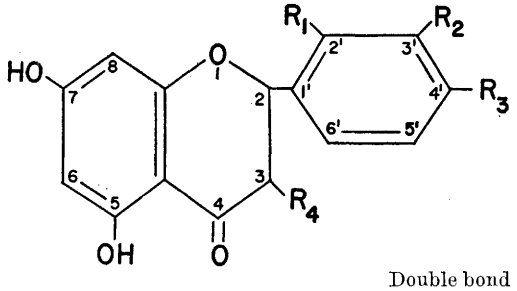
TABLE I. Thymus Gland Weights of Control and Flavonoid-Fed Rats.

Flavonoid fed	No. of rats	Range of final body wt, g	Mean body wt, g	Avg thymus wt, % of body wt		
				Mean ± S.E.	Diff. ± S.E.	P*
None (basal, semi-synthetic diet)	40	67–99	83.7	.350 ± .006		
Hesperetin	10	64–87	78.9	.340 ± .011	.010 ± .013	
Naringenin	10	77–90	84.7	.326 ± .017	.024 ± .018	
Luteolin	9	62–90	77.7	.313 ± .010	.037 ± .012	<.01
Ca-flavonate glycoside	10	74–101	85.2	.319 ± .014	.031 ± .012	.02
Eriodictyol	10	54–81	67.1	.295 ± .012	.055 ± .014	<.001
Quercetin	10	77–98	89.0	.303 ± .008	.047 ± .010	"
None (basal, non-synthetic diet)	5	65–90	79	.297 ± .07		
Morin (non-synthetic diet)	5	69–90	80	.306 ± .08	.009 ± .11	

* P = Probability that such a difference would occur by chance. Only significant values of P are indicated.



	R_1	R_2	R_3	R_4	Double bond 2:3
Hesperetin	H	OH	OCH_3	H_2	No
Naringenin	H	H	OH	H_2	"
Luteolin	H	OH	"	H	Yes
Eriodictyol	H	"	"	H_2	No
Quercetin	H	"	"	OH	Yes
Dihydro-quercetin	H	"	"	H, OH	No
Morin	OH	H	"	OH	Yes
Diosmetin	H	OH	OCH_3	H	"

FIG. 1. Generalized structure of flavonoids. Substituents for individual flavonoids are tabulated above.

various flavonoids to their thymolytic action will be discussed later.

Summary. (1) Hesperidin, hesperetin, naringenin, morin and luteolin were fed to young rats to determine their ability to induce thymus involution. Only luteolin was effective, and in this respect resembles quercetin, dihydroquercetin, and eriodictyol. (2) A commercially available preparation, "Calcium Flavonate Glycoside, Lemon," caused thymus involution. Some relationships between structure and thymolytic action of flavonoids are discussed.

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Demonstration of Glucose-6-Phosphatase in Mammalian Pancreas.* (25108)

SYDNEY S. LAZARUS (Introduced by Bruno W. Volk)
Isaac Albert Research Inst., Jewish Chronic Disease Hospital, Brooklyn, and Albert Einstein College of Medicine, Bronx, N. Y.

Previous workers demonstrated non-specific alkaline phosphatase in pancreatic ductules (1,2,3) and non-specific acid phosphatase in islets as well as in exocrine pancreas(4,5). Glucose-6-phosphatase has been demonstrated in liver, kidney and intestinal tract(6,7) but has been stated to be absent from pancreas (7). This enzyme is heat labile, inactivated at

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pH 5 or below and under some conditions by formalin fixation(8,9). The enzyme is probably concerned with uptake and/or liberation of glucose by cells which contain it. Since the B cell responds to blood concentration of glucose(10,11) it seems reasonable to expect a mechanism controlling movement of glucose into and out of the cell. This viewpoint is, furthermore, consistent with the fact that glycogen deposits, found within pancreatic ductular epithelium and B cells under conditions of protracted hyperglycemia(12,13,14) recede quickly when blood sugar concentration reverts to normal(15). A study of pancreatic glucose-6-phosphatase was undertaken and its distribution compared with that of non-specific acid and alkaline phosphatase.

