COMPARATIVE HISTOCHEMICAL STUDIES ON CESTODES
INCLUDING A REVIEW OF
THE HISTOCHEMISTRY OF ENDOPARASITES

by

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INTRODUCTION AND STATEMENT OF THE PROBLEM

Since numerous physiological studies with endoparasitic organisms have shown differences of considerable magnitude, often between rather closely related forms, taxonomically, a comparative histochemical study was conducted on two cyclophyllidean cestodes, *Hymenolepis diminuta* Rudolphi, 1819, and *Raillietina cesticillus* Molin, 1858. These cestodes were particularly well suited for this purpose since they were readily available and, more importantly, since concurrent studies on their metabolism were being made in the same laboratory.

Since most cestodes are too small or too complex in structure to study various organs or organ systems separately, quantitative measurements of physiological processes must usually be carried out on whole worms or homogenates of them. Cyto- or histochemical techniques provide a means of avoiding some of these difficulties and, in addition, they are semi-quantitative in nature, themselves. In fact, with some methods smaller amounts of substances can be detected with these techniques than with the usual quantitative procedures. The data from histochemical and quantitative methods considered together will undoubtedly do more to increase our knowledge of the metabolism of parasitic animals than either method considered alone.

As a guide to further study of endoparasites by cytochemical and histochemical methods, the following review of the distribution of certain organic constituents of endo-
parasitic protozoa and helminths has been included in this investigation.

PROTOZOA

GLYCOGEN:

The polysaccharides, glycogen and paraglycogen, are widely distributed among the protozoa, the latter, differentiated from true glycogen by its relative insolubility in cold water, being confined to this group. Since in most cytochemical analyses, no distinction between glycogen and paraglycogen is made and since a number of workers (von Brand, 1952) express doubt as to the validity of differentiating between two compounds solely on the basis of degree of solubility in cold water, the word "glycogen" in the following discussion will refer simply to a polysaccharide digested with diastase with no reference to whether the substance was concluded to be glycogen or paraglycogen.

Cytochemical determinations of the glycogen content of a number of protozoa have been made using various staining techniques (iodine, Best's carmine, Bauer-Feulgen, and periodic acid-Schiff techniques). The distribution of glycogen has been most widely studied in the Coccidia, including Klossiella muris and Eimeria stiedae (Lillie, 1947), E. magna (Cheissin, 1940), E. tenella (Edgar et al., 1944; Gill and Ray, 1954a), and E. brunetti and E. acervulina (Pattillo and Becker, 1955). In Klossiella no glycogen could be demonstrated although Bauer-positive material not extractable in diastase was present in some parasites.
However, in *Eimeria* glycogen, in diffuse or finely granular form, occurred more or less evenly distributed in the cytoplasm of late macrogametocytes, non-sporulated oocysts, and in the cytoplasm of the sporozoites, as well as in the spore of *E. acervulina*. None could be demonstrated in the schizonts, microgametocytes, microgametes, very young macrogametocytes, or liberated sporozoites in the intestinal lumen of the host according to Edgar et al. (1944) and Pattillo and Becker (1955). However, Gill and Ray (1954a) reported glycogen in the microgametocytes and microgametes, and an insignificant reaction for the sporozoites of *E. tenella*. Pattillo and Becker (1955) postulated a protein-glycogen combination in the spore of *E. acervulina*, since the color reaction was not extracted with diastase unless pretreated with pepsin. Gill and Ray (1954a) correlated the glycogen deposition in *Eimeria* with the physiological activity of the parasite at its various endogenous stages.

Certain other Sporozoa have been studied to a limited extent. The iodinophilic vacuole in the sporoplasm of *Myxobolus* is an often used character for diagnosis of the genus (Kudo, 1954). Giovannola (1934) reported that the glycogen granules of gregarines occurred primarily within the deutomerite. However, Daniels (1938) pictured a young gregarine under normal conditions in which the glycogen granules were scattered throughout the cytoplasm. No glycogen could be demonstrated by Lillie (1947) in *Plasmodium gallinaceum*. 
The appearance of glycogen deposits in human intestinal amoebae stained with iodine is well known because of its value in diagnosis. Glycogen appears in the young encysted stages but is depleted as the encysted forms mature. In all but Iodamoeba bütschlii and Entamoeba coli, glycogen appears to be rather diffuse with only occasional heavy local concentrations. However, Entamoeba coli generally has a more distinctly limited region of glycogen storage, and I. bütschlii has the characteristic large glycogen vacuole located at the pole opposite the nucleus. Some studies with more specific cytochemical methods have been made by Armer (1944) with Endamoeba blattae and Endolimax blattae of the cockroach, and Lillie (1947) and Hallman et al. (1955) with Entamoeba histolytica. The two cockroach amoebae appeared to contain little glycogen and this was diffusely distributed in the cytoplasm. Lillie (1947) reported a diffuse distribution of glycogen in the tissue stages of E. histolytica, but Hallman and co-workers could find no traces of diastase-extractable polysaccharide in these stages as well as in those on a starch-free medium. On starch-containing medium, amoebae were strongly positive for glycogen.

Of the ciliates, the rumen protozoa have been studied most intensively, primarily by Schulze (1924), Trier (1926), Weineck (1931), Westphal (1934), MacLennan (1934), and Hungate (1943). The general pattern appears to be similar in all the species studied. Westphal noted that glycogen
in *Diplodinium* and *Ophryoscolex* was deposited rather heavily in large granules in the skeletal plates of the middle endoplastic zone. Other than in the skeletal plates, MacLennan (1934) found small ectoplastic granules which were most numerous in the opercular and anal regions of *Polyplastron*. Other than the rumen ciliates, only *Ichthyophthirius* and *Balantidium* have received attention. In the former, there is an increase in glycogen granules persisting into the encysted forms in the same scattered arrangement as in the trophozoites (MacLennan, 1936). Faure-Fremiet and Thaureaux (1944) have described glycogenuous bodies in the cytoplasm of *Balantidium elongatum*.

Among the flagellates, *Trichomonas foetus* (Stewart, 1938), *Nyctotherus ovalis*, *Lophomonas striata*, and *L. blattarum* (Armer, 1944), and *Trypanosoma equiperdum* and *T. cruzi* tissue stages (Lillie, 1947) have been studied. *L. striata* normally contains only traces of glycogen, but there is an increase in the polysaccharide when the host is fed on a high-carbohydrate diet. None at all could be demonstrated in *L. blattarum*. *N. ovalis* contains a fair degree of glycogen occurring as granules and forming a bean-shaped mass between the anterior end of the body and the macronucleus. Some granules are scattered throughout the rest of the cell. Neither of the trypanosomes contained demonstrable glycogen, while *T. foetus* showed diffuse distribution of glycogen inclusions.
OTHER POLYSACCHARIDES:

Polysaccharides other than glycogen have been studied only to a very limited extent. The methods used are generally the treatment of tissues stained for polysaccharide by non-specific methods, such as the periodic acid-Schiff or metachromatic techniques, with various enzyme solutions specific for particular polysaccharides. Also more specific staining methods such as the dialyzed iron method for acid mucopolysaccharides have been used. Gill and Ray (1954b) found mucoid sulfates in the cytoplasm and a hyaluronic acid type of polysaccharide in the nucleus, especially the karyosome, of *Eimeria tenella*. The latter was also found to form the protective covering of the oocyst. Loran et al. (1955) also demonstrated cytochemically the presence of a hyaluronic acid type of polysaccharide in *Entamoeba histolytica*. This substance was present in both the cytoplasm and nucleus of amoebae grown with various mono-associates. Also collagen was demonstrated in the outer coat of one strain when grown with streptobacillus. Variation of the acid polysaccharide content of the cytoplasm and the nature of the outer surface coat appeared to be more closely related to type of associate than to strain differences.

LIPIDS:

The distribution of fatty material has been studied in a large number of protozoa, primarily by use of osmic acid or various fat-soluble dyes. Fat droplets are found fre-
quently and often in great abundance in the Myxosporidia (Cohn, 1896; Doflein, 1898; Erdmann, 1917; Petruschowsky, 1932), Coccidia (Joyet-Lavergne, 1926; Gurwitsch, 1927; Cheissin, 1940; Pattillo and Becker, 1955), and gregarines (Daniels, 1938). In Eimeria brunetti and E. acervulina (Pattillo and Becker, 1955), the indifferent stages, the schizonts containing matured merozoites, and the microgametocytes containing microgametes were devoid of demonstrable fat. The cytoplasm of the growing macrogametocyte exhibited an accumulation of these globules which increased in number and size as it aged, persisting into the walled oocyst, which exhibited an accumulation of these globules on the nuclear boundary. Fat globules were noted in the cytoplasm of many non-sporulated oocysts, and the mature spore contained a large fat globule at each pole between the sporozoites and the spore wall. Also, smaller globules were noted both within the sporozoites and external to them within the spore. In the gregarines, sexual differences in fat content have been observed (Joyet-Lavergne, 1926), the female of an encysted pair containing more than the male. The amount of fat in this group varies between species to some extent but generally occurs irregularly scattered throughout the cytoplasm.

Fat droplets occur to a much lesser extent in parasitic amoebae and flagellates (Sassuchin, 1928, 1930; Armer, 1944). Armer finding none at all in Endamoeba blattae and Endolimax blattae, although occasional sudanophilic droplets were
seen in the latter when the host was fed on a high-fat diet. *Nyctotherus ovalis*, also, accumulated some fat substances in its cytoplasm when the host was fed a high-fat diet, but none at all could be demonstrated in *Lophomonas striata* and *L. blattarum* (Armer, 1944).

Some, but not all, of the ciliates studied store large amounts of fat (Cheissin, 1930; Kedrowsky, 1931; MacLennan, 1936). Kedrowsky (1931) noted a seasonal cycle in the abundance of fat droplets in *Opalina ranarum*, this ciliate containing large amounts in the fall but losing it by the end of winter. In *Ichthyophthirius*, the fat granules show a scattered arrangement in the cytoplasm similar to the distribution of glycogen and, like the latter, increase in number with the age of the ciliate, being present to a considerable extent in mature and encysted forms (MacLennan, 1936).

**PROTEIN SUBSTANCES:**

Although some studies have been made with general protein tests such as mercuric bromphenol blue, ninhydrin, and Million's reagent for tyrosine, by far the most work has been with the Feulgen and methyl-green-pyronin tests for desoxyribose and ribose nucleic acids respectively.

