tients treated with ACTH; this author was of the opinion that hyaluronic acid is a product of the mast cells and that these cells are the peripheral transmitters of the cortisone hormonal action.

In other experiments, we have been able to show that the inhibitory effect of cortisone is well marked on all the cellular constituents of the connective tissue: fibroblast, reticulo-endothelial system, plasma cells(8,9). The reduction in number of mast cells may be regarded as a partial manifestation of this general inhibitory effect of the hormone. In other unpublished experiments we have also observed that in agreement with others(10), the hormone, also when locally applied decreases the mast cells and other cellular constituents in the dermal tissue. It is very likely therefore that the action of cortisone is more pharmacological than hormonal in nature.

Summary. Adult rats, injected with cortisone in various doses and for a different period, constantly showed a marked reduction in the mast cell counts of the dermal, muscular and myocardial tissue, together with a strong decrease of hyaluronic acid of the connective tissue. Similar results were observed in the skin when the hormone was applied locally. It is pointed out that a close correlation exists between the mast cells and the hyaluronic acid and that cortisone acts as an inhibitory factor on these cells, just as on the other cellular constituents of the mesenchymal tissues.

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Received August 29, 1951. P.S.E.B.M., 1951, v78.

Effect of Soybean Sterols in the Diet on Plasma and Liver Cholesterol in Chicks. (19002)

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It is generally agreed that the absorption of sterols from the intestinal tract is highly specific. Cholesterol is readily absorbed by animals, especially in the presence of fat. Schoenheimer and associates(1-3) and others (4) have shown that a number of plant sterols are not absorbed.

The experiments described in this report were suggested by the author's observation that simultaneous addition of soybean sterols and cholesterol to the diet did not produce the rise in plasma cholesterol which was obtained when cholesterol alone or in oil was added to the chicks' diet.

Animals. The experimental animals were 14-day-old single comb White Leghorn chicks that had been maintained on a standard stock diet from the time of hatching. In the preliminary experiments (Fig. 1) chicks of both sexes were used. In the later experiments (Tables I and II) only males were employed.

Diets. The basal diet used in these experiments was the same as that previously reported by Peterson(5). Additions of cholesterol, soy sterols* and cottonseed oil were made by substitution for a like amount of

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* Mixed Soy Sterols containing a minimum of 95% sterols, principally sitosterols and stigmasterol, were supplied through the courtesy of Mr. C. N. Behrndt of Distillation Products Industries.

