

copepods; but as we have just indicated, the conditions are quite different with copepods, because, so far as is now known, these crustacea can not push forward or backward the stage of maturity, which can come only at the 6th copepodid stage.

The form of *C. vernalis* is modified by temperature, at least as regards the relation of width to length of the furcal rami. Length of furca relative to length of body is probably somewhat less in copepods reared at high temperatures than in those reared at low temperatures (slight degree of inverse correlation) but the direct correlation (with temperature) of width of furca relative to length of body and to length of furca is most obvious: temperature influences different qualities (width and length of furca) differentially. There are significant indications of a tendency to increased spination and also to the development of a spine in lieu of a seta on the outer border of the terminal segment of the endopod of the fourth swimming foot when copepods are reared at low temperatures. "Spine formulas" and the substitution of a spine for a seta at the place mentioned on the 4th foot have been used as bases for specific distinctions for *vernalis* Fischer, *robustus* Sars, *parvus* Herrick, *americanus* Marsh and *brevispinosus* Herrick. The results of these experiments support Lowndes' view that the spine formulas are invalid for specific distinctions in this group of copepods, and they go further in suggesting very strongly the immediate (whether direct or indirect) influence of the environment in the development of the characters upon which the four last-mentioned species were based. Although the three species last mentioned are recognized in the most recent American literature, we do not regard them, or Sars' *robustus* either, as entitled even to sub-specific rank.

It was learned that the copepods of each of the three species employed in the experiments (*vernalis*, *serulatus* and *viridis*) have the faculty of becoming dormant at excessively high temperatures (31°–36° C., according to conditions) and of remaining so for hours after exposure to normal temperatures. This condition of dormancy seemed to be different from anything of the kind previously reported for copepods. It was not the "sleep" of Zenker, the kind of dormancy from which the animal can be awakened to immediate liveliness; it was not apparently like the resting condition of encystment in which many copepods survive periods of desiccation. It was a condition induced automatically at certain temperature levels, one from which the animal revived very slowly, one which could be repeatedly induced with an individual animal without obvious injury. During dormancy the animal was to all intents and appearances dead: it would respond to no mechanical stimulus, even though kept at room temperature for

some time—hours in some cases. Upon revival in the course of from 4 to 24 hours or more, the condition of the animal was seemingly quite normal; it might continue to form sacs with fertile eggs.

The temperature at which dormancy ensued varied with the temperature at which the copepod had been reared; copepods reared at low temperatures became dormant at temperature levels several degrees below that at which high-temperature copepods succumbed. Retaining low and high temperature copepods under the same conditions of temperature for a week seemed to have no marked effect on subsequent relative tolerances to high temperature: "acclimation" in the usual sense could hardly be invoked. It appears that the temperatures prevailing during development leave some internal effects which are not essentially modifiable by fairly prolonged "acclimation" at room temperatures. (A week is a rather long period in the life of a copepod, such as *vernalis*, covering the period of a possible cycle from egg to egg at room temperature.)

The copepods reared at low temperatures were different from those reared at high temperatures in size and, to some extent, in form and in physiology. How general are the expressions of the influence of temperature on form and physiology is not yet ascertained. The experiments give no suggestion of heritability of the differences induced by temperature. Detailed reports of the experiments are to appear in the *Internationale Revue der gesamte Hydrobiologie*, the *Journal of the Elisha Mitchell Scientific Society* and possibly elsewhere.

Reference may be made also to our recently published report³ of apparently indefinite inhibition of development without impairment of vitality at ordinary summer temperatures in a new species of copepod.

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AMEBIASIS IN REPTILES

PARASITIC ameba, similar to *Endamoeba histolytica*, occur in the digestive canal of various reptiles and amphibia.^{1, 2, 3, 4} So far as is known these organisms have not been implicated in disease processes affecting the intestines of their hosts, although amebic hepatitis has been described in *Rana temporaria*.⁵

³ "Arrêt du Développement chez les Copépodes," *Bulletin de la Société Biologique de la France et de la Belgique*, 67 (2): 276–287, 1933. Description of the species will appear in an early number of the *Journal of the Elisha Mitchell Scientific Society*.

¹ C. M. Wenyon, "Protozoology," 2 vols., 1926.

² G. Lavier, *Ann. de Paras.*, 6: 152, 1928.

³ R. Knowles and B. M. D. Gupta, *Ind. Jour. Med. Res.*, 18: 97, 1930.

⁴ E. P. Sanders and L. R. Cleveland, *Arch. f. Protist.*, 70: 267, 1930.

⁵ G. V. Epstein, *Arch. Russes Protist.*, 5: 211, 1926.