Most of the studies appear to have been done on the Sporozoa, particularly the malaria organisms. Although Pawan (1931) could demonstrate no desoxyribose nucleic acid in *Plasmodium falciparum* or *P. vivax*, other investigators using the same or similar techniques have demon-
strated DNA in *P. relictum* (Breindl and Jirovec, 1932; Jirovec and Cerny, 1932), *P. gallinaceum* (Ungo-Mugdan, 1938; Lillie, 1947; Lewert, 1952), *P. elongatum* (Chen, 1944), *P. lophurae* (Lewert, 1952), *P. knowlesi* (Deane, 1945) and *P. vivax* (Deane, 1945; Lewert, 1952). Generally, the nuclei of sporozoites, late trophozoites, early schizonts, and gametocytes do not stain or stain only slightly for DNA, while the nuclei of young trophozoites, older schizonts, segmenters, and large exoerythrocytic stages have greater concentrations. Ribose nucleic acid in this group has been studied only by Lewert (1952). He noted an intense cytoplasmic staining of all stages but the gametocytes and sporozoites of *P. gallinaceum*, *P. lophurae*, and *P. vivax*. A marked RNA cycle was noted in the plasmodia and this was associated with a juxtanuclear structure which behaves in a manner similar to that of a nucleolus, dividing prior to nuclear division.

The distribution of DNA in Coccidia has been studied by Sassuchin (1935), Cheissin (1940), Lapage (1940), Lillie (1947), and Pattillo and Becker (1955). In *Klossiella muris* the nucleus is Feulgen-negative in young parasites and stains but slightly in older forms (Lillie, 1947). *Eimeria stiedae*, on the other hand, was demonstrated to be Feulgen-negative when large, while some of the smaller forms were positive. Pattillo and Becker (1955) found DNA to be located in a nuclear ring in sporozoites, merozoites, schizonts, and microgametes of *Eimeria brunetti* and *E. acer-
vulina. A nuclear ring of DNA was evident in the very young macrogametes of *E. brunetti*, but had disappeared in slightly larger forms and did not reappear until after the oocyst wall had formed and the oocyst was liberated into the lumen of the gut. DNA was not observed in the macrogamete or non-sporulated oocyst of *E. acervulina*. Cheissin (1940) obtained a positive Feulgen reaction in the zygote of *E. magna*, but also failed to find DNA in the macrogametes. The concentration of DNA increased in the nuclei of *E. brunetti* and *E. acervulina* immediately preceding and during division. Ribose nucleic acid in *E. brunetti* and *E. acervulina* (Pattillo and Becker, 1955) was located at the nuclear boundary and throughout the cytoplasm of merozoites, schizonts, microgametocytes, macrogametes, and sporozoites, with a decrease in amount as the parasite stages, particularly the gametocytes matured. The karyosomes in these stages were of RNA, and these increased in size as the gametocytes matured and then decreased in size after maturity. Besides the nucleic acids, the walls of both sporulated and non-sporulated oocysts reacted positively with mercuric bromphenol blue, indicating their protein nature (Pattillo and Becker, 1955). Also, refractile globules within the sporozoites and the plastic granules of the macrogametocytes gave positive protein reactions. Gregarines have been studied only by Daniels (1938) who found the nuclei of several species from *Tenebrio* to be Feulgen-negative.
Nucleic acids have been studied in several flagellates, including *Trypanosoma equiperdum* (Lillie, 1947), *T. cruzi* (Pizzi and Diaz, 1955), *T. diemyctyli* (Barrow, 1954), and *Leishmania tropica* (Scoururi, 1955). According to Lillie, the nucleus of *T. equiperdum* was Feulgen-negative and the blepharoplast could not be demonstrated by this technique. The data available on other flagellates show an entirely different situation. *Trypanosoma cruzi*, *T. diemyctyli*, and *Leishmania tropica* all have nuclei which show a peripheral layer of DNA adjacent to the nuclear membrane, and the kinetoplasts characteristically give a much more intense Feulgen reaction than the nucleus. Only crithidial and leishmanoid forms of *T. cruzi* were studied. It was noticed in these that during the mitotic stage the Feulgen-positive material of the nucleus seems to break down into 3-4 chromosome-like bodies. In immune hosts the Feulgen-positive material tends to become more compact and less definite in outline in the nucleus of *T. cruzi*. Besides DNA, Barrow (1954) demonstrated in the cytoplasm of *T. diemyctyli* numerous large metachromatic granules, probably mucoprotein, and numerous euchromatic granules of ribose nucleic acid. The karyosome of the nucleus also contained RNA.

The nucleic acids of one parasitic ciliate, as well as the cytoplasmic protein granules of another, have been studied. In *Balantidium coli* (Auerbach, 1953), DNA was present only in the matrix of the macronucleus (an amicro-nucleate strain was studied), while RNA was found in the
vacuoles of the macronucleus and in the cytoplasm in a diffuse state. MacLennan (1936) noted granules giving protein staining reactions scattered irregularly in the cytoplasm of Ichthyophthirius. The number of these granules increased with age and persisted in the encysted form.

The only study of parasitic amoebae found was that of Lillie (1947) on Entamoeba histolytica. Tissue stages of the amoebae stained only faintly with the Feulgen technique.

ENZYMES:

Alkaline and acid phosphatases have been studied cytochemically in several groups of protozoa using for the most part various modifications of the Gomori technique. Although these techniques are satisfactory for locating the enzyme in tissues, placement within the cell must be regarded as of questionable significance (Pearse, 1953). Thus, the significance of the recent findings of Carrera and Changus (1948) and Carrera (1950) on Entamoeba histolytica, Lewert (1952) on Plasmodium gallinaceum, P. lophurae, and P. vivax, Ray and Gill (1954) and Gill and Ray (1954c) on Eimeria tenella, and Auerbach (1953) on Balantidium coli can not as yet be evaluated with any degree of certainty. Entamoeba histolytica from culture, rectal aspirations, and within guinea pig caecal tissues were demonstrated to give a strong reaction for acid phosphatase activity in their cytoplasm. The distribution of alkaline phosphatase activity closely paralleled that of ribose nucleic acid in Plasmodium gallinaceum, P. lophurae, and
P. vivax (see discussion of RNA, p. 9). No alkaline phosphatase activity could be demonstrated in an amicro-nucleate strain of *Balantidium coli*, but acid phosphatase appeared to occur most heavily in the macronucleus and basal granules of the cilia.

**MISCELLANEOUS:**

Vitamin C has been demonstrated in a number of parasitic protozoa by the acid silver nitrate technique. It must be remembered, though, that the specificity of this technique is not well established (Pearse, 1953). Ascorbic acid granules have been reported in the cytoplasm of *Trypanosoma equiperdum*, the number of these increasing when the host was injected with vitamin solution (Roskin and Nastiukova, 1941). *Opalina*, however, was found to be entirely void of these granules. Smyth et al. (1945) found that vitamin C granules were distributed throughout, but limited to, the endoplasm of *Nyctotherus cordiformis*.

Glutathione has been demonstrated, by various modifications of the nitroprusside reaction, in *Trypanosoma equiperdum* (Voegtlin et al., 1923), in the coccidium, *Aggregata eberthi*, and in several species of mealworm gregarines (Joyet-Laverne, 1927). In gregarine trophozoites, glutathione is especially concentrated within the deutomerite as are the mitochondrial elements, leading the author to assume that glutathione was attached to these. In encysted forms glutathione occurred diffusely scattered throughout the protoplasm.
HELMINTHS

In contrast to the parasitic protozoa which show wide variances in their chemical composition possibly because of their extreme polyphyletic origin, the parasitic helminths are a fairly closely-knit group and reflect this in their histochemistry.

GLYCOGEN:

Various histochemical techniques for glycogen (iodine, Best's carmine, Bauer-Feulgen, and various modifications of the periodic acid-Schiff method) have been used on a wide variety of parasitic helminths.

Glycogen distribution has been studied in a large number of trematodes, including Eurytrema coelomatiolum, E. pancreaticum, Paragonimus westermani, Microcoelium dendriticum (Yamao, 1952e), Uroculeidus principalis, Haemato-locelus medioplexus, Clinostomum attenuatum, Polystomoidella oblongum, Gorgodera amplicava, Gorgoderina attenuata, Fasciolooides magna, Cryptocotyle lingua, Siphodera vinalewardsii (Axmann, 1947), Ostolium sp. (Wilmuth and Goldfischer, 1945), Schistosoma japonicum (Axmann, 1947; Lillie, 1947), S. mansoni (Axmann, 1947; Lillie, 1947; Bueding and Koletsky, 1950), and Fasciola hepatica (Ortner-Schönbach, 1913; Prenant, 1922; Kajiro, 1927; Yamao, 1952e). The general pattern in adult worms is very similar in all forms studied. The cuticle is glycogen-free and the subcuticular zone usually has little or none. The parenchymal cells, however, store most of the polysaccharide, although the
muscles contain some and the suckers, particularly the muscular layers, contain large amounts. The pharynx is somewhat variable, usually containing some glycogen, although none was found in the schistosomes, *Clinostomum*, and *Gorgoderina*. The intestinal cells, nerve cells, excretory ducts, and male reproductive system, other than the mature sperm, are glycogen-free. The sperm, when mature, generally give a positive reaction, although they were glycogen-free in the schistosomes studied. The ovary is without glycogen, but the vitellaria vary in glycogen content between species, generally correlating with similar differences in the glycogen content of the ova. However, even the mature vitellaria of *Haematoloechus*, *Gorgodera*, *Gorgoderina*, *Siphodera*, *Ostolium*, and schistosomes were negative for glycogen, although shelled ova in the uterus gave positive reactions. The developing ova usually do not contain glycogen except in *Polystomoidella*, *Fascioloides*, *Fasciola*, and *Allasostoma*. By the time the ova have matured, those of *Siphodera* and *Cryptocotyle* give positive reactions also, and usually all of the species studied showed variable amounts of glycogen in the shelled ova. The glycogen content of male schistosomes was found to be considerably higher than that of females. This is explained by the less well-developed musculature and parenchyma which is glycogen-positive, and the more abundant sexual apparatus in the female which is glycogen-free (Bueding and Koletsky, 1950).
The distribution of glycogen in the developmental stages of *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium* has been studied by Axmann (1947). The newly-hatched miracidia contained more glycogen than the uterine eggs. It occurred in both a diffuse and granular state in the subepithelial tissue and the parenchyma. The gut, cephalic glands and germ balls were glycogen-free. The primary sporocysts contained no glycogen, while the secondary sporocysts contained a small amount in a diffuse state, and the cercariae accumulated more than the retaining sporocysts, first in a diffuse state and then in a granular state. Glycogen was located in the subepithelial tissue, the suckers, and particularly, the tail-stem but not the tail-forks. The positive reaction in the basophilic, but not the acidophilic, cephalic glands and their ducts may have been due to mucin. The rediae of an unidentified species contained small amounts of diffuse glycogen, while the cercariae and sporocysts gave reactions similar to that of corresponding schistosome stages.