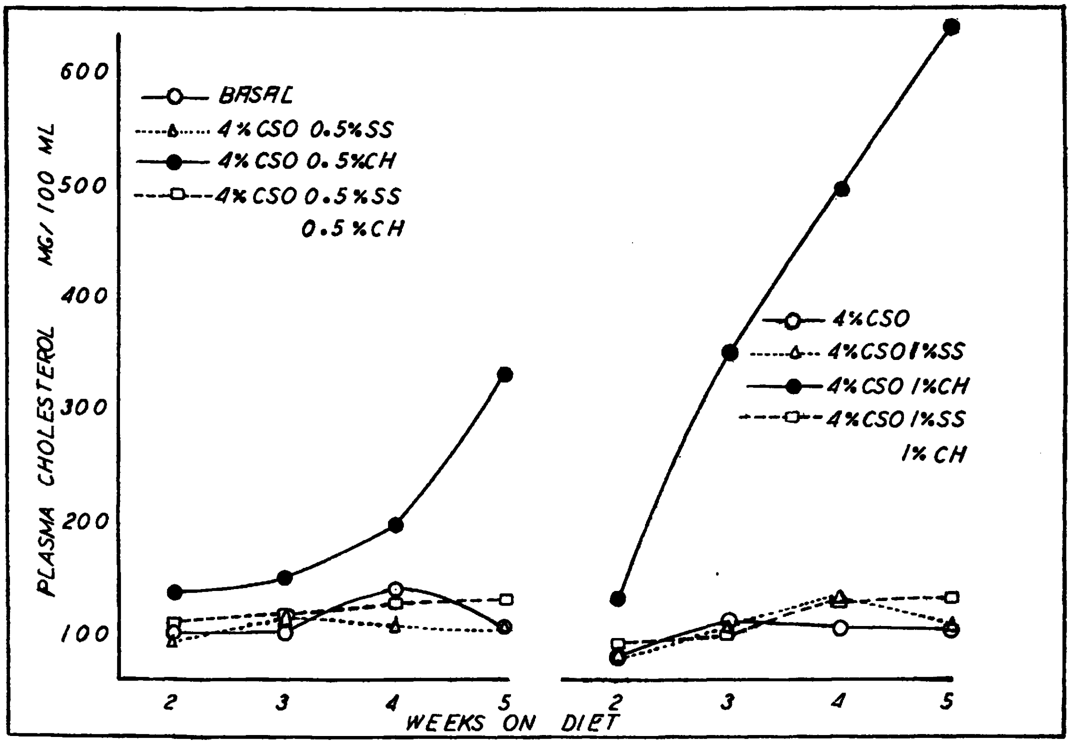

FIG. 1 and 2. Effect of soy sterols on plasma levels of cholesterol. CSO = cottonseed oil; SS = soy sterols; CH = cholesterol.

barley in the basal diet. Cholesterol and soy sterols or mixtures of these were dissolved by warming in cottonseed oil.† The thick paste which resulted on cooling was then mixed in the diet.

Analytical. Samples of heparinized plasma were analyzed for total cholesterol by the method of Schoenheimer and Sperry as modified by Sperry and Webb(6). Technics of sampling varied as indicated below. In the later experiments, livers were also analyzed for total cholesterol. They were taken at autopsy, weighed and frozen immediately. Weighed samples were later homogenized with ground pyrex glass using a mortar and pestle. The homogenate was then transferred to an Erlenmeyer flask with the aid of wet filter paper and covered with 95% ethyl alcohol. The alcohol was removed by evaporation on a steam bath and the residue extracted with several portions of a 1:1 mixture of absolute alcohol and acetone until the extract no longer gave a Liebermann-Burchard reaction. These extracts were then diluted to volume and aliquot samples analyzed by the Schoenheimer-Sperry procedure(6). Moisture determinations were made on each liver sample by drying at 100°C to constant weight.

Results and discussion. Several preliminary experiments showed that the addition of a mixture of soy sterols and cholesterol in cottonseed oil to the diet resulted in considerably lower levels of cholesterol in the plasma than the addition of cholesterol in cottonseed oil. Typical results of such an experiment are shown in Fig. 1.

Feeding cottonseed oil or a mixture of cottonseed oil and soy sterols in the diet did not increase total plasma cholesterol beyond the levels observed in chicks fed the basal diet alone.

Feeding of cholesterol and cottonseed oil produced the typical rise in plasma cholesterol

† Wesson Oil.

6. Sperry, W. M., and Webb, M., *J. Biol. Chem.*, 1950, v187, 97.

TABLE I. Plasma and Liver Levels of Cholesterol in Male Chicks Fed Various Diets.

Diet	3 wk on diet: No. of birds	3 wk on diet: Plasma total cholesterol, mean ± S.D., mg/100 ml	4 wk on diet: No. of birds	4 wk on diet: Plasma total cholesterol, mean ± S.D., mg/100 ml	Liver total cholesterol: Wet wt, mean ± S.D., %	Liver total cholesterol: Dry wt, mean ± S.D., %
S-1 Basal	7	86 ± 34	6	91 ± 14	.295 ± .025	1.12 ± .15
S-2 Basal + 4% cottonseed oil	6	75 ± 9	6	85 ± 8	.275 ± .026	1.01 ± .12
S-3 Basal + 4% cottonseed oil + 1% cholesterol	6	456 ± 147	7	553 ± 95	1.40 ± .238	5.21 ± .78
S-4 Basal + 4% cottonseed oil + 1% cholesterol + 1% soy sterols	7	112 ± 13	7	109 ± 19	.437 ± .099	1.69 ± .39
S-5* Basal + 4% cottonseed oil + 1% cholesterol + 1% soy sterols	7	130 ± 28	7	133 ± 35	.554 ± .116	1.99 ± .41
S-6 Basal + 4% cottonseed oil + 1% soy sitosterols + 1% cholesterol	7	136 ± 21	7	109 ± 9	.462 ± .104	1.65 ± .34

* Cholesterol and soy sterols added separately to diet.

