TABLE II. Comparison of X-Ray Diffraction Patterns.

d, A.U.	Sitosterol	Cholesterol	1:1 mixed xtal standard	*In vivo* centrifugate	*In vitro* centrifugate
18.5	*3*— .6				
5.89	*1*—1.0				
5.72		*1*—1.0	*2*— .3	*2*— .3	*2*— .3
5.48			*1*—1.0	*1*—1.0	*1*—1.0
5.41		*4*— .6			
5.33			*3*— .2	*3*— .2	*3*— .2
5.21		*2*— .8			
4.99		*3*— .7			
4.85	*2*— .8				
4.61	*4*— .4				

Table II presents the interplanar spacings and relative intensities for the strongest lines obtained from x-ray diffraction patterns. Italicized numbers refer to intensity rank of that diffraction line while the following number is relative intensity, I/I_1. The uncertainty of d values varies from $\pm.01$ to $\pm.03$ Angstrom unit depending on sharpness of the line.

villi washings of the second segment in one animal. In no case was a cholesterol pattern obtained. A comparison of the x-ray diffraction patterns obtained *in vivo* and *in vitro* is presented in Table II.

Discussion. Davis(2) previously observed that both sitosterol and crystals of 1:1 cholesterol-sitosterol mixed crystal have only one-third the solubility of cholesterol in methanol, and exhibit similarly reduced solubilization in aqueous sodium oleate or sodium desoxycholate systems. Sitosterol thus physically removes cholesterol from the dispersed state necessary for absorption. These experiments present evidence that formation of a mixed crystal of cholesterol and sitosterol may indeed be a mode of action responsible for the hypo-cholesteremic effect in ingested plant sterols. The relative importance of other proposed mechanisms is undergoing further investigation.

Summary. It was shown that a 1:1 cholesterol:sitosterol mixed crystal are formed not only in aqueous *in vitro* systems, but also *in vivo*.

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Oxidative Pathways in Pancreatic B Cells.*† (25286)

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In previous work(1,2,3) glucose-6-phosphatase was demonstrated within the pancreatic B cells in rabbits, guinea pigs and dogs, as well as in ductular epithelium of the dog. Presence of this enzyme in the B cell as well as known direct relationship between glycemic level and insulin production led to the hypothesis that the net amount of glucose-6-phosphate available for B cell metabolism regulates rate of insulin output. Since glucose-6-phosphate is the first step in entrance of glucose into the metabolic cycle, a histochemical study was undertaken of pathways for glucose oxidation within the B cells. Me-

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† Microphotographs were prepared by Herbert A. Fischler.

tabolism of glucose-6-phosphate could proceed by either the hexosemonophosphate (HMP) pathway or by the Embden-Meyerhoff (E.M.) pathway and the Krebs cycle. For the HMP pathway the triphosphopyridine nucleotide (TPN) linked enzyme glucose-6-phosphate dehydrogenase (G-6-PD) as well as TPN diaphorase (TPND) were studied. Since E.M. enzymes are mostly diphosphopyridine nucleotide (DPN) linked, lactic dehydrogenase as well as DPN diaphorase (DPND) were studied. Succinic dehydrogenase (SD) was utilized as an indicator for Krebs cycle activity.

Material and methods. New Zealand white rabbits of either sex weighing 2.5 to 3.5 kg were sacrificed by overdosage with nembutal. The pancreas was removed immediately and frozen in petroleum ether, kept at −70°C by dry ice(4). The frozen tissue was dried with filter paper and stored in stoppered tubes at −20°C. Cryostat sections at 10 μ were affixed to cover slips and incubated immediately (G-6-PD and SD) or placed in neutral 6% calcium formol at −4°C for 15 minutes prior to incubation (TPND, DPND and LD). Additional blocks of pancreas were placed in Zenker formol solution and processed as described previously(5). A modification of the method of Hess *et al.*(6) was used to demonstrate G-6-PD and LD activity. The incubating solution consisted of:

	ml
pH 7.6 phosphate buffer (0.1 M) with sodium cyanide (2 mg %)	1.5
Nitro B-T (3 mg/ml)	1.5
Magnesium chloride (.05 M)	.6
Glucose-6-phosphate potassium salt (.1 M) or sodium lactate (.5 M)	1.2
TPN (25 mg/ml) or DPN (50 mg/ml)	1.2

