ism. It is probable, therefore, that the doses employed in the present study produced some central depression. Whether this depression was adequate to account for the decreased sensitivity to radiation cannot be stated. However, preliminary results utilizing other depressants have failed to demonstrate a comparable protection against radiation vomiting. The lowered effectiveness of solutions allowed to stand for 2 hours is not in accord with reports on its stability for 24 hours(9). No explanation is available for this discrepancy.

The apparent increase in survival time after chlorpromazine requires confirmation with larger series of animals. Studies with mice and guinea pigs have failed to confirm this prolonged survival following x-radiation(18). Additional dogs have not yet been studied.

Summary. Chlorpromazine-(dimethylamino-1-n propyl-3) - N - (2-chloro) - phenothiazine HCl in doses of 10 mg/kg injected subcutaneously protected dogs against vomiting after 800 r x-radiation. Injections of 5 mg/kg were ineffective as were 10 mg/kg prepared from solutions allowed to stand for 2 hours at room temperature before injection. Cysteinamine (200 mg intravenously) had no or questionable protection whether given before, during, or after radiation. Increased survival time was noted with both chlorpromazine and cysteinamine. The mechanism of protection is discussed.

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Esterification of Soybean Sterols *in vitro* and their Influence on Blood Cholesterol Level. (21077)

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It has been reported recently(1,2) that the feeding of soybean sterols to rats resulted in a lowering of the blood and lymph cholesterol. The decrease was mainly in the ester fraction. An explanation offered(2) for these effects is that the plant sterols interfere with the esterification of cholesterol in its passage from the lumen of the intestine to the lymph. In connection with studies being carried out in these laboratories it has been demonstrated that the enzyme cholesterol esterase and bile salts play an important role in the esterification of cholesterol and in cholesterol absorption(3,4). It became of interest to determine

TABLE I. Blood Cholesterol Levels of Rats Fed Soybean Sterols in a Basal Diet Containing Cholesterol, Bile Salts and Fat.

Group	No. rats	Days on diet	Soybean sterol content, %	Whole blood cholesterol,* mg % Free	Ester	Total
	48	Pre-diet		75.9	34.0	109.9
AS	8	7	.0	108.9	85.4	194.3
	8	14	.0	114.0	144.0	258.1
	8	21	.0	116.2	236.8	353.0
AT	8	7	.5	108.8	84.2	193.0
	8	14	.5	99.9	133.4	233.3
	8	21	.5	93.5	186.4	279.9
AU	8	7	1	98.2	60.2	158.4
	8	14	1	96.2	125.7	221.9
	8	21	1	80.2	124.5	205.1
AV	8	7	2	78.2	73.7	151.9
	8	14	2	86.2	92.7	178.9
	8	21	2	73.0	135.8	208.8
AW	8	7	3	84.0	56.4	140.4
	8	14	3	80.1	85.4	165.5
	8	21	3	77.6	97.6	175.2
AX	8	7	4	89.8	45.7	135.5
	8	14	4	81.3	75.8	157.1
	8	21	4	77.8	64.1	141.9

* Avg stand. dev. of total blood cholesterol was ± 4 mg % in pre-diet period; during experimental period, ± 36 mg %.

if soybean sterols inhibit the marked increase in blood cholesterol of rats which results from a diet containing cholesterol, fat, and bile salts(4) and also if soybean sterols are esterified by pancreatic cholesterol esterase. Such findings might supply additional support for the suggested mechanism of action of dietary soybean sterols on the blood cholesterol.

Methods and materials. In vivo experiments. Forty-eight rats (male and female) from our stock colony, weighing 175-200 g and raised on Purina pellet chow were used. The animals were housed in separate cages and given water *ad libitum*. Following a control period on pellet chow during which the blood cholesterol fractions were determined, the rats were divided into 6 equal groups. The animals were pair-fed according to the following technique: after the first day all rats in all groups received the amount of food ingested the previous day by the rat having the lowest food intake. Each group received the basal diet to which was added either 0, (control group) .5, 1, 2, 3, or 4% soybean sterols. The basal diet had the following composition: 20% casein, 22% starch, 23% glucose, 25% olive oil, 5% Hubbel-Mendel-Wakeman salt mixture, 2% ruffex, 2% cholesterol, 1% sodium taurocholate, and adequate amounts of the crystalline vitamins. The soybean sterols were added at the expense of the starch and glucose. Blood samples were collected directly from the tail vein in .2 ml pipettes and placed in 1:1 acetone alcohol mixture for cholesterol analysis(5). *In vitro experiments.* Substrate mixtures were prepared as described earlier(6) with the exception that 250 mg blood albumin was used in lieu of egg albumin. Cholesterol-oleic acid and soybean sterol-oleic acid were used as the substrates for esterification. One of the important aspects of studies on cholesterol esterase is the uniformity and stability of the emulsification of substrates. Our criterion(6) for uniformity and stability of emulsification has been that the total cholesterol content of duplicate samples removed from the enzyme digests at different times should agree within the limits of error of the analytical method for determining cholesterol. A homogenate of hog pancreas(7) was used as the enzyme source. The reaction time was 1 hour. All calculations were based on the amount of free cholesterol and soybean sterol present at zero time and

TABLE II. Esterification of Soybean Sterols and Cholesterol with Oleic Acid by Pancreatic Cholesterol Esterase. Bile salt, sodium taurocholate at the optimum for each substrate concentration(3). pH of digests, 6.1. Oleic acid conc., 3 equivalents in terms of cholesterol. Enzyme, 1 ml 10% hog pancreas homogenate.

