

Physiological Function of Alpha Cells of the Pancreas.* (25078)

SERGIO A. BENCOSME,[†] JOSE M. TOLEDO AND DENNIS F. CRASTON
(Introduced by Charles L. Yuile)

Dept. of Pathology, Queen's University, Kingston, Ontario, Canada

Much is known of biochemical and physiological actions of glucagon, a substance considered by most to be an alpha cell hormone of the pancreas. However, our knowledge of functions of the alpha cell based on morphological alterations is very confused. Among functions suggested are those linking it with development of diabetes(1,2), production of elastase(3,4), regulation of somatic growth (5) and fat metabolism(6). Non-diabetic dogs devoid of alpha cells can be obtained by selective partial pancreatectomy. Glucagon and alpha cells are permanently absent from remaining pancreas of these animals(7,8). The present report investigates some general metabolic aspects in such dogs devoid of alpha cells.

Materials and method. Eighteen mongrel dogs, kept in individual metabolic cages and weighed approximately twice a week, had free access to measured water and received abundant mixed diet until weight remained stationary for about 1 month. They were then fed a diet of 200 g of bread and 300 g of raw tripe for about 30 days prior to pancreatectomy. Surgical procedures were performed under Nembutal anesthesia. In 10 dogs, body and tail of pancreas were removed, the uncinate process transplanted subcutaneously and a skin pancreatic fistula created. At this time, a complete transverse section of the uncinate process proximal to body of pancreas was taken for histological examination. Because no alpha cells were found in these biopsies, it could be assumed that no alpha cells were present distal to these biopsies(7). Five dogs died of post-operative complications. Five survived experimental period. The first day after surgery, animals received only milk and sugar.

From second day they were given 100 g bread and 300 g raw tripe. All dogs received 100,000 I.U. of penicillin and 5 units of Protamin Insulin Zinc for first 3 days. Blood samples were obtained from leg veins 1½ hours after morning meal and levels of glucose(9), cholesterol(10), NPN(9) and urea (9) were then determined. Glucose tolerance tests (GTT) were done as follows: glucose (0.3 g/kg in 20% solution) was injected intravenously in fasted anesthetized (Nembutal) dog within 20-30 seconds. Blood samples were taken prior to and at 30, 60 and 90 minutes after glucose injections. At least 3 GTT were done before surgery and at least one afterwards. Twenty-four hour urine specimens were collected and specific gravity and pH determined. In addition, concentrations of the following substances were determined at specified periods (Fig. 1 and 2): total nitrogen(9), urea nitrogen(9), ammonia(9), albumin(9), glucose(11), PO₄⁻⁻⁻(9) and Cl⁻(9). Na⁺ and K⁺ concentrations were determined in urine using Coleman flame photometer. Acetone was determined with Acetest, (Ames Co., USA). Dogs were killed by overdose of Nembutal at 71, 49, 59, 50, and 57 days after surgery. Samples of pituitary, thyroid, parathyroid, lung, heart, intestine, liver, pancreas, spleen, adrenal, kidney and gonads were taken for histological examinations. Search was made for any residual pancreatic fragments and all suspected tissue was examined histologically. Tissues were fixed in Zenker formol, processed according to methods previously described and a trichrome stain was used on paraffin sections 2.5 μ thick(12). Pancreas and pituitary were also stained with aldehyde fuchsin(13) and with modification of chrome-alum hematoxylin and Masson's trichrome technics(14). Glycogen was stained in pancreas, liver and kidneys with periodic acid Schiff controlled by diastase digestion. Eight untreated dogs served as morphologic

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[†] Present address: Univ. of California Medical Center, Los Angeles.