For SD the incubating medium consisted of 2 cc each of the buffer, Nitro B-T solution and 0.5 M sodium succinate. For TPND and DPND a modification of Scarpelli's method (7) was used. The solution consisted of 1.25 cc each of buffer and Nitro B-T, to which is added 2.5 cc of distilled water and 30 mg of either TPNH or DPNH. After addition of the reduced pyridine nucleotide the solution is stable for only 1½ hours. For osmotic protection of tissue, 7.5% polyvinylpyrrolidine was added to each medium(6). For DPNH and TPND sections were incubated 15 to 20 minutes. For dehydrogenases 1 to 1½ hours gave best results. Control sections were incubated in media without substrates. After incubation sections were rinsed briefly in distilled water and fixed in 10% neutral formalin overnight. They were then washed in running tap water for 30 minutes and rinsed in distilled water. Alternate sections were mounted in glycerine jelly and the remainder dehydrated in cellosolve, cleared in toluene and mounted in permount.

Results. G-6-PD staining was most marked in B cells of the islets and in the ductular epithelium (Fig. 1). Abundant, small granules could be seen throughout the cytoplasm of these cells (Fig. 2). When slides are mounted in aqueous media the A cells are identifiable, stained pale brownish red by monoformazan and showing refractile globules packed in the cytoplasm. G-6-PD activity is seen as a few sparse coarse blue granules. D cells could not be identified. The reaction in the acinar tissue was similar to that in A cells. The wall of arterioles showed numerous small granules apparently in the cytoplasm of smooth muscle cells.

Distribution of TPND activity differed from that of G-6-PD in that all pancreatic elements showed greater reactivity. The cytoplasmic structures were morphologically similar to those showing G-6-PD activity but in A cells and in acinar cells they were more abundant and of a darker color (compare Figs. 1 and 3). With some exceptions, LD showed distribution and localization of staining similar to DPND (Figs. 4, 5). However, in each instance, DPND activity was greater than that of LD. Both showed marked staining of densely packed, fine cytoplasmic granules in duct epithelium and somewhat less intense staining in the form of fine granules in acinar cells. The islets showed minimal reactivity except for well stained groups of A cells (Fig. 6). Arteriolar walls had numerous reactive granules which were more numerous after DPND staining than after LD staining. In addition, after staining for DPND, acinar cell walls could be seen as blue outlines.

