Acid and Glucose-6-Phosphatase Activity of Pancreatic B Cells after Cortisone and Sulfonylureas.* (25227)

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In previous work(1,2) glucose-6-phosphatase (G-6-Pase) was demonstrated histochemically in mammalian pancreatic B cells. It was also found, contrary to other reports (3,4), that non-specific acid (B-glycero and alpha napthyl) phosphatase (Ac-Pase) was present throughout pancreatic islets as well as in acinar cells. The function of these enzymes in pancreatic hormonal functions is unknown. However, glucose-6-phosphate (G-6-P) available for B cell metabolism may determine insulin output. G-6-P would vary with blood glucose concentration and G-6-Pase activity. On the other hand, Ac-Pase may play a role in protein synthesis(4), specifically in production of pancreatic endocrine and exocrine secretions. Increased insulin output from B cells as indicated by diminution of pancreatic B cell granulation occurs in cortisone-induced experimental diabetes(7) and after administration of hypoglycemic sulfonylureas(8,9). In the former instance, this is mediated by hyperglycemia(5,6,7,10). whereas sulfonylureas have a direct effect on B cells(8,9). A study of G-6-Pase and Ac-Pase content of B cells, under these conditions, may be helpful in assessing previous hypotheses regarding control of B cell secretory activity, and in explaining the pancreatic action of sulfonylureas.

Material and methods. Twenty-four New Zealand white rabbits weighing 2.5 to 3.5 kg were divided into 3 groups: Group I (6 normal rabbits) served as controls. Group II (12 animals) received 5 mg/kg cortisone acetate intramuscularly once daily for 10 to 21 days. Seven developed moderately severe diabetes and were sacrificed from 10 to 21 days. Group III (6 animals) received 125 mg/kg chlorpropamide intramuscularly twice daily and were sacrificed after 8 days. All animals were killed with Nembutal. Pancreas was removed immediately and frozen in petroleum ether kept at −70° by a bath of alcohol and dry ice(11). The frozen tissue was dried with filter paper and stored in stoppered tubes in the deep freeze at −20°C. Cryostat sections at 5 to 10 μ were affixed to slides and stained immediately or placed in neutral 6% calcium formol at −4° for 10 minutes prior to staining. Additional blocks of pancreas were placed in Zenker-formol solution and processed as previously described(12). To demonstrate G-6-Pase activity, the previously described technics(13,14) were modified(1). Slides were incubated for periods of 10 to 30 minutes in a mixture consisting of 2 cc of 0.5% solution of potassium glucose-6-phosphate, 20 cc of 0.2% tris maleate buffer, pH 6.7, 3 cc of 2% lead nitrate and 7 cc of distilled water. Control sections were incubated without substrate as well as after iodine treatment. Ac-Pase was demonstrated by Gomori's method(15,16) utilizing B-glycero phosphate as substrate at pH 5.2 with acetate buffer substituted for tris maleate buffer and incubation times of 30 to 60 minutes. The Zenker fixed tissue was stained by the periodic acid Schiff trichrome method(12) and a modified aldehyde fuchsin (17) trichrome method.

Results. G-6-Pase activity in normal rabbit pancreas was confined to B cell cytoplasm which frequently appeared to be more heavily stained in the perinuclear area(1,2). Areas of islets presumably occupied by A and D cells showed no precipitate. There was only minimal diffuse staining of the exocrine parenchyma (compare Fig. 1 and Fig. 2). Ac-Pase activity was found throughout the rabbit pancreas, but was greatest as secretory poles of acinar cells and at vascular poles of islet cells (Fig. 3). Morphologic alterations in the pancreas after cortisone treatment (Fig. 4) included marked and frequently complete degranulation of B cells as well as glycogenic vacuolization of duct epithelium and frequently also of B cells(7,10,18). In animals

* Supported by Grant from National Science Fn., Washington, D.C.

