Pancreatic islet from the region of pancreas devoid of alpha cells. Dark cells with rather coarse granules are X cells and stained deep red in the original preparation. Zenker-Formol, Masson's Trichrome × 1045.

one instance (L 9), where it was only 5 mg%. The 10-minute value was only 4 to 12 mg below the fasting level. By 15 minutes the insulin effect was somewhat more obvious with values of 12 mg% to 25 mg% and at 25 minutes the values varied from 24 to 45 mg% below the fasting level.

In view of the differences in islet volume observed morphologically 1/10 g equivalents of extracts from alpha cell containing portions and 4 g equivalents from the uncinate process which is devoid of alpha cells were also tested.

With the 1/10 g portions from the body, the initial hyperglycemia was less, ranging from 22 to 30 mg% above the fasting level and the peak was reached at 5 to 10 minutes after the injection. Thereafter, the blood sugar declined so that at 25 minutes the value ranged from 10 to 22 mg% below the initial level (Table I). The 4 g portion, from the areas devoid of alpha cells, also elicited a very mild initial blood sugar rise of 6 and 13 mg% above the fasting value at the 5 minutes specimen. This was followed by a decline of the blood sugar of 37 mg% and 32 mg% respectively at the 25 minutes specimens.

Discussion. The results of these experiments demonstrate an almost complete lack of hyperglycemic effect of extracts from the portion of pancreas devoid of alpha cells as compared to the remainder of the pancreas. However, the fact that a significant decline of the blood sugar did not occur until the 15 minutes specimen when 1 g portions of uncinate process were used, suggests the presence in the sample of an anti-insulin factor. The very mild initial hyperglycemia obtained with 4 g portions leads to a similar inference. Whether despite the lack of alpha cells this anti-insulin effect is due to glucagon, is open to question. Since the whole area of pancreas used for the extract cannot be examined in each case, it might be argued that some alpha cells were present. However, in other work it was demonstrated by consecutive longitudinal sections that the transition between the area devoid of alpha cells and the alpha cell containing area is quite abrupt(25). Therefore complete cross sections of the pancreas as performed in this study enable the separation of these two areas. It may be that the moderate hyperglycemia in this instance is a non-specific effect due to the large amount of foreign protein present in crude extracts.

Because of the interest in the argentaffin cells as a possible source of glucagon, these were also studied. However, only very occasional Fontana silver positive cells which

identify the argentaffin cells, were found randomly distributed throughout the entire pancreas. It is therefore most unlikely that these cells do serve as a source of glucagon.

In view of the blood sugar changes obtained with the extracts from the uncinate process, it is not possible to conclude definitively that glucagon is derived from alpha cells. However, the clear differences in the results when 1 g aliquots from 2 portions of the pancreas were used, does provide evidence for the view that alpha cells produce glucagon. At the present time, no other explanation is available for these differences.

Similar conclusions have been drawn from quantitative assays of extract of cobalt treated guinea pig pancreas which showed a 60% reduction in glucagon content(31). In another study using a qualitative method of assay, a mean diminution of 10% in the hyperglycemic activity of extract of cobalt treated dog pancreas, as compared with the normal was reported(19). On the other hand no significant difference in the glucagon content of extract of cobalt treated and normal dogs were found by other workers(5,20,21). These workers stated that since many alpha cells were still left in the pancreas after cobalt treatment, and since the method of assay was qualitative rather than quantitative, a moderate reduction in glucagon content may not have become apparent. In the present study although there was a marked reduction of glucagon content in extracts from the uncinate process, the blood sugar curves from this area still indicate a minimal anti-insulin effect. This may have been a non-specific action because of the crudity of the extracts. Further assays of purified material seem to be necessary to resolve the problem of whether small amounts of glucagon are actually present in the area devoid of alpha cells.

Summary. Most of the uncinate process of the dog pancreas is devoid of alpha cells, whereas these are found in the remainder. On the other hand argentaffin cells are sparsely but evenly distributed throughout the pancreas. Extracts of the portion of pancreas devoid of alpha cells show no hyperglycemic activity when 1 g aliquots are used. When 4 g portions are employed, a very mild initial hyperglycemia does occur. These findings are interpreted to support the opinion that glucagon originates from the alpha cells but are not definitive.

