TABLE II. Effect of Adrenalectomy on Carbon Clearance of Mice Treated with Hydroxyzine.

Treatment	Dose, mg/kg	No. of animals	T/2 ± S.E.	P value
A. Normal, saline		9	27.0 ± 2.6	
B. Adrenalectomy, saline		9	24.9 ± 2.5	.6
C. Normal, hydroxyzine	100	10	59.6 ±10.7	.01
D. Adrenalectomy, hydroxyzine	"	9	25.1 ± 2.2	.6
E. Sham operation, hydroxyzine	"	9	39.5 ± 3.5	.01

susceptibility to infections as well as certain other side effects observed after administration of large doses of certain tranquilizers, may be due to an excess of steroid hormones in the body. However, the data presented here indicate that tranquilizers given in the usual clinical doses would not be likely to affect the reticulo endothelial system.

Several tranquilizers when given in large doses produced a marked depression of the central nervous system, manifested by deep sedation and loss of the righting reflex. The possibility that this central nervous system depression may affect phagocytic activity of the reticulo endothelial system was tested by determining clearance rates in animals treated with pentobarbital 50 mg/kg and amobarbital 140 mg/kg. These hypnotics which produced deep central nervous system depression with loss of righting reflex did not inhibit phagocytic activity of the reticulo endothelial system.

Summary. Administration of excessive doses of certain tranquilizers decreased the phagocytic activity of the reticulo endothelial system. This decrease of phagocytic activity did not occur in adrenalectomized animals given hydroxyzine. The significance of this finding is discussed in relation to the strong RES inhibition produced by corticosteroids (6,2).

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Received June 25, 1959. P.S.E.B.M., 1959, v102.

Serum Lipid Analyses in Rats Fed Natural and Hydrogenated Cottonseed Oil with Cholesterol and Cholate.* (25153)

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In a recent study we recorded some measurements of rat blood lipid concentrations when dietary cholesterol was present or absent in rations with varying degrees of saturation of vegetable fats(1). The results indicated that serum cholesterol is raised by increasing saturation of dietary lipids and that dietary cholesterol augments this effect for more saturated fats. In this experiment, we extended our observations to include lipoproteins and fatty acid compositions of serum cholesterol esters when saturated (hydroge-

* This investigation supported by grant from Nat. Inst. Health, U.S.P.H.S.

nated) and unsaturated vegetable fats are fed to rats with or without sodium cholate and dietary cholesterol.

Methods and materials. Animals. Sixty, 3-month-old albino male Sprague-Dawley rats were marked, weighed and bled for serum lipid determinations. Animals had average weight of 344 g (310-399 g) and had been maintained on Purina Laboratory chow. They were divided into 6 groups comparable with respect to distribution of initial weight and serum lipid concentrations. All rats were kept in a thermostatically controlled room (76°F), in large wire-bottomed cages with 10 rats/cage. *Diet.* A liquid ration, described by Moskowitz, *et al.*(2) of 50% fat, 28% carbohydrate, 5% protein, and 1% choline (on dry weight basis) and containing adequate minerals, vitamins, and roughage was used. Three diets were made with each of 2 fats: cottonseed oil, iodine number (I# 107) and hydrogenated cottonseed oil (I # 78) and each was fed to 10 rats. Detailed analyses of these fats have been presented(1). One diet with each fat was fed with 0.4% sodium cholate, or with 0.4% sodium cholate and 1% cholesterol, or without these additions. Diets were prepared and fed by stomach tube as previously described(1). *Serum lipids.* Methods described earlier were used for serum cholesterol and lipid phosphorus(1). Lipoproteins were analyzed by a standard method of paper electrophoresis(3). Total serum cholesterol was separated into free and esterified parts by alumina column chromatography using a modification of Kerr and Bauld's technic(4). Silicic acid column chromatography yielded pure cholesterol ester fractions using method of Riemenschneider(5). Cholesterol esters were then saponified and methylated using methanol and sulphuric acid. Methylated fatty acids obtained from cholesterol-ester serum fractions were analyzed by gas chromatography for chain length and unsaturation as described by James(6). *Histopathological technic.* All animals were autopsied when they died or when sacrificed at end of experiments. Rats were exsanguinated from abdominal aorta under ether anesthesia. Organs were examined grossly and tissue samples of heart (transverse and frontal blocks) as well as all other organs were fixed in neutral formalin-saline. Opened aortas were fixed flat in buffered formalin-saline, and stained for gross neutral fat with Sudan IV. Duplicate blocks were taken of aorta (both ends) for frozen sections stained for neutral fat with Sudan IV. Other tissue blocks were embedded in paraffin, cut at 6 μ, and stained with hematoxylin-eosin.

