FIG. 1.
Cross section through the gonads and gonoducts of *A. tigrinum* control female. Note unstimulated Mullerian ducts (m) and Wolffian ducts (w).

FIG. 2.
Cross section through the gonads and gonoducts of *A. tigrinum* female treated with testosterone propionate for 171 days. Note absence of Mullerian ducts and stimulated Wolffian ducts (w) and close resemblance of the right duct (left in the picture) to the frog seminal vesicle.

FIG. 3.
Cross section through the gonads and gonoducts of *A. tigrinum* female treated with estradiol dipropionate and theelin for 171 days. Note stimulated Mullerian ducts (m) and unstimulated Wolffian ducts (w). All figures ×25.

original formation of gonoducts; they stimulate only the secondary (functional) enlargement of parts of ducts already present. It has been shown by means of parabiosis that the testes of males release some inductive substance which inhibits the development of the ovaries of female cotwins and indirectly may cause some genetic females to continue development in a male direction.[6] On the other hand, in the male-female parabiotic combinations there is no precocious stimulation of either gonoducts or cloacal glands. These fundamental differences in the observed reactions prove that the crystalline sex hormones used in this experiment cannot be identical with the substances which normally act as inductors of sex differentiation.

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Effect of Foods on Serum Esterase of Rats.

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In a previous publication[1] it was reported that the tributyrin splitting property (here called esterase concentration) of the serum of rats is markedly increased after poisoning with carbon tetrachloride and after feeding a high fat diet. On the other hand, substances such as xanthine, which exert a protective action on the liver against carbon tetrachloride, will, when subcutaneously injected into rats, definitely lower the serum esterase concentration. Before proceeding further with the investigation into the possible significance of the serum esterase change in the animal's resistance to carbon tetra-

[6] Witschi, Emil, *Allen's Sex and Internal Secretions*, Baltimore, 1939, 2nd ed., 145.

[1] Forbes, J. C., and Outhouse, E. L., *J. Pharm. and Exp. Therap.*, 1940, **68**, 185.

chloride, it was necessary to study in greater detail the effect of various foods on the esterase concentration, laying particular emphasis on the changes resulting from the administration of single doses of fatty substances. In our previous recorded experiments the animals were kept on the high fat diet for a number of days and were allowed food up to the time of sacrificing.

The results herein presented deal with the effect of single doses of various foods on previously starved animals. They show that non-lipoid food stuffs, sucrose, glycerol and proteose-peptone exert no demonstrable effect on the serum esterase while neutral fats or fatty acids markedly increase it. No significant change was obtained in any case in the neutral fat, phospholipid or cholesterol content of the liver.

Experimental. Rats weighing approximately 200 g, after being starved for the previous 24 hours, were given the various supplements by stomach tube, except in the case of the Purina and Purina plus 40% butter fat diets. In these cases the required amount of food was fed early in the morning and only animals which consumed practically the whole amount in at least an hour and one-half were used for the various studies. The serum esterase, liver neutral fat, total phospholipid and cholesterol were determined by the methods previously described.[1, 2] The experimental results are shown in Tables I, II, and III.

It will be seen from the results recorded in Tables I, II and III that the administration of non-lipoid foods does not measurably affect the tributyrin-hydrolyzing power of the rat's serum. Glucose has also been used and found to be inactive. The administration of fats, oleic and palmitic acids, on the other hand, resulted in a marked rise in the serum esterase concentration. Since glycerol is inactive, while fatty acids have a pronounced effect, it would appear that the increase in esterase value is in some way related to an increase in some phase of fatty acid metabolism. The lack of apparent response following the feeding of Purina dog chow itself, which contains approximately 4% of ether extractable materials, indicates that a fair amount of fatty acids must be absorbed in order to obtain a definite response by the analytical method employed.

In a previous publication[1] it was shown that the serum esterase concentration of rats fed a high fat-low choline diet for several weeks, at which time a high fat content of the liver was produced, did not apparently differ from that obtained by feeding a high fat-Purina dog chow diet on which the deposition of fat in the liver was quite moderate. Other experiments in which sufficient choline was added to a high fat-low choline diet to prevent fat deposition in the

[2] Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, in press.

TABLE I.

Effect of Feeding Fat and Oleic Acid on the Serum Esterase and Liver Lipids of Rats.

No. of rats	Serum esterase			Liver, %			Remarks
	Max.	Min.	Avg	N.F.	P.L.	Chol.	
9	25	14	20	0.9	4.13	0.25	Controls.
4	31	17	25	2.0	4.06	0.28	Killed 1 hr after feeding by stomach tube 1 cc of butter fat per 100 g.
5	34	18	30	1.5	4.19	0.27	" 2 " " " as above.
2	33	30	31	1.0	4.06	0.29	" 3 " " " " "
5	36	28	33	1.2	4.18	0.26	" 4 " " " " "
2	25	20	22	0.8	3.83	0.25	" 5 " " " " "
5	28	21	25	2.0	4.23	0.27	" 6 " " " " "
2	24	22	23	1.1	3.78	0.28	" 7 " " " " "
2	23	21	22	2.2	4.36	0.27	" 8 " " " " "
1	—	—	19	1.1	4.77	0.24	Control.
2	26	23	24	1.0	4.45	0.26	Killed 2 hr after feeding by stomach tube 1 cc of olive oil per 100 g.
2	29	26	27	1.2	4.79	0.29	" 4 " " " as above.
2	37	22	34	1.7	4.78	0.29	" 6 " " " " "
2	37	26	31	1.7	4.58	0.25	" 8 " " " " "
2	28	26	27	—	—	—	" 10 hr 30 min after feeding as above.
1	—	—	22	1.3	4.58	0.26	Control.
1	—	—	26	1.6	4.38	0.26	Killed 1 hr after feeding by stomach tube 1 cc of oleic acid per 100 g.
3	33	28	30	1.2	4.35	0.27	" 2 " " " as above.
3	36	34	35	1.7	4.24	0.25	" 4 " " " " "
3	44	38	41	1.4	4.29	0.25	" 6 " " " " "
2	35	28	31	—	—	—	Killed 3 hr 20 min after feeding by stomach tube 0.4 g sodium palmitate per 100 g.

