THE SEASONAL INFESTATION OF *NASSA OBSOLETA* (SAY) WITH LARVAL TREMATODES.

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The purpose of this investigation has been to make a twelve months' survey of large numbers of a single species of marine mollusk, to determine what different larval trematodes parasitize it, and especially to determine and to try to interpret the seasonal fluctuations in the degree and character of the infestation. The study was begun at the Marine Biological Laboratory, Woods Hole, Massachusetts, in August, 1924, when several common species of gastropod were collected in Quamquisset Harbor. Examination at that time showed *Nassa obsoleta*, the common mud snail, to be the most heavily parasitized, and therefore this species was chosen as the one for study throughout the year. The biology and ecology of this snail have been studied by a number of investigators (see especially Dimon, 1905; Sumner, Osburn, and Cole, 1913; Allee, 1923*a*, 1923*b*). The work was continued at the Zoölogical Laboratory of Washington University with snails shipped periodically from Woods Hole, and the twelve months' survey was completed at Woods Hole during the summer of 1925. All collections were made from a fifty yard area in the part of Quamquisset Harbor known as Gansett; a total of 8,875 individuals of *Nassa obsoleta* were examined. In addition to the data on the seasonal infestation brief descriptions of the larval trematodes are also included.

HISTORICAL.

Relatively little work has been done on marine larval trematodes, and that chiefly by European investigators. Among these Pelseneer (1906), Sinitsin (1911), and Lebour (1905–1912) have

[1] The routine examination of snails and collection of data for descriptions of the trematodes are almost wholly the work of the junior author, as are all drawings except numbers 2, 9, 11 and 17.

contributed most extensively. There are only a few scattered references on North American cercariæ. Fewkes (1882) briefly described a cercaria with caudal setæ found free near Newport, Rhode Island. Tennent (1906, 1909), worked out the life history of *Bucephalus haimeanus* and described its gasterostome cercaria. Linton (1915*a*, 1915*b*) found three species of cercariæ in the Woods Hole region: two furcocercous forms, one from *Hydroides dianthus* and one from *Pecten irradians*, and one tailless larva from *Nassa obsoleta* which has been designated *Cercariæum lintoni* in this paper. More recently one of us (Miller, 1925*a*, 1925*b*) has made surveys of the larval trematodes infesting marine gastropods from Puget Sound and from the Dry Tortugas.

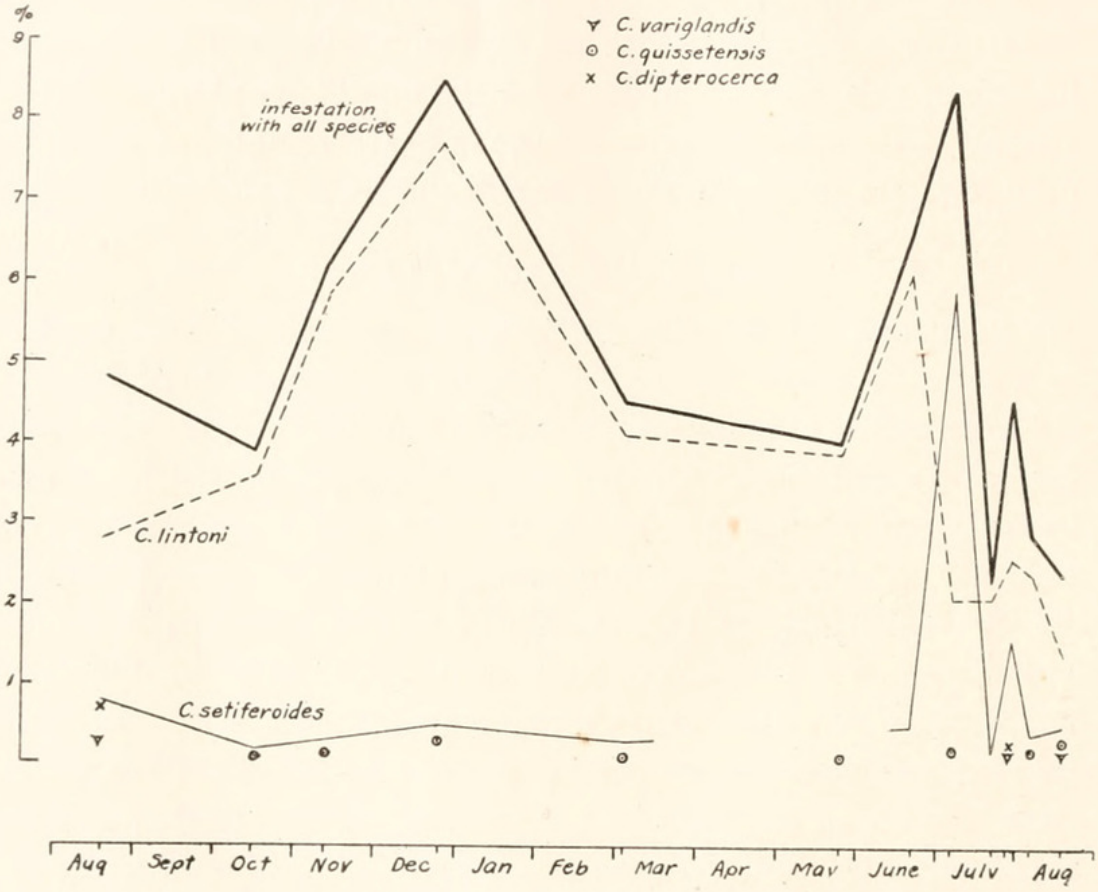
Material and Methods.

Snails received at Saint Louis were maintained until used in synthetic sea water made up according to the formula of Ditmar. All individuals of a collection, except that of May, were isolated for forty-eight hours to determine those from which mature larvæ were emerging. These infested snails were placed in separate aquaria as a constant source of living cercariæ for study. At convenient times the remaining snails were crushed individually, and their tissues examined under a binocular dissecting microscope in order to find non-emerging cercariæ and their parthenitæ; these were usually present in the digestive gland. After the living material had been studied, the infested viscera, freed cercariæ, and parthenitæ were fixed in Bouin's, or hot corrosive sublimate fluid. Cercariæ and parthenitæ were stained with Ehrlich's acid hematoxylin and mounted in Canada balsam. Unless otherwise noted, all measurements recorded were taken from these preserved specimens. Sections of snail viscera, two and one half and five micra in thickness, were variously stained.

Seasonal Infestation.

The graph for total infestation (Text-fig. 1) with all five species of larvæ has two maxima, practically equal, occurring in December and in July. Between these maxima are low areas, in each of which the percentage of infestation averages not more than one half that of the maximum. This plainly shows a seasonal fluctuation in the presence of larval trematodes in this

particular host. Before discussing this, the frequence and nature of the infestation with each of the five larvæ found will be taken up. Two of them were present only infrequently and in low percentages, and may be dismissed with brief statements. *Cercaria dipterocerca* sp. nov. was found in only four specimens, three in August, 1924, and one in late July, 1925; and similarly, *C. variglandis* sp. nov. was found in one host in each of these



TEXT-FIGURE 1. Graphs showing seasonal fluctuation in total infestation of *Nassa obsoleta*, and infestation with each species of trematode.

same collections, and once in August, 1925. There is no obvious explanation for the slight and infrequent occurrence of these two trematodes in *Nassa obsoleta*. It may be that they are normally parasitic in some other mollusk host, but are capable of developing occasionally in this species of *Nassa;* or the final hosts may be migratory.

A third species, *C. quissetensis* sp. nov., was found in eight of the twelve collections, always in very few hosts, and never emerging after isolation of the snail. In the October collection the rediæ were filled with mature cercariæ; in November and

December only immature rediæ were present, while in the succeeding months there were always some apparently mature, but non-emerging cercariæ. In July and August, 1925, many fully developed cercariæ together with a few early germ balls were present in all of the rediæ. A fourth species, *C. setiferoides* sp. nov. was found in every collection but that of May. Although usually less than one per cent. of *Nassa* was infested, in early July (1925) 5.9 out of a total of 8.4 per cent. infestation was due to this species. Some mature, or at least apparently fully formed, cercariæ were found in every collection, which would seem to indicate that *Nassa obsoleta* is continually being infested by the miracidia of this trematode. There was no emergence of *C. setiferoides* from isolated snails until June and July, 1925; this seems to be the season of maturity of this larva, although some infestations with immature rediæ were found both in late July and in August, 1925.

Much the greatest part of the total infestation was due to the presence of a tailless larva, *Cercariæum lintoni* sp. nov., which Linton (1915*b*) briefly described but did not name. It was present in every collection, and except in that of July 6, 1925, the percentage of infestation with this species was greater than the total for all other trematodes present (Text-fig. 1). Although fully developed larvæ were found throughout the year, there was a striking seasonal variation in the percentage of snails infested with mature [1] larvæ. In Text-figure 2, the total percentage of infestation of *Nassa obsoleta* with *Cercariæum lintoni* and the percentage of snails from which mature larvæ emerged are shown graphically. The two maxima of the total infestation graph might be ascribed to the semi-annual visitation of Woods Hole by the definitive vertebrate hosts, whether migratory birds or fishes. It is presumed that the majority of the definitive hosts of marine cercariæ are fishes, concerning the migrations of which

[1] The emergence of fully formed larvæ from the snail when isolated in sea water for forty-eight hours is taken as evidence of the full maturity of the trematode. In some cases the larvæ in crushed snails were apparently fully formed, but did not emerge, for reasons unknown. Whether or not emergence is a fair test of maturity, the same procedure has been followed with all collections (except the May shipment, when they were not isolated due to lack of time). It is possible that in the case of some snails only a few larvæ emerged and that these escaped observation.

relatively little is known (Meek, 1916; Bigelow and Welsh, 1925).

Why a certain percentage of infestation, such as the maximum of December or June, once reached is not maintained would seem to be due directly to two factors, the death of heavily infested snails, and the recovery of some of the snails because of the maturing and complete emergence of the larval trematodes. The serious and often fatal effects of larval trematode parasites on the snail host have been studied by a number of investigators.

TEXT-FIGURE 2. *Cercariæum lintoni;* graphs showing total infestation and percentage of emerged (mature) cercariæ.

The very rapid increase in actively metabolizing trematode tissue, which frequently results in the destruction of most of the visceral mass of the snail, probably causes the death of large numbers of them. As to recovery, Sewell (1922: 17) and others have noted a condition of degeneration of liver or gonad apparently attributable to previous trematode infestation; the present author has also found this condition in some individuals of each of a number of species, such that at first glance the liver appeared to be parasitized, but no trematodes were present. Sewell included also the factor of natural death at certain seasons of the year as one which might explain seasonal fluctuations in percentage of

infestation. This operates, according to him, to raise the percentage of infestation by removing old snails which had formerly been infested and had recovered and become immune. He believes that the majority of fresh water mollusks may die from natural causes in June and July. This is borne out by the observations of Manson-Bahr and Fairley, Annandale, and others. This factor is probably an important one among the fresh water snails of India which Sewell studied, for the percentage of infestation was frequently very high and the number of old, immune individuals was probably relatively quite large. But among marine gastropods, where usually only a small percentage harbors larval trematodes, it would not be expected that this factor

TABLE I.