Results. Body and liver weights. All groups gained weight during experiment. There were identical slight weight increases in groups fed the natural oil (I# 107) with cholate or cholate and cholesterol. However, when cholate was aded to the hydrogenated fat (I# 78) weight increase was markedly diminished. This decrease in weight gain was accentuated when cholate and cholesterol were added together. For unsaturated dietary oil, added cholate caused increased liver weights. Combination with dietary cholate and cholesterol further increased liver weight. For saturated dietary fat, only the combination with both cholesterol and cholate resulted in increased liver weight and then not to levels seen with these additions to unsaturated oil.

Histopathology. Study of the histological sections in this experiment revealed no atherosclerotic lesions in coronary arteries, aortic or mitral valves or proximal or distal aorta.

Serum lipid determinations. Fig. 1 shows results of serum total cholesterol and phospholipid determinations. For all groups serum cholesterol rose to a peak at 9 or 11 weeks. There was a fall in values after achieving this peak. For both unsaturated and saturated lipid, added dietary cholate did not alter serum cholesterol levels from those achieved by the oil fed without additions. However, when both dietary cholate and cholesterol were added together, serum cholesterol rose. This effect was far greater for saturated fat. Phospholipid values and C/PL† ratios were closely parallel to serum cholesterol values. Increasing saturation from I# 107 to I# 78 or adding 0.4% cholate to either fat did not increase the C/PL ratio. However, the ratio was slightly elevated when both cholate and cholesterol

† Serum cholesterol/phospholipid ratios.

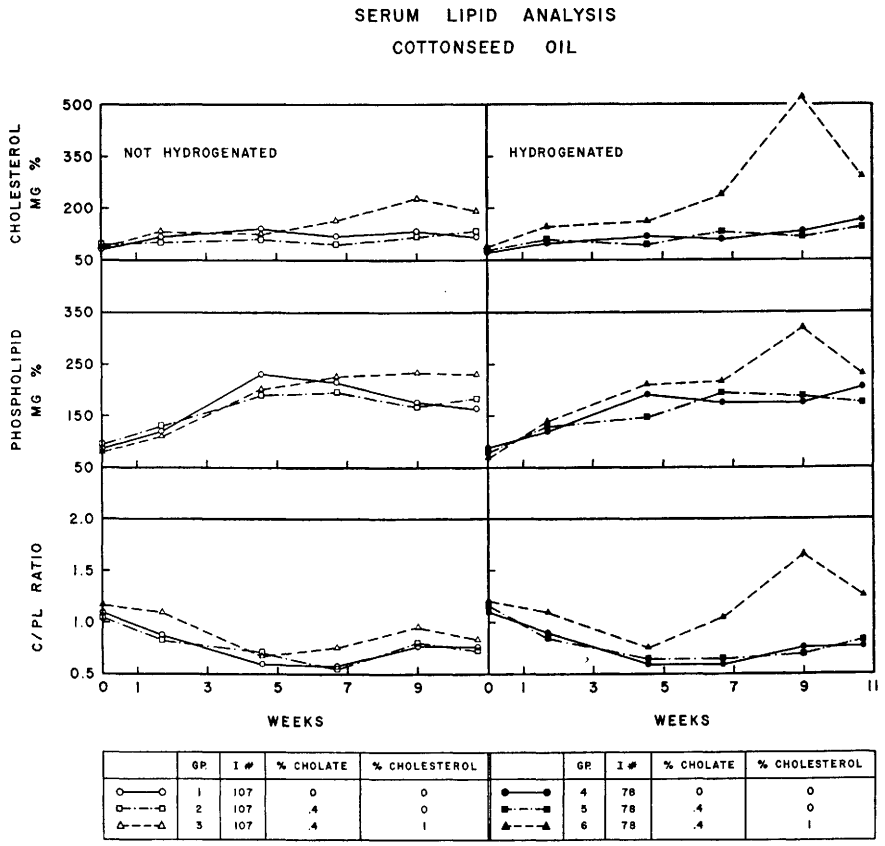


FIG. 1. Mean serum lipid analyses.