Glycogen distribution in cestodes has been studied by Brault and Loeper (1904a,b), Busch (1905a,b), Ortner-Schönbach (1913), von Brand (1933), Wardle (1937), Smyth (1947, 1949), and Yamao (1952e). The most extensive study was that of Ortner-Schönbach on *Anoplocephala plicata*, *A. perfoliata*, *A. mamillana*, *Calliobothrium coronatum*, and *Caryophyllaeus mutabilis*, while the cestode most studied was *Moniezia expansa* (von Brand, 1933; Wardle, 1937; Yamao,
The general pattern of distribution is similar in all the species studied. The cuticle is glycogen-free and subcuticular tissue contains little or none. Most of the glycogen storage is in the parenchymatous tissue, although there is very little in the scolex parenchyma, except the suckers of *Anoplocephala* and the bothria of *Calliobothrium* which contain considerable amounts. The muscle-fibers are glycogen-free but difficult to distinguish because they are surrounded by glycogen-rich tissue. The nervous, excretory and reproductive systems are almost free from glycogen, although the vitelline glands vary to some extent, containing small amounts in *Caryophyllaeus* and none in *Moniezia*. The sperm generally give a positive reaction, while the ova vary in glycogen content. Generally, there is no glycogen in the ovary. In *Anoplocephala* spp. a small amount was present in the uterine eggs, while in *Moniezia* the uterine eggs contained none. Smyth (1949), studying the plerocercoid larva of *Ligula intestinalis*, found glycogen distributed primarily in the parenchymal area although the intercellular spaces of both circular and longitudinal muscle layers contained large amounts. Small globules were present in the small lumina of the longitudinal muscles as seen in transverse section, and the subcuticular spaces were also filled with glycogen. The distribution of glycogen in the plerocercoid of a diphyllobothriid cestode was primarily in the intercellular spaces of the parenchyma and the outer periphery of the
longitudinal muscles (Smyth, 1947).

In Acanthocephala, the distribution of glycogen has been studied in *Macracanthorhynchus hirudinaceus* (von Brand, 1939a, b, 1940), *Leptorhynchoides thecatus* (von Brand, 1939b), *Echinorhynchus coregoni* (von Brand, 1939b; Bullock, 1949b), *E. gadi*, *Pomporhynchus bulbocollis*, *Neoechinorhynchus cylindrus*, and *N. emydias* (Bullock, 1949b). As with the trematodes and cestodes, there is a general pattern of distribution which is followed by all the species studied. The cuticle contains no glycogen. Most of the glycogen storage is in the tissues of the body wall, particularly in the subcuticular muscle fibers and the lacunar system. Most of the storage in the muscles of the body wall is in the non-contractile parts of the cells. However, the muscles in the body cavity (retractor muscles of the proboscis, etc.) also contain large amounts and the glycogen is also distributed in the contractile parts of the cells. The muscular layers of the inner wall of the apical part of the receptacle are rich in glycogen, while those in the distal portion are glycogen-poor. The muscular wall of the bursa copulatrix contains much of the polysaccharide. The floating ovaries contain varying amounts and this is confined to the stroma, while the young embryos contain little or none. The mature embryo, however, contains large amounts in the region of the "Embryonalkern". The testes, seminal reservoir, and sperm contain little or none, while the cement glands may contain considerable amounts. The
lemnisci and various structures of the excretory system contained little or no glycogen. Besides the above observations, Meyer (1931) casually mentioned glycogen in the lacunar system of *Neoechinorhynchus rutili*.

The literature on the distribution of glycogen in nematode parasites is relatively voluminous and no attempt has been made to collect all of it, particularly in respect to those nematodes which parasitize invertebrates. The adults of such species as *Rhabdias ranae*, *Ancylostoma caninum*, *A. duodenale*, *Necator americanus*, *Oxyurus curvula*, *Ascaris lumbricoides*, and *A. megaloccephala* have been studied by Busch (1905a), Faure-Fremiet (1913), Giovannola (1935, 1936), Hirsch and Breitschneider (1937), von Kemnitz (1912), Martini (1916), Payne (1923), Weinland (1901a,b), Weinland and Ritter (1903), Toryu (1933), and Yamao (1952e).

The general pattern of distribution can be summarized as follows. The cuticle is generally glycogen-free. The subcuticle, especially its enlargement along the lateral lines, contains much glycogen and is the most important storage place. The muscles characteristically contain large amounts of glycogen, particularly in the large plasmatic bulbs of the cells. The intestinal cells vary to some extent in glycogen storage, but often contain large amounts. The nerve cells generally are glycogen-free. In the ovary, the oocytes contain only small amounts of glycogen. However, the oogonia contain relatively large amounts which are somewhat decreased after formation of the egg-shell.
In the testes, the spermatocytes are glycogen-free, while later stages of spermatogenesis contain some glycogen. The bursa generally gives a heavy reaction for glycogen.

The eggs of *Rhabdias ranae*, *Ancylostoma caninum* and *Ascaris lumbricoides* have been studied by Giovannola (1936), while Izumi (1953) studied those of *Ascaris megaloecephala*. Giovannola reported a general decrease in the glycogen content as the eggs are fertilized, the shell formed, and cleavage begins. Izumi carried the description from there, stating that in the early developmental stages of the embryo, glycogen was located between the fat droplets, first scattered in location and later, in the late morula, at one pole. In the larval stage, the anterior portion of the animal pole contained most of the glycogen granules, with local increases occurring where cell division and tissue formation were taking place.

The free-living larvae of *Ancylostoma duodenale*, *Necator americanus*, *Ancylostoma caninum*, and *Nippostrongylus muris* have been studied by Busch (1905a), Payne (1923), and Giovannola (1936), while the tissue forms of the larvae of *Trichinella spiralis*, *Acanthocheilonema perstans*, *Necator americanus*, *Ancylostoma caninum*, and *Nippostrongylus muris* have been studied by Brault and Loeper (1904b) and Giovannola (1936). These authors found that glycogen is usually absent from the free-living stages, although a small amount can be demonstrated in the lumen of the intestinal tract and the body cavity in the rhabditiform larvae of some.
None could be demonstrated in the filariform larvae. No glycogen could be demonstrated in the microfilariae of Acanthocheilonema, but encysted Trichinella gave positive reactions. Nippostrongylus and hookworms in lung migration showed glycogen in the lumen of the digestive tract and in the body cavity, particularly in the anterior and posterior extremities.

LIPIDS:

The distribution of fats has been studied in a large number of parasitic worms, particularly nematodes, using various fat-soluble dyes and the less specific osmic acid method. In some Acanthocephala, phospholipids have been demonstrated by dichromate oxidation and subsequent staining with Sudan dyes or osmic acid, and the Smith-Dietrich test. Cholesterol has also been demonstrated by the Schultz test in a few helminths.

The distribution of fat in trematodes has been studied most extensively on Fasciola hepatica (von Brand and Weinland, 1924; Prenant, 1922; Vogel and von Brand, 1933; Stephenson, 1947). In the adult worm, very few fat droplets occurred in any organ other than the excretory system. Fat globules evidently are produced in the walls of the excretory system and were observed to pass into the lumen and thus to the exterior. Microchemical analyses of these globules have established that they do not contain appreciable quantities of cholesterol, cholesterides or lipins, but may be triglycerides containing some unsaturated fatty
acid. No fat excretion could be demonstrated in the miracidia, rediae, or cercariae of *Fasciola*, although some fat droplets could be demonstrated within the tissues other than the excretory system. However, differences in fat distribution have been observed between various species. von Brand (1934) could demonstrate no fat excretion in *Dicrocoelium lanceatum*, although its habitat is the same as that of *Fasciola*. Cholesterol could not be demonstrated in the vitellaria of *Fasciola*, although Schmidt (1930) demonstrated large amounts in the vitellaria and eggs of *Gorgodera cygnoides*.

The distribution of lipids in cestodes has been studied by Brault and Loeper (1904a,b), Schiefferdecker (1874), Pinter (1922), Arndt (1922), Coutelen (1931), von Brand (1933), and Smyth (1947, 1949). The most extensive studies were made by von Brand on *Moniezia expansa*, *M. denticolata*, *Taenia saginata*, *T. marginata*, and *Dipylidium caninum*. The distribution of fat appears to closely parallel that of glycogen. The cuticle, subcuticle, and muscle fibers did not contain any, while the parenchyma contained much fat in the form of small droplets. Only traces were found in the suckers, reproductive system, embryos and nerve cords. In the eggs, the lipid droplets were generally located between the embryo and the shell. In *Dipylidium caninum*, large droplets were located in the uterus, and Coutelen (1931) and von Brand (1933) observed lipid globules in the excretory systems of *Echinococcus granulosus* and
Moniezia expansa respectively. Smyth (1947, 1949) found that the plerocercoids of Ligula intestinalis and a diphyllobothriid cestode, in vitro, produced large amounts of cytoplasmic fat, although the fresh, uncultured larvae were nearly fat-free. These observations, and the observations of Reid (1942) that the fat content of Raillietina cesticillus was the same in worms taken from fed and starved hosts, although the parasites from the latter had lost most of their glycogen, have led von Brand (1952) to assume that some fat is a metabolic end-product rather than an energy reserve, as already observed in Fasciola. This view has been questioned because of the work of Smorodincev and Bebesin (1935) who showed that the anterior end of Taenia saginata contained over twice as much fat percentage-wise than the middle and posterior regions.

Lipid droplets in the lacunar system of the Acanthocephala Echinorhynchus and Neoechinorhynchus rutili have been mentioned by Hamann (1891), Saefftigen (1885), and Meyer (1931). More detailed studies have been made by von Brand (1939a) on Macracanthorhynchus hirudinaceus and Bullock (1949b) on Echinorhynchus coregoni, E. gadi, Pomporhynchus bulbocollis, Neoechinorhynchus cylindratus, and N. emydis. In Macracanthorhynchus, considerable amounts of fat were present in the deeper regions of the hypodermis, occurring in various sized droplets between the fibers. There was less in the anterior end, in the female, the posterior ends. No fat could be demonstrated in the lemnisci, body fluid,
nervous system, suspensory ligament, and excretory system. A few droplets occurred in the non-contractile portions of the muscle cells. The cement glands and testes contained little fat, but considerable amounts occurred in the modified hypodermis covering the bursa copulatrix. Considerable amounts were also noted in the vagina, the inner layer of the uterus, and the ovaries, closely-packed droplets occurring in the stroma of the latter. The young eggs contained some fat, but none could be demonstrated in the older embryos. However, Bullock (1949b) found that the muscles of the species he studied contained high concentrations of fat, particularly those in the body wall, although those of the heavy muscular receptacle contained only little. The fat in these muscles was distributed in both the contractile and non-contractile portions of the cells. The subcuticula of *Neoechinorhynchus* and *Pomporhynchus* contained very large amounts, particularly in the innermost layer, but the condition in *Echinorhynchus* was more like that of *Macracanthorhynchus* where there was somewhat less fat deposition in this region. The subcuticula of the proboscis and neck regularly contained more fat than elsewhere and the pattern was diffuse with a few larger droplets occurring at the bases of the hooks. Both diffuse and globular deposits were noted in the lemmisci, with a gradual increase in concentration toward the free ends in *Neoechinorhynchus* and *Pomporhynchus*. A marked fat content was noted in the posterior end of the apical organ of *Neoechinorhynchus* with a gradual decrease anteriorly
to the subcuticula. All of the organs contained some deposits, although little was observed in the bursa copulatrix. The uterine bell and uterus contained moderate amounts, and the vaginal glands stained heavily. The ganglia contained some fat. As von Brand (1939a) had noticed, the germ balls contained considerable amounts of fat, but little could be demonstrated in the embryo. On the basis of the technique used, Bullock concluded that the fatty material discussed above was true fat. With more specific techniques, he demonstrated phospholipids in the muscles, receptacle, ganglia, cement glands, and subcuticula, as much occurring in the latter as true fat, especially adjacent to the lacunae of the lacunar system and those in which the nuclei lie. Cholesterol was limited to the outer layer of the subcuticula in most forms studied.

