later used by Longenecker (*5*), who elaborated the general equation $[Y] = k[X]^3$ to include specific glycerides of all pos-

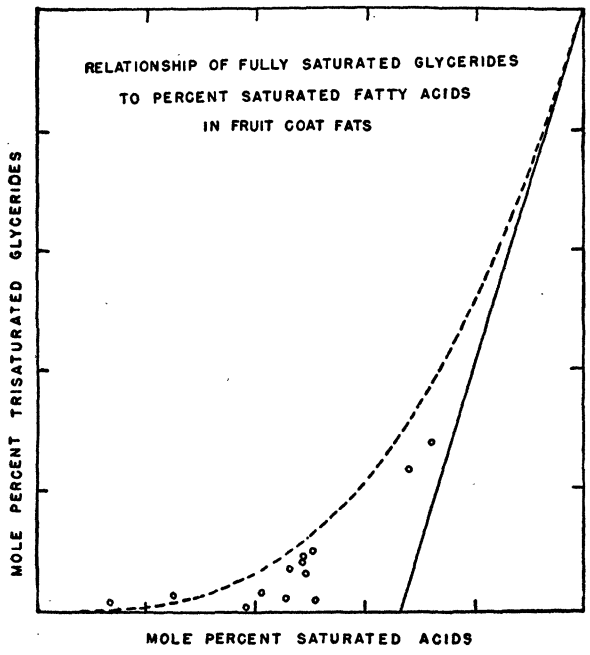

FIG. 3

sible configurations. Similar equations have recently been published by Bailey (*1*).

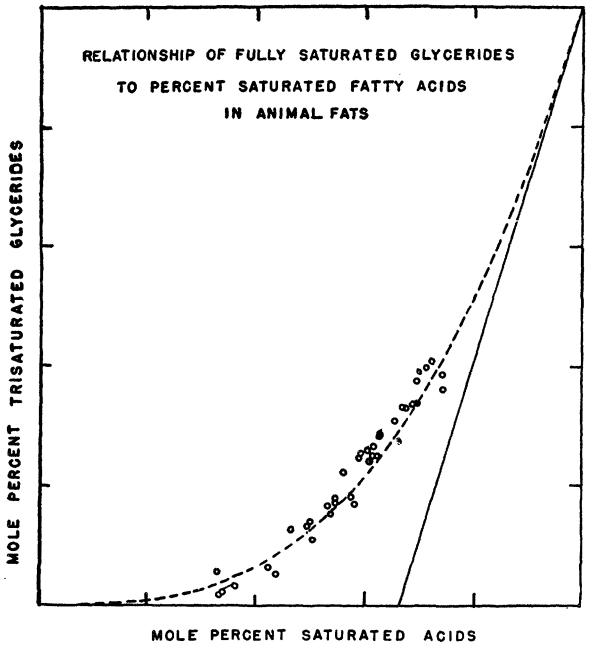

FIG. 4

In summary, an analysis of representative data showing the relationship of the molar concentrations of trisaturated glycerides to those of saturated acids in natural fats reveals that in the case of animal fats the glycerides are formed by a "random" or nearly "random" distribution of fatty acid radicals among the glycerol molecules. It has been shown that the "even distribution hypothesis" does not represent truly "even" distribution, but rather is an approach to the latter, being more or less arbitrarily fitted to experimental data. No general class of fats adheres to truly "even" distribution. Seed fats appear to approach the trisaturated glyceride contents anticipated by the "even distribution hypothesis," but even here the data are inconclusive.

References

1. BAILEY, A. E. *Industrial oil and fat products*. New York: Interscience Publishers, 1945.
2. DEAN, H. K. *Utilization of fats*. New York: Chemical Publishing Co., 1938.
3. HILDITCH, T. P. *The chemical composition of natural fats*. New York: Wiley, 1941.
4. LONGENECKER, H. E. *Chem. Rev.*, 1941, **29**, 201.
5. LONGENECKER, H. E. Paper presented before the American Oil Chemists' Society, October 1942.

