growth of 3 strains of *B. coli* on 1% glucose-agar plates. These were incubated at various temperatures for a period of 5 days before the type of growth was recorded as in Table I.

The marked correlation between incubative temperature and mucoid growth of *B. coli* indicates that the thermal factor is important in the production of mucoid dissociants of this organism.

9328 P

Bacterial Nutrition of the California Mussel.

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The sea mussel, *Mytilus californianus,* like other lamellibranchs, derives its nourishment from the microörganisms and finely divided detritus which it swallows.[1] It has been suggested that bacteria may constitute a very important part of the food of such plankton- and detritus-feeders.[2,3] *M. californianus* was selected for experimentation along this line because of its abundance in the vicinity, its convenient size and the ease with which it can be maintained in the laboratory.

Specimens measuring 6-9 cm. in length and weighing 20-50 gm. were scraped clean of organisms attached to their shells and placed singly in liter jars of aërated sea water in the dark. After 2 weeks' inanition a bacterial suspension was added to each jar. A red coccus and a spore-forming bacillus which were morphologically and culturally distinctive enough to be readily recognized were used. They were grown on nutrient agar in Blake bottles and washed in 2 changes of saline by centrifuging. The mussels removed most of the bacteria from suspension within 2 hours as indicated by the clearing of the water,[4] whereas in control jars lacking mussels the water remained turbid for several hours. Three hours after the addition of about 200 million bacteria per cc., plate counts revealed the presence of less than ten thousand bacteria per cc. in jars containing mussels. That many of these bacteria were actually ingested and not merely clumped or rejected as pseudo-feces[4] was proved by

1 Fox, D. L., *Bull. Scripps Inst. Oceanogr.,* Tech. Ser., 1936, **4**, 1.

2 MacGinitie, G. E., *Science,* 1932, **76**, 490.

3 Baier, C. R., *Arch. f. Hydrobiol.,* 1935, **29**, 183.

4 Fox, D. L., Sverdrup, H. U., and Cunningham, J. P., *Biol. Bull.,* 1937, **73**.

microscopic and cultural demonstration of bacteria in the stomachs, especially on the head of the style, of dissected mussels soon after receiving a bacterial meal.

The disappearance of bacteria from the digestive tract within 6 hours after the bacterial meal and their paucity in the animal's feces were regarded as evidence that the bacteria were digested by the mussels. Further proof was obtained by demonstrating the occurrence of digestive enzymes which lyse bacteria. The viscera, including the digestive diverticula, stomach, style and intestine of several large well-fed mussels were ground with sand and the resulting extract pressed through cheesecloth. A few drops of this extract were added to toluene-preserved suspensions of bacteria buffered at pH 7.0 in small test tubes and held at 25°C. After different intervals smears of the mixture were stained and examined microscopically. Although much of the enzyme was probably lost by this technic, there was enough to dissolve the bacteria rapidly. The test has been made on 18 different marine bacteria[5] which were lysed by the extract. There was little or no lysis in the controls in which the extract was inactivated by heat.

Finally, the assimilation of the ingested and digested bacteria was proved by other feeding experiments. Small mussels 2-4 cm. long, weighing 2-5 gm. each, were used because small ones would be expected to grow more rapidly than larger ones. After an initial quarantine and inanition period of 2 weeks they were placed in groups of 3 per liter of filtered sea water. The water was aërated in the dark at 14-15°C. Three times each week bacteria were added in sufficient numbers to give about 200 million per cc. Eight jars received the red coccus, 8 received the spore-forming bacillus and nothing was added to the 8 control jars. Twice each month the mussels were weighed. Individuals were identified by depositing a drop of colored sealing wax on the shell. Once a month the water was replaced by filtered sea water. After 9 months, 47 of the original 72 mussels were still alive, including 17 of the 24 fed the coccus, 21 of the 24 fed the bacillus, and 9 of the 24 controls. Some of the survivors gained weight and others lost but a statistical treatment of the data revealed that the coccus-fed mussels gained an average of 0.46 gm. each or 12.4%, the bacillus-fed mussels gained an average of 0.39 gm. or 9.7%, and the fasting controls lost an average of 0.21 gm. each or 6.8%. It is estimated that during the 9-month period the mussels received 24 gm. of bacteria each and about

[5] ZoBell, C. E., and Feltham, C. B., *Bull. Scripps Inst. Oceanogr.*, Tech. Ser., 1934, **3**, 279.

2.4 gm. of solid material, since the bacteria contain about 90% water. Mussels have been maintained in this laboratory for 16 months on an exclusive diet of bacteria. Professor T. Kincaid[6] has maintained oysters for several months with nothing to eat except bacteria. The oysters appeared to develop normally and their glycogen-content increased.

To date a total of 30 different new and undescribed species of marine bacteria[5] have been filtered from prepared sea-water suspensions by mussels, ingested and probably digested. In general, bacteria washed free of their metabolites do not seem to injure mussels unless so many are added that their respiration vitiates the water. In the presence of appreciable concentrations of bacterial nutrients such as peptone, the products of bacterial metabolism are usually toxic for the mussels.[1] The peptone itself is not toxic as indicated by the fact that mussels survive for several days in peptone solutions at refrigerative temperatures which retard bacterial activity.*

Most of the bacteria fed to mussels were alive. Heat-killed bacteria were also ingested by the mussels but they were not tolerated in as large doses as the living ones. This is attributed to the vitiation of the water by other bacteria which are always associated with the mussels and which multiply at the expense of the organic matter from the heat-killed bacteria. Bacteria known to be pathogenic for animals have not been fed to the mussels.

9329 P

Augmentation of the Gonadotropic Hormone from the Pregnant Mare.*

ALLEN LEIN. (Introduced by Bennet M. Allen.)

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Several investigators have described the so-called augmentation phenomenon, which has recently aroused considerable interest in its applications to the study of the gonadotropic hormone obtained from the blood and certain other tissues of the pregnant mare. Cole and

[6] Personal communication, University of Washington, Seattle.

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