Among reptiles dying in the Philadelphia Zoological Garden, acute intestinal disease associated with amebic infection was found in the following: 1 *Varanus varius*, 1 *Tiliqua scincoides*, 1 *Pseudoboa clelia*, 1 *Lampropeltis getulus*, 1 *Natrix sipedon* and 5 *Natrix rhombifer*. In *N. rhombifer* the enteritis was accompanied by liver abscesses and, in one of this group, by gastric ulcers as well. Another of this species had gastric ulcers without intestinal or liver involvement. Amebas also were associated with the lesions of the liver and stomach.

Disease changes in the lower digestive tract were limited to the colon and the adjoining small intestine. The gut wall was injected, thickened, inelastic and turbid. The mucosa of the colon was covered by adherent, friable, blood-stained exudate which sometimes filled the lumen. In the small intestine, changes were not so severe and ulcers were seen as circumscribed depressions of the mucosa filled with exudate and necrotic debris and surrounded by hyperemic zones. Microscopic examination of the wall of the colon revealed extensive superficial necrosis of the mucosa and occasional limited areas in which this extended into the deeper tissues. The more circumscribed lesions in the small intestine consisted of areas of necrosis and ulceration involving the mucosa. Leukocytic infiltration was much more pronounced in these areas than in those in which the disease was more advanced.

Ulcers in the stomach usually were circumscribed. They occurred in all parts of the organ and were accompanied by acute inflammatory response. Microscopically they consisted of localized areas of necrosis and ulceration of the mucosa and occasionally the submucosa. Abscesses in the liver were multiple and usually circumscribed. They were larger in the cephalic half of the organ and in one case were limited to this region. Whether they involved one or several lobules of the liver the more advanced areas of degeneration were about the central veins.

Amebas were seen in sections of all these organs. They were often associated with the lesions, but in the wall of the intestine they were much more numerous deep in the tissue, especially in lymph vessels. Disease changes, similar to these, have been found occasionally in reptiles that were not infected with ameba, but there has been a difference in the degree of tissue damage, this being greater in the presence of ameba.

Five strains of ameba have been isolated from *N. rhombifer* and are growing and producing cysts at room temperature in 0.5 per cent. saline-horse serum 9:1 plus rice starch. Cultures are transferred at 7- to 10-day intervals. The first two strains were also grown on liver-infusion agar slants covered with

0.8 per cent. saline-horse serum 6:1 plus rice starch,⁶ and on 0.8 per cent. saline-horse serum 9:1 plus rice starch. Abundant growth and cyst formation occurred on these media, but seemed best maintained on the first and second mentioned. Use of the first of these has been continued because of its simplicity.

In the saline preparations from the intestines, stomach and liver, the amebas were actively motile in the slug-like manner of *E. histolytica*, clear ectoplasmic pseudopodia being formed on change of direction. Cysts also were recovered from the digestive tract and from the liver. The greater number of cysts from the liver were uninucleate.

Smear preparations of the organisms from the intestine and liver were fixed in warm Schaudinn's fluid plus 5 per cent. glacial acetic acid, in one half strength Schaudinn's fluid plus 2 per cent. glacial acetic acid and in Bouin's fluid, and stained with Heidenhain's hematoxylin. Measurements of ameba from six *N. rhombifer* were as follows: 250 trophozoites; range 10–25 μ $\times$ 8–22 μ , average diameter 14.16 μ ; nucleus, range 3.5–6 μ , average 4.5 μ ; ratio of average diameter of nucleus to that of trophozoites 0.31; 100 cysts, range 11–19 μ , average diameter 13.05 μ .

Morphology of the trophozoite ameba is strikingly similar to that of *Endamoeba histolytica*. The cytoplasm is dense, the nucleus has the typical karyosomal granule or granules in the center and the thin layer of discrete peripheral chromatin granules evenly distributed against the nuclear membrane. The cytoplasm often contains ingested bacteria. The cysts are also very similar to those of *E. histolytica*. The size range of both phases of this organism is within that of the human parasite. There is also close similarity to several species of *Endamoeba* of reptiles and amphibia, but, since cysts have not been described in a number of cases, synonymy or differences may not be established until further data are at hand. For the present the organism found to be associated with intestinal, hepatic and gastric disease in the hosts mentioned herein will be designated *Endamoeba sp.* of reptiles.

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THE SEPARATION OF CAROTENES BY ADSORPTION ON MAGNESIUM OXIDE

It has recently been shown that carotene as isolated from many plants, especially carrots, is a mixture of several isomeric hydrocarbons. In view of the importance of this observation to the chemistry and

⁶ L. R. Cleveland and E. P. Sanders, *Arch. f. Protist.*, 70, 223, 1930.