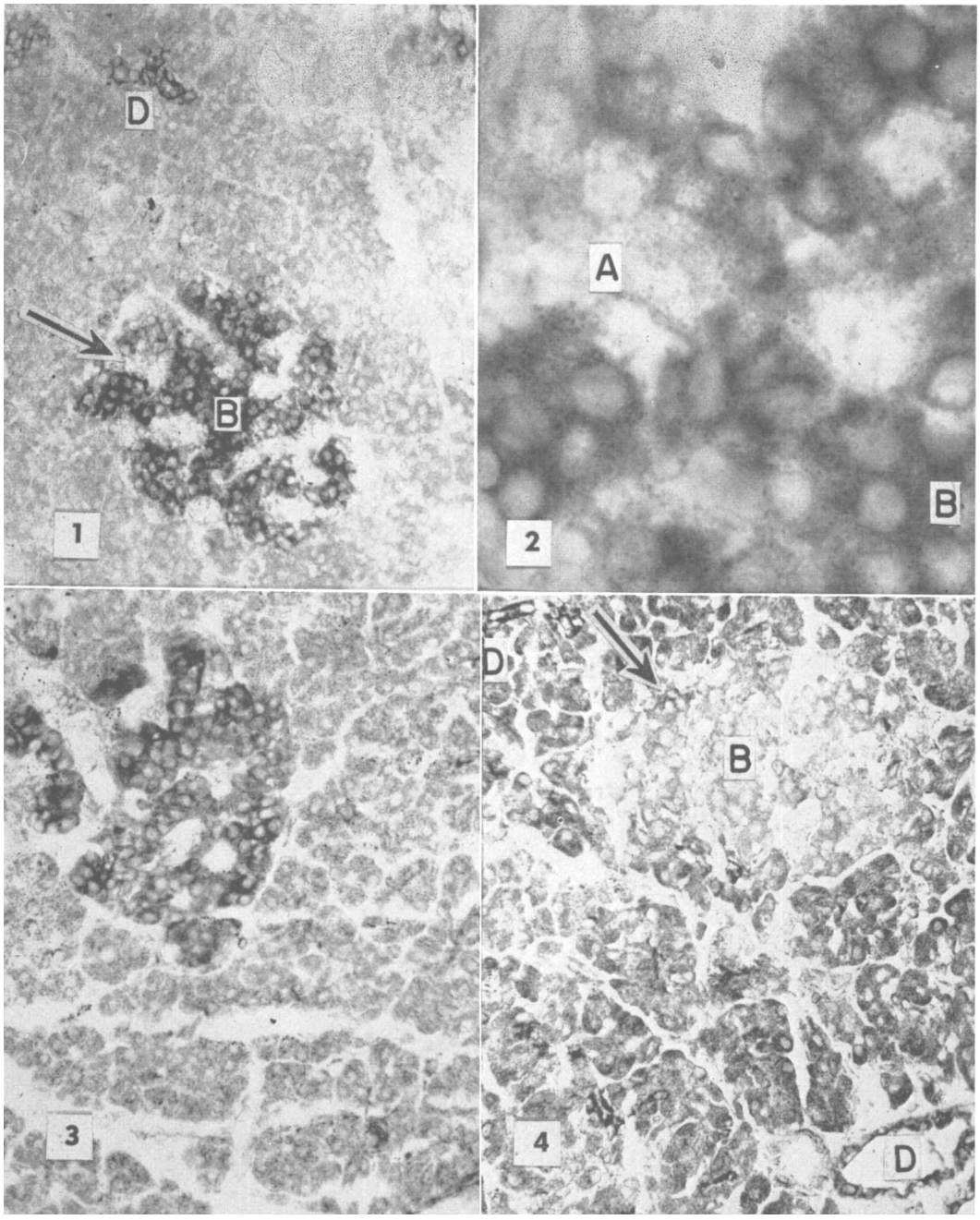

FIG. 1. Rabbit pancreas showing distribution of glucose-6-phosphate dehydrogenase. There is marked activity in B cells (B) as well as in duct epithelium (D). Areas of islets occupied by A cells (arrow) as well as the acinar tissue show limited staining. × 100.

FIG. 2. High power view of same pancreas as in Fig. 1 showing densely packed cytoplasmic granules in B cells (B) and sparsely staining granules in A cells (A). × 960.

FIG. 3. Rabbit pancreas showing distribution of triphosphopyridine nucleotide diaphorase. Note similarity of localization to that of glucose-6-phosphate dehydrogenase. × 150.

FIG. 4. Rabbit pancreas showing distribution of lactic dehydrogenase. Most marked activity is in duct epithelium (D), acinar tissue and A cells (arrow). B cells (B) stain very lightly. × 150.