1. Sutherland, E. W., *Recent Progress in Hormone Research,* 1950, v5, 441. Academic Press Inc. New York, N. Y.

2. Foa, P. P., *Advances in Internal Medicine* 1954, v6, 29, The Year Book Publishers Inc.

3. Cavallero, C., *Rev. Canad. de biol.,* 1953, v12, 509.

4. De Duve, C., *Lancet,* 1953, v2, 99.

5. Volk, B., Lazarus, S. S., and Goldner, M. G., *Arch. Int. Med.,* 1954, v93, 87.

6. Thorogood, E., and Zimmermann, B., *Endocrinology,* 1945, v37, 191.

7. Marks, H. P., and Young, F. G., *J. Endocrinol.,* 1939, v1, 470.

8. Gaston, E. A., *New England J. Med.,* 1948, v238, 395.

9. Mirsky, I. A., Futterman, P., Wachman, J. and Perisutti, C., *Endocrinology,* 1951, v49, 73.

10. R-Candela, J. L., Tamarit, J., R-Candela, R., and Cristensen-Konig, W., *Arch. Med. Exp.,* 1951, v19, 11.

11. Durieux, J., and Perruchio, P., *Biologie Medical,* 1953, v42, 562.

12. Weisberg, H. F., and Schaefer, G. L., *Am. J. Clin. Path.,* 1952, v22, 1169.

13. Fodden, J. H., *Am. J. Clin. Path.,* 1953, v23, 994.

14. Davis, J. S., *J. Path. and Bact.,* 1952, v64, 575.

15. Avezzu, G., and Luise, R., *Boll. Soc. ital. biol. sper.,* 1951, v27, 1515.

16. Mayer, J., Andrus, S. B., and Silides, D. J., *Endocrinology,* 1953, v53, 572.

17. Volk, B. W., Lazarus, S. S., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 406.

18. Lazarus, S. S., Goldner, M. G., and Volk, B. W., *Metabolism,* 1953, v11, 513.

19. Dorner, M., and Stahl, J., *Comp. rend. Soc. de biol.,* 1954, v148, 590.

20. Lazarus, S. S., Volk, B. W., and Goldner, M. G., *Metabolism,* 1954, v3, 449.

21. Goldner, M. G., Volk, B. W., and Lazarus, S. S., *J. Clin. Endocrinol.,* 1954, v14, 184.

22. Fodden, J. H., and Read, W. O., *Endocrinology,* 1954, v54, 303.

23. Van Campenhout, E., Cornelis, G., and Deulin, Th., *Ann. d'endocrinol.,* 1954, v15, 89.

24. Bencosme, S. A., personal observation.

25. Bencosme, S. A., and Liepa, E., *Endocrinology,* in press.

26. Best, C. H., Haist, R. E., and Ridout, J. H.,

J. Physiol., 1939, v97, 107.
27. Bencosme, S. A., *Arch. Path.*, 1952, v53, 87.
28. Gomori, G., *Am. J. Clin. Path.*, 1950, v20, 665.
29. Thomas, T., *Am. J. Anat.*, 1937, v62, 31.
30. Staub, S., and Behrens, O. K., *J. Clin. Invest.*, 1954, v33, 1629.
31. Hawk, P. B., Oser, B. L., and Sommerson, W. H., *Practical Physiological Chemistry*, 13th Edition 1954, Blakiston Co. Inc., Toronto.
32. Vuylsteke, C. A., Cornelis, G., and De Duve, S., *Arch. Internat. Physiol.*, 1952, v60, 128.

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Phagocytosis by HeLa Cells and Their Susceptibility to Infection by Human Tubercle Bacilli.* (22043)

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In order for an infectious agent to multiply within its host cell it must, of course, enter the cell by passing through the cell boundary. Since this portion of the cell is relatively impermeable even to molecules of moderate size, the passage of a particle as large as a bacterium or virus might be expected to be an unusual process. In the case of some of the bacteriophages it is apparently necessary only for the nucleic acid portion of the viral particle to enter the cell, the structural material of head and tail remaining on the outside of the cell wall(1). Such dissociation of viral nucleic acid from protein may later be found also in the animal viruses, yet it seems certain that with some of the larger infectious agents which multiply intracellularly, such as the rickettsiae and the leprosy bacillus, that the infectious agent enters the cell in intact form. The mechanism by which the infectious agent passes the cell membrane would thus appear to be an important factor in determining whether or not a cell is susceptible, that is, whether the agent can multiply within the cell. One such mechanism by which the infectious agent could pass the cell membrane could be the engulfment of the particle by the host cell, and it is possible that many of the differences between the growth of bacterial and animal viruses depend upon this activity by animal cells. The engulfment of droplets (pinocytosis) has been observed in "phagocytic" cells and cells of malignant origin, including the HeLa cell, by Gey *et al.*(2) and the engulfment of particles (phagocytosis) has also been described for many animal cells. The categoric separation of cells into "phagocytes" and "non-phagocytes" is unfortunate in this connection, because the difference between cells such as macrophages and fibroblasts is in degree of engulfing activity, rather than presence or absence of this activity.

Experiments described below deal with HeLa cells and the relationship between their phagocytic activity and the culture medium placed on them. It is seen that when HeLa cells are made more phagocytic by the use of media containing certain horse serums, human tubercle bacilli quickly enter the cell where they then grow luxuriantly, even when the medium is subsequently changed to one containing human serum, in which phagocytosis is at a minimum. If one examines a HeLa cell culture a few days after it was infected with the tubercle bacilli, large increases in the amount of intracellular growth are observed to result from the use of suitable horse serum media during the first day of infection, so that in effect the HeLa cells have been rendered more susceptible to this infection.

Materials and methods. The HeLa cells were grown on cover slips in flat-sided tubes. The cultures of the HeLa cells were started from cultures in 400-ml square bottles, the growth being washed 3 times with BSS (Hanks' balanced salt solution without sodium bicarbonate) then pushed off with a rubber policeman into 5 ml of the growth medium of

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