Effect of Vitamin B Complex on Inactivation of Estrone *in Vivo* and *in Vitro*

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In 1934 Zondek (*6*) found that estrone is inactivated by animals and plants. In experiments carried out *in vivo*, rats were injected with estrone and killed a few hours later. The bodies were finely minced, extracted, and the extract assayed as to estrogenic activity. Only about 2–4 per cent of the estrogenic activity of the injected estrone solution was recovered. Subsequent hydrolysis improved the recovery to 6–20 per cent. The major part of the inactivation was therefore not due to esterification. In numerous experiments inactivation of estrone by rats *in vivo* was consistently observed. Over a period of many years we have not chanced upon a single case in which such inactivation failed to occur.

In experiments carried out *in vitro*, pulps of various organs were incubated for some hours with estrone at 37°C., the mixture then being tested for estrogen activity. It was found that liver brei inactivates estrone to an extent of about 90 per cent, while of other organs tested only spleen inactivates estrone to a small degree. Hydrolysis of the inactive mixture does not liberate any estrogenic activity. It should be mentioned that not all livers tested inactivate estrone: for some unexplained reason, liver brei of normal rats occasionally fails to mediate this reaction.

Since heating inactivates liver pulp estrone, it may be concluded that the inactivation is enzymatic. This enzyme, which has been designated by one of us (B. Z.) as estrinase, has been obtained in cell-free extracts of liver and of various plants (*6*, *7*). The plant enzyme is closely associated with tyrosinase and inactivates estrone presumably by oxidation (*5*).

Recently Biskind and Biskind (*1*) have pointed to the existence of a relationship between the amount of vitamin B com-

[1] Aided by a grant from the Rockefeller Foundation.

plex in liver and the ability to inactivate estrone. These authors implanted pellets of estrone into spleens of adult castrated female rats. On a normal diet the animals remained anestrous. When the rats were given a vitamin B-complex-free diet, vaginal estrus occurred. In another series of experiments Biskind and Shelesnyak (*2*) castrated female rats and transplanted one ovary into the spleen. On a normal diet they remained anestrous, but when kept on vitamin B-complex-free diet, estrus appeared.

TABLE 1

INACTIVATION OF ESTRONE BY VITAMIN B-COMPLEX-DEFICIENT RATS *in Vivo*

Rat No.	Duration of vitamin B-complex-free diet (days)	Wt. of animal (grams)		Time elapsed between injection of estrone and killing of rat (hrs.)	Amount of estrone injected (I.U.)	Recovery of estrone (%)
		Start	Final			
1	33	25	23	5	2,000	0–10
2	30	25	20	5	1,600	10
3	30	25	25	5	2,000	10
4	30	40	32	4	1,000	10
5	30	45	36	4	1,000	10
6	30	42	35	4	1,000	25
7	30	40	27	4	1,000	0–10
8	30	40	29	4	1,000	0–10
9	32	47	35	2	2,000	25
10	32	47	37	2	2,000	50

Shipley and György (*3*) observed an impairment of ability to inactivate estrone *in vivo* in rats maintained on diets low in protein and high in fat. Vitamin B-complex deficiency induced similar but less constant response. Not all strains of rats tested by these authors reacted to the vitamin B-complex deficiency with less ability to inactivate estrone.

Singher, *et al.* (*4*) examined the dependence of inactivation of estrogens on vitamin B-complex deficiency and have shown that liver slices of riboflavin- and thiamine-deficient rats are unable to inactivate estradiol.

In experiments carried out on 28 vitamin B-complex-deficient female rats we have found that vitamin B deficiency has no influence on the inactivation of estrone by rats *in vivo*. Table 1 summarizes the results obtained with 10 rats kept for one month on a vitamin B-complex-free diet.[2] Most of the animals were near death after three weeks of the experiment, and a few died from cachexia caused by vitamin B-complex deficiency before the inactivation test could be performed. In the inactivation experiments, vitamin B-complex-deficient rats were injected with 1,000–2,000 I.U. of estrone.[3] These were killed 2–5 hours following the injection, and the minced bodies were analyzed as to their estrogenic content by the Allen-Doisy method. The inactivation after 2 hours was of the order of 50–70 per cent; after 4–5 hours, 75–100 per cent.

