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 10–25μ × 8–22μ, average diameter 14.16μ; nucleus, range 3.5–6μ, average 4.5μ; ratio of average diameter of nucleus to that of trophozoites 0.31; 100 cysts, range 11–19μ, average diameter 13.05μ.

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.

HERBERT L. RATCLIFFE
QUENTIN M. GEIMAN
ZOOLOGICAL SOCIETY OF PHILADELPHIA AND UNIVERSITY OF PENNSYLVANIA

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.

physiology of the carotenoids, much effort has been expended in developing methods whereby the components of naturally occurring carotene mixtures may be separated and recovered in crystalline form.

The experience in this laboratory indicates that carotenes are most easily separated by passing a solution of the mixture over columns composed of suitable adsorbents (Tswett column). Under these conditions α-carotene moves through the column faster than the other carotenes associated with it. The β-carotene in turn moves faster than the remaining carotenes and lycopene, which are held most strongly by the adsorbent at the top of the column. By separating the column and eluting the pigments from each portion, the individual carotenes may be obtained in crystalline form.

It has also been found that α-carotene may be separated from carotene mixtures by passing solutions of the latter over columns containing slightly less than enough adsorbent to retain all the carotene with the result that α-carotene accumulates in the filtrate. After removing the top portion of the adsorbent, the β-carotene can be eluted from the bulk of the adsorbent without removing the latter from the column.

Of the numerous adsorbents tested for the separation of carotenes, magnesium oxide possesses the greatest number of desirable properties. This oxide is white, so that the positions of the adsorbed constituents are readily visible. Unlike some of the heavy metal oxides magnesium oxide does not affect the carotene through oxidation. It exhibits a highly specific adsorption for each carotene. Magnesium oxide is readily obtained in a high state of purity and in an extremely active form so that only small amounts of adsorbent (0.1–0.3 gm per mgm carotene) are required, thus effecting an economy in solvent (petroleum ether) and in time. In this respect magnesium oxide is a better adsorbent than the samples of calcium oxide and hydroxide which have been used in this laboratory and which have been recommended as adsorbents for carotene by other workers. Moreover, the adsorbed carotenes may be removed by eluting with petroleum ether containing ethanol, which is not the case with charcoal and fuller's earth. After eluting the carotene, the magnesium oxide can be recovered by drying in an oven at 110–150°. It should be pointed out that the adsorptive powers of magnesium oxide are largely dependent on its method of preparation. Most of the magnesium oxide preparations used in the separation of carotenes were manufactured by the low-temperature decomposition of magnesium hydroxide and were supplied to us through the courtesy of Mr. Max Y. Seaton, of the California Chemical Corporation, Newark, Calif. By mixing the magnesium oxide with heat-treated siliceous earth (Hyflo Super Cel) the columns were found to filter much more evenly and rapidly.

Carrot root carotene has been separated into its major constituents by means of magnesium oxide with a recovery of from 30 to 40 per cent. of the β-carotene and from 40 to 70 per cent. of the α-carotene originally present. The α-carotene prepared by this method was identical with α-carotene separated from carrot root carotene by other methods,[1] as determined by its melting point, optical rotation, solubility and absorption maxima in ethanol and carbon disulfide. The β-carotene also proved to be identical with that isolated from carrot root carotene and from leaf carotene,[2] as determined by similar physical properties.

α–Carotene
Melting point, 184° corrected.
Specific rotation, $[\alpha]_{6678}^{20°}$ +351° (benzene)
Absorption maxima, (Ångström units)

Ethanol	4457	4760
Benzene	4587	4881
Carbon disulfide	4764	5071

β–Carotene
Melting point, 178° corrected.
Specific rotation, $[\alpha]_{6678}^{20°}$ 0° (benzene)
Absorption maxima, (Ångström units)

Ethanol	4520	4818
Carbon disulfide	4850	5114

HAROLD H. STRAIN
CARNEGIE INSTITUTION OF WASHINGTON
STANFORD UNIVERSITY
DECEMBER 5, 1933

[1] P. Karrer and O. Walker, *Helv. chim. Acta*, 16: 641, 1933.
[2] R. Kuhn and E. Lederer, *Ber. deutsch. chem. Ges.*, 64: 1349, 1931.

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