Soybean sterol, mg/digest	Esterification, mg/esterified/hr	Cholesterol, mg/digest	Esterification, mg/esterified/hr
14	7.0	14	7.8
29	15.2	29	17.2
43	19.7	39	22.0
55	21.8	55	31.5
86	26.8	82	34.0
86	.0*		
86	.0†		

* No bile salts present in enzyme digest.
† No fatty acid " " " " .

at the end of the incubation period. Soybean sterols were determined in the same manner as cholesterol, *i.e.* digitonin precipitation followed by the Lieberman-Burchard color reaction(5) and comparison with standard solutions of soybean sterols.

Results. Table I, Group AS, shows the effect of the basal diet containing 2% cholesterol plus 1% sodium taurocholate on the free, total and ester cholesterol of blood. Both total and ester fractions increased markedly and after 21 days on the basal diet; the total and ester fractions were 353 and 237 mg % respectively. The addition of soybean sterols to the diet (Groups AT-AX) resulted in a lesser rise of the blood cholesterol, especially in the ester fraction, than occurred in the group not receiving soybean sterols. The higher the concentration of soybean sterols in the diet, the greater was the difference. At a concentration of soybean sterols of 4%, (Group AX), the total blood cholesterol level after 3 weeks was 142 mg % which is 148% lower than the group not receiving soybean sterols and only 30% above the control level when the animals were on pellet chow. Thus the addition of 4% soybean sterols practically completely inhibited the increase in the blood cholesterol, which occurred in the animals on the basal diet.

Since esterification(9) is presumed to play an important role in the absorption of cholesterol, it was desirable to ascertain if soybean sterols were esterified *in vitro* by pancreatic cholesterol esterase and, if so, under what conditions. Table II shows the enzymatic activity obtained with soybean sterols and cholesterol as substrates. Both sterols are esterified with oleic acid in the presence of the enzyme. However, cholesterol is esterified somewhat more rapidly. It was also observed that bile salts and fatty acid are required for the esterification of soybean sterols. Similar findings have been reported with respect to cholesterol(6).

Discussion. Plant sterols have been used to lower the blood cholesterol of animals and humans(1,10), but their practicability seems questionable because of the necessity of feeding relatively large amounts. The results of the feeding experiments described above give support to the suggestion(2) that soybean sterols interfere with the absorption of cholesterol. It is also shown in this study that plant sterols and cholesterol are esterified under the same conditions by pancreatic cholesterol esterase *in vitro*. Hence, since the enzyme does not show any specificity towards these substrates, it may be presumed that it acts similarly in the intestine. It is probable that the soybean sterols compete with the cholesterol for the enzyme, bile salts, and fatty acids. That would impair the absorption of cholesterol by inhibiting its esterification. It is interesting to note that even though plant sterols are esterified, they are not absorbed(8).

Summary. Soybean sterols inhibit the increase in blood cholesterol of rats receiving a diet containing 2% cholesterol and 1% sodium taurocholate. The degree of inhibition increased as the soybean sterol concentration of the diet was increased. It was also observed that pancreatic cholesterol esterase catalyzes the esterification of soybean sterols albeit at a slower rate than with cholesterol. Bile salts and a fatty acid source are required for their esterification. The mechanism of action of soybean sterols in inhibiting cholesterol absorption is discussed.

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Serum Lipoproteins in Experimental Diabetes. II. Action of Heparin *in vivo* on Lipoproteins of Depancreatized Dogs.* (21078)

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Clearing of lipemic serum by heparin was first observed by Hahn(1) and by Weld(2). Further study of the phenomenon demonstrated that heparin combines with a "coprotein" supplied by normal plasma to form a "clearing factor"(3-5), capable of causing profound changes in the lipid and lipoprotein composition of normal human and animal serum and of the serum of patients with atherosclerosis or hypercholesterolemia(6-10).

The object of this investigation was to compare the changes caused by heparin in the serum lipoproteins of normal lipemic dogs with those caused in the serum lipoproteins of diabetic lipemic dogs.

Materials and methods. Two normal dogs, kept on a commercial dog food diet, were fasted for about 20 hours and, after taking a control sample of venous blood, fed a meal consisting of 25 g of lard, 2 egg yolks (dog W), and a small amount of commercial dog food. Serum samples were obtained 3 hours after the meal, at which time heparin sodium (2 mg/kg) was injected intravenously;‡ other blood samples were obtained 10 minutes (dog W) and 30 minutes after the injection. Five depancreatized dogs were allowed to develop mild to severe ketosis and lipemia by the withdrawal of insulin. After securing a fasting blood sample, heparin was injected intravenously and another blood sample obtained 30 minutes after the injection. Serum was separated by centrifugation and analyzed for total cholesterol and lipid phosphorus according to the methods of Bloor, Pelkan and Allen (11) and of Horwitt(12), respectively. Serum aliquots were subjected to electrophoresis on filter-paper, following a previously described technic(13,14) and the cholesterol and lipid phosphorus content of the resulting fractions was determined. A semi-quantitative pattern of the total lipid distribution along the strip of filter-paper was obtained by staining the strip with Sudan III(13) and measuring the density of the stain with a spectrophotometer at a wave length of 520 mμ. A curve was obtained by plotting color density against the distance along the strip.

Results. Normal dogs. The ingestion of a fatty meal caused a small rise in the total cholesterol and lipid phosphorus content of serum. This was accompanied by a rise in the slow-migrating lipoprotein fractions (β_1, β_2), while the cholesterol and lipid P content of the fast-moving components decreased slightly. These changes were not statistically significant. On the other hand, the total lipid content of the non-moving band ($\beta_2\gamma$) increased sharply, probably as a result of neutral fat load. This is indicated also by the intensity of the color in the strips stained with Sudan III (Fig. 1). The administration of heparin

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