INFESTATION RECORD OF 8,875 SPECIMENS OF *Nassa obsoleta*.

Date.	Number Collected.	Died before Examination.	Total Per Cent. of Infestation.	Infestations.	Per Cent. of Infestation.
8/17/24	353	0	4.8	10, *C. lintoni* (7, emerged).	2.8
				3, *C. setiferoides* (2, immature rediæ only).	0.8
				3, *C. dipterocerca* (1, emerged).	0.8
				1, *C. variglandis*.	0.3
10/14/24	869	2	3.9	31, *C. lintoni* (13, emerged).	3.6
				2, *C. setiferoides* (1, immature rediæ only).	0.2
				1, *C. quissetensis*.	0.1
11/11/24	995	3	6.2	58, *C. lintoni* (20, emerged).	5.8
				3, *C. setiferoides* (2, immature rediæ only).	0.3
				1, *C. quissetensis* (immature rediæ only).	0.1
12/24/24	947	9	8.5	73, *C. lintoni* (15, emerged).	7.7
				5, *C. setiferoides* (2, immature rediæ only).	0.5
				3, *C. quissetensis* (immature rediæ only).	0.3
3/3/25	1,153	9	4.5	47, *C. lintoni* (9, emerged).	4.1
				4, *C. setiferoides*.	0.3
				1, *C. quissetensis*.	0.1
5/22/25	727[1]	8	4.1	29, *C. lintoni*.	4.0
				1, *C. quissetensis*.	0.1

[1] Not isolated for emergence.

TABLE I.—(*Continued*).

Date.	Number Collected.	Died before Examination.	Total Per Cent. of Infestation.	Infestations.	Per Cent. of Infestation.
6/20/25	649	1	6.6	40, *C. lintoni* (2, emerged). 3, *C. setiferoides* (1, emerged).	6.2 0.5
7/6/25	509	0	8.4	11, *C. lintoni* (0, emerged). 31, *C. setiferoides* (7, emerged). 1, *C. quissetensis*.	2.1 5.9 0.2
7/21/25	575	0	2.3	12, *C. lintoni* (3, emerged). 1, *C. setiferoides*.	2.1 0.2
7/29/25	504	0	4.6	13, *C. lintoni* (6, emerged). 8, *C. setiferoides* (3, emerged, 4, immature rediæ only). 1, *C. dipterocerca*. 1, *C. variglandis*.	2.5 1.6 0.2 0.2
8/ 6/25	546	0	2.9	13, *C. lintoni* (9, emerged).	2.4
8/15/25	423 [2]	0	0.7	3, *C. lintoni* (2, emerged).	0.7
8/18/25	625	0	2.4	9, *C. lintoni* (6, emerged). 3, *C. setiferoides* (very immature). 2, *C. quissetensis*. 1, *C. variglandis*.	1.4 0.5 0.3 0.2
Total...	8,875				

would play a considerable part. There are no data in Dimon's (1905) study of the biology of *Nassa obsoleta* on the life span of this species, nor whether large numbers die at any particular season of the year. The question of age immunity does not enter into the present study, as only data for large specimens of *Nassa* were included in the graphs; one collection of small individuals showed an infestation of only 0.7 per cent. on August 15, 1925, in contrast to 2.4 per cent. (Aug. 6) and 1.4 per cent. (Aug. 18) for two collections of large ones.

Inspection of the graph for emerged cercariæa (Text-fig. 2) shows that in the different collections there is variation in the percentage of snails harboring mature larvæ. There does not seem to be any correlation between these percentages and the

[2] Small snails; data not included in any graph.

fluctuations in the total percentage of infestation; the relative percentage of mature larvæ to total infestation is shown by the dotted line. It is unfortunate that the May collection was not tested for emergence; but from the available data it is seen that the occurrence of mature larvæ continues high from August to December, and then apparently is lower until late July and August, at which time it approximates its former values. The high percentages of emerged *C. lintoni* in July and August, 1925, are supported by the fact that during these months most of the sporocysts from crushed snails contained fully formed larvæ. Plotting of the temperature readings taken at the Fish Commission Wharf,[1] probably only in a general way comparable to that at Quamquisset Harbor, seems to show a relation between maturity and temperature; the maturity graph apparently lags behind that of temperature, remaining high while the temperature is dropping in November and December, and low for a considerable period after the temperature has increased in the spring and early summer months. Other factors which have already been discussed as affecting total infestation might also affect the percentage of mature cercariæa.

It is interesting to note that there is a close general similarity between the graphs shown by Sewell (1922: Chart 1) for two species of fresh water snails from India and the total infestation graph for *Nassa obsoleta* in the present study. The maxima for Sewell's examination of *Melanoides tuberculatus* from the Museum tank, fall in July and December, as they do for *Nassa obsoleta*, and the partial graph for *M. tuberculatus* from the Zoölogical Gardens follows the same general trend, with summer maximum in August rather than in July. Sewell's two graphs are based on 139 and 53 mollusk individuals respectively, while in the present study 8,452 large, and 423 small, specimens of *Nassa obsoleta* were examined.

[1] Temperature data were secured from the records of the Bureau of Fisheries for the 1st and 15th of each month of the period from August, 1924, to August, 1925; the figures are the mean of three daily readings taken at the U. S. Fisheries Station at 8 A.M., 12 M., and 4 P.M. The highest is 71° F. for August 1, 1924, and the lowest 30° F., just six months later, February 1, 1925. Thus there was at least an annual range of 41° Fahrenheit.

Summary.

From the data resulting from the examination of almost nine thousand specimens of *Nassa obsoleta* Say it seems clear that there is a semi-annual rise and fall in the larval trematode infestation. In view of the fact that none of the adults of these larvæ are known it is difficult to explain these phenomena. In all probability migrations of the definitive hosts, and the degree of their infestation, affect the seasonal fluctuations; and other factors are probably the life span of *Nassa*, and the effect of parasitism upon it. The relative importance of these factors is not clear.

Brief Description of New Species Cited in this Paper.

Cercaria setiferoides sp. nov.

(Figs. 1–3, 6, 10.)

Trichocercous distome cercaria with opaque yellowish body and characteristic excretory vesicle. Contractile body varying from 140 μ to 268 μ in length and 104 μ to 156 μ in width; average length 187 μ and width 126 μ; average tail length 486 μ. Oral sucker slightly elongate, 64 μ in average length and 34 μ in width; ventral sucker smaller and oval, 40 μ in width and 34 μ in length. Surface of body finely pebbled, and completely covered with short, regularly arranged spines. Conspicuous pigmented eye spots, composed of large spherical granules and a so-called lens. No spines on tail, but on either side of it rows of long setæ; usually seven setæ per group, the longest in the center; about thirty pairs of setæ groups arranged along sides of tail opposite to each other. Well developed digestive system clearly seen in both living and preserved material; mouth antero-ventral in oral sucker; short prepharynx followed by large oval pharynx, 24 μ in length and 16 μ in width; short esophagus, and two wide intestinal ceca extending to posterior end of body; jelly-like contents of ceca with great affinity for intra-vitam neutral red, in contrast to all other structures in body. Eleven pairs of larval glands, preacetabular in position, arranged in three groups; no observable ducts from the most posterior six glands; glands of all groups strongly acidophilic in all combinations of stains;

lightly stained with intra-vitam neutral red, but not with toluidine blue. Large excretory vesicle, the most conspicuous of internal structures, slightly to right of median line normally, and extending nearly to region of bifurcation of gut; very wide and bent more or less in shape of Z; filled with spherical refractile concretions, ranging from very small ones up to 3 μ in diameter. Main lateral collecting tube of either side entering excretory vesicle at a point about two thirds of distance from anterior end; entrance of anterior and posterior branches posterior to level of ventral sucker. Succession of single excretory flagella in wall of main lateral collecting tube; many flame cells observed, but exact pattern of excretory system not solved; apparently more highly developed posteriorly. Several cell masses present in anlage of reproductive system; no interpretation ventured as to parts of adult system represented. Development of cercariæ in rediæ in visceral mass of *Nassa obsoleta*. Immature rediæ slender, without locomotor appendages; average length 440 μ, and diameter at widest part 74 μ; long coiled gut extending beyond middle of redia. Germinal epithelium localized in posterior end. More mature rediæ with both mature cercariæ and germ balls.

C. setiferoides is similar to *C. setifera* Joh. Müller 1850 (described by Monticelli, 1914, and redescribed by Odhner, 1914, from one of Monticelli's slides), but differs in a number of important structures. It is obviously different from *C. lutea* Giard 1897, *C. pectinata* Huet 1891, and *C. setifera* Pelseneer, 1906; *C. fascicularis* Villot 1875 is not completely described. The cercaria with caudal setæ which Fewkes (1882) found free near Newport, R. I., may be identical with *C. setiferoides;* a detailed description of it was not published. The present species may also be identical with the larval form of *Pharyngora bacillaris*, recorded by Nicoll (1910) and Lebour (1917) as free in the plankton from Plymouth. The latter investigator (1916, 1917) found the metacercarial stage in various medusæ and in *Sagitta bipunctata;* her brief description and single figure of the trichocercous cercaria (1917) are not sufficiently detailed to determine whether it and the present species are the same.

Cercaria dipterocerca sp. nov.

(Figs. 4, 5, 7, 8.)

Distome larva with prominent lateral cuticular fins extending along entire length of tail. Average body length 145 μ, varying between 109 μ and 212 μ, width 50 μ, varying between 47 μ and 65 μ. Body, but not tail, uniformly covered with small spines; double row of large ones around mouth. Pigment present only in two large eye spots, composed of very coarse granules and a "lens." Oral sucker 24 μ in diameter, larger than ventral sucker; subterminal mouth, prepharynx, pear-shaped pharynx; ceca not seen posterior to ventral sucker. Nine pairs of three different kinds of larval glands: on either side two pairs (Fig. 7, *a*), dorsal, lateral to pharynx, with coarsely granular, yellowish cytoplasm; stain with intra-vitam neutral red; eosinophilic in sections; second group of four pairs (*b*), laterally situated, with finely granular, greyish cytoplasm; basophilic with Ehrlich's acid hematoxylin; third group of three pairs (*c*), not observed in living cercaria; slightly basophilic in sections; no ducts found. Large thick walled excretory vesicle, varying from elongate oval to triangular in outline; main lateral collecting tubes and positions of some flame cells shown. Reproductive system represented by conspicuous mass of cells dorsal to ventral sucker. Tail with a pair of lateral convoluted cuticular fins extending the entire length; smaller median fin on ventral side of distal fourth of tail, extending around end up on to dorsal side; maximum tail length 320 μ, average 250 μ. Development of cercaria within elongate rediæ, averaging 795 μ in length and 92 μ in diameter; pharynx large; more or less cylindrical redia constricted at irregular intervals.

