the saponin, but after the cholesterol is exhausted inhibition is apparent.

Effect of Saponin on Phosphatases. The effects of saponin on blood phosphatase as well as on partially purified fecal, upper and lower intestinal phosphatase (kindly supplied by Dr. Harris of these laboratories) were determined. The method of determination was that suggested by Bodansky[3] and the inorganic phosphate liberated was estimated by the method of Fiske and Subbarrow.[4] As much as 15 mg. of saponin in 10 cc. of reacting mixture caused no inhibition of any of the phosphatase preparations used. It appears, therefore, that phosphatase activity is not appreciably influenced either by the action of saponin or the highly reduced surface tension of the reacting mixture solution.

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Cholesterol and Fatty Acids in Blood Plasma of Male and Female Rats.

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In the course of an investigation of cholesterol metabolism in the rat, a survey of the literature indicated a paucity of knowledge concerning the normal fluctuations of blood cholesterol in this species. The present communication deals with such values in normal adult albino rats of both sexes. Inasmuch as there is a large residue of free cholesterol in the erythrocytes, a circumstance essentially vitiating the determinations on whole blood, the necessity of using plasma or serum should be emphasized.

The rats used in this study were breeding stock and were of the Osborne and Mendel strain obtained from the Connecticut Agricultural Experiment Station. The females were not used until one month or more had elapsed after weaning of litters. All animals were fed a stock diet which includes a mixture of 97% G.L.F. calf meal[1] and 3% cod liver oil. During the lactation period the

3 Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

4 Fiske, C. H., and Subbarrow, Y., *J. Biol. Chem.*, 1925, **66**, 387.

* Sterling Fellow (1933-35).

† Commonwealth Fellow (1934-36).

1 Maynard, L. A., *Science*, 1930, **71**, 192.

females received in addition, a paste food consisting of whole milk powder 25%, casein 25%, wheat germ 20%, and lard 30%. The food was removed approximately 15 hours before killing the animals.

Under ether anesthesia, the blood was drawn from the abdominal aorta into oxalated centrifuge tubes. All blood samples were secured within 5 minutes after the animal was anesthetized. Promptness in obtaining blood samples is necessary as is emphasized by the results of Mahler[2] who showed a definite rise in blood cholesterol, after 5 to 7 minutes, proportional to the duration of the anesthesia. With few exceptions 4 cc. or more of plasma were obtained from each animal; the plasma of 2 rats was combined in cases where it was impossible to obtain a volume of 4 cc. from an individual animal. The plasma was extracted with alcohol-ether (3:1) mixture and the precipitated protein washed with portions of boiling ethyl ether to insure complete extraction of the cholesterol. Total lipids were determined by Bloor's oxidative procedure.[3] The total and free cholesterol were determined by Okey's method of oxidizing the cholesterol digitonide as outlined by Boyd,[4] with an additional modification which will be described in a later publication.

In Table I are presented the concentration of the fatty acids in the plasma, and the percentage of free cholesterol in total cholesterol. The concentrations of the various cholesterol fractions are not reported in view of the work of Sperry and Schoenheimer,[5] which was recently published. According to these investigators oxalated blood plasma contains significantly smaller amounts of total and free cholesterol than either serum or heparinized plasma. However, they state that the total and free cholesterol are reduced in the same proportion. Therefore, it appears that the use of oxalated plasma has not affected the present results with respect to the relation of free and combined cholesterol.

Whereas in male rats, the free cholesterol in total cholesterol averaged 32%, with a range of 23 to 49%; in female rats the average value was 30% with a range of 20 to 38%. The average concentration of fatty acids in the plasma of the male rats was found to be 121 mg. per 100 cc. with an individual variation of 87 to 183 mg. per 100 cc.; and in the plasma of the female animals an average value of 150 mg., with a variation of 122 to 186 mg. per 100 cc.

2 Mahler, A., *J. Biol. Chem.*, 1926, **69**, 653.

3 Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

4 Boyd, E. M., *J. Biol. Chem.*, 1933, **101**, 323.

5 Sperry, W. M., and Schoenheimer, R., *J. Biol. Chem.*, 1935, **110**, 655.

TABLE I.

The Concentration of Fatty Acids and the Percentage of Free in Total Cholesterol in the Blood Plasma of Male and Female Rats.

Males					Females				
Rat No.	Age (days)	Wt. (gm.)	Fatty Acids (gm./100 cc.)	Free in Total Cholesterol %	Rat No.	Age (days)	Wt. (gm.)	Fatty Acids (gm./100 cc.)	Free in Total Cholesterol %
1	268	336	183	23	22	440	332	176	24
2	180	514	150	49	23	439	251		
3	184	538	166	33	24	373	290	186	30
4	346	438	129	34	25	253	340	167	22
5	281	457	126	32	26	377	267	170	29
6	194	468	122	40	27	358	267	170	37
7	180	395	123	26	28	409	260		
8	181	487	125	35	29	415	330	156	37
9	268	371	112	25	30	330	341	154	24
10	359	580	117	34	31	176	358	142	23
11	175	485	123	34	32	369	306		
12	333	376	113	27	33	371	209	147	27
13	194	537	109	32	34	342	308	145	33
14	383	406	116	34	35	317	287		
15	352	480	103	33	36	395	299	138	27
16	311	443	98	30	37	597	261	136	25
17	550	360	102	33	38	379	254		
18	345	558	87	24	39	223	325	122	20
19	383	327	89	31	40	202	264		
20	381	313			41	395	237	128	34
21	182	435	—	35	42	485	257		
Average			121	32	43	281	246	136	37
					44	360	237		
					45	477	322	128	36
					46	489	230	—	33
					47	360	218		
					48	448	256	—	38
					49	395	276		
					Average			150	30

The relation of the cholesterol in the free state to that combined as ester appears to be the same in both the female and male animals, and is similar to that reported for human subjects,[6] although the concentration of total cholesterol in the plasma of rats is only approximately one-half that found in human subjects. The values for fatty acids indicate a higher concentration in the plasma of breeding female rats than in male animals.

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Phage-Specific Heat-Labile Factors in *B. dysenteriæ* Sonne.

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It was previously reported[1] that from a certain multivalent Shiga phage, most Sonne strains absorbed distinctly but only the rough fraction, *i. e.*, detectable only when a rough Shiga strain or a susceptible Sonne strain was employed in the test for residual phage. In these and earlier experiments[2] the bacteria were heated for 2 hours at 70°C.

Subsequent studies were then made with phages derived from chicken-stools and propagated with Sonne organisms. Although these phages gave equally strong reactions on all (15) Sonne cultures available, curiously enough none of our strains heated to 70°C. showed distinct absorptive effects. This result seemed to indicate the presence of heat-labile factors. So, we compared organisms heated at 56°C. and at 85°C. for one hour. Absorption was obtained only with some strains subjected to the milder degree of heat. The nature of absorbing and non-absorbing strains could not be correlated with the above-mentioned differences in ability to absorb the rough fraction of the Shiga phage, nor with the quality of smoothness or roughness, nor with the direct titer. Indeed, from one of the absorbing strains, smooth and rough variants were obtained and both exhibited thermal lability.

In view of the stability to formalin of heat-labile antigenic (flagellar antigens) and/or phage-specific factors (*V.* antigen of

6 Sperry, W. M., *J. Biol. Chem.*, 1936, **114**, 125.

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1 Levine, Philip, and Beerman, P., *J. Immunol.*, 1936, **30**, 377.

2 Levine, Philip, and Frisch, A. W., *J. Inf. Dis.*, 1935, **57**, 104.