Again, in regard to fat distribution in nematodes, the literature is voluminous and no attempt has been made to collect all of it, particularly in respect to those parasitizing invertebrates. For fat distribution in many of these forms, see Goodey (1930). The adult worms have been studied by von Kemnitz (1912), Faure-Fremiet (1913), Mueller (1929), Giovannola (1935), Hirsch and Bretschneider (1937), Chitwood and Chitwood (1938), and Chitwood and Jacobs (1938). Of these, *Ascaris lumbricoides* and *A. megalocephala* have been most studied. The pattern of fat distribution is similar in both species. The subcuticula, especially the chords and non-contractile portions of the body wall muscles,
is the most important place for fat deposition. Also, the intestinal cells, ganglion cells, and various structures of the reproductive system contained fat globules. The eggs of *Ascaris lumbricoides*, *A. megalocephala*, *Toxascaris limbata*, *Toxocara cati*, *T. canis*, *Ascaridia galli*, *Enterobius vermicularis*, and *Rhabdias ranae* have been studied by various authors, including Brault and Loeper (1904b), Busch (1905a), Zawadowsky (1928), Giovannola (1936), Wottge (1937), B. G. Chitwood (1938), Jacobs and Jones (1939), Timm (1950), and Izumi (1953). Most of these workers made particular reference to the vitelline membrane of the egg which they believed to be lipid in nature. Timm (1950) concluded that the membrane was myricyl palmitate on the basis of the melting point and mixed melting point of isolated membrane substance from *Ascaris* eggs. M. D. Chitwood (1951) came to a similar conclusion for the vitelline membrane of the eggs of *Meloidogyne javanica*, a plant parasite. The ova itself is almost fat-free in the early stages, but when mature but not yet fertilized fat droplets are readily perceptible. These decrease in number after fertilization. Izumi (1953) found the fat distribution in the early embryo of *Ascaris megalocephala* to correspond to the distribution of glycogen, i.e. scattered evenly and densely in the body, but in the tadpole or larval stage the fat granules remained only around the digestive organs.

The free-living larvae of *Necator americanus*, *Ancylo-
stoma caninum, Nippostrongylus muris, Strongyloides rüeborni, Rhabdia ranae, and Haemonchus contortus have been studied by Payne (1922, 1923), Giovannola (1936), Rogers (1939, 1940), and Weinstein (1949). In the young rhabditiform larvae, the fat occurs in the lumen of the intestinal tract, while in later stages, deposition is primarily in the wall of the intestine and the mesodermal tissues of the body wall. With age, there is a decrease in fat content and, at the same time, a more or less parallel decrease in activity. Rogers (1939, 1940), however, did not find a direct correlation between fat content, activity, and infectivity, although some correlation evidently existed in that only a small number of worms were infective when their fat reserves were completely exhausted. Fat distribution in the larval stages within the tissues has been studied by Giovannola (1936) on Trichinella spiralis and Necator americanus. He found encysted Trichinella to contain some fat and observed an increase in the fat content of Necator larvae in the lung. Two days after infection, the latter showed a small amount in the body cavity, but older larvae showed large quantities of fat present, particularly in the intestinal tract.

ENZYMES:

Phosphatases have been studied in a number of trematodes, cestodes, acanthocephalans, and nematodes, using various modifications of the Gomori technique. Yamao (1952a) has studied both alkaline and acid phosphatases in Eury-
Eurytrema, alkaline phosphatase was located in the body wall and the walls of the excretory canals, giving strongly positive reactions, while acid phosphatase was demonstrable in most tissues but gave only weak reactions. In Dicrocoelium, alkaline phosphatase was distributed in a manner similar to Eurytrema, but the acid phosphatase gave a much stronger reaction and was particularly concentrated in the mesenchymatous fibers and their nuclei. Yamao claims that the distribution and intensity of acid phosphatase in Clonorchis sinensis was very similar to that in Fasciola hepatica, but no reference for the latter could be found. Alkaline phosphatase could not be demonstrated in Clonorchis.

The distribution of phosphatases has been studied in a fair number of cestodes, both larval and adult. In the adults of Moniezia expansa (Rogers, 1947; Lefevere, 1952; Yamao, 1952c), M. benedeni (Lefevere, 1952; Yamao, 1952c), Multiceps serialis, Taenia saginata (Lefevere, 1952), T. taeniaeformis (Yamao, 1952c), Davainea proglottina, Raillietina cesticillus (Lefevere, 1952), Anoplocephala perfoliata, and A. magna (Yamao, 1952c), the pattern of alkaline phosphatase is almost identical. All contain alkaline phosphatase activity in the cuticle and subcuticular layer, the latter giving the strongest reaction in the immature proglottids. Yamao found that all but T. taeniaeformis of the species he studied showed concentrations of the enzyme
in the excretory ducts, and Rogers found evidence of some activity in the developing eggs. Acid phosphatase in the adults was studied only by Yamao, who found it also to be present in the cuticular and subcuticular layers. Lefevere (1952) found alkaline phosphatase activity in the form of granules in the large parenchymal cells of Nybelinia larvae. No alkaline phosphatase activity could be demonstrated by Yamao (1952d) in the body proper of Cysticercus bovis, although the lining of the excretory ducts in both C. fasciolaris and larval Echinococcus cysticus fertilis showed enzyme activity. At the site of the future cuticular surface, acid phosphatase activity only could be demonstrated in C. bovis, while hydatid cysts showed concentrations of both alkaline and acid phosphatases. The pattern of distribution of both enzymes in C. fasciolaris was identical to the adult. Where the larvae were attached to the cyst wall, strong positive reactions for both enzymes occurred. Lefevere (1952) could find only small amounts of alkaline phosphatase activity in C. pisiformis, although acid phosphatase was present in large amounts. Although the significance of the localization of phosphatases is not at all understood, some of the conclusions drawn by these authors are interesting. Regarding the cuticular and subcuticular concentrations in adult worms, all authors agree that these may be correlated with the absorption of nutrient materials from the environment. Lefevere also thought perhaps these concentrations might be correlated with excretion of meta-
bolic wastes through the cuticle. In regard to the larvae, Yamao concluded that the heavy concentrations at the attachment point with the cyst wall might be correlated with absorption of nutrient also. The strong acid phosphatase activity in *Cysticercus pisiformis* was thought by Lefevere to be correlated with the metabolism of endogenous food reserves.

Yamao (1951, 1952b) has studied the phosphatases of *Ascaris lumbricoides* and *A. megalocephala*, but neither these papers nor abstracts of them were available for this review. However, Rogers (1947) noted that small spherical bodies in the intestinal cells of *Ascaris lumbricoides* gave positive reactions for alkaline phosphatase. No other tissue of the worm gave a positive reaction. Bullock (1953) could demonstrate no alkaline phosphatase activity in encysted *Trichinella spiralis* larvae.

Alkaline phosphatase and lipase both have been demonstrated by Bullock (1949a) in certain Acanthocephala using the Gomori techniques. *Echinorhynchus coregoni, Pomporhynchus bulbocolli, Neoehinorhynchus cylindratus*, and *N. emydias* were used in this study. Definite evidence of lipase activity was found in the subcuticula of the trunk and slight activity of this enzyme was detected in the lemnisci. The role of lipase is unknown, but possible correlation with the hydrolysis or synthesis of fat as an energy reserve, a waste product, or both is suggested. Alkaline phosphatase was located in the subcuticula of the trunk with the great-
est concentration in the outer layer and a lesser but marked concentration in the inner layer of all but Neo-echinorhynchus, where neither alkaline phosphatase nor lipase could be demonstrated. A correlation of alkaline phosphatase distribution with the passage of metabolites or waste products to or from the worm is suggested.

MISCELLANEOUS:

Vitamin C has been demonstrated histochemically in two trematodes, Opisthioglyphe ranae (Smyth et al., 1945) and Fasciola hepatica (Stephenson, 1947) by the acid silver nitrate technique. The specificity of this technique has not as yet been proved and is somewhat in doubt, but the observations of these authors are interesting and possibly of some significance. In Fasciola as well as Opisthioglyphe, the greatest concentrations occurred in the walls of the excretory system. In addition the submuscular tissues of the body wall in Fasciola and the subepidermal tissues of Opisthioglyphe contained moderate amounts. Other studies have been made on nematodes, using the same technique. The predominant localization in Toxocara canis (Smyth and Hill, 1944; Smyth et al., 1945) and encysted larval Trichinella spiralis (Bullock, 1951) is within the intestinal cells. This is also true of Ascaris megalocephala, although the muscles and reproductive organs contain moderate amounts as shown quantitatively by Giroud and Rakota-Ratsimamanga (1936). Rogers (1945) located vitamin C in the body fluid of Ascaris lumbricoides.
Desoxyribose nucleic acid has been demonstrated in the nuclei of the cells of the "Embryonalkern" of *Macrocanthorhynchus hirudinaceus*, *Leptorhynchos thecatus*, and *Echinorhynchus coelegoni* (von Brand, 1940), and the nuclei of the embryo of *Capillaria hepatica* and *Onchocerca volvulus* (Lillie, 1947) by the Feulgen technique. Also, nuclei within the adults and embryos of *Schistosoma japonicum* and *S. mansoni* give positive reactions (Lillie, 1947). Ogren (1952) demonstrated the presence of ribose nucleic acid in the cytoplasm of the subcuticular cells, muscle cells, reproductive cells, uterine cells, and parenchymal cells of the adult *Mesocestoides variabilis* with methyl-green-pyronin staining. RNA was also demonstrated in the embryonic plastin, or germinative cells, of the onchosphere.

Smyth (1954) has reviewed the evidence, histochemical and otherwise, for the view that the egg-shell in trematodes, certain cestode groups, and in turbellarians, is a quinone-tanned protein secreted by the cells of the vitelline glands. The vitelline cells, in addition to giving positive reactions for proteins and phenols, give a strongly positive reaction for polyphenol oxidase, an essential constituent of a quinone-tanning system. *Fasciola hepatica*, *Haematoloechus* sp., *Dolichosacculus rastulus*, *Diciidophora merlangi*, *Schistocephalus solidus* and *Proteocephalus filicollis* were studied in this respect. Also, Monné and Borg (1954) concluded that the egg-shells of two other trematode species, *Tetrastes bonasia* and *Dicrocoelium lanceatum*, and several
nematode species, *Syngamus trachea*, *Passalurus ambiguus*, *Metastrongylus elongatus*, *Dictyocaulus viviparus*, and *Trichurus* spp., consisted of quinone-tanned proteins. These shells were Gram-positive and able to reduce the ammoniacal silver nitrate solution upon application of the Fontana method. Ascarid eggs which are generally considered to be chitin did not react in this manner.
MATERIALS AND METHODS

*Hymenolepis diminuta* and *Raillietina cesticillus* were raised in the laboratory in white rats and chickens, respectively. The male Sprague-Dawley rats used for *Hymenolepis* infections were given two cysticercoids generally 2 to 3 months, and not less than about three weeks, previous to their sacrifice for experiments. The one exception to the rule is the experiment with normal and starved rats which had been given ten cysticercoids each. Generally, both worms matured in the two cysticercoid infections. The rats were maintained on a diet of "Purina Laboratory Chow", and weighed between 300 and 500 gms. at sacrifice.

