ment. A clear-cut demonstration of retardation of the homograft response was achieved. An interval of more than a week supervened after the slough of the last homograft in the untreated group and the slough of the first homograft in the treated group. Mortality due to treatment was significantly reduced. Nonetheless, all homografts were rejected between the 26th and 28th day following grafting. Hence, it is apparent that additional treatment was required in order to maintain suppression of the homograft rejection response.

Discussion. These results clearly established that 6-Mercaptopurine in the dose levels employed produced an inhibitory effect on the immune response to skin homografts in rabbits. The lethality of this treatment with 6-Mercaptopurine has also been demonstrated. That lethality was not always a result of bone marrow suppression is indicated by the relatively large number of animals dying prior to development of leukopenia. The unusually large number of animals dying prior to slough of the skin homograft did not permit determination of maximum survival time if treatment had been continued indefinitely. If treatment were discontinued, as in Group III, all grafts eventually sloughed. Hence, it appeared that sustained treatment was necessary to maintain suppression of the homograft response. It was also of interest to note that profound leukopenia was present in those animals showing the longest graft survival. This was interpreted as suggesting the importance of using doses sufficiently large to achieve hemopoietic suppression in order to obtain this effect with this antagonist of purine metabolism. Nonetheless, interference with nucleic acid synthesis(6) may be inferred to be the predominant action of this drug and the means by which it presumably suppresses production of humoral antibody and the homograft reaction.

Summary. 6-MP, a powerful metabolic antagonist to nucleic acid synthesis, prolongs survival time of skin homografts in rabbits when administered after grafting. Treatment must be given continuously to maintain graft viability. However, toxicity of 6-MP limits the length of time treatment may be given.

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Formation of Mixed Crystals of Cholesterol and Sitosterol *in vitro* and in Rabbit Intestine. (25285)

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Many investigators have observed that simultaneous feeding of plant sterols and cholesterol to animals prevented hypercholesteremia obtained when only cholesterol was fed. These researches demonstrated the effectiveness of sitosterol as a hypo-cholesteremic agent in man. The following modes of action have been proposed for hypo-cholesteremic effect of sitosterol: (1) Formation of a mixed crystal of sitosterol and cholesterol, from which cholesterol is less readily dispersed into the colloidal form(1,2); (2) competition between sitosterol and cholesterol for esterification(3); and (3) competition with cholesterol for acceptor sites on or within the intestinal mucosal cell(4,5). Dispersion into colloidal

TABLE I. *In Vitro* Conversion of Mixed Aqueous Suspensions to Mixed Crystals.

Composition of equilibration mixture	Time of equilibration (hr)	Identity of precipitate by x-ray diffraction
.25 mg/ml sitosterol (parent suspension)	9	Sitosterol + mixed crystal (weak)
.25 mg/ml cholesterol (parent suspension)	20	As above
5 mg/ml sodium desoxycholate		
.1 M phosphate buffer (pH 8.8)		
.20 mg/ml sitosterol (representing parent suspension with largest 20% of particles removed)	9	Sitosterol (weak) + mixed crystals (weak)
.20 mg/ml cholesterol (parent suspension)	20	Sitosterol (weak) + mixed crystal
5 mg/ml sodium desoxycholate		
.1 M phosphate buffer (pH 8.8)		
.12 mg/ml sitosterol (representing parent suspension with largest 52% of particles removed)	9	Mixed crystal (weak)
.12 mg/ml cholesterol (parent suspension)	20	Mixed crystal (strong)
2.5 mg/ml sodium desoxycholate		
.1 M phosphate buffer (pH 8.8)		

form and esterification are presumed to be requirements for sterol absorption. In our experiments it was possible to demonstrate in a model colloidal aqueous system and in intestines of rabbits conversion of mixtures of sitosterol and cholesterol to the characteristic 1:1 mixed crystals previously described(2).

Methods. In vitro experiments: Aqueous sterol suspensions were prepared as previously described(2). Sitosterol suspensions were much less stable than cholesterol suspensions of the same concentration. In our experiments particle size of sitosterol was a consideration since rapid equilibration was desired. Consequently, the largest particles were removed by degrees from initial sitosterol suspension by centrifugation. After gravimetric analysis of the supernatant fine suspension, the stated mixed suspensions (Table I) were placed in rubber-stoppered test tubes and gently rocked in a bath at 38°C. Samples for x-ray diffraction were obtained by centrifuging at 60,000 × G for 30 minutes, washing with water to remove soluble materials, and re-centrifuging. Diffraction patterns were compared with those of pure compounds and of an equimolar composition crystallized from the molten state or from methanolic solution. *In vivo* experiments: To minimize interference in x-ray diffraction patterns by insoluble solids present in a natural diet, a purified diet described by Thacker(6) was modified by excluding fiber. Two % cholesterol and 8% sitosterol were added to the diet in finely ground form, and mechanically mixed with the diet. Male, adult albino rabbits were allowed access to the diet and water *ad lib* for 2-3 weeks. Animals were then sacrificed by inducing an air embolism. The small intestine was immediately removed and divided into 2 equal segments. The lumen of each segment was stripped of contents and the intestine then slit longitudinally to wash the villi with water. Thus, 2 separate specimens were obtained from each intestinal segment.

Results. With preliminary experiments, the pellet obtained by vigorous centrifugation of intestinal contents or washings from the villi, yielded x-ray diffraction patterns of crystalline sitosterol or no pattern at all. Initial failure to obtain a pattern may be attributed to presence of large amounts of amorphous material, *e.g.*, cellular debris, mucosa, etc. Subsequent experiments in which a preliminary, mild centrifugation was used to remove large particles and in which the pellet used for diffraction analysis was obtained from a vigorous centrifugation of the resulting supernatant were more successful.

A 1:1 cholesterol-sitosterol mixed crystal pattern was obtained from either intestinal contents or villi washings irrespective of the intestinal segment in several of the animals. Mixed crystal patterns ranged from strong to weak and, also, were seen in some specimens in the presence of the sitosterol pattern. A particularly strong mixed-crystal pattern was obtained from both the intestinal contents and

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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