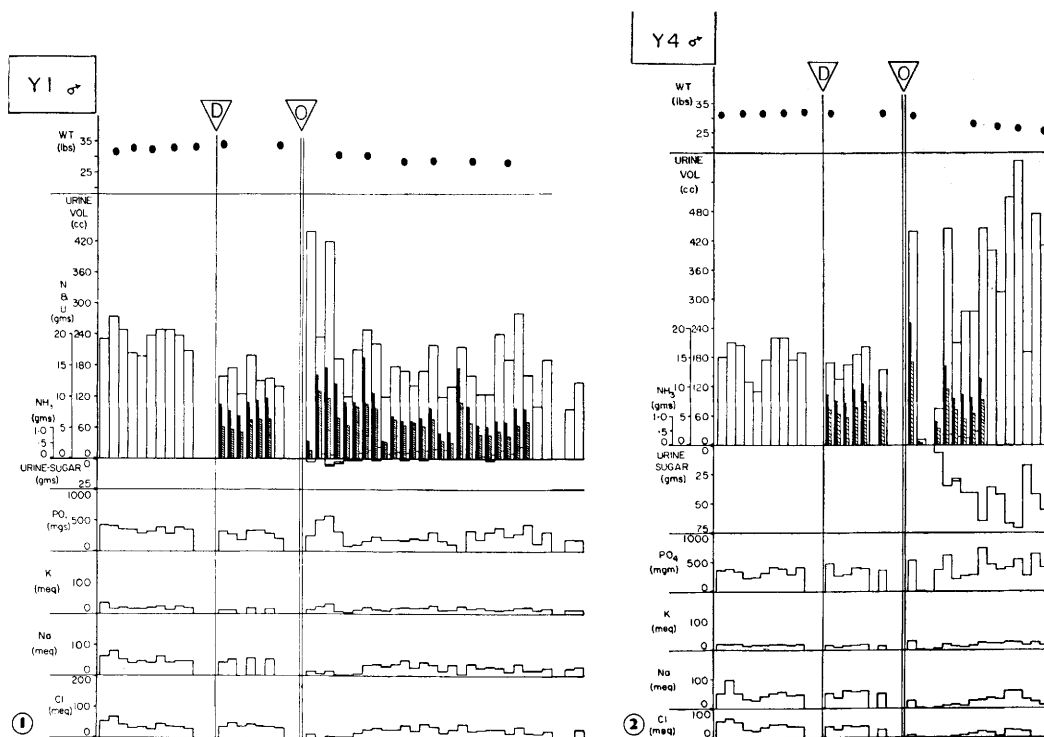


FIG. 1 and 2. Summary of data pertinent to one non-diabetic animal, Fig. 1, and one diabetic animal, Fig. 2. Determinations of urinary volume and nitrogen may be interpreted as follows:

Daily Observations

- | | |
|--------------------------------|-------------------------------|
| □ Vol of urine/24 hr | ▨ Urinary urea nitrogen/24 hr |
| ■ Total urinary nitrogen/24 hr | □ Urinary ammonia/24 hr |

In the 269-day period before diet ∇D , graphs show data for about 10 consecutive days while animals were on unrestricted food intake. In the 30-day period before operation $\nabla D - \nabla O$, there is another set of data for a given number of consecutive days while animals were on restricted diet. After operation ∇O , a number of values were obtained on consecutive post-operative days whereas the last 2 sets of determinations, when done, were taken from 15 to 30 days later and a few days before termination of experiment.

controls only. For biochemical aspect, each operated animal served as his own control during period before operation.

Results. Before operation, the GTT were normal except for first one which usually was of subdiabetic type, probably because of initial poor nutritional state of animals. Average pre-operative blood levels in mg% were: glucose 86; cholesterol 223; urea 17; and NPN 29. Results of examining urine before operation during pre-diet and diet periods showed no significant difference (Fig. 1 and 2).

Shortly after surgery, animals lost some weight but this loss did not progress except in 2 which developed diabetes, as indicated

by hyperglycemia and marked, persistent glycosuria. The other 3 dogs, while showing a diabetic type GTT, were normoglycemic and had only occasional slight glycosuria. Slight ketonuria was seen occasionally in 2. NPN increased in all dogs reaching an average of 82 mg %. There was no change in blood cholesterol and urea. During first week after surgery, there was marked reduction in urine Na^+ and Cl^- but no change or slight increase in content of K^+ and PO_4^{--} . By tenth day after operation all electrolytes had reached pre-operative levels. Except for 2 dogs with glycosuria and one, which had transient hyperglycemia, urine volume did not change appreciably. Water intake before and after

operation ranged from 50-1,000 and averaged about 250 ml. At all times urine volume was correlated closely with fluid intake. Total nitrogen and urea in urine decreased slightly for first 48-72 hours after operation but maintained normal values thereafter. The very few determinations of ammonia were within normal range. No changes were observed in pH (6.2-8.8) nor in specific gravity (1.045-1.068) of urine between pre- and post-operative periods. In a few instances, traces of albumin were found in urine of some dogs before and after pancreatectomy. No alpha cells were found in the remnant of the pancreatic transplant obtained at autopsy nor was the structure of the delta and X cells altered. Minimal degranulation of beta cells was present in 3 dogs whereas marked beta cell degranulation was present in the 2 with diabetes. No islet tissue was present in periduodenal tissues. As in previous work, no specific changes were found in the extrapancreatic tissues of these dogs devoid of alpha cells(7).