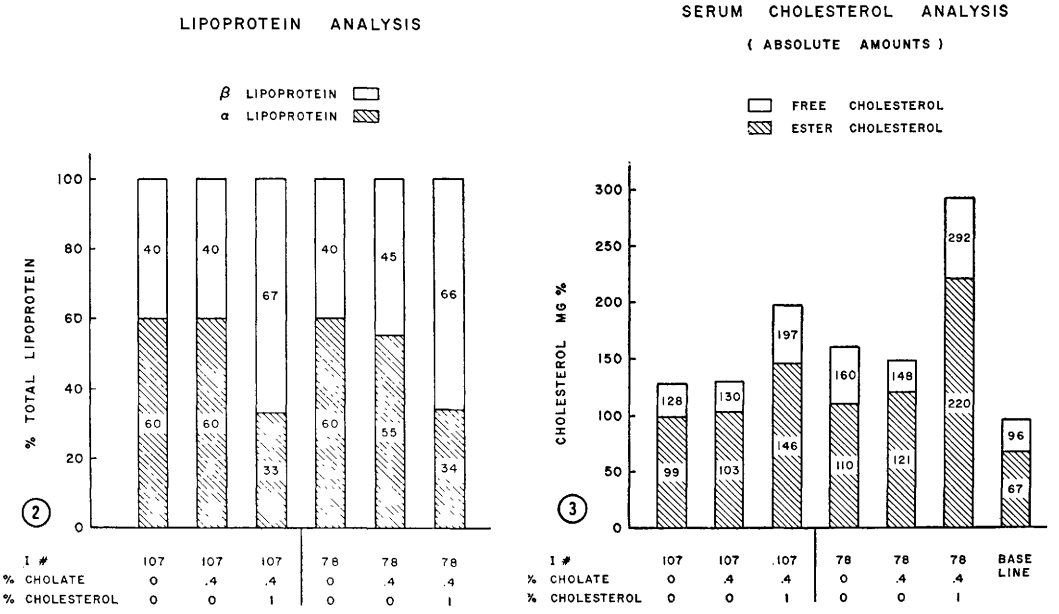
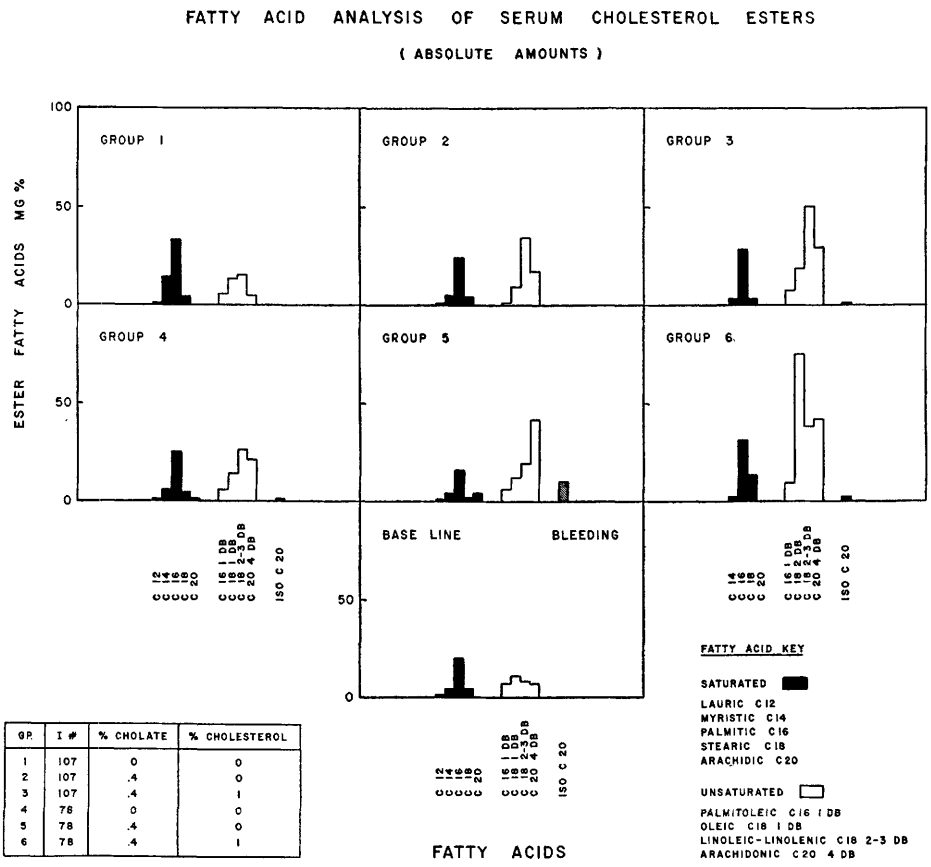