Material and methods. Pancreatic tissue was obtained from normal rabbits, guinea pigs and dogs and also portions of liver from rabbits. All animals were sacrificed by overdosage with nembutal. Tissues were removed immediately and frozen in petroleum ether, kept at −70° by bath of alcohol and dry ice (16). The frozen tissue was dried with filter paper and stored in stoppered tubes in deep freeze at −20°C. Cryostat sections at 5 or 10 μ were affixed to slides and stained immediately or were placed in neutral 6% calcium formol at −4°C for 15 minutes prior to staining. Additional blocks of pancreas were placed in Zenker-formol solution and processed for study of cell types(17). To demonstrate glucose-6-phosphatase activity, previously described technics(6,18) were modified. Slides were incubated for 10 to 30 minutes in a mixture consisting of 20 cc of 0.02 M solution of potassium glucose-6-phosphate, 20 cc of 0.2 M 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 and also at pH 5 and pH 9.2. For pH 5, acetate buffer was substituted for tris maleate buffer. Additional sections were incubated in the same substrate at pH 6.7 but with 20 cc of either 0.02 M B-glycero-phosphate, 0.02 M glucose-1-phosphate, 0.002 M adenosine phosphate or 0.002 M adenosine triphosphate substituted for glucose-6-phosphate. Non-specific acid and alkaline phosphatase were demonstrated at pH 5 and pH 9.2 respectively, utilizing B-glycero-phosphate as substrate as well as by azo dye method using alpha-napthyl-phosphate(19,20). For Zenker fixed tissue the periodic acid Schiff trichrome method(17) was used as well as modified aldehyde fuchsin trichrome method(21).

Results. In unfixed rabbit liver glucose-6-phosphatase activity was present only in cytoplasm of hepatic cells. No enzyme was present in blood vessels, canalicular system or Kupfer cells. After fixation in cold 6% calcium formol similar staining was obtained, with very slight, if any, diminution in enzyme activity (Fig. 1). This applied for pancreas as well, and since the tissue was better preserved after short formalin fixation this was used exclusively.

In the rabbit pancreas, with glucose-6-phosphate as substrate and 10 minute incubation, the precipitate was confined to B cell cytoplasm which frequently appeared to be more heavily stained in the perinuclear area (Fig. 2). Areas of islets presumably occupied by A and D cells did not stain (Compare Fig. 2 and Fig. 3). There was only very minimal diffuse staining of the exocrine parenchyma. Longer incubation times increased the precipitate in B cells but did not show staining at any other site. Control sections treated with Lugol's solution or incubated without substrate showed complete absence of staining. At pH 5 with 10 minute incubation there was markedly reduced B cell staining while at pH 9.2 almost no staining was observed.

Equivalent localization or intensity of staining as with glucose-6-phosphate was not obtained in the rabbit pancreas with any other substrate at pH 6.7 and 10 minutes incubation. Thus, with B-glycero-phosphate or alpha-napthyl-phosphate there was faint staining of the entire islet as well as the acinar tissue. Glucose-1-phosphate showed very faint islet staining and almost no staining of exocrine parenchyma. With adenosine phosphate there was very faint diffuse staining throughout. Adenosine triphosphate stained the cytoplasm and nuclei of islet and acinar cells. Cell borders and nuclear membranes stand out, as well as perivascular and periductular tissue. In addition, there was more intense

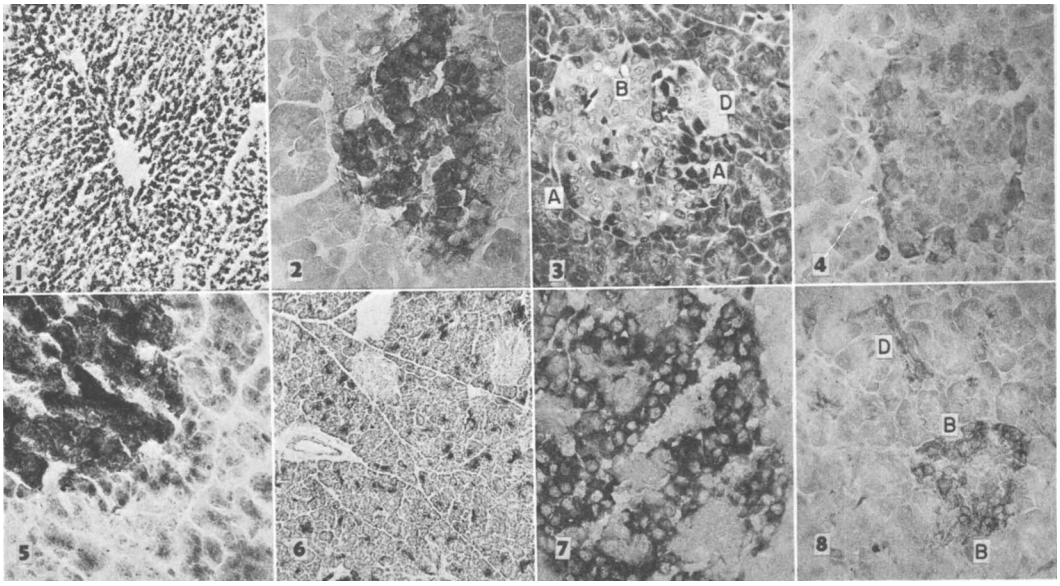

FIG. 1. Distribution of glucose-6-phosphatase in cryostat sections of rabbit liver fixed 10 min. in cold neutral 6% calcium formol, then incubated for 10 min. with glucose-6-phosphate at pH 6.7. Enzyme activity confined to cytoplasm of hepatic cells. × 32.