TABLE II. Plasma and Liver Cholesterol Values for Male Birds Fed for 10 Weeks.

Diet	Plasma total cholesterol (composite samples from 3 chicks) Weeks on diet (mg/100 ml): 4	6	8	10	Liver total cholesterol 10 wk on diet: Wet wt, %	Dry wt, %
No. 1						
Basal +	82	78	84	108	.309	.970
4.5% cottonseed oil	78	82	72	88	.330	1
	82	95	83	121	.304	.937
Avg	81	85	80	106	.314	.969
No. 2						
Basal +	542	517	719	766	2.48	8.37
4.5% cottonseed oil +	542	554	1503	534	2.49	8.07
1% cholesterol	876	1138	576	1526	2.88	12
Avg	653	736	933	942	2.61	9.48
No. 3						
Basal +	108	121	138	192	.407*	1.28*
4.5% cottonseed oil +	124	121	124	138	.552	1.75
1% cholesterol +	139	131	121	143	.572	1.72
1.3% soy sterols						
Avg	124	124	128	158	.510	1.62

* Values averaged from 2 livers. All other values are averages of determinations on 3 individual livers.

obtained with continued cholesterol feeding(7). This rise was more rapid when cholesterol was fed as 1% of the diet than 0.5%, however there was a significant rise with 0.5% cholesterol. Addition of the soy sterols to the cottonseed oil-cholesterol diet prevented the rise in plasma cholesterol over the 5-week feeding period (Fig. 1). In this experiment cottonseed oil was employed at the 4% level. Cholesterol and soy sterols were used at 2 levels: 1% and 0.5% of the diet. Each week heparinized blood samples were taken by heart puncture from chicks which had been anesthetized with nembutal. It was necessary to sacrifice the chicks in order that

7. Horlick, L., and Katz, L. N., *Am. Heart J.*, 1949, v38, 336.

sufficiently large samples could be obtained for individual analyses. Each point on the curve represents an average value for 4 chicks.

The results of an experiment using male chicks are shown in Table I. Blood samples were taken by heart puncture when the chicks were sacrificed. Half of the chicks were sacrificed after the third week on the diets; the remainder after the fourth week on the diets when liver and blood samples were taken for analysis. The groups fed diets S-1 to S-4 present results essentially the same as those previously obtained. Diet S-5 was identical in composition with diet S-4 with the exception of the method of addition of the fat and sterols. Here the cholesterol was dissolved in half the cottonseed oil and added to the diet. Soy sterols were dissolved in the remainder of the cottonseed oil and added to the diet. The purpose of this procedure was to eliminate the possibility that the results obtained might be due to changes occurring while heating the mixture of soy sterols and cholesterol. It can be seen from Table I that plasma cholesterol remained low on this diet (S-5) regardless of the method of mixing the sterols.

Diet S-6 contained 1% of a purified sitosterol fraction (m.p. 128-130°C) of soybean sterols obtained from a commercial source. The results obtained with the diet containing this fraction were similar to those obtained with the mixed soy sterols. Further work using purified sterols will be necessary in order to determine whether the effect obtained is a characteristic of plant sterols in general or whether it is a property of sitosterols in particular.

The values for total liver cholesterol are given for both the wet and dry basis (Table I). Feeding of cholesterol and cottonseed oil produced the usual deposition of cholesterol in the liver. Livers of these birds were grossly fatty in appearance. Livers of birds on the control diets (S-1 and S-2) and of the birds on the phytosterol-cholesterol diets were normal in appearance. Livers of the birds on the soy sterols diet, although slightly higher in cholesterol than the controls, were much lower than those fed cholesterol alone. This was possibly due to decreased absorption of cholesterol owing to interference by one or more constituents of the mixed sterols.