Discussion. We wished to determine what biochemical alteration, if any, would result from a complete lack of alpha cells. Except for NPN, which showed a moderate rise in all cases, and blood glucose, which increased in some animals, no significant persistent changes were found. We are not certain about the reasons for the NPN increase. However, lack of significant increase in urinary N and lack of continued weight loss, except in presence of diabetes, suggest that the blood NPN increase may be due to inhibition of protein synthesis.

Because in dogs, glucagon has a marked enhancing effect on renal excretion of Na^+ , K^+ , Cl^- and PO_4^{--} (15), urinary electrolytes were studied. The changes here reported are probably not due to lack of alpha cells. Similar excretion patterns have been described following surgical trauma(16); moreover, urinary electrolytes returned to normal levels within 2 weeks after operation.

An alpha cell hormone regulating fat metabolism(6) has been postulated in the rabbit on the basis of hypercholesterolemia following cobalt chloride treatment. Hypercholesterolemia was probably not due to al-

pha cell damage because, as is now generally accepted, cobalt-treated rabbits do not show alpha cell degeneration(17,18). Moreover, the present experiment does not support the idea that alpha cells regulate blood cholesterol levels.

Many investigators have suggested a close relationship between anterior pituitary growth hormone and alpha cells(1,5,19). Lack of changes in the pituitary in our experiment, as well as in pituitaries of dogs with experimentally altered pancreatic alpha/beta cell ratio(2,7,8), does not favor such relationships.

The diabetic aspect of some of these operated dogs is dependent upon number of beta cells left in transplanted pancreas as shown previously(2,7,8).

Lack of specific extra-pancreatic morphological alterations in dogs devoid of alpha cells is in agreement with previous work where no histological change was found that could be attributed to the absence(7), absolute decrease(8) or relative increase(2) of alpha cells. Possibly alpha cell hormone, not necessarily glucagon, acts only during period of growth. Studies are now in progress using puppies devoid of alpha cells to investigate this possibility.

Summary. 1) A study of levels of several electrolytes and various other substances concerned with protein, lipid and carbohydrate metabolism has been made in blood and urine of dogs subjected to removal of alpha cell containing part of pancreas by selective, partial pancreatectomy. 2) Despite previous suggestions by others, there was no definite evidence in our work of a relationship between functional activity of alpha cells and any of the substances studied, with the possible exception of blood non-protein nitrogen. Since this was significantly elevated in all experiments and was not associated with a significant increase in urinary nitrogen excretion nor loss of weight, it is possible that absence of alpha cells exerts a slight inhibitory effect on protein synthesis.

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Glycoprotein Content of Serum Lipoproteins.* (25079)

FREDERICK H. EPSTEIN[†] AND WALTER D. BLOCK

*Depts. of Epidemiology, School of Public Health, and of Dermatology, Medical School,
University of Michigan, Ann Arbor*

There is evidence to suggest a relationship between glycoprotein content of arterial wall on the one hand and aging process and arterial disease on the other. Thus, the arterial intima and media stain increasingly for mucopolysaccharides with age and in presence of atherosclerosis(1,2), even though chemical determinations have not shown an increase in hexosamine concentration of arterial tissue with age or arterial disease(3-5). Another possible link between glycoproteins and arterial disease is provided by relationship between lipemia clearing factor, probably a heparin-containing protein(6), and lipid metabolism. An inhibitor of lipemia clearing factor containing hexosamine has also been demonstrated(7). Hexosamine and glycoprotein content of serum itself is said to increase with age and in presence of atherosclerosis(8). Finally, glycoproteins of ground substance appear to be in equilibrium with the serum(9). Since not only glycoproteins, as discussed,

but also lipids play a part in processes associated with atherosclerosis and do, indeed, coexist in the lesion itself, it seemed of interest to study their interrelationship in serum from which the arterial deposits may be in part derived. Present studies were undertaken to determine glycoprotein content, expressed as hexosamine concentration, in lipoproteins of human serum. If glycoproteins and lipoproteins were linked in the serum, these substances might be simultaneously deposited in the arterial wall after infiltration. The current studies suggest that serum glycoproteins and lipoproteins are not linked to any appreciable extent. It would seem likely, therefore, that glycoproteins in the arterial wall are either deposited from serum independent of lipids, or are derived wholly or in part from local alterations in the arterial wall.

Methods. Blood was obtained from 4 healthy blood donors A, B, C, and D. Blood was allowed to clot in dry flasks and serum separated. All operations were carried out in the cold as far as possible during all stages. Lipoproteins were separated in

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[†] Special Research Fellow, Nat'l Heart Inst.