Cercaria dipterocerca differs from *C. hymenocerca* Villot 1875, *C. quadripterygia* Sinitsin 1911, and *C. lophocerca* (in Lebour, 1912) in significant details of structure.

Cercaria variglandis spec. nov.

(Figs. 15–17.)

Binoculate furcocercous distome cercaria most closely resembling members of the Elvæ group (Miller, 1923:44). Average

body length 262 μ and width 77 μ; tail approximately same length as body; ratio of tail stem to furcæ about 3 : 2. Anterior penetrating organ, a highly modified oral sucker, 51 μ in greatest diameter; ventral sucker much smaller. Eosinophilic head gland observed in sections of anterior organ; ventral capillary mouth and esophagus as in other members of group. Body covered with short spines uniformly distributed; absent from tail and furcæ. Two eye spots composed of large pigment granules, in posterior connection with nervous system. Most of body filled with three differentiated sets of larval penetration glands: one pair of cells, posterior to eye spots, with finely granular cytoplasm, chromophobic in sections; two pairs of glands, dorsal to ventral sucker, with coarsely granular eosinophilic cytoplasm; three pairs of glands in posterior part of body, with rod-filled cytoplasm, homogeneous in sections, staining deeply with iron hematoxylin. No rapid selection of intravitam neutral red or toluidine blue shown by any set; anterior pair deeply stained, the two middle pairs chromophobic, and the three posterior pairs lightly stained in very strong solution of either dye. Five pairs of flame cells in body and one pair in proximal tail stem; exact connections of two posterior body flame cells not determined, but judging from fresh water larvæ for which excretory system is known pattern is probably like that of *C. wardi* (Miller, 1923: Text-fig. 4); excretory system in tail opening at tips of furcæ. Cell mass representing future reproductive system ventral and posterior to ventral sucker. Development of cercaria within long sporocysts of uniform diameter throughout, measuring on average 1.3 mm. in length and 0.37 mm. in width and containing between twenty and thirty cercariæ.

Cercaria variglandis is different in a number of respects from the few described marine furcocercous larvæ: *C. dichotoma* Müller 1850, *C. discursata* Sinitsin 1911, and *C. syndosmyæ* Pelseneer 1906.

Cercaria quissetensis sp. nov.

(Figs. 9, 11–14, 18, 19.)

Echinostome cercaria with twenty-seven spines on collar. Body averaging 290 μ in length and 130 μ in width (extreme

extension of living cercaria 630 μ); tail about 330 μ in length. Oral sucker 42 μ, ventral sucker 64 μ in diameter. Mouth subterminal, short prepharynx, pharynx, wide esophagus bifurcating into ceca extending to extreme posterior part of body. Granular cytoplasm of small glands around esophagus and portions of contents of esophagus and ceca stained deeply with neutral red; dorsal gland cells eosinophilic and ventral ones chromophobic in sections; fine bundles of minute ducts passing through oral sucker and opening on anterior end. Many rod-filled cystogenous glands [1] in dorsal part of body; stained deeply with iron hematoxylin in sections. Excretory vesicle averaging 36 μ in diameter; arms filled with refractile concretions, of double coffee bean shape. Excretory system pattern not completely worked out; of type of *C. complexa* Faust (1919); twelve to fourteen flame cells observed on one side. Irregular masses of reproductive system cells posteriorly located, with a row of nuclei extending to a small mass anterior to ventral sucker. Average length of sausage-shaped redia 1.1 mm., width 0.2 mm.; orange-yellow pigment in wall; birth pore and two posterior locomotor appendages observed only in immature rediæ. Cercariæ encyst readily on glass slide, cysts averaging 142 μ in diameter, with two diametrically opposite projections.

Cercaria quissetensis differs in a number of respects from the six species reviewed by Lebour (1912), and from *C. proxima* and *C. sagitata* Lespès.

Cercariæum lintoni sp. nov.

(Figs. 20, 21.)

Tailless larva, properly designated Cercariæum; original description by Linton (1915*b*), supplemented and emended in a few details by this study. Average length of a number of emerged larvæ, killed without pressure, 230 μ, width 84 μ. Very

[1] There is a possibility that the October and November infestations represent a second species of echinostome. The second infestation consisted of immature rediæ only, but in October the rediæ were filled with apparently fully formed cercariæ (Fig. 12). These differ from the mature cercariæ of the succeeding infestations chiefly in that they are of somewhat smaller size and different shape (Fig. 13), and lack cystogenous material. This is not considered as sufficient to differentiate them into two species.

narrow intestinal ceca, traceable only in serial sections, reaching almost to excretory vesicle. One pair of prominent larval gland ducts, with granular contents, on either side of body, mistaken by Linton for excretory vesicles; four large eosinophilic larval glands, staining also with intra-vitam neutral red, but clearly observable only in sections. Large excretory vesicle, much constricted posteriorly, and surrounded by a sphincter muscle; opening in center of adhesive disc. A few flame cells observed, but exact pattern of excretory system obscured by numerous refractile globules distributed throughout body. Future reproductive system represented by a number of cell groups; two spherical masses, just posterior to ventral sucker, probably testes, with female complex anterior to excretory vesicle. Inchworm locomotion of this species effected by successive attachment of posterior end of body, modified by invagination into an adhesive disc, then extension of body and attachment by ventral sucker.

Cercariæum lintoni is obviously different from *C. dentali*, *C. giardi*, and *C. crispata* of Pelseneer (1906), and from the five species described by Lebour (1912) in character of the digestive and excretory systems, or in the parthenita.

REFERENCES CITED.

Allee, W. C.

'23*a* Studies in Marine Ecology. I. The Distribution of Common Littoral Invertebrates of the Woods Hole Region. BIOL. BULL., 44: 167–191.

'23*b* Some Physical Factors Related to the Distribution of Littoral Invertebrates. BIOL. BULL., 44: 205–253.

Dimon, A. C.

'05 The Mud Snail: *Nassa obsoleta*. Cold Spring Harbor Monogr., V., 48 pp., 2 pl.

Bigelow, H. B., and Welsh, W. W.

'25 Fishes of the Gulf of Maine. U. S. Bur. Fish., 40 (Part I.): 1–567.

Faust, E. C.

'19 The Excretory System in *Digenea*. II. BIOL. BULL., 36: 322–339; 10 text-figs.

Fewkes, J. W.

'82 A Cercaria with Caudal Setæ. Amer. Jour. Sci., 23: 134–135; 1 text-fig.

Lebour, M. V.

'12 A Review of the British Marine Cercariæ. Parasitol., 4: 416–455; 4 pl.

Lebour, M. V.

'16 Medusæ as hosts for larval trematodes. Jour. Marine Biol. Assn., 11: 57–59; 1 text-fig.

Lebour, M. V.

'17 Some parasites of *Sagitta bipunctata*. Jour. Marine Biol. Assn., 11: 201–206; 6 text-figs.

Linton, E.

'15*a* Sporocysts in an Annelid. BIOL. BULL., 28: 115–118; 5 text-figs.

'15*b* Note on Trematode Sporocysts and Cercariæ in Marine Mollusks of the Woods Hole Region. BIOL. BULL., 28: 198–209; 8 text-figs.

Meek, A.

'16 The Migrations of Fish. London. 427 pp.

Miller, H. M., Jr.

'23 Notes on Some Furcocercous Larval Trematodes. Jour. Paras., 10: 35–46; 1 pl.; 5 text-figs.

'25*a* Larval Trematodes of Certain Marine Gastropods from Puget Sound. Publ. Puget Sound Biol. Sta., 5: 75–89; 2 pl.

'25*b* Preliminary Report of the Larval Trematodes Infesting Certain Mollusks from Dry Tortugas. Carnegie Inst. Wash., Year Book, No. 24: 232–238.

Monticelli, Fr. S.

Recerche sulla *Cercaria setifera* di Joh. Müller. Atti. Accad. Sc. fis. e. mat. Napoli, 15: 48 pp., 5 pl.

Nicoll, W.

'10 On the entozoa of fishes from the Firth of Clyde. Parasitol., 3: 322–359; pl. 29.

Odhner, T.

'11 *Cercaria setifera* von Monticelli. Zool. Beit. Uppsala, 3: 247–255; 1 pl.

Sewell, R. B. S.

'22 Cercariæ Indicæ. Indian Jour. Med. Res., 10: 1–372; 32 pl.; 7 text-figs.

Sinitsin, D. Th.

'11 La génération parthénogénétique des Trématodes et sa descendance dans les mollusques de la Mer Noire. Mem. Acad. Sci. St. Petersbourg, 30: 1–127; 6 pl. Russian text.

Sumner, F. B., Osburn, R. C., and Cole, L. J.

'11 A Biological Survey of the Waters of Woods Hole and Vicinity. Bull. Bur. Fish., 31: 1–860.

Tennent, D. H.

'06 A Study of the Life History of *Bucephalus haimeanus;* a Parasite of the Oyster. Quar. Jour. Micr. Sci., 49: 635–690.

'09 An Account of Experiments for Determining the Complete Life History of *Gasterostomum gracilescens*. Science, N.S., 29: 432–433.

Plate I.

All drawings were made with the aid of a camera lucida, except where otherwise stated.

Fig. 1. *Cercaria setiferoides;* general view. × 150.

Fig. 2. Free-hand diagram of excretory system pattern in redia of *C. setiferoides.* × 40.

Fig. 3. Immature redia of *C. setiferoides,* showing pharynx and gut. × 92.

Fig. 4. *C. dipterocerca;* immature redia. × 88.

Fig. 5. General view of *C. dipterocerca.* × 56.

Fig. 6. Nearly mature redia of *C. setiferoides,* containing germ balls and almost fully formed cercariæ. × 72.

Fig. 7. *C. dipterocerca;* dorsal view of body showing eye spots, digestive and gland systems, and locations of most easily observable flame cells. Reproductive system cell mass between ventral sucker and excretory vesicle. × 415.

Fig. 8. Nearly mature redia of *C. dipterocerca.* × 138.

Fig. 9. Outline of body of *C. quissetensis,* when in extreme extension. × 42.

Fig. 10. *C. setiferoides;* dorsal view of body showing eye spots, digestive and gland systems, and cell masses representing future reproductive system. Only excretory vesicle and entrance of main lateral collecting vessels are shown. × 342.