FIG. 2. Terminal lipoprotein analyses of pooled serum (relative values only).
FIG. 3. Pooled serum cholesterol in absolute amounts. Top figure is total serum cholesterol. Bottom figure is amount of esterified portion.



GP.	I #	% CHOLATE	% CHOLESTEROL
1	107	0	0
2	107	.4	0
3	107	.4	1
4	78	0	0
5	78	.4	0
6	78	.4	1

FIG. 4. Baseline and terminal fatty acid analyses of serum cholesterol esters.

were added to natural cottonseed oil (I# 107) and greatly raised when these additions were made to saturated fat (I# 78). The latter was the only dietary group which maintained C/PL elevation at end of experiment.

Fig. 2 shows relative values for Alpha and Beta lipoprotein (LP) analyses done at termination of experiment. Alpha LP predominated. For both lipids, when combination of dietary cholate and cholesterol was added, the previous relation reversed, and Beta LP became dominant fraction. In work previously published(1) we found that only extremely hydrogenated cottonseed oil (I# 43) fed with cholesterol would cause Alpha to Beta LP ratio to reverse and Beta LP to become dominant. In contrast to this result, combination of dietary cholate and cholesterol permits reversal of Alpha to Beta LP ratio with moderate or no hydrogenation of dietary fat.

Fig. 3 shows fractionation of terminal serum cholesterol into free and esterified portions. High fat feeding for both unhydrogenated and hydrogenated fat caused an increase in serum esterified cholesterol. Increased saturation of hydrogenated lipid slightly increased ester content. Added cholate with both fats further increased ester values; more so for saturated fat.

Fig. 4 shows cholesterol ester fatty acid analyses in mg %. For both unhydrogenated and hydrogenated fats with addition of cholate or cholate with cholesterol, there was a stepwise tendency for esterification to occur more with unsaturated fatty acids than with saturated acids. Considering saturated serum esters, with either dietary lipid, C-14 myristic acid declined with added cholate or cholate and cholesterol. For all groups C-16 palmitic acid was the saturated ester acid in greatest amount. Addition of cholate alone to both fats caused a decrease in C-16 saturated acid

but added cholate with cholesterol raised values to previous levels. This effect was more pronounced with saturated dietary fat. Of the unsaturated acids, C-18 oleic acid was altered in a way similar to C-16 palmitic acid, with some decline with added cholate and restoration with cholate and cholesterol. In the groups fed natural oil (I# 107) linoleic-linolenic acids were the most abundant unsaturated ester acids. Added cholate increased these acids. There was another increase when both cholate and cholesterol were fed. For the group fed hydrogenated lipid alone, linoleic-linolenic was most abundant of unsaturated esters. When cholate was added, unlike its unhydrogenated counterpart, esterification pattern shifted so that arachidonic acid was most abundant unsaturated acid. When cholesterol with cholate was fed, esterification again shifted and now was greatest with oleic acid. This was the group with highest values for serum cholesterol, phospholipid, and C/PL.

