The gonads, accessories and adrenal cortex undergo regression regardless of the female hormone present. The resorption of the pubic symphysis does not depend on the presence of anterior lobe material. A small fragment of prehypophysis enables the administered female hormone to induce growth of the mammary gland. Such a small pituitary fragment permits regression of the gonads, accessories and adrenal cortex. Caution must be urged in use of the term "physiologically hypophysectomized" animals.†

9204 P

Effects of Saponin and Digitonin on Lipase and Phosphatase Action.

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In the course of the investigation of the effects of various snake venoms on lipolytic action controls were run to determine whether any of the observed effects might be due to the decreased surface tension caused by the venoms. Two of the agents so employed were "purified" saponin, Merck, and Hoffman-LaRoche digitonin. The following communication is a report on the rather interesting observation that the former markedly inhibits the activity of pancreatic lipase but inhibits the lipase activity of blood only slightly, while digitonin in very low concentrations increases the lipolytic action of pancreatic lipase and has little or no effect on blood lipase action. That the activity in each case seems to be due to some factor apart from the effect on surface tension is indicated by the fact that both of these reagents are very powerful surface tension depressants. The inability of both to show the same marked effects on blood lipase seems to indicate that they are inactivated or removed by some constituent in blood serum. It is likely that the serum cholesterol combines with the saponin or the digitonin to form a very slightly soluble digitonide, thus removing the inhibiting or stimulating factor.

† Theelin was kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co., and progynon-B by Dr. Schwenk of Schering Corp.

* Contribution No. 89 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

Lipolytic activity was determined by the method of Sure, *et al.*,[1] with olive oil as the substrate and gum arabic, U.S.P., as the protective colloid. The enzymes employed were serum from fresh defibrinated horse blood, Eimer & Amend Lipase "pure", and Difco steapsin. The saponin solution contained 5 mg./cc.; lipase, 2.5 mg./cc.; steapsin, 2.5 mg./cc.; digitonin solution prepared according to the method of Schoenheimer and Sperry,[2] 1.4 mg./cc. The amount of lipolytic action was determined after 24 hours at 37° by titration with N/100 NaOH in alcoholic solution, using phenolphthalein as an indicator. The increase in acidity between 0 time and 24 hours is taken as a measure of activity. The results are tabulated as cc. of N/100 alkali used.

Tables I and II give the results of a few typical runs illustrating the relative effects of saponin inhibition and digitonin acceleration.

TABLE I.
Effects of Different Concentrations of Saponin on Blood and Pancreatic Lipase.

Saponin	Blood Lipase*	Eimer-Amend Lipase (cc. N/100 NaOH used)	Steapsin
0.0	4.2	12.45	1.80
0.5	4.1	8.65	0.35
1.0	4.0	7.95	0.10
2.0	4.1	6.85	0.10
3.0	3.9	6.25	0.10
4.0	4.0	5.55	0.05
5.0	3.4	4.50	0.10
	Final volume, 10 cc.		

*1.0 cc. blood serum used or 1.0 cc. 0.25% enzyme sol.

TABLE II.
Effects of Different Concentrations of Digitonin on Blood and Pancreatic Lipase.

Digitonin (mg.)	Blood Lipase (2 cc. used)	Eimer-Amend Lipase (cc. N/100 NaOH used)	Steapsin (at 20°)
0.0	10.6	8.1	0.6
0.7	10.6	5.8	0.6
1.4	10.5	11.0	1.0
2.8	10.6	14.5	1.7
4.2	10.4	15.1	1.9
	Final volume, 10 cc.		

From these results it can be concluded that Merck's saponin "purified" shows marked inhibiting powers toward pancreatic lipase, while digitonin shows marked accelerating action. The effects are not apparent with blood lipase except when very high concentrations are used, which seems to indicate that blood cholesterol precipitates

1 Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 27.
2 Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, **106**, 745.

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.