The laboratory hosts for *Raillietina* were White Rock chickens of mixed sexes, either 8 or 22 days of age at infection. The latter group was used exclusively for Sudan Black, acid-hematin, and succinic dehydrogenase tests, the former for alkaline glycerophosphatase and glycogen tests. They were maintained on a diet of standard commercial growing mash. The chickens were given 10 cysticercoids each, but the worms recovered from them after 19 to 37 days of infection varied from 2 to 9.

Only normal-appearing mature worms which showed evidence of apolysis were used in this study. Particularly in *Raillietina* infections it was necessary to choose the worms carefully, since the worms recovered from the chickens varied considerably in size and degree of maturation.
However, *Hymenolepis* infections did not present as much of a problem.

To prepare the animals for the castration studies, the testes were removed both before and after the administration of the cysticercoids of *Hymenolepis* to the rats.

In the starvation experiments, food was removed from the cages about 12:00 PM and the rats were sacrificed and the worms recovered after appropriate periods of starvation. The duration of starvation denoted in following discussions is accurate within one or two hours. No rats died, so cannibalism did not occur.

The various methods of fixation, staining, etc. that were used will be discussed along with the results obtained. In all cases except the succinic dehydrogenase technique, the worms were fixed by laying them outstretched on a strip of filter paper and applying the fixative to them with a medicine dropper. Then they were removed to a petri dish containing the fixing solution for an appropriate amount of time. Whenever possible, the histochemical treatment of the worm was begun within 5 or 10 minutes after removal from the gut. Tissues of both worms fixed in picroformalin-alcohol were stained with hematoxylin and eosin as a guide to the general morphology of the worms. Also, some sections stained with the periodic acid-Schiff method were counter-stained with hematoxylin to aid in the identification of structures. Sections of paraffin-blocked tissues were cut at 7μ, while those cut on the freezing-microtome
were 10\(\mu\) in thickness. The latter method required gelatin embedding.
RESULTS

GLYCOGEN:

The worms were fixed in picro-formalin-alcohol and embedded in paraffin. Sections were stained by the McManus' (1946) periodic acid-Schiff method, using the Schiff reagent of de Tomasi (1936) and covering the sections with celloidin before hydration. Control sections were extracted in several changes of saliva for 1/2 to 1 hour at room temperature, dehydrated and then carried through the staining procedure with the non-extracted sections. Some preliminary studies were done with the Bauer-Feulgen method (after Bensley, 1939).

Six Hymenolepis diminuta from normally-fed, intact rats were studied for glycogen distribution in the normal worm. Four of these worms were from rats given an initial dose of 10 cysticercoids, while two were from rats with two-worm infections. The distribution and relative amounts of glycogen seemed to be identical, regardless of whether the worm came from a ten-worm infection or a two-worm infection. Glycogen in the scolex was concentrated primarily in the medullary region, i.e. the region inside the ring of parenchymal musculature, although the cortical region contained a small amount (Plate 1). However, the glycogen concentrations in the medullary region were somewhat more irregular than in the region just behind the scolex where a more even medullary distribution occurred. Heavier deposits occurred in the sucker cup than elsewhere, particularly at the base
of the radial musculature. Toward the posterior end of the scolex where the many tiny osmoregulatory canals fuse to form four larger longitudinal canals, heavy concentrations of glycogen occurred adjacent to the inner walls of the two larger ventral canals.

In the immature region, i.e. the region of strobilization behind the scolex in which no differentiation of organ systems had taken place, the general pattern consisted of heavy deposits of glycogen in the medullary region, especially adjacent to the osmoregulatory canals, and only scattered deposits in the cortical region (Plate 2a). Although it was somewhat difficult to ascertain, it appeared that most of the glycogen storage was intracellular, large globules appearing in the parenchymal cells of the center of the proglottid. The glycogen globules decreased in size toward the periphery, the subcuticular layer containing only a very small amount of glycogen in the form of small scattered droplets. Throughout the parenchymal regions, scattered small droplets also occurred between the larger globules. The parenchymal muscles contained some glycogen as will be seen when the results of the starvation study are discussed, but because of the heavy concentrations in adjacent cells, they were difficult to distinguish. The subcuticular muscle layer, the cuticle, and the walls of the osmoregulatory canals appeared to be entirely glycogen-free. This was the general pattern throughout the tapeworm body, only the various organ systems modifying the
picture somewhat in more mature proglottids. In the region of developing organ systems and in the mature region, glycogen was not found in any of the organ systems except the vitellarium, although strands of parenchyma between the lobules of the ovary contained small deposits and the parenchyma immediately adjacent to the ovary contained large amounts (Plate 3a). When the vitellarium first appeared, it was completely free of glycogen, but as it matured small diffuse deposits of glycogen appeared in the cells and these deposits increased to a point at which the organ appeared mature and the general picture of the entire proglottid was that of maturity. In the mature proglottid and extending for some distance posteriorly a structure, thought to be the shell gland, located just dorsal to the vitellarium and consisting of a group of cells clustered about the oviduct, contained a PAS-positive material, not glycogen, diffusely distributed within the cytoplasm of the cells with particularly heavy concentrations adjacent to the lumen of the oviduct. The walls of the cirrus pouch, cirrus, ejaculatory duct, internal seminal vesicle and vagina appeared to contain no glycogen, while those of the sperm duct, vasa efferentia, seminal vesicle, seminal receptacle and seminal canal contained at least small amounts. The walls of the oviduct were glycogen-free, but the limiting membranes of the vitellarium, ovary and testes appeared to contain large amounts as did the sperm in both the seminal receptacle and seminal vesicle.
In ripening proglottids, the sperm continued to stain heavily, only losing their glycogen stores in the terminal stages of degeneration. In late mature proglottids, it was noticed that some sperm still within the testes stained for glycogen, but this was generally not the case in younger proglottids.

In the ripening proglottids, non-shelled ova in the uterine cavity contained only small amounts of glycogen at first, and this was located in the peripheral cells. More posteriorly (Plate 4a), a heavy layer of glycogen appeared about the embryos, and in the shelled eggs, this layer assumed a position between the embryo and the embryophore. Also, the area between the embryophore and the egg-shell appeared to contain some glycogen in a diffuse state, although some polysaccharide not extractable with saliva appeared to be present also.

On the whole, the glycogen picture in the two Raillietina cesticillus studied appeared to be similar in most respects to that in Hymenolepis, although several differences of some magnitude were noted. The general pattern of glycogen storage in the entire worm can be seen in the immature region (Plate 2b). It consisted of several zones of heavy glycogen deposition with areas of lesser concentration in between. The area from the cuticle to the base of the subcuticular cells had less glycogen than any other part of the worm, excluding certain organ systems, although there was a rather diffuse placement of small droplets of glycogen.
Central to this was an area of heavy deposition of large globules. This area corresponded in location to the inner portion of the cortical parenchyma and the outer portion of the medullary region, therefore, centering around the bands of longitudinal parenchymal musculature, although these could not be distinguished. The area just inside this band contained a rather diffuse distribution of smaller globules of the polysaccharide, showing less glycogen than adjacent regions but considerably more than the subcuticular region. Lastly, there was a high concentration of large globules of glycogen in the center of the medullary region adjacent to and between the osmoregulatory canals in the immature region of the worm and between the proglottids in the mature region. In the presence of the organ systems, the latter zone of concentration was manifested by heavy concentrations about the various organs. The subcuticular muscles could not be distinguished with certainty in the experimental preparations. However, in the controls it was evident that these, the parenchymal muscles, and certain areas in the center of the medullary parenchyma contained a diffuse distribution of polysaccharide other than glycogen. No glycogen was demonstrated in the walls of the osmoregulatory system or the cuticle. Here, as in Hymenolepis, the bulk of the glycogen storage appeared to be intracellular.

In the mature region of Raillietina, with the exception of the vitellarium, glycogen did not occur within any of the organs (Plate 3b). The vitellarium, when first evi-
dent, did not contain an appreciable amount of positive material, but as it matured it began to acquire glycogen in a diffuse state within the cytoplasm of the cells. The shell gland, as in *Hymenolepis*, stained a bright pink and the color reaction remained after saliva extraction. The positive material had an identical location within the cells as it was described for *Hymenolepis*. The walls of the vasa efferentia, sperm duct, seminal vesicle, vagina, seminal receptacle, and seminal canal did not contain glycogen, although the sperm contained within them stained heavily. The ejaculatory duct, cirrus, and cirrus sac stained a light pink and the color remained after extraction with saliva. The walls of the oviduct other than at the position of the shell gland did not stain. As the proglottids ripened, sperm still within the testes stained for glycogen to some extent, and as the various organ systems degenerated, the sperm which were contained within the persisting sperm tracts of both the male and female reproductive systems retained their glycogen. Eventually in the gravid proglottids, these organs disintegrated and the sperm were still PAS-positive although they were recognizable only by reference to preceding sections.

When the embryos first appeared in the uterus in the ripening proglottids, a small amount of glycogen was demonstrable in the yolk cells surrounding them. From this region posteriorly, the embryos gained in glycogen content. In the shelled eggs (Plate 4b), a heavy layer of glycogen
surrounded the embryo and the embryophore gave a positive reaction for this substance where it did not in *Hymenolepis*. Occasionally, large globules of glycogen occurred in the parenchymatous pouches external to the embryo. In the gravid region of the worm, large amounts of glycogen were concentrated around the parenchymatous pouches, thus obliterating the zoned aspect of parenchymal distribution described for the more anterior regions. The subcuticular zone, however, remained essentially the same. The control sections showed that much polysaccharide, other than glycogen, occurred in the walls of and within the lumen of the parenchymatous pouches, as well as within the egg. A small amount of similar non-glycogenous material occurred within the embryo, itself.

Three *Hymenolepis* from normally-fed castrate rats were studied for glycogen distribution. In general, they appeared to be very similar to those from normal hosts. However, three slight but consistent differences were noted. First, the parenchymal musculature appeared to contain less glycogen than normal, therefore being more easily distinguished in an area of heavy glycogen deposition in adjacent cells. This difference was not noted in the very anterior portion of the worm, but became evident in the mature region. More posteriorly the muscles became less distinguishable because the adjacent cells had apparently lost some glycogen. Secondly, there appeared to be more cortical glycogen than normal in all sections examined and this became more pro-
nounced in the posterior region of the worm. Lastly, small droplets of glycogen were scattered within the ovarian cells.