N.F. = Neutral fat plus cholesterol.
P.L. = Total phospholipids.

TABLE II.

Effect of Purina Dog Chow, and 60% Purina Plus 40% Butter Fat on the Serum Esterase and Liver Lipids of Rats.

No. of rats	Serum esterase			Liver, %			Remarks
	Max.	Min.	Avg	N.F.	P.L.	Chol.	
4	24	15	19	1.5	3.89	0.29	Controls.
5	24	15	19	1.2	3.89	0.28	Killed 2 hr 30 min after feeding 5 g Purina.
5	23	15	18	1.1	3.58	0.27	" 4 " 30 " " " as above
5	24	13	18	1.3	3.73	0.27	" 6 " 30 " " " " "
3	16	16	16	1.4	3.76	0.25	" 8 " after feeding as above.
4	22	17	19	0.9	3.78	0.29	Controls.
2	34	32	33	1.1	3.85	0.31	Killed 2 hr 45 min after feeding 3 g of 60% Purina + 40% butter fat.
2	32	32	32	1.4	3.18	0.25	Killed 4 hr after feeding as above.
2	20	16	18	0.8	3.75	0.26	" 5 " " " " "
4	24	14	20	1.6	3.37	0.27	" 7 " " " " "
4	29	18	25	1.5	3.50	0.23	" 9 " " " " "
2	20	10	15	2.2	3.16	0.25	" 25 " " " " "
4	24	21	22	1.1	3.74	0.36	Controls.
4	38	26	31	1.3	3.50	0.37	Killed 2 hr 45 min after feeding 5 g of 60% Purina + 40% butter fat.
4	34	26	30	1.2	3.67	0.36	Killed 4 hr 45 min after feeding as above.
4	34	34	34	—	—	—	" 5 " after feeding as above.
5	27	23	25	1.1	3.56	0.35	" 7 " " " " "
4	23	22	22	1.0	3.48	0.31	" 9 " " " " "

N.F. = Neutral fat plus cholesterol.
P.L. = Total phospholipids.

TABLE III.

Effect of Feeding Non-lipid Substance on the Serum Esterase and Liver Lipids of Rats.

No. of rats	Serum esterase			Liver, %			Remarks.
	Max.	Min.	Avg	N.F.	P.L.	Chol.	
1	—	—	18	1.3	3.90	0.28	Control.
1	—	—	17	1.0	4.05	0.23	Killed 1 hr after 0.8 g of sucrose per 100 g by stomach tube.
2	21	15	18	1.7	3.78	0.24	" 2 " " feeding as above.
2	25	19	22	1.0	3.81	0.23	" 4 " " " " "
2	22	16	19	1.4	3.42	0.22	" 6 " " " " "
3	20	17	18	1.3	4.11	—	Controls.
1	—	—	15	1.4	4.40	—	Killed 1 hr after 0.5 g glycerol per 100 g by stomach tube.
4	23	12	18	1.6	4.04	—	" 2 " " feeding as above.
4	20	13	15	2.0	4.02	—	" 4 " " " " "
4	26	12	19	2.1	3.74	—	" 6 " " " " "
2	16	16	16	—	3.70	—	" 7 " 20 min. after feeding as above.
1	—	—	20	0.9	4.00	0.29	Control.
1	—	—	19	0.9	4.15	0.29	Killed 1 hr 30 min after 0.75 g proteosepeptone per 100 g by stomach tube.
2	21	18	19	1.1	4.32	0.29	Killed 2 hr 30 min after feeding as above.
2	25	25	25	1.0	4.29	0.29	" 4 " " " " "
2	18	15	16	1.1	4.00	0.26	" 6 " " " " "

N.F. = Neutral fat plus cholesterol.
P.L. = Total phospholipids.

liver have been found not to affect the esterase response. A lack of correlation between the degree of visible lipemia and the esterase value has been noticed. In many cases the lipemia would have practically disappeared while the esterase value still remained high, while in others a marked lipemia was associated with a relatively low esterase value.

In considering the results herein presented it should be realized that only changes in the tributyrin hydrolyzing power of the serum are under consideration. Mosters[3] did not find any definite correlation between the ability of blood to hydrolyze methyl butyrate and tributyrin. He found that ascorbic acid administration increased the blood's power to hydrolyze methyl butyrate but the effect on tributyrin hydrolysis was very indefinite, the two effects being sometimes in opposite directions. Gajdos,[4] however, found the subcutaneous administration of ascorbic acid to invariably increase the serum's power to hydrolyze either substrate.

Any discussion as to the possible significance of serum esterase in fat metabolism and in determining an animal's resistance or susceptibility to halogen hydrocarbons seems unjustifiable at the present time. However, further data is being accumulated in an attempt to definitely determine the significance of this enzyme in these conditions.

Summary. It has been shown that the oral administration of nonlipoid foods, glycerol, sucrose, glucose and proteose-peptone exert no demonstrable effect on the serum esterase of rats. The administration of neutral fats, oleic and palmitic acids leads to a marked increase which lasts for several hours.

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[3] Mosters, J., *Klin. Wochschr.*, 1936, **15**, 1557.

[4] Gajdos, A., *Compt. rend. soc. biol.*, 1939, **131**, 59.