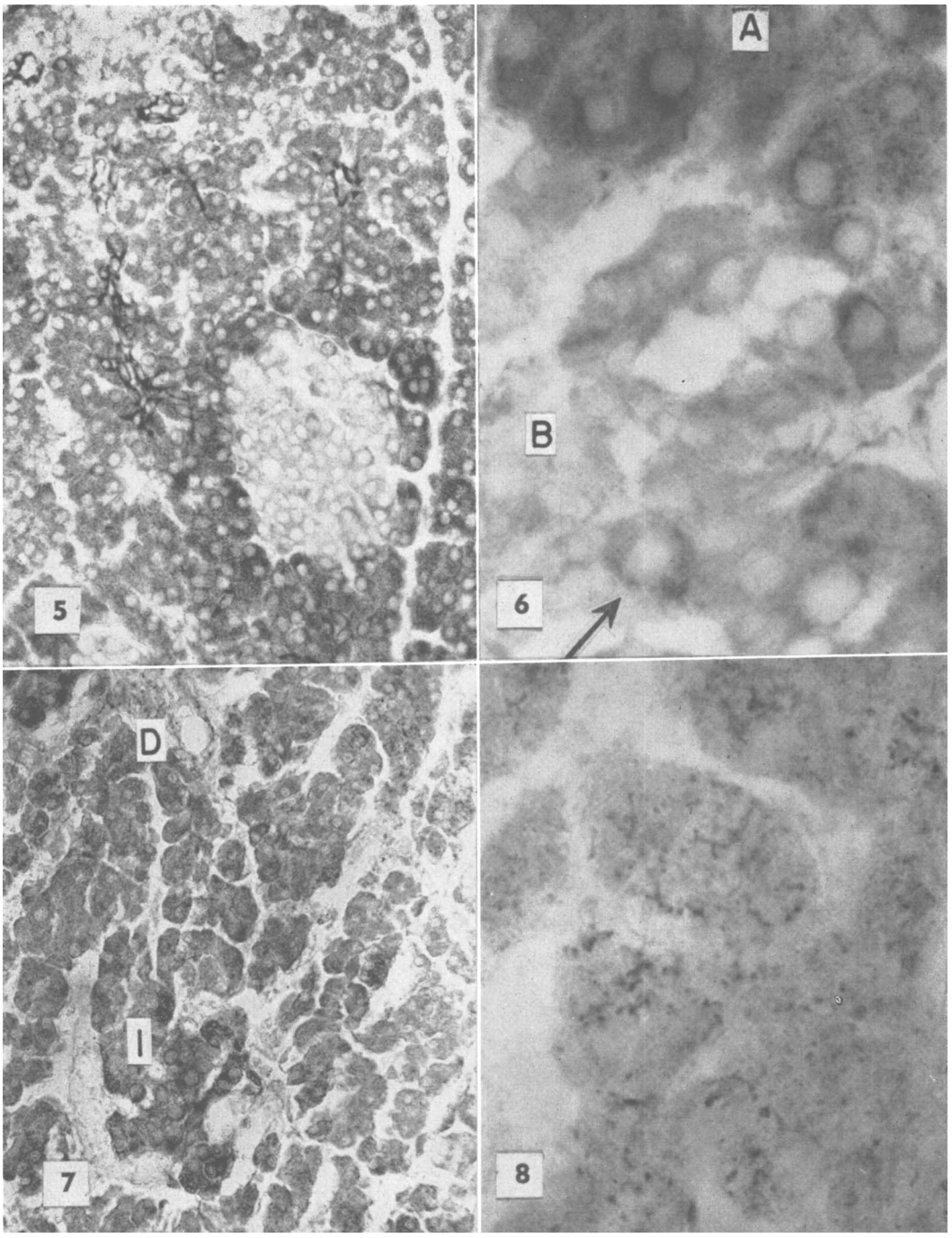

FIG. 5. Rabbit pancreas showing distribution of diphosphopyridine nucleotide diaphorase. Note similarity of localization to that of lactic dehydrogenase. × 150.

FIG. 6. High power view of same pancreas as in Fig. 5 showing faintly staining B cells (B), heavily stained A cells (arrow) and well stained acinar tissue (A) . × 960.

FIG. 7. Rabbit pancreas showing distribution of succinic dehydrogenase. There is good activity throughout but most marked in duct (D) and islets (I). × 150.

FIG. 8. High power view of same pancreas as in Fig. 7 showing mitochondria-like appearance of the reactive structures. × 1280.

S.D. activity was distributed throughout the pancreas. It was most intense in duct epithelium and in B cells, less so in other cells (Fig. 7). The walls of arterioles contained numerous rather coarse granules. The intracellular localization of S.D. differed from that of other enzymes studied. The reactive structures appeared as varying sized rods as well as spheres suggestive of mitochondria (Fig. 8).

Discussion. Our results indicate that G-6-P oxidation in rabbit pancreatic B cells is predominantly *via* the HMP shunt while the EM pathway plays a secondary role. The well marked S.D. activity found in the islets is at variance with results of biochemical studies in toadfish(8). The low ratio of S.D. to cytochrome oxidase is probably accounted for by the limited metabolic role which the EM pathway and the Krebs cycle plays in B cells as compared with the HMP shunt.