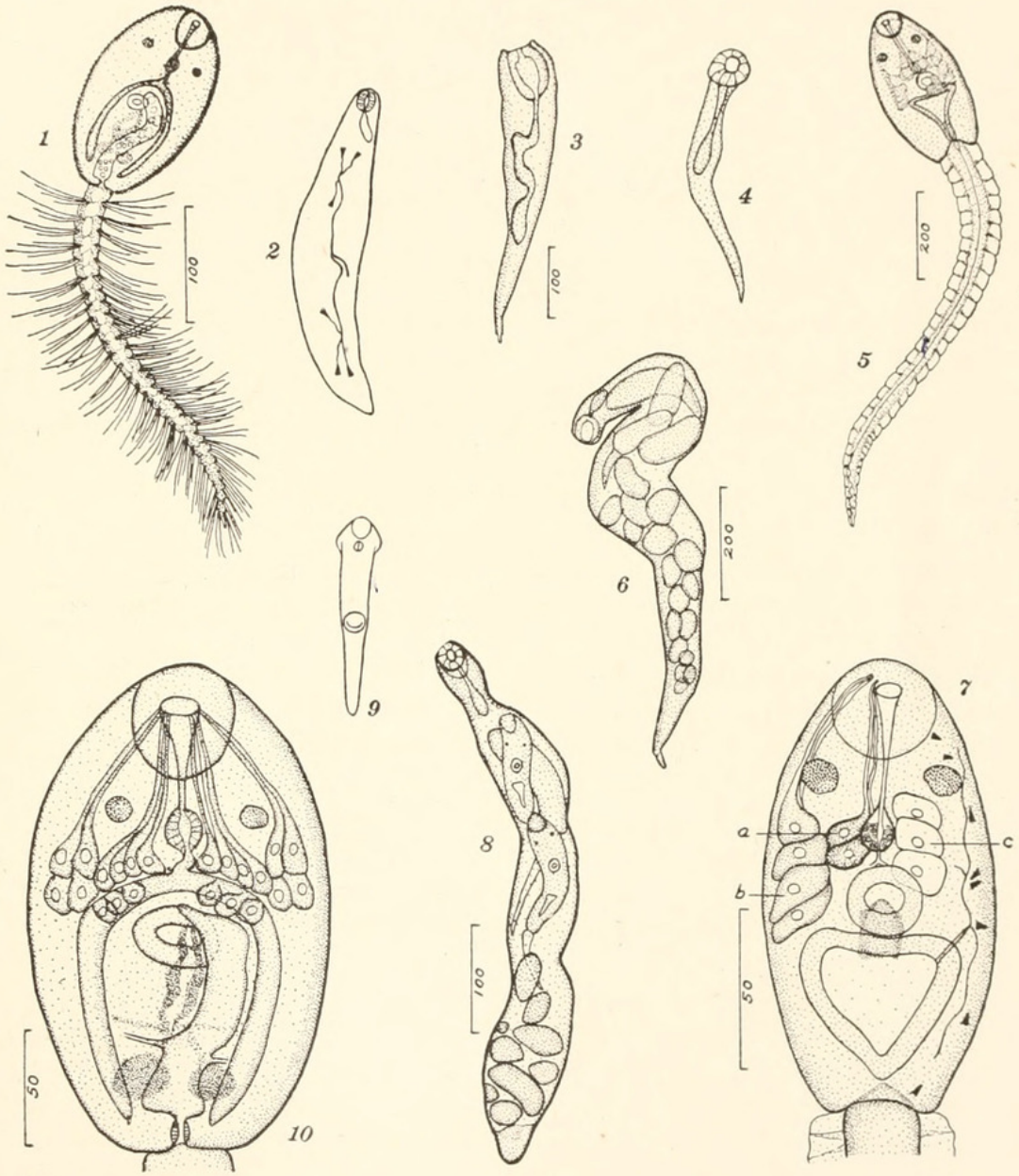


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Plate II.

Fig. 11. *C. quissetensis;* redia with gut and birthpore, containing only one fully formed cercaria. × 53.

Fig. 12. Redia of *C. quissetensis*, packed with mature cercariæ. × 47.

Fig. 13. Ventral view of body of *C. quissetensis;* digestive and gland systems; main trunks of excretory system; cell masses of future reproductive system. × 162.

Fig. 14. General view of *C. quissetensis.* × 93.

Fig. 15. *C. variglandis;* dorsal view showing anterior organ (highly modified oral sucker), eye spots, and three differentiated sets of gland cells. × 120.

Fig. 16. Sagittal section of body of *C. variglandis*, showing especially the different gland cells. × 385.

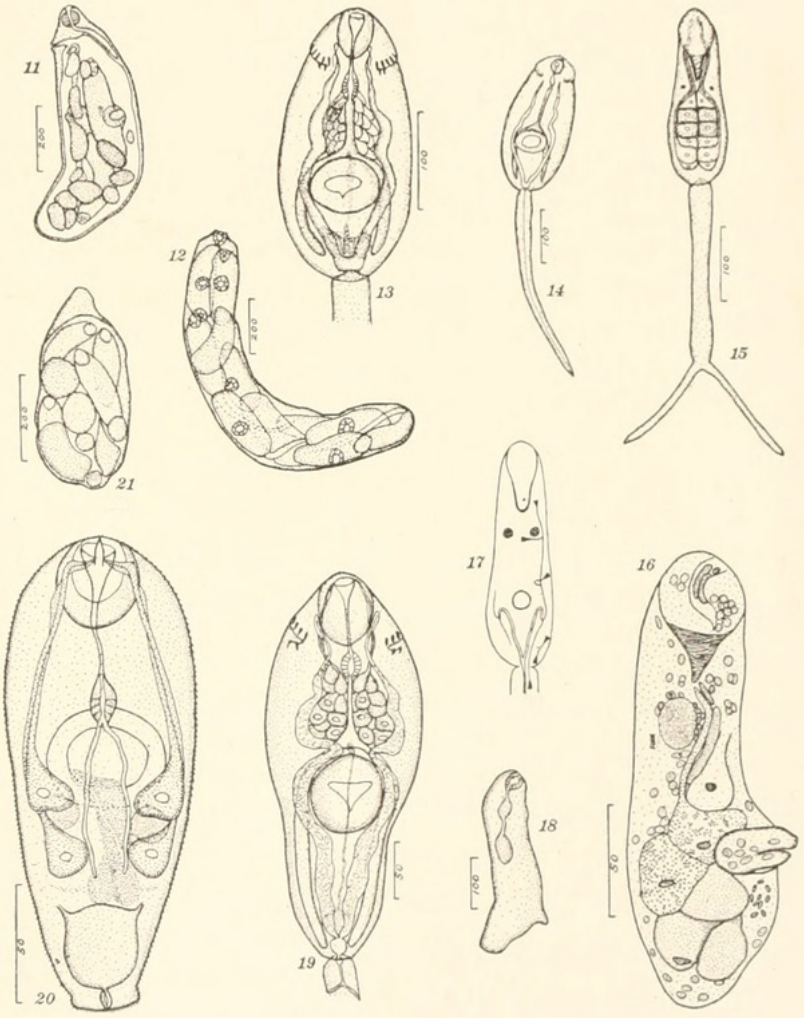
Fig. 17. *C. variglandis;* free-hand diagram of flame cell pattern in body. × 190.

Fig. 18. *C. quissetensis;* immature redia, showing pharynx and gut, and posterior locomotor appendages (on one side only). × 83.

Fig. 19. Extended body of *C. quissetensis*, showing digestive, gland and excretory systems, and reproductive system cell masses. × 200.

Fig. 20. *Cercariæum lintoni;* dorsal view showing digestive and gland systems, cell masses of reproductive system, and excretory vesicle. × 398.

Fig. 21. Sporocyst of *C. lintoni*, containing germ balls and mature cercariæ. × 70.



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CORRELATIONS AND VARIABILITY OF THE CENTRAL NERVOUS SYSTEM AND BODY SIZE OF THE ALBINO RAT.

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A knowledge of inter-organ weight relationships gives data which assist in the making possible a more exact conception of differential development. The presence or absence of a statistical association may indicate the presence or absence of a community of growth response to general developmental factors; it may indicate the presence or absence of a specific conditioning of the one organ by the other; or it may indicate the play of reciprocal influence.

The use of the coefficient of correlation in an investigation of this problem is marked by its neglect. What little the literature does contain, is, with one or two exceptions, sketchy and inadequate. It is usually a mere record of figures which in many cases are of meagre value because of the paucity of raw data.

Realizing the need for a more systematic survey of inter-organ weight relations a beginning was made somewhat over a year ago with a biometrical analysis of the weight interrelations of the glands of internal secretion. A preliminary note was published in Endocrinology (1) in anticipation of full presentation in the *Journal of Metabolic Research* at some future date. In the latter there will be found the conditions which should govern a study of this nature. The present paper is an extension of the analysis to the brain and spinal cord, using as raw data the body weights, body lengths, brain weights and spinal cord weights of the same rats the organ weights of which served for the earlier study. These animals were the controls of the studies of the thyroid apparatus (2). They were 150 days of age at the time the organs were removed for weighing. Both sexes were used, 121 rats of each. They all came from the Experimental Colony Stock of The Wistar Institute. The values thus represent the associations found in the mature male and female albino rat of this stock.

Turning now to the method of analysis I can do no better than to quote directly from Miner (3): "A coefficient of correlation measures the degree to which two variables are associated, taking the value + 1 when a deviation of one variable from its mean is always associated with a proportional deviation of the other in the same direction, decreasing as the intensity of the association decreases until for complete independence of the two variables it takes the value 0, and again increasing in numerical value, but with a negative sign for increasing intensity of association where deviations of one variable in one direction are coupled with deviations of the other variable in the opposite direction. Thus the absolute magnitude of a coefficient of correlation measures the intensity of the association of two variables, while its sign indicates whether as one variable changes the average values of the other variable change in the same or opposite directions. The possible range of values is from + 1 to − 1."

The coefficient of correlation does not of itself indicate which is the dependent and which the independent variable. This must be determined by other means. The simple or zero order correlation between any pair of variables is not representative of their association uncomplicated by assumed interferences derived from the other variables. In order to obtain such an uncomplicated index it is necessary to determine the correlation between any given pair of variables when the others are held constant. This is done by the method of partial correlation.

Now it is conceivable that the weight association between brain and spinal cord is factored by the general size factors carried by the body weight and possibly by the body length, particularly in the case of the spinal cord. Hence the elimination of these general factors for size by stabilization for body weight and length, is essential if we are to obtain the uncomplicated association between the brain and the spinal cord. That is to say the association free from the general body size factors. This has been done as succeeding paragraphs will show.

In order that the values obtained by the method of partial correlation be valid it is necessary that all the zero order regressions be linear, and that the number of observations in each of the zero order tables be fairly large as compared with the

number of variables dealt with. The test for linearity is made through a determination of the correlation ratio and a comparison of this with the coefficient of correlation r. The methods used for the determination of the coefficient of correlation, the correlation ratio and the partial correlation coefficient are those described by Pearl (4).

The raw data and the correlation tables which serve as the basis of this study are not given because of space limitations. They are on file at The Wistar Institute.