FIG. 2. Rabbit pancreas showing distribution of glucose-6-phosphatase. Staining almost entirely confined to B cells and more intense in perinuclear areas. × 183.

FIG. 3. Rabbit pancreas showing an islet with A, B and D cells. Periodic Acid Schiff Trichrome. × 183.

FIG. 4. Rabbit pancreas showing distribution of precipitate when adenosine triphosphate is substituted for glucose-6-phosphate in incubation mixture. Intense staining of peripheral islet cells, which are either A or D cells. × 125.

FIG. 5. Rabbit pancreas showing distribution of non-specific acid (B-glycero-) phosphatase at pH 5 after 45 min. incubation. Activity is present throughout pancreas. Staining greatest at vascular pole of islet cells and at secretory pole of acinar cells. × 183.

FIG. 6. Rabbit pancreas showing distribution of non-specific alkaline (B-glycero-) phosphatase at pH 9.2 after 45 min. incubation. Activity present mostly in ductular system. × 48.

FIG. 7. Guinea pig pancreas showing distribution of glucose-6-phosphatase. Staining confined almost entirely to B cells. × 183.

FIG. 8. Dog pancreas showing distribution of glucose-6-phosphatase. Marked staining present in cytoplasm of ductular epithelium (D) and in B cells (B). × 125.

staining of groups of islet cells which, from their localization, are thought to be A or D cells (Fig. 4). Non-specific acid phosphatase was found throughout rabbit pancreas when either B-glycero-phosphate or alpha-napthyl-phosphate was used at pH 5. Its activity was greatest, however, at the secretory poles of acinar cells and at vascular poles of islet cells (Fig. 5). Non-specific alkaline phosphatase as demonstrated by either method was mostly present in the ductular system (Fig. 6).

In guinea pig pancreas distribution of glucose-6-phosphatase was the same as in rabbit (Fig. 7). In the dog, however, staining was also present in cytoplasm of ductular epithelial cells (Fig. 8).

Discussion. Glucose-6-phosphatase activity has been demonstrated within the cytoplasm of pancreatic B cells in rabbit, guinea pig and dog. In the dog, in addition, some activity also was observed in ductular epithelium. Specificity of histochemical localization of this enzyme is demonstrated by the differences in distribution or the limited activity when rabbit pancreas was incubated at pH 6.7 for equally short times with other substrates and by the fact that maximum staining was obtained at optimum pH for this enzyme with loss of activity at pH 5 and pH 9.2(22,23). Inability of a previous worker (7) to demonstrate glucose-6-phosphatase activity in pancreas may have been due to differences in incubation mixture or to the fact

that unfixed frozen pancreatic tissue may autolyze rapidly when incubated.

Contrary to reports that formalin inactivates glucose-6-phosphatase(6,7,9) we found that short fixation in cold neutral 6% calcium formol does not appreciably alter its activity or distribution and gives better tissue preservation.

The known relationship of insulinogenesis to blood sugar concentration(10,11) suggests that B cell glucose-6-phosphatase might function in relation to the mechanism of insulin release. Perhaps the net amount of glucose-6-phosphate available for B cell metabolism is the controlling factor in limiting rate of insulin output. The net rate of formation of glucose-6-phosphate would depend on relative velocities of hexokinase and glucose-6-phosphatase reactions and velocity of glucose phosphorylation by hexokinase would depend upon blood sugar concentration. Thus an increase in blood sugar concentration or decrease in glucose-6-phosphatase activity would increase available glucose-6-phosphate and increase insulin output. Conversely, lowering blood sugar or increasing glucose-6-phosphatase would diminish available glucose-6-phosphate and also diminish insulin output.

Summary. Glucose-6-phosphatase has been demonstrated in pancreatic B cells of rabbit, guinea pig and dog, and in canine ductular epithelium. Short fixation of fresh frozen cryostat sections in cold neutral 6% calcium formol does not inhibit its activity or alter its distribution in liver or pancreas. Furthermore, specificity of histochemical reaction for glucose-6-phosphatase was demonstrated by differences in staining when other substrates were incubated at same pH for equal time. In view of known relationship of blood sugar concentration to rate of insulin secretion, a hypothesis is advanced relating presence of this enzyme in B cells to control of insulin secretion. It is suggested that net rate of glucose-6-phosphate formation is the controlling factor and that this would be influenced by any increase or decrease of glucose-6-phosphatase activity.

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