chloride. Hence it is believed that the substance present in extracts of invertebrate tissues is acetylcholine. The content in the organs of *Octopus* ranges from 0·1–0·2γ per gm. (skin, median ventricle) to 3γ per gm. (arms without skin) of wet tissue. The stomach and posterior salivary glands contain a fair amount (1·3γ per gm.). The blood and testicles have very little or none. The apparent acetylcholine content of *Sipunculus nudus* and of the longitudinal muscles of *Holothuria tubulosa* ranges from 0·9γ to 1·7γ per gm. Choline esterase is present in the blood, not only of *Octopus vulgaris*, but also of *Aplysia depilans* and *Murex*.

It thus seems that, in at least some invertebrates, the conditions for cholinergic nervous action are realised. This second point is now being investigated in *Octopus*.

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May 15.

[1] Dale, H. H., "Nothnagels Vorlesung", Urban and Schwarzenberg, Vienna, 1935.
[2] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, **79**, 255; 1933.

P.S. The above letter had been sent to the Editor before I saw an advance proof of the communication by Mr. C. F. A. Pantin on the response of the leech to acetylcholine (NATURE, May 25, p. 875). *Hirudo* does not seem to be suited for detailed physiological analysis; *Octopus*, on the contrary, is a remarkable animal for experimental work. In addition to the facts already mentioned above, I wish to add the occurrence of a very large amount of acetylcholine (77γ per gm.) in the cerebral ganglia; this fact suggests that at the central synapses of this cephalopod a cholinergic mechanism is probably involved.

Z. M. B.

May 23.

Dietary Hæmorrhagic Disease in Chicks

A NUTRITIONAL disease of chicks characterised by subcutaneous, intramuscular and abdominal hæmorrhages, prolonged blood-clotting time and erosions of the gizzard lining has been described in detail by Holst and Halbrook[1] of this laboratory. They were able to cure the disease by the use of fresh cabbage. The gizzard erosions and bleeding tendencies have been noted by McFarlane, Graham and Hall[2]. Dam and Schönheyder[3] have also produced the hæmorrhagic symptoms in chicks, and have shown that the disease is not caused by lack of any of the known vitamins. The same finding has been obtained by Halbrook[4].

Dam[5] has reported that the disease is caused by lack of an organic substance which was found present in hog-liver fat, hemp seed, tomatoes, kale and, to a less degree, in many cereals. The anti-hæmorrhagic factor was found to be present in the fat-soluble, unsaponifiable, non-sterol fraction.

Since the first publication from this laboratory[1], additional findings have been made. The disease can be prevented by so little as one half per cent of dehydrated alfalfa, and the anti-hæmorrhagic factor is located in the unsaponifiable, ether-extractable portion of alfalfa. Completely extracted alfalfa, chlorophyll and the saponifiable fraction of alfalfa ether extract fail to prevent the disease. The factor can be adsorbed from its ether solution by activated carbon. It is stable to heating at 120° C. for 24 hours.

The disease can be prevented by a concentrate prepared from alfalfa and fed at the level of 1/1,000 per cent of the basal diet. This basal diet consists of fish meal 20, dried brewer's yeast 12, polished rice 65, limestone 1, cod liver oil 1 and salt 1. Birds on the basal diet develop the disease to a severe extent, comparable with that described by Dam and Schönheyder[3].

In addition, the fish meal used in our basal diets can protect against the disease if allowed to remain in a wet condition for several days, thus affording opportunity for the action of micro-organisms. Rice bran treated in the same way will also afford complete protection, while the untreated rice bran fails to prevent the disease. A sample of the fish meal, completely extracted with ethyl-ether and kept in a moist condition for several days, will, after drying, yield a potent ether extract effective in small amounts when added to our basal diet containing the untreated fish meal.

For these reasons, anti-hæmorrhagic power cannot be attributed specifically to any feed ingredient unless the possibility of action upon it by micro-organisms has been guarded against. These results offer an explanation for the failure of Cribbett and Correll[6] to obtain symptoms of the disease on the Holst and Halbrook diet, and for the fact that samples of fish meals, meat scraps and commercial casein have often failed to produce the disease.

In an experiment to determine a possible causative factor in fish meal, the fish meal was diluted with varying amounts of a mixture of purified casein and bone ash compounded so as to resemble the fish meal. The sample of fish meal used in this case was a different lot from that formerly used although obtained from the same manufacturer. When this fish meal alone was used as the animal protein, the symptoms were only moderately severe. However, the severity and early onset of the disease markedly increased as the proportion of purified casein replacing the fish meal increased. The severity of the disease was also noticeably greater on lower levels of dried brewer's yeast. The results indicated the presence of small but inadequate amounts of the anti-hæmorrhagic factor in both of these samples of fish meal and yeast, rather than a specific disease-promoting factor in the fish meal.

Replacement of 50 parts of polished rice by wheat or yellow corn fails to prevent the disease. The results indicate very little, if any, of the anti-hæmorrhagic factor in these cereals.

In regard to the chemical and physical properties of the anti-hæmorrhagic factor, our work closely agrees with that of Dam[5]. The nature of this substance is being actively investigated.

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[1] W. F. Holst and E. R. Halbrook, *Science*, **77**, 354; 1933.
[2] W. D. McFarlane, W. R. Graham, Jr., and G. E. Hall, *J. Nutrit.*, **4**, 331; 1931.
[3] H. Dam and F. Schönheyder, *Biochem. J.*, **28**, 1355; 1934.
[4] E. R. Halbrook, Thesis, University of California; 1935
[5] H. Dam, NATURE, **135**, 652, April 27, 1935.
[6] R. Cribbett and J. T. Correll, *Science*, **79**, 40; 1934.