In Table I. are given the zero order coefficients of correlation (r_0), the correlation ratios (η), the values for ($\eta^2 - r^2$), the probable errors for r_0 and ζ, and the quotients r/E_r and ζ/E_ζ of the several comparisons made in this study. In addition to these values there is to be found in the literature the following figures: Donaldson (5), (6) in a series of rats of scattered ages and with the sexes combined found the correlation between body weight and brain weight to be 0.764: the correlation between body weight and cord weight to be 0.856: between body length and brain weight to be 0.86: between body length and cord weight to be 0.99. Hatai (7) records a value of 0.516 in the male and of 0.692 in the female for the coefficient of correlation between body weight and cranial capacity in the mature albino rat. Jackson's (8) values are based on too few observations to be any more than suggestive. For adult man, Boas (9) records a correlation between body weight and head length of 0.43 in the male and 0.41 in the female; and between body weight and head width of 0.32 in the male and 0.33 in the female. Blakeman (10) found the coefficient of correlation between body length and brain weight to be 0.289 in the male and 0.367 in the female; while Pearl (11) got values ranging from 0.166 to 0.183 in the male and from 0.183 to 0.349 in the female for this association, and 0.167 in the male and 0.226 in the female for the degree of association between body weight and brain weight.

The values for man are of little help in the problem because the raw data from which they were derived are complicated by a multiplicity of interfering variables which vitiate any comparison with the figures obtained from the more uniform population of albino rats.

TABLE I.

THE COEFFICIENTS OF CORRELATION, THE CORRELATION RATIOS AND THE QUOTIENTS OF THE ERRORS OF THE SEVERAL COMPARISONS.

Sub-script.	Male.					Female.				
	r_0.	r/E_r.	η.	ζ.	$\zeta/E\zeta$.	r_0.	r/E_r.	η.	ζ.	$\zeta/E\zeta$.
12	0.872 ± 0.015	58.1	0.882	0.0175 ± 0.0161	1.1	0.783 ± 0.024	32.6	0.811	0.0446 ± 0.0255	1.8
13	0.676 ± 0.033	20.5	0.725	0.0686 ± 0.0310	2.2	0.710 ± 0.030	23.7	0.755	0.0659 ± 0.0305	2.2
14	0.809 ± 0.021	38.5	0.821	0.0195 ± 0.0170	1.2	0.726 ± 0.029	25.0	0.770	0.0658 ± 0.0305	2.2
23	0.650 ± 0.035	18.6	0.701	0.0689 ± 0.0310	2.2	0.571 ± 0.041	13.9	0.632	0.0734 ± 0.0316	2.3
24	0.778 ± 0.024	32.4	0.798	0.0315 ± 0.0215	1.5	0.719 ± 0.030	24.0	0.751	0.0470 ± 0.0260	1.8
34	0.800 ± 0.022	36.4	0.813	0.0210 ± 0.0176	1.2	0.779 ± 0.024	32.5	0.799	0.0316 ± 0.0215	1.5

(1) Body Weight; (2) Body Length; (3) Brain Weight; (4) Spinal Cord Weight.

Notwithstanding the non-elimination of the age factor, the associations reported by Donaldson (6) between body weight and brain weight, body weight and spinal cord weight, and the brain and spinal cord in his series of rats are of the same order of magnitude as those found in this study. The association between cranial capacity and body weight in the female as recorded by Hatai (7) is also of the same order of magnitude as my value for body weight and brain weight correlation. Aside from these similarities the data are rather widely divergent.

Turning now to the analysis of my data it is evident that there is a high degree of positive correlation between the several pairs of variables under investigation. Neglecting the brain-spinal cord weight correlation ($r_{3\ 4}$) it is seen that the order of increasing degree of association is the same in both sexes. This indicates that the association of the brain and spinal cord with the body as a whole is governed by factors which are largely independent of sex determinants of association. These independent factors are probably specific in origin.

The degree of association between brain weight and spinal cord weight ($r_{3\ 4}$) is practically the same in the female as in the male. This indicates that the correlation is independent of the sex differences in body size which exist in animals of the same age. That is to say the association between these two parts of the central nervous system is independent of sex factors contributive to differences in differential development. It is, however, dependent on other factors. While it is possible that specificity plays an important part in the determination of the association, I am inclined to believe that the basis of the reaction lies rather in the community of characteristic chemical make-up of the two organs, with the consequent similarity in response or resistance to extraneous influences. Not to be neglected is the idea that the chemical similarity conditions a similarity in the processes of growth and hence association in weight.

The association between body weight and body length ($r_{1\ 2}$) is consistently greater than that between the other pairs of variables. Nevertheless, the superiority is statistically valid in but 50 per cent. of the comparisons. The general trend of difference is uniform, however, and if accepted as significant is

suggestive of a greater interdependence or dependence on a common factor. Body weight and body length are thus more closely related in differential development, than is the brain or spinal cord related to either of them individually. That is to say, body weight and body length follow more nearly parallel courses during differential development than do brain and body weight or body length, and spinal cord and body weight or body length. The difference can be attributed to the probability that the type metabolism productive of body weight is more nearly like that productive of body length, than is the type metabolism of body weight and body length like that which characterizes brain and spinal cord. In the case of body weight and body length the reactions are essentially increments in protoplasm, in the case of the brain and spinal cord the differential development is characterized by lipoid accretions.

The correlation between brain weight and body weight ($r_{1\ 3}$) is less than that between the other pairs of variables with the exception of brain weight and body length ($r_{2\ 3}$). The degree of difference is valid in 50 per cent. of the cases. The difference if accepted as significant is suggestive of a lesser dependence of brain weight and body weight on a common factor. That is to say brain weight and body weight are less related in differential development than are spinal cord weight and body weight and length, and than brain weight and spinal cord weight with each other. From which it can be inferred that during differential development brain weight deviates more from the course followed by body weight, than it does from that followed by the spinal cord, or than does the spinal cord deviate from the course followed by body weight and body length.

The greater association between brain and spinal cord ($r_{3\ 4}$) is undoubtedly due to the community of type metabolism as compared with the disparity between brain and body weight. The higher degree of association between spinal cord and body length ($r_{2\ 4}$) is justly attributed to the structural relations existing in this case. Why cord weight should be more closely associated with body weight ($r_{2\ 4}$) than is brain weight is a question. It may be that the preference is a consequence of the structural relationship of the cord with the body length of high degree of correlation with body weight.

Both brain and spinal cord are more closely associated with body weight than with body length. Although the differences are slight and not statistically valid, the consistency of their direction and their presence in both sexes puts into the relation a significance that cannot be denied. It is a relation that might be expected by virtue of the fact that the developmental processes productive of weight in the parts of the organism are more closely allied than the developmental processes productive of weight are allied to those of length. This result would seem on the face of it to detract somewhat from Donaldson's (5) dictum that "body length is a better datum than body weight from which to infer the weight of the brain or spinal cord." The objection is negatived, however, by the fact that in a normal population the variability in body weight is greater than that in body length. This will be discussed presently.

The high degree of positive correlation of brain weight and spinal cord weight with body weight and body length allows the extension of Donaldson's (6) conclusion that the weight of the spinal cord can be inferred from body length or body weight with a high degree of accuracy, to include the brain.

With one exception ($r_{1\,3}$) the degree of association between the several pairs of variables is greater in the male than in the female rat. The degree of difference is, however, statistically valid only in the case of the body weight-body length correlation ($r_{1\,2}$). These figures in Table I., it must be remembered, are indices of the degree of association between pairs of variables, when interfering influences assumed to be exerted by the other variables are still present. Such being the case, and if the general trend of sex difference is accepted as significant because of its consistency, it is suggestive of a greater independence of the central nervous system of the female from the general factors contributive to interstructural and inter-organ association as carried by the body as a whole. This inference is supported by the results of the analysis by the method of partial correlations. The use of this method is allowable here because the regressions are in all cases essentially linear.

In Table II. are given the correlation coefficients of the several pairs of variables after the removal of the assumed influences

exerted by the others by statistical treatment. Continuing the phase of comparison dealing with the sex differences. If we take as a measure of this relation the sex difference in degree of change in association of the first and second order coefficients from the zero order correlation between brain weight and spinal cord weight ($r_{3\,4}$), *i.e.*, when first body weight ($r_{34\,1}$) or body length ($r_{34\,2}$) are held constant, and then when the body weight and body length are both held constant ($r_{34\,12}$), it is seen that in general the reduction in value is percentagely greater in the male than in the female. This indicates that the conclusion drawn in the preceding paragraph is justified.

Table II.

The (Partial) Correlation Coefficients.

Subscript.	Male.	Female.
r_{12}	0.872	0.783
r_{13}	0.676	0.710
r_{14}	0.809	0.726
r_{23}	0.650	0.571
r_{24}	0.778	0.719
r_{34}	0.800	0.779
$r_{12\,3}$	0.773	0.654
$r_{12\,4}$	0.659	0.546
$r_{13\,2}$	0.293	0.515
$r_{13\,4}$	0.082	0.334
$r_{14\,2}$	0.425	0.377
$r_{14\,3}$	0.606	0.391
$r_{23\,1}$	0.169	0.034
$r_{23\,4}$	0.074	0.025
$r_{24\,1}$	0.253	0.353
$r_{24\,3}$	0.566	0.532
$r_{34\,1}$	0.584	0.545
$r_{34\,2}$	0.616	0.644
$r_{12\,34}$	0.655	0.573
$r_{13\,24}$	0.043	0.384
$r_{14\,23}$	0.325	0.069
$r_{23\,14}$	0.027	0.201
$r_{24\,13}$	0.193	0.399
$r_{34\,12}$	0.567	0.570

(1) Body Weight; (2) Body Length; (3) Brain Weight; (4) Spinal Cord Weight.

Further confirmation of the conception is had from the fact that the growth of the brain and spinal cord of the female albino rat follows the changes in growth retardation in body weight and

body length which are caused by thyroid and parathyroid removal at different ages, to a lesser degree than does that of the male.

A general analysis of the progress of partial correlation would naturally only be an extension and confirmation of the comparisons made from the zero order values. As an example: the association between brain weight and spinal cord weight is conditioned to a lesser degree by body length than by body weight. This is shown by the fact that the percentage reduction in degree of association between brain and spinal cord weight from the zero order value ($r_{3\ 4}$), is greater when body weight is held constant ($r_{34\ 1}$) than when body length is held constant ($r_{34\ 2}$). The difference is what is to be expected from the fact noted earlier that brain weight and spinal cord weight are more closely associated with body weight than with body length.

It is important to note that there is a high degree of positive correlation between brain weight and spinal cord weight ($r_{34\ 12}$) which is independent of the general factors for size carried by the body as a whole. Indeed this value is much higher than that found for any of the pairs of organs so far studied (thyroid, adrenals, hypophysis, gonads, thymus and pancreas (1)). Between many of these no correlation was present at all after stabilization for body weight. Such being the case it is evident that the uncomplicated weight association between brain and spinal cord is peculiar. The phenomenon can be attributed to the community of characteristic chemical (lipoid) make-up of the two organs as already noted. As far as simple correlation with body weight is concerned, the brain and spinal cord have also a higher degree of association than any of the above, save the hypophysis and the pancreas in the male. The value of r_0 is 0.701 ± 0.031 for the hypophysis, and 0.600 ± 0.039 for the pancreas. It might be noted that since the same animals served as original sources of the material used in both studies, the value of the comparisons is enhanced.