On the other hand, liver pulps of avitaminotic rats, contrary to normal liver, in a majority of cases uniformly fail to inactivate estrone. Table 2 shows results obtained with liver brei of 7 vitamin B-complex-deficient rats incubated with 250–500 I.U. of estrone in phosphate buffer pH 7.3 at 37°C. for 3–5 hours. The recovery of estrone was quantitative, and only in one case was there a measurable loss (25 per cent) of original estrogenic activity.

Hence, (1) the vitamin B-complex-deficient rats are able to inactivate estrone *in vivo* as are normal rats; (2) whereas the liver of normal rats inactivates estrone *in vitro* in a high percentage of cases, liver of vitamin B-complex-deficient rats does not inactivate estrone *in vitro*.

TABLE 2

INACTIVATION OF ESTRONE BY LIVER BREI OF VITAMIN B-COMPLEX-DEFICIENT RATS

Rat No.	Duration of vitamin B-complex-free diet (days)	Wt. of animals (grams)		Wt. of liver (grams)	Time of incubation with estrone at 37°C. (hrs.)	Amount of estrone used (I.U.)	Recovery of estrone (%)
		Start	Final				
11	25		40	1,600	5	500	100
12	22	40	30	1,700	4	500	100
13	22	40	32	1,650	4	500	100
14	35	45	40	1,800	4	500	100
15	28	45	36	1,550	3	500	75
16	28	48	37	1,700	3	500	100
17	23	45	24	1,800	3	250	100

The question as to whether the inability of liver of vitamin B-deficient rats to inactivate estrone *in vitro* is due to vitamin B-complex deficiency is under investigation.

References

1. BISKIND, M. S., and BISKIND, G. R. *Endocrinology*, 1942, **31**, 109.
2. BISKIND, M. S., and SHELESNYAK, M. D. *Endocrinology*, 1942, **30**, 819.
3. SHIPLEY, R. A., and GYÖRGY, P. *Proc. Soc. exp. Biol. Med.*, 1944, **57**, 52.
4. SINGHER, H. O., KENSLER, C. J., TAYLOR, H. C., RHOADS, C. P., and UNNA, K. *J. Biol. Chem.*, 1944, **154**, 79.
5. WESTERFELD, W. W. *Biochem. J.*, 1940, **34**, 51; GRÜNBARD, M., and PINCUS, G. *Endocrinology*, 1942, **30**, 265; ZONDEK, B., and FINKELSTEIN, M. *Ibid.*, 1945, **36**, 291.
6. ZONDEK, B. *Skand. Arch. Physiol.*, 1934, **70**, 133; *Lancet*, 1934, **227**, 356.
7. ZONDEK, B. *Hormone des Ovariums und des Hypophysenvorderlappens.* (2nd. ed.) Vienna: J. Springer, 1935; ZONDEK, B., and SKLOW, J. *Proc. Soc. exp. Biol. Med.*, 1942, **49**, 629.

Feeding of Oysters in Relation to Density of Microorganisms

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Regardless of extensive studies on the physiology of oysters (*Ostrea virginica*), many problems concerned with their food and feeding are still unsolved and several are vigorously debated. For example, biologists do not agree on the effects of different quantities of material suspended in water upon the efficiency of the feeding of oysters. One group of workers, headed by Kellogg (*2*), maintains that oysters and other lamellibranchs are able to feed only when the water is comparatively clear, while another, represented by Grave (*1*), states that they can feed even in very turbid water. To clarify at least certain aspects of this problem we have made a study of the effect of different concentrations of microorganisms upon the rate of water pumping and, therefore, feeding of oysters.

[2] We are obliged to Dr. M. L. Tainter, Winthrop Chemical Company, Rensselaer, New York, for supplying us with this material.

[3] We are indebted to Dr. B. J. Brent, Roche Organon, Inc., who supplied the estrone.