Two starvation experiments were carried out to determine the effect upon the glycogen stores in *Hymenolepis diminuta*. A total of 23 worms from 18 rats were examined, five from rats given initial doses of 10 cysticercoids, and 18 from rats with two-worm infections. The glycogen content of worms from rats with ten-worm infections starved for periods of 12, 24, 36, 48 and 60 hrs. showed a rapid decline in amount until the worms contained almost no parenchymal glycogen and few could be found remaining in the gut. This occurred at about 60 hrs. of starvation, only one worm being recovered at that time. Several trends in glycogen loss were noted in starved worms. Glycogen loss occurred quite rapidly in the anterior end and the area of glycogen depletion gradually progressed posteriorly until little parenchymal glycogen was demonstrable in the entire worm. The first evidence of glycogen loss appeared in the region lateral to the organ systems, although this region in normal worms also appeared to contain less of the polysaccharide than the regions dorsal and ventral to the organ systems. In those regions where almost no parenchymal glycogen remained, the little that was left was usually located in the center of the medullary region next to the organs, particularly the ovary. The greatest tenacity for glycogen seemed to exist in the parenchymal mus-
culature, which stood out more clearly as the peripheral regions of the medulla were depleted. They retained their glycogen even though all of the parenchymal glycogen had been lost. Also containing normal amounts of glycogen in advanced stages of starvation were the sperm and vitellarium (Plate 5a). In all cases where shelled eggs occurred in the uterus, the eggs appeared to contain a normal amount of glycogen as can be seen in Plate 5b. However, the gravid region had been lost from the worm removed from the rat starved 60 hrs.

Rats with two-worm infections starved for periods of 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168, 192, and 216 hrs. yielded worms which did not completely follow the pattern seen in the ten-worm starvation experiment. The general pattern was the same in the early stages, the worms showing a glycogen loss first in the anterior immature region and then progressing gradually to the posterior. Also, the vitellarium, sperm and muscles were extremely tenacious of their glycogen stores. At 36 hrs. of starvation, there was almost no glycogen present in the immature region, and at 48 hrs. this loss had extended to the mature region (Plate 5a), some areas showing none at all in the parenchyma. However, in the 60 hr. starved worm, the anterior end appeared to contain a normal amount of glycogen, both the anterior and posterior boundaries of the former area of glycogen depletion having moved posteriorly with a new low point in the region just behind the mature
proglottids. The gravid region of the worm still contained normal amounts of glycogen. A pattern of posterior movement of the depleted area and a posterior progression of the new glycogen stores in the anterior end characterized the 72, 84, and 96 hr. starved worms. However, in the 120 hr. starved worm, the glycogen content of the anterior end began to decrease again, and a pattern of four successive waves of glycogen concentration moving posteriorly down the length of the tapeworm characterized this and succeeding periods of starvation, the initial wave of depletion having reached the early gravid proglottids in the 168 and 192 hr. starved worms. After 216 hrs. of starvation, the glycogen content was noticeably decreased throughout, with almost no glycogen at all found in sections made from the region behind the mature proglottids to the very terminal gravid proglottid (Plate 5b). It is of interest to note that the one worm recovered from the rat initially infected with 10 cysticercoids and starved 60 hrs. also showed a slight increase in glycogen content in its immature region over that of the 48 hr. starved worm.

NEUTRAL FATS:

For demonstration of neutral fats, the worms were fixed in formal-calcium, sectioned on the freezing-microtome, and stained with Sudan Black B. Control sections were extracted in acetone at room temperature. One *Hymenolepis diminuta* from a normally-fed intact rat was studied for fat distribution in the normal worm. The immature region
of this worm contained only a very small amount of fat, this being distributed around the osmoregulatory canals and between them in the center of the medullary region. The number and size of fat globules increased in the region of developing organ systems and the mature region (Plate 6a), the newly appearing fat being generally confined within the outer ring of parenchymal musculature. There were heavier deposits in the center of the medullary region around the organ systems and the osmoregulatory canals. There were no deposits in any of the organ systems, although deposits in the interlobular parenchyma of the vitellarium and ovary occurred. The walls of the osmoregulatory canals and the male and female sperm tracts contained no significant amount of lipid, and the parenchymal muscles also were fat-free. More posteriorly, as the proglottids became more gravid, fat droplets also occurred between the bundles of parenchymal muscle and in the inner region of the cortical parenchyma. In the terminal proglottids, there was a considerable amount in the cortex although the subcuticular tissue rarely contained any. Heavy concentrations were noted adjacent to the uterus containing the eggs (Plate 7a). In the eggs (Plate 8a), fat droplets were located within or just beneath the limiting membrane of the embryo and a more or less diffusely positive reaction was given by the embryo itself. Also, finely granular lipid material was present in the membrane just beneath the egg shell. It could not be determined with certainty whether the fat droplets were locat-
ed intra- or extracellularly, but it is believed that at least some of the fat was contained within the cells. All positive material was extracted with acetone.

In Raillietina, the distribution of neutral fat was studied in one worm. The scolex was not examined but in the very immature regions just behind the scolex as well as more posteriorly, no fat could be demonstrated. As the organ systems began to develop, however, a small amount was accumulated as small globules in a zone just external to the parenchymal muscles. In the premature regions, this deposition occurred only in two parallel bands, one in the dorsal cortex and another in the ventral cortex. Very little occurred laterally. A few globules occurred in the medullary parenchyma, but not in the developing organs. As the proglottids became more mature a greater amount of deposition occurred, eventually connecting the two bands of lipid in the lateral areas of the proglottid (Plate 6b). Also, more fat occurred in the medullary region, but again not in any of the organs. Fat deposition was found to increase as the proglottids became more gravid, but the increase was primarily in the cortical ring of fat concentration. In the gravid region there appeared to be less medullary lipid, mainly because of the medullary location of the parenchymatous pouches containing the eggs (Plate 7b). Little fat could be demonstrated between the cortical ring and the parenchymatous pouches and rarely did any occur in the subcuticular region, although in the gravid proglottid
the cortical ring of concentration extended to the base of these cells. The eggs were very difficult to section and few remained on most of the preparations. Of those that remained, some showed a diffuse Sudan-positivity, others did not. No demonstrable fat occurred in the walls of the osmoregulatory or the various sperm tracts and associated structures. At least some of the fat is believed to be intracellular, although this was difficult to ascertain. Again, all positive material was extracted in acetone.

Four Hymenolepis from rats starved 72, 130, 168, and 216 hrs. were examined. Fat could not be demonstrated in the immature region of any of these worms, but otherwise no significant difference from the normal worm could be determined.

PHOSPHOLIPIDS:

The acid-hematin method (Baker, 1946) was used to study the distribution of phospholipids in two Hymenolepis and two Raillietina from normally-fed intact hosts. Controls were extracted by incubation in pyridine, and rat adrenals were carried through the procedure along with the worm tissues as an additional control. The adrenals gave the same staining reactions as obtained by others in this laboratory.

In Hymenolepis diminuta, characteristic reactions for phospholipid occurred in the subcuticular layer as numerous elongate oval or slightly irregular bodies with a small amount of diffuse positive material between them. In both cortical and medullary parenchyma, similarly-shaped masses
of positive substance were noted but their concentration and staining intensity did not approach those of the subcuticular layer. The walls of the osmoregulatory canals, as well as those of the seminal vesicles (both external and internal), ejaculatory duct, vagina, and seminal receptacle appeared to contain phospholipid. Neither the cirrus, ovary, nor the vitellarium gave positive reactions, although scattered cells in the testes evidently contained phospholipid (Plate 9a). The sperm did not stain, nor did the parenchymal muscles. In the gravid proglottid, some of the embryos occasionally stained intensely, but most of them did not. Sudan-positive material showed up as light blue globules with the acid-hematin method. However, the membrane beneath the egg-shell stained an even light blue color with no indication of the granular lipid deposits present in the Sudan-stained material (Plate 11). The subcuticular and parenchymal pattern did not change throughout the length of the worm as can be seen in plates 9a and 10a. Pyridine extracted material showed no positive reactions whatsoever.

In Raillietina, the distribution of phospholipid was somewhat similar. Very heavy concentrations occurred in the subcuticular cells, but not in the subcuticular muscles (Plates 8b and 9b). Also, there were scattered deposits in both the cortical and medullary parenchyma. In the gravid proglottid, the parenchymal concentrations were particularly evident and stained intensely in the medullary region ad-
The walls of the osmoregulatory canals gave a positive reaction, but the walls of both the female and male sperm tracts, as well as the ovary, vitellarium, and sperm, apparently did not contain phospholipid. Occasional cells in the testes gave positive reactions, however. No phospholipid could be demonstrated in the muscles. Conclusions could not be drawn on the eggs since few remained after sectioning.

**ALKALINE GLYCEROPHOSPHATASE**

A modification of the calcium-cobalt method of Gomori (1949) was used. The worms were fixed 24 hours in 80% ethanol at 4°C, dehydrated 24 hours in 95% ethanol at that temperature and absolute ethanol at room temperature for one hour, cleared in chloroform, and blocked in paraffin. The sections were deparaffinized in xylene, hydrated to water in ethanol, and incubated in a solution of 20 pts. 2% sodium $\beta$-glycerophosphate, 10 pts. of 2% calcium chloride, 20 pts. 2% sodium barbitol, and 50 pts. distilled water, for one hour at 37°C. After incubation, the sections were transferred directly to two changes of 1% calcium chloride, treated with 2% cobalt chloride, washed in distilled water, treated with a dilute (about 1%) solution of yellow ammonium sulfide, and washed under the tap for about 10 minutes. Dehydration and clearing were done in ethanol and xylene, but the slides were removed from the latter within 10 or 15 minutes since the color reaction fades. Controls were
carried through the same procedure, but the substrate in
the incubation medium was replaced with distilled water.
A total of three Hymenolepis and two Raillietina from nor-
mally-fed intact hosts were studied in this manner.

Throughout the length of both species the cuticle gave
a strongly positive reaction that was not evident in con-
trol sections. Plates 12a, 13a, and 14a illustrate the
distribution of alkaline glycerophosphatase activity in
the immature, mature, and gravid portions of Hymenolepis,
respectively. Although alkaline glycerophosphatase activity
was evident in the cytoplasm and nuclei of all cells to some
extent, slightly heavier concentrations were noted in the
subcuticular cells of the immature and mature regions of
Hymenolepis, particularly in the former. As the proglottids
matured, larger amounts of enzyme activity were concentrated
adjacent to the organ systems, particularly in the limiting
membranes of the testes and to a lesser extent the ovary and
vitellarium. The walls of the seminal vesicles, ejaculatory
duct, cirrus pouch, vagina, seminal receptacle, and seminal
canal showed considerable enzyme activity, while the sperm,
the parenchymal muscles, and the cells of the testes, vi-
tellarium, and ovary stained only slightly. The dorsal
osmoregulatory canal showed a large amount of enzyme activity
in its walls in the mature and more posterior regions, while
the walls of the ventral canal stained only slightly. Both
ventral and dorsal canal gave only slight reactions in the
immature region of the worm. In the gravid regions, the
heaviest parenchymal concentrations of the enzyme appeared in the medullary region adjacent to the walls of the uterus which stained heavily. The cells of the embryo, the embryo-phore, and the membrane just beneath the egg-shell gave moderate reactions for the enzyme, while the egg-shell stained heavily. Control sections of *Hymenolepis* showed almost no staining reaction.