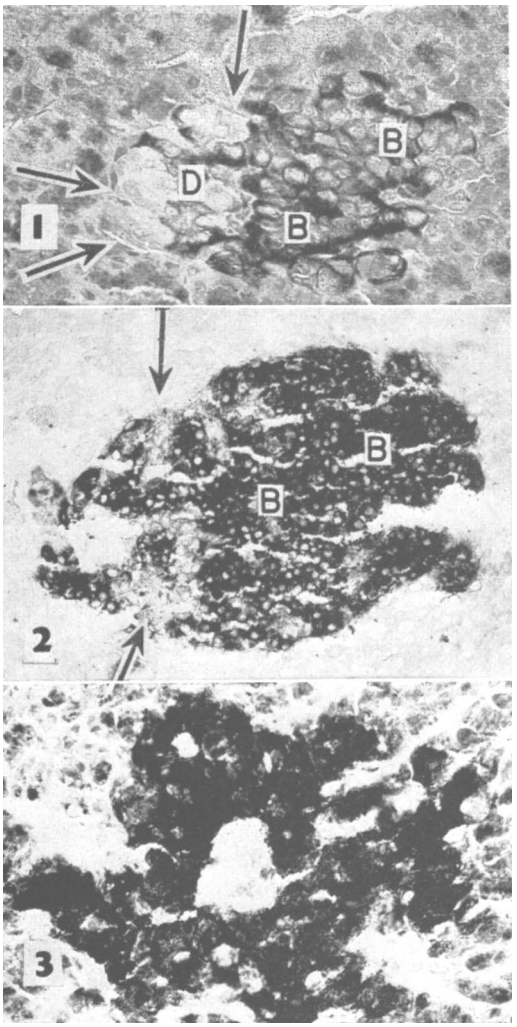

FIG. 1. Pancreas of untreated rabbit showing islet with A (arrows), B and D cells. Aldehyde Fuchsin Trichrome Stain. × 375.

FIG. 2. Pancreas of untreated rabbit showing distribution of glucose-6-phosphatase. Staining almost entirely confined to B cells. Sites of A and D cells (arrows) and acinar tissue are unstained. × 190.

FIG. 3. Pancreas of untreated rabbit showing distribution of non-specific acid phosphatase. Activity is present throughout the pancreas and most intense at vascular pole of islet cells and at secretory pole of acinar cells. × 375.

treated longest, there was diffuse proliferation of intercalated ducts, occlusion of small ductules by inspissated secretion and intraislet proliferation and dilatation of ductules. Despite these changes, no definite alteration in enzymatic staining was found, except that areas of islets occupied by proliferating duct epithelium did not show any G-6-Pase or Ac-Pase activity. After treatment with chlorpropamide as with other sulfonylureas(8,9,19,20), marked to complete B cell degranulation occurred (Fig. 5), but Ac-Pase activity was similar to normal. On the other hand, intensity of G-6-Pase staining at 10 minutes incubation was considerably less than in normal controls (Fig. 6), but this difference became less apparent after longer incubation.

Discussion. Hyperfunctioning rabbit pancreatic B cells degranulated by cortisone-induced hyperglycemia or by sulfonylurea administration showed no grossly visible changes in Ac-Pase staining. If this enzyme is important for protein synthesis in the B cell, an increase in its activity might have been expected. However, it must be remembered that histochemical methods give rough estimates of intracellular enzyme activity.

Lack of alteration of B cell G-6-Pase after cortisone and diminution in activity after sulfonylurea are consistent with the postulated role of this enzyme in controlling insulin output. High blood sugar levels after cortisone treatment would make increased G-6-P available for B cell metabolism despite intact G-6-Pase activity and thereby increase insulin output. On the other hand, diminution in G-6-Pase activity after sulfonylurea would accomplish similar results despite lowered blood sugar level.

Biochemical studies in liver homogenates have shown a slight(21) or marked(22,23,24) increase in G-6-Pase activity after cortisone and a diminution after chronic sulfonylurea administration(25). On the other hand, both *in vivo* and *in vitro* experiments have failed to demonstrate an acute effect of the sulfonylureas on liver G-6-Pase activity(25,26), suggesting that diminished hepatic G-6-Pase activity after chronic sulfonylurea administration is an adaptive response similar to that observed after insulin administration and not due to a direct action of the drug on this enzyme. This alternative hypothesis could also apply to the reduced B cell G-6-Pase observed in the present study after sulfonylurea administration.