There is no sex difference in the degree of association between brain weight and spinal cord weight freed from the general factors for size ($r_{34\ 12}$). The significance of this relation has been discussed in an earlier paragraph.

Another statistical value of assistance in an estimation of the

forces concerned in differential development is the coefficient of variability when used comparatively. This figure is the quotient times 100 of the mean of the variates into their standard deviation, or, C.V. $= \sigma/M \times 100$ per cent. It is an abstract value which makes possible inter-group, inter-sex, inter-structural and inter-organ comparisons of sensitivity to the totale of forces contributive to variation which play upon the organism. In a study such as this, where a comparison is being made of the organs as parts of a whole, the differences in the coefficient of variability exhibited are indices of differences allied to differential development. They are worthy of investigation because they represent deep-seated biological relationships.

In Table III. are given the coefficients of variability and their probable errors of the body weight, body length, brain weight and spinal cord weight of the male and female albino rats at 150 days of age.

TABLE III.

COEFFICIENTS OF VARIABILITY OF THE BODY WEIGHT, BODY LENGTH, BRAIN WEIGHT AND SPINAL CORD WEIGHT OF THE MALE AND FEMALE ALBINO RATS 150 DAYS OF AGE.

Structure.	Male.	Female.
Body Weight	14.19 ± 0.63	11.39 ± 0.49
Body Length	4.42 ± 0.19	3.40 ± 0.15
Brain Weight	5.19 ± 0.22	4.92 ± 0.21
Spinal Cord Weight	6.69 ± 0.29	6.25 ± 0.27

The values show definitely that body weight in the female is less variable than in the male. This difference has already been noted by King for albino stock (12), for inbred albino stock (13) and for Norway rats (14). Jackson's (8) values show a like direction of difference in the albino rat.

This sex difference is not exhibited in adult man (11). Nevertheless it must be remembered that our data are derived from a racially homogeneous stock, while that of Pearl were not so constituted. It would be rash to state that the results of biometrical analysis of groups of humans is inadequate to divulge such relations. It is better to say that the lack of sex difference in value in a heterogeneous stock is no indication of its non-existence in an homogeneous population. While it would also be rash to generalize from rat to man, the fact that three observers

of five different rat populations have obtained sex-differences in body weight variability in the same direction, is indication that we are dealing with a biological sex-difference of response to factors contributive to variability, and that studies on man should be so planned as to eliminate the possible interfering factor of racial heterogeneity which might well mask a basic sex difference. Further evidence supporting this view is the fact that the body weight variability of the rat of homogeneous stock is generally considerably less than that of man of heterogeneous stock. The values recorded by Pearl (11) for man are 21.3 for the male and 24.7 for the female.

The female albino rat is also less variable than the male in body length to a statistically valid degree, and shows a like tendency in brain and spinal cord weight, though the degree of difference here is too small to be valid. At this time I do not want to go into a comparison of the entire array of organs in the rat, but might point out that the sex difference is not uniform in direction for all the organs, which fact has interesting implications as a later study will show.

However, the fact that in body weight and in body length the female is less variable than the male albino rat indicates in this species, at least, a greater stability of the female organism as a whole to outside forces tending to disturb body size equilibrium. Teleologically this might be considered an expression of a protective mechanism, tending to enhance resistance and thus favor the essential purpose of the female, namely reproduction.

From Table III. it is seen that body length is much more stable than body weight. The same holds true for man. The C.V. for stature in the male was found to be from 3.8 to 4.3 (Pearl) and 3.6 to 4.5 (Blakeman): and in the female from 4.0 to 4.7 (Pearl) and 3.8 to 4.2 (Blakeman). Attention is directed to the fact that the values for man are of the same order of magnitude as those for the rat.

It is hardly necessary to point out that the lesser variability of body length or stature is a consequence of the greater inherent metabolic stability of the skeleton as the chief component determinative of this measurement, as compared with that of the body weight with its predominant element of metabolically

variable tissue. The one is, by virtue of its chemical make-up, a relatively fixed structure, the other a fluctuating mass freely subject to many influences.

The fact that the body length variability of the rat is of the same order of magnitude as that of man is indicative of a biological similarity in inherent structural response to factors contributive to variability, which might well have been predicted from the very nature of the structures involved, and which is support for the idea expressed in a preceding paragraph that the species difference in body weight variability exhibited here is factored in part by difference in racial uniformity of population from which the data were derived.

The lesser variability of the body length combined with the fact that the variability in brain weight and spinal cord weight is closer in degree to that of body length than to that of body weight establishes the conclusion postulated by Donaldson (5) that body length is a better datum from which to infer brain and spinal cord weight, than is body weight.

Both brain and spinal cord are less variable than the body in weight. This also holds for man in the case of the brain. Pearl (11) records a C.V. value of 7.5 to 8.8 for the male brain, and 7.1 to 8.7 for the female, while Blakeman (10) found 7.8 for the male and 8.2 for the female.

The lower variability of the central nervous system is obviously again an expression of an inherently more stable chemical make-up. Evidence for this is had both in the fact that the brain and spinal cord are more resistant to conditions of malnutrition and inanition than is the body as a whole (15), and in the fact that the growth of these organs is more resistant to the metabolic upsets incident to thyroid and parathyroid deficiencies than is that of the body in weight (16). This has been discussed in another paper. All that need be pointed out here is that the high content of the central nervous system in characteristically stable lipoids determines in it a resistance to metabolic disturbances which primarily affect the more readily utilizable tissue components, such as occurs in inanition, thyroid deficiency and individual dietary idiosyncrasies affecting body weight.

As one after another point of view is used in the attack on the

problem of differential development, there emerges a unanimity of inter-relationship which substantiates the premises on which the interpretations are based.

The variability in brain weight is greater in man than in the rat. This is probably another expression of the difference between heterogeneous and homogeneous material. It is, therefore, not essentially a real difference. Contributive to, it may be the greater variability in human body weight as compared with rat body weight, and the high correlation between brain weight and body weight in the rat. The low association between this pair of variables reported for man rather negatives this idea, however.

Both brain and spinal cord are more variable than body length. In man also brain weight is more variable than stature. This may be taken as an indication that skeletal composition is more fixed and metabolically stable than is that of the central nervous system. Such a conclusion is in accord with the chemical and physiological data so far available.

The brain appears to be less variable than the spinal cord. This is consistent with the fact that brain weight is less highly correlated with body weight of high variability than is the spinal cord. On the other hand when body length variability is eliminated from brain and spinal cord variability free from the assumed influence of body weight variability, during the computation of the actual or reduced variability of these organs according to the usual statistical procedure, there is no reduction in value (of brain and spinal cord variability) below that which obtains when the variability has been stabilized for body weight. A different result would be expected if the respective brain and spinal cord associations with body weight and body length were dominant factors in the variability coefficients. Moreover, since the spinal cord has a relatively higher percentage of the stable (non-readily utilizable) lipoids characteristic of the central nervous system, and a relatively lower percentage of the labile readily utilizable elements than the brain, it would be expected that its (the spinal cord) variability would be less than that of the brain, if this compositional difference is the factor of importance. All the evidence so far accumulated gives an affirma-

tive answer to this last point (17), and hence the reversal of expected relationship is either a real objection to the theory or else some other factor has intruded to mask the normal reaction. Such a factor is present in the technic of removal of the spinal cord for weighing. This is no place to describe the matter, nor is it necessary, for a little consideration will show that when the removal of an organ is accompanied by the cutting of many connections, the technic (no matter how much care is exercised) is bound to give a higher weight variability, than when the removal is merely a matter of shelling an organ out of its envelope. In view of this fact the brain-spinal cord difference in variability gives no basis for an inter-organ biological interpretation.

In a preceding paragraph mention was made of "reduced variability." This is a statistical value purporting to show the variability of a variable freed from influences assumed to be exerted by the other variables being studied. I have calculated these values for the body weight, body length, brain weight and spinal cord weight, and have arranged them in the order of decreasing variability in Table IV. as a matter of record. While in every case the variability is less than that which obtains when all factors of influence are in play, the relative position of any variable in the general scheme is unchanged.

TABLE IV.

THE REDUCED VARIABILITY OF THE SEVERAL VARIABLES DISCUSSED IN THIS STUDY.

Structure.	Male.	Female.
Body Weight	11.41	9.86
Spinal Cord	5.28	5.06
Brain	4.35	4.08
Body Length	3.65	2.93

In the earlier study (1) on the weight inter-relationships of the glands of internal secretion a similar computation was made of the reduced variability of that particular array. It was found that the thyroid has the highest variability of all the organs so far studied, and that the thymus comes next. Both of these organs are much more variable than the body as a whole. The adrenals, gonads, hypophysis, and pancreas give lower figures, but in all cases values considerably higher than those for brain or spinal cord.

This generally lesser variability of the central nervous system is a demonstration that it is much more metabolically stable than are the other organs of the body so far studied. The interpretation is based on the difference in chemical make-up already discussed, in which differences in type metabolism participate. A more extended discussion of the problem is reserved for a later paper.

Summary and Conclusions.

The brain and spinal cord of the albino rat show a high degree of positive weight correlation, free from the influences assumed to be exerted by the other variables studied (body weight, body length). The value for the male is 0.567, and for the female 0.570.

The weight variability of the brain and spinal cord is less than that of any of the other organs so far studied. It is greater than that of the body in length.

The analysis and interpretation of these and other relations are given in detail in the text.

The computations upon which this study is based were made by Miss Mildred Wilson.

References.

1. **Hammett, F. S.**
'25 Interglandular Associations (A Statistical Study). Preliminary report. Endocrinology, *9*, 297–300.

2. **Hammett, F. S.**
'23 Studies of the Thyroid Apparatus. IX. The Effects of the Loss of the Thyroid and Parathyroid Glands at 100 Days of Age on the Growth in Body Length, Body Weight and Tail Length of Male and Female Albino Rats. Am. J. Physiol., 63, 218–244.

3. **Miner, J. R.**
'22 Suicide and Its Relation to Climatic and Other Factors. Am. J. Hyg. Monograph, No. 2, 1–146.

4. **Pearl, R.**
'23 Medical Biometry and Statistics. W. B. Saunders Co. Philadelphia.

5. **Donaldson, H. H.**
'08 A Comparison of the Albino Rat with Man in Respect to the Growth of the Brain and Spinal Cord. J. Comp. Neurol. and Psychol., *18*, 344–392.

6. **Donaldson, H. H.**
'09 On the Relation of the Body Length to the Body Weight and to the Weight of the Brain and of the Spinal Cord in the Albino Rat. J. Comp. Neurol. and Psychol., *19*, 155–168.