Discussion. In view of results of Hegsted *et al.*(15) under experimental conditions similar to ours, we expected to observe cardiovascular sudanophilia in our animals. However, microscopic lesions were not seen, possibly because of differences in the detailed design of the experiments. For example we employed whole egg as dietary protein while they used casein. Furthermore their dietary choline and fat levels were much lower. The fatty acid composition of esterified serum cholesterol is of interest in view of suggestions made about effect of saturated versus unsaturated acids on atheromatous deposits. It has been stated that predominant saturation of dietary lipid may cause a prepondernace of saturated cholesterol esters in blood and tissues(8). These sterols esterified with saturated acids have higher melting range, are less soluble, and possibly less compatible in blood. Of more importance, they are turned over more slowly in tissues than are unsaturated essential fatty acid esters(9). Thus saturated sterol esters may be relatively inert and have greater tendency to accumulate in artery walls. There is evidence that sterol esterified oleic acid is found in greater abundance in atheromatous plaques than in serum(10). Possibly then, oleic acid is a partially unsaturated acid which when incorporated in cholesterol esters is metabolically equivalent to saturated acids and thus more likely to accumulate in vessel walls. In this experiment oleic acid rose markedly in the group which had highest serum cholesterol, Beta lipoprotein and C/PL. James described an increase of oleic acid in serum esterified cholesterol and triglycerides of coronary patients(11) thus supporting the idea that a fault in lipid metabolism involving oleic acid contributes to coronary artery disease. Our experiment shows that dietary fat saturation and additions of cholesterol with cholate or cholate alone influence esterification of serum cholesterol. There is evidence that this is true for other serum lipids and for liver and depot fat(12,13,14).

Our results show that not only serum cholesterol level but also qualitative and quantitative composition of cholesterol esters, as influenced by dietary factors, is likely to be significant in understanding elevation of serum lipids and deposition of lipids in arteries.

Summary. 1. Dietary cholate with cholesterol added to natural and hydrogenated cottonseed oil raised serum cholesterol and phospholipid. This effect was augmented with saturated fat. Dietary lipid saturation or cholate alone had no effect on serum cholesterol. 2. C/PL ratio was elevated only when both dietary cholate and cholesterol were added to hydrogenated fat. 3. In the rat, highly saturated fat (I# 43) fed with cholesterol reverses the Alpha to Beta LP ratio with Beta LP becoming dominant. Combination of dietary cholate with cholesterol permits reversal of Alpha to Beta LP with moderate (I# 78) or no hydrogenation (I# 107) of dietary fat. 4. Serum cholesterol ester fatty acid composition changes with greater saturation of dietary fat and added cholate and cholesterol. High oleic acid levels were noted in serum cholesterol ester fraction of rats fed saturated fat (I# 78) with cholate and cholesterol.

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Received June 25, 1959. P.S.E.B.M., 1959, v102.

Quantitative Determination of C^{14}-Tyrosine Absorption in Melanated and Non-Melanated Mouse Tissues.* (25154)

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Historically the study of tyrosine metabolism is coincident with the study of genetically controlled alcaptonuria, a condition characterized by excretion of a tyrosine derivative, homogentisic acid(1). Many investigations as reviewed by Knox(2), and Meister(3), have been carried out on pathways of tyrosine metabolism. More recently, radioactively labelled tyrosine has been employed, with excellent results, in the study of the tyrosine metabolism mechanism(4,5,6). The role of tyrosine in melanosarcoma formation has also received considerable attention(7,8), and in addition the selective absorption of this tagged amino acid into various tissue proteins has been established(9). It is our purpose to study selective absorption of C^{14}-labelled tyrosine by both melanotic and non-melanotic tissues of the mouse by means of quantitative uptake determinations. S91 melanoma tissue is also included in the analyses. Since the method utilized provides a uniform assay procedure and geometry, it is felt that many technical quantitative problems are eliminated; *e.g.*, variation in self-absorption between dissimilar tissue samples.

* This work supported in part by research grant from Am. Cancer Soc. (Mass. Division).

Materials and methods. A. The mice used were of the dba/1 Jackson strain subsequently inoculated with Cloudman S91 melanoma. All animals were of approximately the same age and weight (6 mo., 15 g). B. *Radioisotope administration.* Five microcuries of dl-tyrosine-2-C^{14} (Tracerlab, Inc.) were prepared and administered according to standard procedure(7) to each animal. 5 weeks after tumor inoculation. Twelve animals were used during the investigation. C. *Tissue preparation.* Each animal was sacrificed 7 days after radioisotope injection with immediate removal of melanoma, liver, kidney, brain, adrenal, thyroid, and combined skin and hair tissue samples. Each sample was placed in a small pyrex tube, quick frozen by immersion into acetone-dry ice mixture, and stored at 0-4°C until time of analysis. D. *Tissue analysis.* To eliminate variabilities encountered by use of wet weights as measurement of tissue sample and to put all tissues used on a comparable and accurate assay basis, a modification of the Van Slyke-Folch(10,11) tissue combustion procedure for determination of tissue carbon content was employed. The manometrically determined quantity of CO_2 produced, was introduced into barium hydroxide solution