In some respects, the distribution of the enzyme activity in *Raillietina* was similar to that in *Hymenolepis*, but several differences of some magnitude were noted (Plates 12b, 13b, and 14b). Here also there appeared to be some activity in all cells, and those in the anterior and mature regions gave stronger reactions than those in *Hymenolepis*. However, the posterior regions of *Raillietina* generally contained considerably less enzyme activity. The heaviest cellular concentrations in the mature region, as well as in more posterior regions, including the gravid proglottids, were in the subcuticular cells. In the mature region, as in *Hymenolepis*, and for some distance posteriorly, the medullary parenchyma adjacent to the organ systems contained heavy diffuse concentrations of alkaline glycerophosphatase, but neither the limiting membranes about the organs nor the walls of the male and female sperm tracts stained as distinctly. This is perhaps due to the more intense staining reaction of the parenchyma in the immature and mature proglottids. Also, the walls of neither osmoregulatory canal showed distinctly from the surrounding parenchyma.
In the gravid proglottid, the walls of the parenchymatous pouches stained but slightly and the diffuse heavy enzyme concentration noted adjacent to the uterus of *Hymenolepis* did not occur. Instead, the enzyme activity in the medullary parenchyma occurred in blotches between and around the parenchymatous pouches. In fact, the entire distribution of the heavier concentrations of enzyme activity in the gravid proglottid appeared to coincide almost exactly with the distribution of phospholipid in the worm. The eggs, however, stained in a manner identical to those of *Hymenolepis*. The numerous calcareous corpuscles stained heavily in experimental but not in control sections. Enzyme activity elsewhere in the controls was also reduced a considerable extent.

SUCCINIC DEHYDROGENASE:

For demonstration of this enzyme, a modification of the technique of Rutenburg, Wolman, and Seligman (1953) was used. Portions of the worm about one centimeter in length were placed in an incubation medium consisting of 10 ml. 0.2% neotetrazolium chloride, 10 ml. 0.2 M sodium succinate, 10 ml. 0.2 M phosphate buffer at pH 7.6, 0.2 ml. 0.33 M calcium chloride, 0.2 ml. 0.005 M magnesium sulfate, 2 ml. 0.6 M sodium bicarbonate, 0.8 ml. 0.01 M aluminum chloride, and 6.8 ml. distilled water, for 24 hours at 4°C. The incubation medium was identical to that of Rutenburg et al. except ditetrazolium chloride was replaced with the chloride salt of neotetrazolium. It was made partially anaerobic by
boiling the solution, placing it in a small flask set with a rubber stopper, and cooling it rapidly under the tap. After 24 hours in the medium at 4°C., the tissues were removed to a new flask of the solution at 37°C. and incubated for 2 hours. The tissues were then washed in 0.9% saline, fixed in formol-calcium, embedded in gelatin, and sectioned at 10μ on the freezing-microtome. The sections were mounted in glycerine-jelly and the slides stored in the dark, since the color reaction is reputed to fade in strong light. Control portions of the worm, incubated in medium in which the sodium succinate was replaced by distilled water, showed almost no reduction of the dye when examined microscopically. Three *Hymenolepis* and two *Raillietina* were studied for succinic dehydrogenase activity.

The general pattern of distribution was quite similar in both species. Succinic dehydrogenase activity was located intracellularly as small rods and spheres frequently clustered into groups. Generally, the largest concentrations occurred in the cortical and subcuticular regions, but more posteriorly the greater concentration became confined largely to the subcuticular layer and the peripheral layer of the cortex. In the gravid proglottids, only the subcuticular layer contained appreciable amounts of the enzyme activity. In *Hymenolepis*, very heavy concentrations occurred in a double ring of blotches corresponding in position to the double ring of parenchymal musculature. This condition was most pronounced in the immature region (Plate
15a), and became less and less pronounced posteriorly and was almost absent in the gravid region. In these latter regions, it could be seen that, although the double ring of blotches persist, the parenchymal muscles contained little, if any, of the enzyme. This double-ringed condition was present to some extent in *Raillietina* but was not as striking (Plate 15b). Elsewhere, than in the heavy cortical concentrations, the enzyme was rather diffusely distributed in the medullary parenchyma of both species with occasional heavier concentrations scattered here and there. The enzyme activity in the osmoregulatory canals appeared to be slightly heavier than in the surrounding parenchyma, although this condition was not as pronounced in the immature region of *Raillietina*. The ventral canal appeared to concentrate slightly more of the enzyme than the dorsal one. In the mature regions of both worms (Plates 16a and 16b), the walls of the male and female sperm tracts stained moderately well except for the cirrus which did not appear to contain appreciable amounts of activity. The sperm, also, did not stain well. In the region of the developing organ systems, the testes and ovary of both species stained heavily. However, except for scattered areas of enzyme concentration, the testes of *Hymenolepis* lost most of their staining reactions shortly after becoming mature. The opposite was observed in *Raillietina*, the ovary losing most of its staining qualities after becoming mature. These structures retained their heavy enzyme activity until degeneration of
the organs was well advanced. The vitellarium of both worms stained slightly more heavily than the surrounding parenchyma. In the gravid regions (Plates 17a and 17b), occasional heavy concentrations of enzyme activity bordered the walls of the uterus and those of the parenchymatous pouches. In the eggs of both species, the enzyme was located only in the membrane just beneath the egg-shell (Plate 18).
DISCUSSION

The distribution of glycogen in *Raillietina cesticillus* and *Hymenolepis diminuta* from normal hosts agrees generally with histochemical studies of other cestodes. Both of these worms appeared to contain moderate amounts in the vitellarium and large amounts in the eggs. In this respect, there is a considerable variation within the cestodes in general. The main difference between the two species appeared to be in the non-glycogenous polysaccharides, *Raillietina* containing, in contrast to *Hymenolepis*, considerable amounts in the parenchymal and subcuticular musculature, the medullary parenchyma, and the walls of the sperm tracts. In both worms, a considerable amount of reserve material evidently is wasted, since the terminal gravid proglottids contain considerable amounts of glycogen, which is evidently not available to the embryos.

The most important storage place for glycogen in both worms was the medullary parenchyma. Organs other than the vitellarium contained no demonstrable polysaccharide. Thus it seems fairly clear that the parenchymal stores probably serve as reserve food materials that can be readily drawn upon by the various organs. The heaviest deposition of glycogen occurred immediately adjacent to the organ systems, particularly the ovary which, although large, is multilobular in structure, possibly as an adaptation to the mobilization of nutrient substances from the parenchyma.
The sperm characteristically contained large amounts of glycogen, and their stores did not diminish significantly during starvation or aging within the seminal receptacle or vesicle. These heavy glycogen stores are possibly correlated with the strenuous flagellar activity probably necessary to travel up the female sperm tract to the ova. There is, in addition, the possibility that the sperm may contribute significantly to the glycogen stores of the fertilized ovum. A low metabolic rate in the sperm within the vesicles and receptacle would explain the persistence of their glycogen stores in the nearly gravid proglottids and in the starved worm.

During starvation in *Hymenolepis*, a normal amount of glycogen was found within the sperm and the cells of the vitellarium. Thus it appears that the reproductive processes are put foremost in the starved worm, both the testes and vitellarium utilizing a normal amount of carbohydrate possibly at the expense of the parenchymal stores of glycogen. All the shelled eggs, even in proglottids almost entirely depleted of parenchymal glycogen, contained a normal amount of the polysaccharide. In addition to the reproductive structures, the parenchymal muscles seem to retain their glycogen at the expense of the surrounding parenchyma. On the other hand, it is possible that glycogen is present in these structures in a form that is not readily metabolized. Kent and Macheboeuf (1947) isolated protein-glycogen complexes from *Moniezia expansa* and it is possible
that similar substances may occur in the parenchymal muscles of *Hymenolepis*. However, no pretreatment with proteolytic enzymes was necessary to extract the glycogen with saliva. In *Raillietina*, however, a polysaccharide non-extractable with saliva was found in the parenchymal and subcuticular muscles.

The effect of starvation on the glycogen content of *Raillietina cesticillus* and *Dibothriocephalus latus* has been studied quantitatively by Reid (1942) and Markov (1939), respectively, and by Daugherty (1955b, 1956) and Daugherty and Taylor (1956) in *Hymenolepis diminuta*. All found that the glycogen content decreased sharply in worms from starved animals. Daugherty (1955b) and Daugherty and Taylor (1956) analyzed separate 10 cm. regions of *H. diminuta* from both normal and starved rats for glycogen content. They also found that the anterior region of the worm decreased most rapidly in glycogen content during starvation, while the decrease in more posterior regions was less pronounced. It was further noted that in worms from starved rats, no significant change in glucose synthesis could be demonstrated *in vitro* (Daugherty, 1956a). This would indicate that not only do the vitellarium, testes, and perhaps the parenchymal muscles draw upon the glycogen stores of the starved tapeworm, but a constant percentage of the glycogen remaining would be converted to glucose according to the principle of mass action. A higher metabolic rate of the cells in the anterior regions of the tapeworm would explain the anterior
to posterior progression of glycogen depletion noted in this study. That such a metabolic gradient does occur has been shown by the higher rate of oxygen uptake in the anterior regions of *H. diminuta* (Daugherty; unpublished research). Also, the marked increase in the glycogen content of the anterior region of the worm over that of more posterior regions in alloxan-treated starved rats (Daugherty and Taylor, 1956) may be correlated with a higher metabolic level of activity in the anterior regions.

Although Daugherty (1955b) and Daugherty and Taylor (1956) also used two worm *Hymenolepis* infections in starved rats for their studies, they were seldom able to recover worms from rats starved over 100 hrs., and did not quantitatively demonstrate the recovery phenomenon of new glycogen storage in the anterior part of the worm. During the course of this study, a total of 21 worms were recovered from 13 rats starved over 100 hrs., and four were recovered from two rats starved 216 hrs. Those studied for glycogen content showed the increase in stored material in the immature and mature regions over that of the worms starved for shorter periods. It is possible that the tapeworm is able to adapt itself to the utilization of a new type of nutrient not capable of being metabolized in normal hosts, or a new type of nutrient not before available is utilized. This would be in agreement with earlier findings in this laboratory (Daugherty, 1955a) which showed that the enzyme mechanisms of *H. diminuta* can be altered to suit the availability of
substrate, glutamic dehydrogenase activity being significantly higher than normal when the rat hosts were fed on a high-protein diet. Further studies, particularly histochemical tests for alkaline phosphatase and phosphorylase activity, on starved worms may yield interesting results.