7. **Hatai, S.**
'08 Studies on the Variation and Correlation of Skull Measurements in Both Sexes of Mature Albino Rats. Am. J. Anat., *7*, 423–441.

8. **Jackson, C. M.**
'13–'14 Postnatal Growth and Variability of the Body and of Various Organs in the Albino Rat. Am. J. Anat., *15*, 1–68.

9. **Boas, F.**
'05 Statistics of Growth. Rept. of the Commissioner of Education. U. S. A., 1905. Chap. 2, pp. 25–132.

10. **Blakeman, J.**
'05 A Study of Biometric Constants of English Brain Weights and their Relationships to External Physical Measurements. Biometrika, *4*, 124–160.

11. **Pearl, R.**
'05 Biometrical Studies on Man. Variation and Correlation in Brain Weight. Biometrika, *4*, 13–104.

12. **King, H. D.**
'15 The Growth and Variability in the Body Weight of the Albino Rat. Anat. Rec., *9*, 751–776.

13. **King, H. D.**
'18 Studies on Inbreeding. I. The Effects of Inbreeding on the Growth and Variability in Body Weight of the Albino Rat. J. Exp. Zoöl., *26*, 1–54.

14. **King, H. D.**
'23 The Growth and Variability in the Body Weight of the Norway Rat (*Mus norvegicus*). Anat. Rec., *25*, 79–94.

15. **Jackson, C. M.**
'25 The Effects of Inanition and Malnutrition upon Growth and Structure. P. Blakiston's Son & Co. Philadelphia.

16. **Hammett, F. S.**
'26 Studies of the Thyroid Apparatus. XXXI. The Rôle of the Thyroid and Parathyroid Glands in the Growth of the Central Nervous System. J. Com. Neurol., in press.

THE NUTRITION OF THE OVUM OF *HYDRA VIRIDIS*.

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The incipient ovary of *Hydra viridis* is represented by a mass of proliferated interstitial cells. At a very early stage in the development of the ovary some of its cells become much larger than the others. There seems to be a struggle on between these cells; for, as development of the ovary proceeds, but one of the larger ones remains whole, and the smaller ones perish. The cells, that are directly involved in this struggle, are enclosed in a thin wall of slightly chromatic, modified epithelio-muscular cells. Kleinenberg (72), Brauer (91), Downing (08) and Tannreuther (08) agree in the statement that one of the enlarged interstitial cells gets the ascendency over the others and grows at their expense. If there be in the incipient ovary more than one greatly enlarged interstitial cell, these may fuse to form the oögonium as over against the cells of what Tannreuther (08) designates "the cells of the peripheral region which contribute to the formation of the yolk," p. 274. These peripheral cells are not taken into the growing oögonium's cytoplasm bodily as Brauer (91) described. They disintegrate at the periphery of the oögonium and are then resorbed. The relation of these disintegrating cells to the cytoplasm of the final oögonium are shown in Fig. 1. Figs. 3 and 4 show phases of disintegration in these neighboring enlarged interstitial cells. As their cytoplasm breaks down, the nuclei display disintegration features. Eventually the entire cell disintegrates. The substance of these disintegrated cells is absorbed by the oögonium, as Kleinenberg (72) described. The material thus obtained results in the oögonium growing greatly to become a conspicuously large amœboid cell (Text-figure 2). This cell, by means of radiating stout pseudopods, spreads out over one third or more of the mesoglea's outer surface. This amœboid gamete was first figured by Fewkes and Mark in 1884. This pseudopodial cell has now attained its maximum size and is, therefore,

the primary oöcyte. The feeding of the oögonium, of the final oögonial generation, upon the neighboring, enlarged interstitial cells represents the first phase of the nutrition of the ovum of *Hydra viridis*. Nutrition, in this phase, is referred to the growth of the final oögonium into the primary oöcyte.

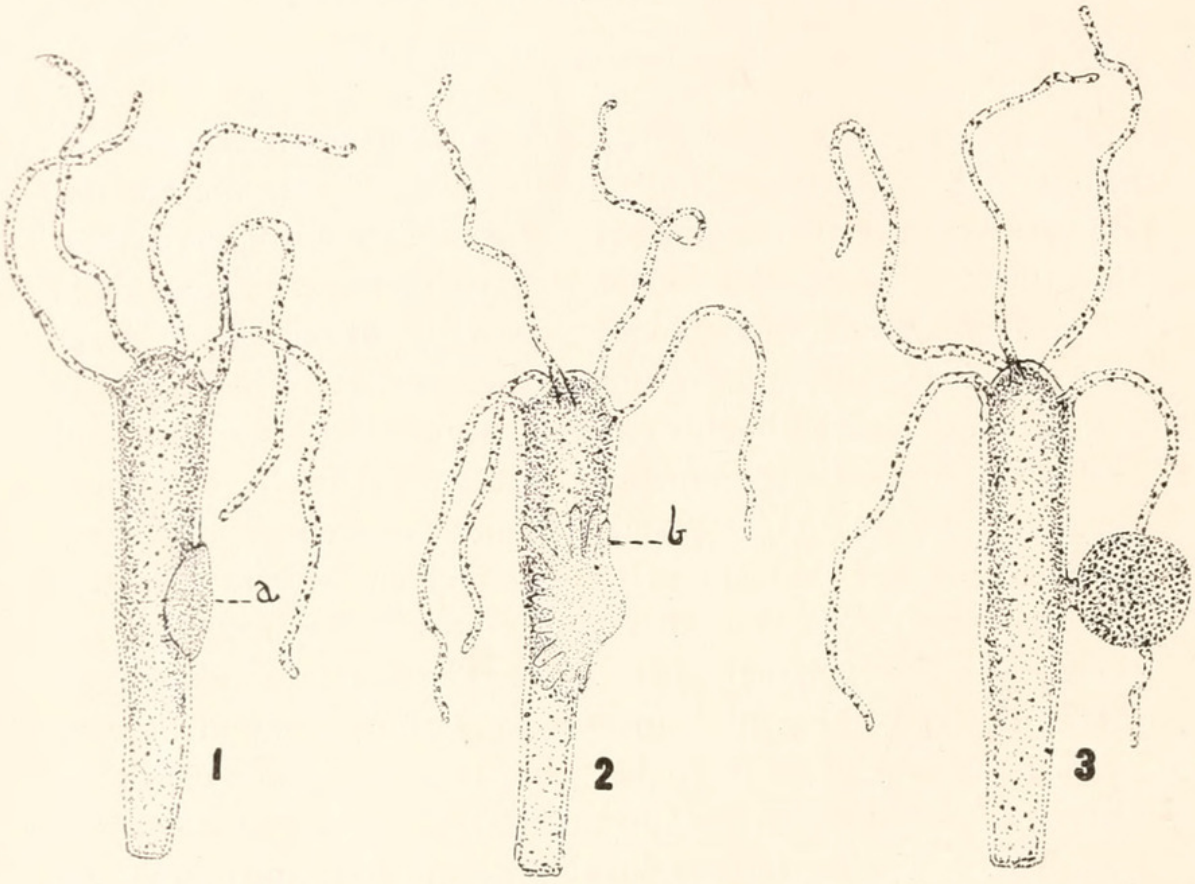


FIG. 1. Oögonium before pseudopodia are thrown off. *a* shows plane through which section, shown in figure 1 of plate, was taken.

FIG. 2. Oöcyte with maximum number of pseudopodia. *b* shows plane through which section, shown in figure 5 of plate, was taken.

FIG. 3. Advanced primary oöcyte with a full complement of deutoplasm.

Previous investigators have failed to recognize that there are two phases in the nutrition of *Hydra's* ovum. Perhaps failure, on their part, to recognize the dual nature of the nutrition of the ovum of *Hydra* arises out of the resemblance of the disintegrating interstitial nuclei to deutoplasmic granules (Fig. 3). Brauer (91) held that the nuclei of the cells, that were being ingested by the egg, became the yolk granules or his "Pseudozellen." Downing (08) says the "interstitial cells adjacent to the egg in the fairly mature ovary have their walls in contact with the egg resorbed and the content of the cell becomes part of the egg (Nusbaum).

The greatly enlarged nuclei, gorged with lecithin, also become yolk granules or "Pseudozellen," p. 66. Tannreuther (08) also looks upon the yolk granules as arising from the nuclei of the interstitial cells. He says: "When the pseudopodia are completely formed, the nuclei of the interstitial cells forming the ovary are taken up by the amœboid egg and become changed into the yolk or pseudo-cells of the egg. . . . After the yolk or pseudo-cells are formed they divide amitotically" (p. 264).

We have been unable to bring the details of *Hydra viridis* ovary in line with the above interpretations and facts.

To begin with, up until the time that the growing, amœboid oögonium has made maximum contact with the mesoglea—thus placing it in relation to the endoderm—there has been no yolk formation. In the meantime, however, many of the attending, peripherally disposed cells have disintegrated and have been resorbed. Sometimes this disintegration and resorption has gone so far that, before the oögonium has well advanced in growth, the attending cells lie only at its margin (Fig. 1). In a typical example, by the time the oögonium has reached its maximum growth, there is but a thin tissue of highly modified epidermis covering its general surface (Fig. 5). In such example, there may yet be disintegrating cells at the tips of the pseudopods or even beyond them. Thus it is to be emphasized that many of the enlarged interstitial cells have disintegrated (perhaps most of them) and have been resorbed during the oögonium's growth. And yet, up until maximum surface contact with the mesoglea has been established, no yolk formation has resulted. It should be further indicated that we have found no enlarged interstitial cells to be taken up bodily by the amœboid egg-cell. These two features of the nutrition of ovum of *Hydra* stand in contrast with what Tannreuther (08) describes. He says: "When the egg has reached its growth, it is amœboid in form with the nucleus near the center. The egg at this stage of development contains no yolk, . . . but when pseudopodia are completely formed, the nuclei of the interstitial cells forming the ovary, are taken up by the amœboid egg and become changed into yolk or pseudo-cells of the egg. Fig. 6 represents a cross-section of several pseudopodia into which the nuclei of the interstitial cells of the ovary

are passing" (p. 263–264). The contrast between what Tannreuther herein describes and our observations appears in two ways. In the first place, if yolk formation depends upon the disappearance of the interstitial cells of the ovary, then yolk should appear when these cells disappear. They are clearly seen to disappear throughout the growth of the oögonium and yet until the latter has reached its full growth no yolk has appeared. In the second place, we find that no interstitial cells have been bodily taken up or ingested as shown by Tannreuther in his Fig. 6. His Fig. 6, however, is not, in itself, convincing; for he shows the so-called nuclei leaving only two interstitial cells. Moreover, the cells from which these nuclei are migrating show no marked cytoplasmic change. Likewise, his written observations are not convincing with reference to the manner in which yolk arises. He makes the significant statement that "The pseudopodia do not grow out between the cells of the ovary, but rather between the ovary as a whole and the mesoglea" (p. 263). If, now, the pseudopodia were sent out with reference to yolk formation, dependent upon the interstitial cells of the ovary, they would "grow out between the cells of the ovary" and not "between the ovary as a whole and the mesoglea."