The high level of all enzyme activity in duct epithelium is surprising in view of their lack of secretory activity. However, it would be in accord with the fact that these are comparatively primitive cells, as demonstrated by neogenesis of islet cells and acinar tissue from ducts in both the adult animal and the embryo(9,10,11). The relatively greater activity and wider distribution of TPND than of G-6-PD denotes the presence of additional TPN-linked enzymes both within the G-6-PD containing cells and at other sites(6,12,13,14). The relative activity and distribution of LD and DPND are similarly interpreted.

Both biochemical and histochemical studies (7,15,16,17,18,19) have shown that S.D. activity is limited to mitochondria. Morphology of the reactive structures for this enzyme in the pancreas are in accord with this viewpoint. On the other hand, the intracellular localization of the other 4 enzymes has not been clarified. Some have claimed that these oxidative enzymes also are localized to mitochondria(6,7,18,20). However, human erythrocytes which lack mitochondria metabolize glucose by the HMP pathway(21,22,23,24). Furthermore, in studies on cell homogenates these enzymes have been reported in both mitochondrial and extramitochondrial fractions(25,26). In pancreatic cells they showed up as abundant, generally small, closely packed granules diffusely distributed in the cytoplasm. Furthermore, DPND showed intense staining of acinar cell borders. These findings suggest that they are mostly extramitochondrial.

High HMP activity is found in other cell systems which are actively synthetic, as in liver, lactating mammary gland and adrenal cortex(27,28,29). This pathway may serve, not only to supply energy for metabolic processes but, also, as a source of TPNH. It has been claimed that cell synthetic activity may depend on generation of TPNH(30,31) and a role for it in insulin synthesis may be postulated. It is also feasible that TPNH production controls insulin output by influencing the biological activity of the many enzymes which depend on presence of free sulfhydryl groups, or by maintaining glutathione in the reduced state(27). The possible importance of sulfhydryl groups and of glutathione in B cell metabolism has been stressed in connection with the toxic action of alloxan (8). Furthermore, it has been shown in red blood cells that high levels of G-6-PD are necessary for the stability of reduced glutathione(24).

Summary. 1) A histochemical study has been conducted of the distribution of glucose-6-phosphate dehydrogenase (G-6-PD), triphosphopyridine nucleotide diaphorase (TPND), lactic dehydrogenase (LD), diphosphopyridine nucleotide diaphorase (DPND) and succinic dehydrogenase (SD) in the pancreas of the rabbit. G-6-PD was found principally in B cells and duct epithelium; LD in duct epithelium, acinar tissue and A cells. TPND was present throughout, with greatest activity in B cells and in duct epithelium and good activity in other areas. SD activity was present throughout the pancreas with greatest staining in B cells and duct epithelium. The reactive structures for SD seemed to be mitochondrial while the other enzymes apparently are mostly extramitochondrial. 2) These findings demonstrate that glucose metabolism in B cells is predominantly *via* the hexose monophosphate shunt, and indicates a role for it in insulin synthesis. A relationship between TPNH generation by this pathway and avail-

ability of reduced glutathione or of free SH groups in enzymes is considered as a possible mechanism controlling B cell insulin output.

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Essential Growth Factor in Serum Dialysate for Chick Skeletal Muscle Fibroblasts.* (25287)

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Growth requirements for mammalian cells in tissue culture have been investigated extensively by Eagle and his associates(3). A number of established cell lines will proliferate indefinitely as monolayers in the presence of 13 essential amino acids, 8 vitamins, 6 ions, and a suitable carbohydrate source, provided that dialyzed serum is added to the culture medium(5,6). Freshly isolated monkey kidney cells have similar short term requirements although indefinite cultivation on Eagle's medium and dialyzed serum has not been achieved(4). Our comparable experiments on chick cells have shown that monolayer cultures obtained from skeletal muscle fail to grow or survive in dialyzed serum supplemented by Eagle's medium. Subsequent work as detailed below indicates that these cells require one or more additional factors present in serum dialysate but not occurring in Eagle's medium or other synthetic nutrients presently available.

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