The results shown in Table II show plasma and liver cholesterol levels for male chicks fed 3 diets over a period of 10 weeks. All diets contained 4.5% added cottonseed oil, diet 2 contained in addition 1% cholesterol, diet 3 1% added cholesterol and 1.3% added soy sterols. Chicks were anesthetized with nembutal and heparinized blood samples were taken from the wing veins every 2 weeks beginning with the fourth week on diet. Analyses for total cholesterol were made on composite samples from 3 chicks. The chicks were sacrificed after 10 weeks on the diets and liver samples analyzed for total cholesterol. The values in Table II represent averages of determinations on 9 individual livers for Diets 1 and 2 and 8 livers for Diet 3. These data show again the pronounced rise in total plasma cholesterol of the group fed cholesterol and cottonseed oil. Plasma cholesterol was again markedly low in those birds fed a mixture of cholesterol and soy sterols. Plasma from the birds fed Diet 2 was hyperlipemic in appearance. Plasma from birds fed Diet 3 presented a normal clear appearance, in general, and only in a few instances was a slight lipemia noticeable. The livers of chicks fed Diet 2 showed, on a dry weight basis, an average cholesterol content almost 10 times that of the controls whereas those birds fed Diet 3 had liver cholesterol values less than twice as great as the controls. Livers from birds fed Diet 2 were extremely fatty in appearance, whereas Diet 3 livers were normal (non-fatty) in appearance.

The mechanism whereby these results are obtained is not apparent. The data would appear to suggest, however, that one or more of the constituents of the mixed soybean sterols interferes with absorption of cholesterol. The results obtained with the sitosterol fraction are identical with those obtained with the mixed soy sterols (Table I). Sperry and Bergmann(4) have noted that total cholesterol levels were lower in the livers of mice fed sitosterol than in control mice and suggested that perhaps sitosterol might interfere with the reabsorption of cholesterol secreted into the intestinal tract. It should be noted that normal growth was obtained on all diets used in these experiments.

Analytical data were obtained on a representative sample of mixed soy sterols by the Schoenheimer-Sperry procedure, using a sample of pure cholesterol‡ as a standard of reference. The color intensity of the soy sterols was found to be, on the average, 77.3% of that of an equal weight of cholesterol. When saponification and the digitonin precipitation was used an average of 89% recovery was obtained with the mixed soy sterols. Thus, if any appreciable absorption of the mixed soy sterols occurred it would probably have been detected by the analytical method used. The data shown above (Fig. 1) indicate that there could be very little absorption of the constituents of the soy sterols in their original state.

‡ Difco Standardized Cholesterol m.p. 149.5-150°C (corr.)

The data presented in this report do not exclude the possibility that the effects described may be produced by some minor constituent of the sterol mixtures fed.

Summary. Evidence is presented that the addition to the chicks' diet of mixed soybean sterols and of purified sitosterols in the presence of cottonseed oil and cholesterol causes markedly lower plasma and liver levels of cholesterol than the addition to the diet of a mixture of cholesterol and cottonseed oil alone. The possibility is discussed that one or more of the constituents of the mixed soybean sterols interferes with the absorption of cholesterol.

The author wishes to acknowledge his indebtedness to Dr. Ruth Okey of the University of California for her interest and suggestions.

Received September 4, 1951. P.S.E.B.M., 1951, v78.

Quantitative Estimation of Antispasmodic Potency Using Isolated Guinea Pig Gall Bladder—Common Duct Preparation.*† (19003)

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Reuse(1) evaluated the antihistaminic activity of pyrilamine and Phenergan with the isolated guinea pig gall bladder-common duct preparation. The data presented indicated that the log-dose response was linear but the method was not developed as a quantitative procedure for estimating antispasmodic potency.

In view of the fact that the antihistaminic drugs have varying degrees of antispasmodic activity(2), we have investigated their effects upon acetylcholine spasm of the guinea pig gall bladder-common duct *in vitro*. Statistical evaluation of the results indicates that this tissue preparation is useful for screening drugs for antispasmodic activity.

Experimental. Guinea pigs, of either sex, weighing 343 to 958 g (average 607 g) were killed by a blow on the head. The gall bladder and common duct were removed, emptied of bile and arranged for recording contractions by the Magnus method. The bathing fluid

* The authors wish to thank Parke, Davis and Co. for the Benadryl and Ambodryl; Merck and Co. for the Phenergan; Bristol Laboratories for the C-5581-H; Winthrop-Stearns for the Thenfadil; Burroughs-Wellcome and Abbott Laboratories for the Chlorocyclizine; Schering Corp. for the Chloro-Trimeton; and Medico Chemical Specialties Corp. for the Anthallan used in this investigation.

† This article is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

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