Summary. Glucose-6-phosphatase (G-6-Pase) and acid phosphatase (Ac-Pase) activi-

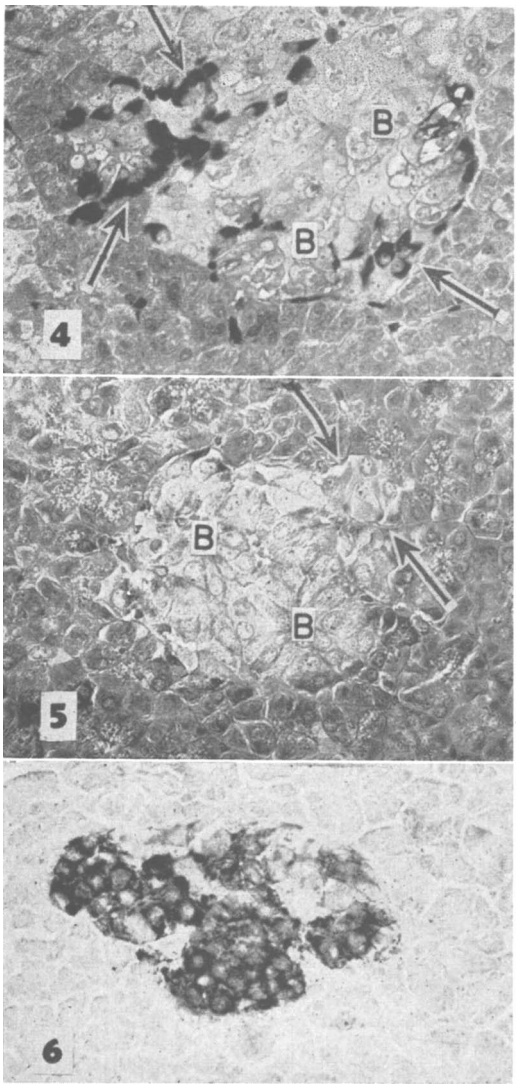

FIG. 4. Pancreas of cortisone treated rabbit showing islet with degranulated and partly vacuolated B cells and intact A cells (arrows). Aldehyde Fuchsin Trichrome Stain. × 375.

FIG. 5. Pancreas of chlorpropamide treated rabbit showing islet with degranulated B cells and intact A cells (arrows). Aldehyde Fuchsin Trichrome Stain. × 375.

FIG. 6. Pancreas of same rabbit as in Fig. 5, showing islet with reduced glucose-6-phosphatase activity. × 375.

ties have been followed histochemically in hyperfunctioning rabbit pancreatic B cells in cortisone diabetes and after administration of hypoglycemic sulfonylureas. Cortisone diabetic rabbit pancreas with B cell degranulation and glycogenic vacuolization of ductules and B cells showed no definite changes in B cell Ac-Pase or G-6-Pase activity. Hypoglycemic sulfonylureas administered over period adequate to degranulate B cells, seemed to diminish their G-6-Pase content without effecting Ac-Pase. Although B cell degranulation was, in each of these instances, indicative of increased insulin output, lack of alteration of histochemically demonstrable B cell Ac-Pase activity was not considered to be at variance with the view that this enzyme may be important for insulin synthesis. Inhibition of B cell G-6-Pase activity by chronic sulfonylurea administration could be the mechanism of pancreatic action of these drugs. However, the alternative explanation that reduced G-6-Pase activity was a secondary adaptive response cannot be entirely excluded.

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Received July 1, 1959. P.S.E.B.M., 1959, v102.

Purine Antagonists and Growth of Tetrahymena.* (25228)

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The demonstration that a purine analog interferes with the metabolism of acetate and with sterol synthesis in *Tetrahymena*(1) led to a reexamination of the effects of other structural analogs of purines. These compounds may be divided into groups on the basis of the nature of structural changes embodied in them, nature of substances bringing about reversal and whether or not the inhibitors are able to bring about death of the cultures. These features can be shown to have a certain degree of correlation to one another.

Material and methods. Strain W of *Tetrahymena pyriformis* was grown in medium A (2) containing Tween 80 at a concentration of 10 mg/ml. From this medium acetate and adenylic acid were omitted as indicated. In certain experiments β-sitosterol or adenine was added. Conditions of growth and incubation were as previously described(2). The purine analogs 8-azaguanine (8AG), 8-azaadenine (8AA), 6-methylpurine (6MeP), 6-chloropurine (6ClP) and purine (Pu) were commercial products. One sample of 6-methoxypurine (6MeOP) was prepared in the Biological Laboratory at Amherst College and another obtained commercially. The sample of 6-methyl-1-deazapurine (6MeDAP) was also prepared in the Amherst College laboratory. Through the kindness of Dr. K. Pfister of Merck and Co. we obtained a sample of 2-azaadenine (2AA).

* Supported by research grants (A-1005 and CY-2924) from the National Institutes of Health, U. S. Public Health Service and a grant (CH-27) from the American Cancer Society.

Results. Table I shows the response of *Tetrahymena* to the various inhibitors in the basal medium and also with addition of acetate, β-sitosterol or adenine or combinations of these substances. The first 3 inhibitors represent the group of analogs in which changes in ring structure have been made and all (Figures in parentheses) are most readily reversed by adenine. Addition of acetate, sterol or both have relatively little influence on the inhibition. The next group of 4 analogs differs from adenine only in the nature of the substituent replacing the amino group in the 6 position. Two of these compounds, 6MeP and 6ClP are reversed best by sterol and synergistic reversing effects are shown by combinations of adenine with sterol or acetate. The other 2 compounds, Pu and 6MeOP, are almost equally well reversed by either adenine or sterol and the effects of combinations of material are not so striking. The last analog (6MeDAP) has changes both in the ring and in the substituent group. It resembles the first group of analogs in its reversal by adenine. With regard to 1-deazaadenine (DAA) it can only be said that it is reversed by adenine(3). There are no data on the effects of the other compounds considered here.

Certain of these purine analogs have another peculiarity in common. They permit normal growth of the organisms up to a point. The organisms then lose motility, settle to the bottom of the tubes and autolyze. This process releases fat droplets, glycogen granules and other particulate material into the medium, thus making it more, rather than