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.

Experiments in which oysters were fed *Chlorella* sp., *Nitzschia closterium*, *Euglena viridis*, other plankton forms, and a common variety of yeast have shown that there are rather definite concentrations above which the density of the microorganisms begins to interfere with the feeding of oysters. These concentrations corresponded to approximately 2,000,000 *Chlorella*, 70,000 *Nitzschia*, and 3,000 *Euglena*/cc. of water. As may be seen, the size of the cells played an important part because a much higher number of small cells, such as *Chlorella*, was needed to produce the same effect as that caused by a smaller number of larger organisms.

In concentrations higher than those given above, the rate of feeding was reduced, and the character of the shell movement of the oysters changed noticeably. In many experiments a correlation was noticed between the density of the microorganisms and the rate of feeding. When the plankton cells were too abundant, little or no food was swallowed by the oysters and the crystalline style was usually absent. Under such conditions the oysters ejected large quantities of pseudofeces to cleanse their gills and palps of an excessive accumulation of plankton. Sometimes the shells remained open for periods of several hours, although no water was pumped. If the oysters were kept in heavy concentrations for a long time, they became sluggish, their responses to stimuli diminished, and the tonus of the adductor muscle was partially lost.

Both the filtrate of the cultures, containing metabolic products of the cells, and the cells themselves affected the oysters because the rate of feeding was reduced or even entirely stopped when they were subjected to strong concentrations of either component.

When, after exposure to heavy concentrations of microorganisms, the oysters were again subjected to a flow of sea water, their rate of feeding usually showed a marked increase. The intensive pumping of water indicated an attempt to remove the microrganisms which had accumulated in the gills and mantle chamber.

In light concentrations, which contained fewer cells than those mentioned above, the rate of feeding and the shell movements remained normal. In many instances the rate of feeding was even greater than when the oysters were kept in running sea water. The possibility is not excluded that the presence of small quantities of plankton in sea water stimulates the pumping activities of the oysters.

The quantities of pseudofeces formed by the oysters were usually roughly proportional to the quantities of plankton present in the water, whereas a reverse relationship existed in the formation of true feces. The presence of large quantities of pseudofeces usually indicated that feeding proceeded under the relatively unfavorable conditions caused by heavy concentrations of plankton in the water. Small quantities of pseudofeces, or their absence, in the presence of large quantities of true feces showed that the oysters were feeding efficiently.

The results of our experiments indicate that both Kellogg and Grave were only partially correct in their conclusions. The opinion advanced by Kellogg, that oysters feed most efficiently only if the water contains small quantities of suspended matter, was corroborated by our studies. Contrary to his opinion, nevertheless, we found that oysters can also feed in water containing a relatively large number of microorganisms, although under such a condition the rate of feeding is decreased. It is true, however, that when the plankton is too heavy, the oysters cease feeding. On the other hand, Grave's conclusion may be considered correct only if it was qualified with the statement that the efficiency of feeding of oysters decreases in water rich in microorganisms. Since no such statement was made by Grave, his conclusion creates the impression that it is unimportant whether or not the water is relatively clear or heavily laden.

A full description of these studies will appear in the official publication of the U. S. Fish and Wildlife Service.

References

1. GRAVE, C. *Science*, 1916, **44**, 178–181.
2. KELLOGG, J. L. *J. Morphol.*, 1915, **26**, 625–701.

Antagonistic Effect of *Corynebacterium diphtheriae gravis*

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The object of this note is to report the occurrence of an antibiotic effect produced by *Corynebacterium diphtheriae gravis* against several organisms. This strain of *C. diphtheriae gravis* was isolated in a culture from a diphtheritic throat and was identical in all particulars to the requirements of this organism. It was the specimen used in instruction of medical bacteriology at the Naval Medical School.

In checking its characteristics on potassium tellurite blood agar, the plate containing 15 mg. potassium tellurite/100 ml. blood agar became contaminated with *Bacillus subtilis*. The result is shown in Fig. 1. The black, clearly defined colonies are

FIG. 1

C. diphtheriae; the confluent growth, *B. subtilis*. About many of the *C. diphtheriae* colonies is a clearly defined zone where growth of *B. subtilis* has been inhibited.

Using moist blood agar plates containing the same amount of potassium tellurite, the strain of *C. diphtheriae gravis* was tested against *Proteus vulgaris*, *Salmonella typhimurium*, and *S. paratyphi A*. The same inhibitory effect was observed.