There is no meaning in the extensive application of the primary oöcyte's pseudopodia to the mesoglea, if the yolk granules are derived from the interstitial cells. On the other hand, we see in this spreading out of the primary oöcyte over the mesoglea a method of making maximum contact with a source of food material upon which to draw for the elaboration of deutoplasm.

In *Hydra*, the endoderm is the source of food supply. Kepner and Hopkins (24) observed that, as a diploblastic animal, *Hydra* cannot transport widely material absorbed by the endoderm. For example, chloretone injected into the enteric cavity of *Hydra* effects only the adjacent ectoderm of the body proper. The sphincters at the bases of the tentacles prevented the injected chloretone entering the latter and the compression of the walls of the peristome prevented chloretone entering its lumen. It was thus of interest to observe that the tentacles and peristome received none of the chloretone that had been absorbed by the general endoderm, for they became unusually active in contrast

to the quieted body proper. Just as chloretone could not be sent to the closed tentacles, so it appears the endoderm of *Hydra* cannot send food-material along a narrow channel to its oögonium. The growing oögonium must, therefore, come to the endoderm. As a result of this imposition, by the time the final oögonium has become, through growth, a primary oöcyte, an extended relation between the latter and the endoderm has been established. This relation established marks the inception of the second phase of the nutrition of the ovum of *Hydra viridis*. At the beginning of this second nutritional phase, there are no deutoplasmic inclusions within the cytoplasm. Soon, however, yolk is formed within the cytoplasm of the oöcyte (Fig. 5). This deutoplasm is elaborated by the oöcyte out of material taken over in solution from the endoderm and assimilated by the female gamete. Thus the deutoplasm may be looked upon as material elaborated by the oöcyte. The deutoplasmic granules are not to be considered the lineal descendants of original nuclei of neighboring interstitial cells that have come to be more and more numerous through amitosis. This position seems logical when we bear in mind the fact that, though many interstitial cells have disintegrated (perhaps most of them) and have been resorbed during the egg's growth, yet, up until maximum surface exposure to the endoderm has been made, no yolk-formation has resulted. Our interpretation is further strengthened by the observation that so long as yolk is making its appearance within the primary oöcyte a maximum surface relation to the endoderm is maintained; but when the maximum amount of yolk has been formed the egg retreats from the endoderm as Tannreuther (08) indicates: "After the amœboid egg becomes filled with yolk, the pseudopodia are drawn in and the egg becomes nearly spherical" (p. 264), (Text-figure, 3). The second phase of the nutrition of *Hydra viridis*, therefore, ends with the retreat of the primary oöcyte from the mesoglea after it has become filled with deutoplasm. No deutoplasm is formed thereafter. This phase of nutrition is referred to the development of the zygote.

Summary.

The nutrition of *Hydra viridis* is a dual process, there being two phases.

The first phase has reference to the nutrition of an oögonium of the final generation. This oögonium is nourished through the disintegration and resorption of adjacent interstitial cells. Through the nourishment, thus obtained, the final oögonium grows into a large pseudopodial cell, the primary oöcyte. The first nutritional phase is referred to the growth of the final oögonium into a primary oöcyte. It does not involve yolk-formation.

The second phase of nutrition begins with the primary oöcyte lying, as a pseudopodial cell, in extended relation to the endoderm. Yolk is elaborated by the oöcyte from material handed over by the endoderm and the protoplasm of interstitial cells is not involved. The second nutritional phase is referred to the development of the zygote.

LITERATURE.

Brauer, A.

'91 Ueber die Entwicklung von *Hydra*. Zeit. f. Wiss. Zool., Bd. 52, 2.

Downing, Elliot R.

'08 Ovogenesis of *Hydra Fusca*. BIOL. BULL., Vol. 15.

Fewkes, J. W., and E. L. Mark.

'84 Embryological Monographs, III. Acelephs and Polyps. Memoirs of the Mus. Comp. Zoöl., Vol. IX., No. 3, Harvard College, Cambridge, Mass.

Kepner, Wm. A., and D. L. Hopkins.

'24 Reactions of *Hydra* to Chloretone. Journ. Exp. Zoöl., Vol. 38.

Kleinenberg, N.

'72 Eine anatomisch-entwicklungsgeschichtliche Untersuchung. Leipsig.

Tannreuther, G. W.

'08 The Development of *Hydra*. BIOL. BULL., Vol. 14.

EXPLANATION OF PLATE.

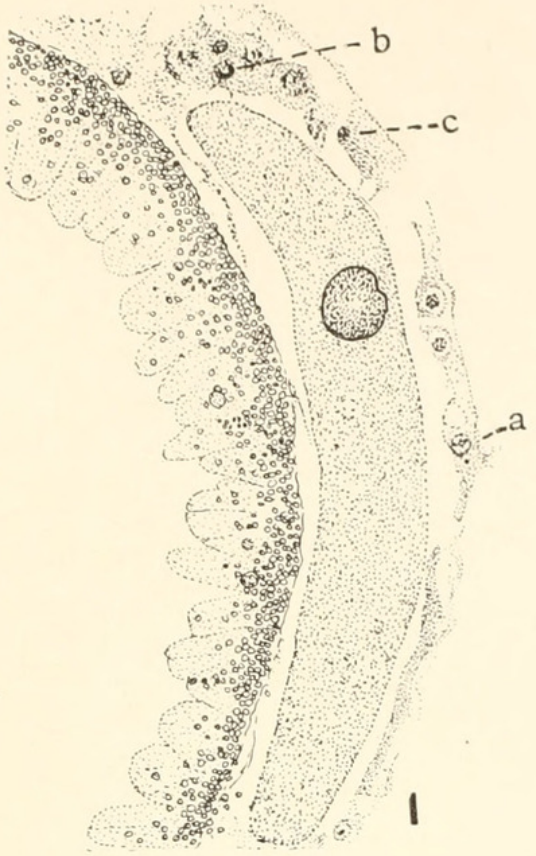
FIG. 1. Part of a section taken through plane indicated at *a* in text-figure. A group of disintegrating interstitial cells is shown at *b*, *c*. *a* shows that already, in this particular example, the superimposed ectoderm was but a single layer thick. × 250.

FIG. 2. Cell *a* of Fig. 1, magnified to indicate the character of cell that forms ovarian wall. × 1,250.

FIG. 3. Cell *b* in Fig. 1. Shows a disintegrating interstitial cell in which the nucleus resembles a deutoplasmic granule. × 1,250.

FIG. 4. Cell *c* in Fig. 1. An attending interstitial cell in a more advanced phase of disintegration than cell shown in Fig. 3. The nucleus no longer resembles a deutoplasmic granule. × 1,250.

FIG. 5. Section taken through plane indicated at *b* in text-figure. Shows eleven pseudopodia closely applied to mesoglea. Yolk-formation has begun; it is not, however, completed though all enlarged interstitial cells have disappeared. × 250.



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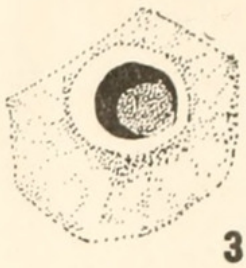

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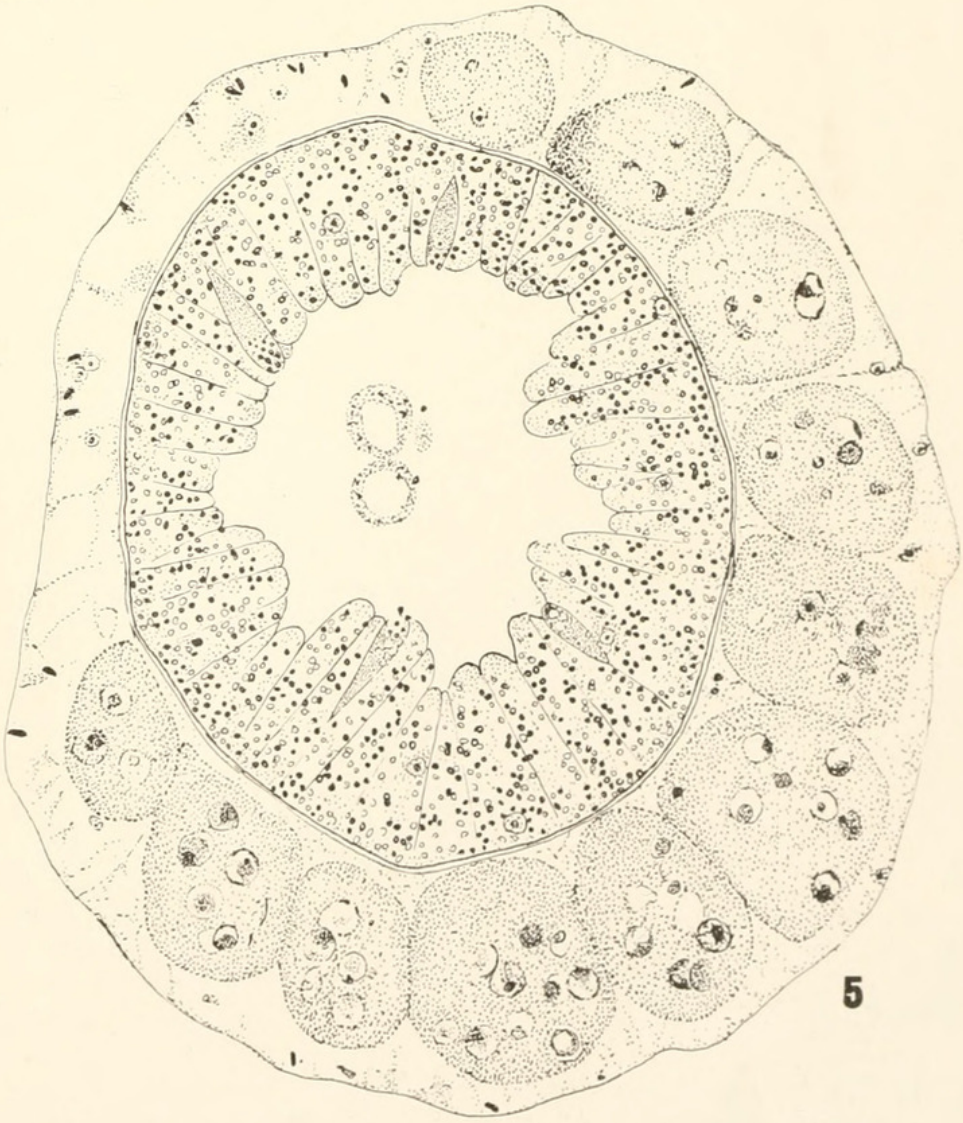

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