That the physiology of the rat tapeworm is significantly altered by castration of the host has been shown by Addis (1946), Beck (1952), Aldrich et al. (1954), and Daugherty (1956). The stunting effect noted by Addis (1946) and the decrease in egg production noted by Beck (1952) were counteracted by injections of testosterone. A reduction in transaminase activity and an increase in fat content was noted by Aldrich et al. (1954). Also, castration of the host caused a decline in the rate of glycogen synthesis from both glucose and pyruvate (Daugherty, 1956). In this study, H. diminuta from castrate hosts contained less glycogen in the muscles and more in the cortical region than normal, and even glycogen storage in the ovary was noted. No explanation for these differences is apparent at present.

The distribution of Sudan-positive material in the two worms studied was in general agreement with the findings of earlier workers, although no lipid droplets were observed in the osmoregulatory system (Coutelen, 1931, and von Brand, 1933, in Echinococcus granulosus and Moniezia expansa). Since the phospholipids demonstrated with the acid-hematin test did not correspond to the distribution of Sudan-positive material, it was concluded that the Sudan-positive droplets
probably consisted of neutral fats, and perhaps cholesterol. No attempt was made to demonstrate the latter. The only difference in the distribution of neutral fats in the two worms studied was in the placement of the heaviest concentrations in the parenchyma. *Hymenolepis* contained most of the fat in the medullary parenchyma, while *Raillietina* showed a ring of concentration just peripheral to the parenchymal muscles.

The neutral fats of these tapeworms can probably be regarded as end-products of carbohydrate metabolism. Some of the evidence for the excretion of fatty substances by cestodes has been summarized in the review. Further evidence that this is the case in *Hymenolepis diminuta* is afforded by this study and quantitative studies (Warren: unpublished research) which show that there is no demonstrable decrease in fat content during starvation. Read (1952b) could attribute only a small amount of the carbohydrate used by *H. diminuta* in *vitro* to acetic acid production either under aerobic or anaerobic conditions. This observation would be in accord with the idea that neutral fats are the end-products of metabolism in cestodes since acetic acid in such a system would be only an intermediate step in the synthesis of the higher fatty acids from which neutral fats are synthesized. Acetic acid, itself, would not be excreted nor accumulated in large amounts.

No references to the histochemical demonstration of phospholipids in cestodes could be found and the only reference to their distribution in helminths was Bullock's
(1949b) work on several species of Acanthocephala. In the present study the major region of phospholipid accumulation in both worms occurred in the subcuticular cells. The major difference between the two worms was seen in the medullary parenchyma of the gravid proglottids, where the distribution of the substance, in darkly staining blotches, in Raillietina was much more marked than in a comparable area of Hymenolepis.

Little is known about the function of phospholipids. Baldwin (1952) correlates the increase in the phospholipid content of the intestinal lymph and blood of mammals after a fatty meal with the absorption of fatty acids and cholesterol and subsequent formation of phospholipids. Whether the same phenomenon is important in cestodes is difficult to say.

The results obtained by the Gomori method for alkaline glycerophosphatase agree very closely with the majority of former studies on cestodes. The cuticle stained most intensely in both worms studied. Subcuticular concentrations and concentrations of enzyme activity in the medullary parenchyma also characterize both worms. However, only in Hymenolepis did the limiting membranes of the various organ systems and the walls of the dorsal osmoregulatory canal stain distinctly. The gravid regions of the two worms, however, were considerably different, the cells with the highest enzyme activity occurring adjacent to and within the walls of the uterus of Hymenolepis, while the cellular area that stained most intensely in Raillietina was located just beneath the cuticle, the medullary parenchyma and the walls
of the parenchymatous pouches staining only slightly. Lefevere (1952), who also studied *Raillietina cesticillus*, noted very light staining reactions about the parenchymatous pouches. From his figures and description, it appears that he obtained a much lighter staining reaction in general than was obtained in this study. He does not mention the staining reactions of the osmoregulatory canals, although Yamao (1952c) found concentrations of enzyme activity in these structures in all but one of the species he studied (see review). Protoplasmic extensions of the subcuticular cells into the cuticle as described by Wardle and McLeod (1952) may contain high concentrations of the enzyme and result in the heavy cuticular staining.

The function of phosphatases in the animal body does not appear to be well understood. Danielli (1953) reviews the evidence for correlation of the distribution of alkaline phosphatase with certain sites of physiological activity, namely in the differentiation of tissues, the secretion of glandular cells, the action of contractile proteins, and at sites of excretion or absorption. The major correlation could possibly be made with phosphorylase activity, since, in the author's opinion, a high degree of phosphorylative processes would necessarily accompany these phenomena. With little known about the function of the enzyme in mammalian tissues, any correlations made about its presence in invertebrate tissues would be entirely speculative. However, the distribution of heavy enzyme concentrations in the
subcuticular cells and in the medullary parenchyma adjacent to the glycogen-free organs would suggest a correlation in the former case with absorption of nutrient materials from the environment into the worm, and in the latter, with a passage of nutrient material into the organ systems. From this same point of view, the heavy staining of the walls of the dorsal osmoregulatory canals might be correlated with a passage of materials to or from the parenchymal tissues. The gravid regions of the two species were quite different in enzyme activity. The relation between the high activity adjacent to the uterus of Hymenolepis and the low activity in this region of Raillietina is hard to reconcile. It is interesting to note, however, that the distribution of the enzyme concentrations in the gravid region of the latter was almost identical to the distribution of phospholipid in that region, although the medullary blotches of activity did not stain darkly. Additional studies with the histochemical technique for phosphorylase developed by Goldberg et al. (1952) may throw additional light on the subject.

As far as the author is aware, no previous histochemical studies of succinic dehydrogenase in endoparasites have been made. The staining reactions of both worms were very similar. Both contained high concentrations of the enzyme activity in the peripheral regions of the worm, with a progressive decrease in the thickness of the peripheral layer of concentration from anterior to gravid regions of the worms. Several explanations for this can be postulated on
the basis of existing information. A simple surface-volume ratio will explain the progressive decrease in the zone of enzyme activity from anterior to posterior regions, a relatively greater surface of the immature regions being subject to the diffusion and, therefore, availability of oxygen within the worm. That this may not be the only limiting factor in the availability of oxygen to the tissues is evident from the work of Daugherty (unpublished research) who showed that the uptake of radioactive methionine in the anterior regions of the worm is significantly greater than in the more posterior regions, indicating a greater permeability in the anterior end. The heavy concentrations within the cells of the ovary and testes are more difficult to explain, although it will be noted that these structures have access to the environment indirectly through the various ducts of the reproductive system. The reasons for the different staining reactions of these two organs in the two species can not be ascertained at present. In both worms considerable enzyme activity was apparent in the membrane just beneath the egg-shell, but nowhere else in the egg. This may manifest a pre-adaptation of the egg for an aerobic existance outside the host or within the hemocoele of the arthropod intermediate host.

The enzyme does not appear to be inhibited significantly in an incubation medium with all the ingredients plus sodium malonate in the same concentration as the sodium succinate. This observation does not conform to those of Read (1952a)
using cell-free homogenates of *Hymenolepis diminuta* and Goldberg and Nolf (1954) who studied the reduction of 2,3,5-triphenyltetrazolium chloride and methylene blue *in vitro* by *H. nana*. However, this observation is supported by Daugherty (unpublished research) who found that the addition of sodium succinate to the incubation medium increased the uptake of radioactive methionine by significant amounts in *H. diminuta in vitro*, and the addition of sodium malonate to this fortified medium did not diminish the effect of the succinate.

Considerable improvement over the earlier techniques for the demonstration of succinic dehydrogenase activity were noted. Previous techniques consisted of incubating sections in the medium. This generally resulted in rather patchy distribution of the staining reaction (Seligman and Rutenburg, 1951; Padykula, 1952; Rutenburg et al., 1953), probably due to the diffusion of the enzyme into the medium from the cut ends of cells, since it was noticed that the color reaction was more uniform in thicker sections (30-40μ). The incubation of larger portions of tissues whenever possible eliminates this difficulty. The reaction, wherever it occurred in the tapeworm tissues, showed uniform distribution and, as evidenced by the staining of various organ systems and the eggs, penetration of the dye was not a problem. These earlier workers noted a red staining reaction in structures rich in lipid material. Similarly, the Sudan-positive material of tapeworm tissues stained a red color.
A comparative histochemical study of Raillietina cesticillus and Hymenolepis diminuta has been made. Determination of the content and distribution of glycogen, neutral fats, phospholipids, alkaline glycerophosphatase and succinic dehydrogenase in the worms showed that the two species are similar in most respects although some differences do exist.

The periodic acid-Schiff method for glycogen, with saliva-extracted controls, showed that both worms store large amounts of the polysaccharide in the parenchyma, primarily in the medullary region adjacent to the osmoregulatory canals and the organ systems. The sperm, also, gave a positive reaction. Except for the vitellarium, which contained glycogen when mature, the organ systems were entirely free of polysaccharide.

Staining frozen sections with Sudan Black B demonstrated a constant, though slight, increase in neutral fats from immature to gravid regions, the former containing little or none at all. In Hymenolepis the distribution of the lipid material was primarily within the medullary parenchyma, while in Raillietina, the greatest concentrations occurred in a ring just peripheral to the ring of parenchymal musculature.

The distribution of phospholipid in the immature and mature regions of both worms was very similar, the heaviest concentrations being located in the subcuticular cells.
However, in the posterior regions, *Raillietina* showed heavy blotches of phospholipid material about the parenchymatous pouches, while *Hymenolepis* showed only slight positive reactions in this region. Baker's acid-hematin method was used.

Evidence of alkaline glycerophosphatase activity, demonstrated by a modification of the Gomori method, was found in both species. Throughout the length of the worms, the cuticle gave the strongest reaction. Also, concentrations of activity occurred in the subcuticular layer in the mature and immature regions, and in the medullary parenchyma of the mature region and early gravid proglottids of *Raillietina*, and both mature and gravid proglottids of *Hymenolepis*.

Succinic dehydrogenase activity was demonstrated by a modification of the technique of Rutenberg, Wolman and Seligman (1953). It appeared to have similar sites of activity in both species. The subcuticular layers contained the heaviest concentrations although heavy concentrations within the developing ovary and testes occurred. In mature and more posterior regions, the ovary of *Raillietina* and the testes of *Hymenolepis* lost most of their staining reactions. In addition, the membrane just beneath the eggshell gave a positive reaction.

In addition, *Hymenolepis diminuta* from starved and castrate hosts were studied. During starvation of rats with ten-worm infections, there was a steady decline in glycogen
within the worms, terminating with their loss from the gut at about 60 hours. However, in two worm infections, a recovery phenomenon was noted in which the glycogen content of the immature and mature regions increased after the initial 60 hours of starvation and the worms were recovered from the gut of the host as long as 216 hours after the rats had been removed from food. The parenchymal muscles, sperm, and vitellarium retained their glycogen stores during starvation and the shelled eggs in the uterus appeared to contain normal amounts. Worms removed from castrate hosts showed less glycogen in the parenchymal muscles and more in the cortical region than normal. Also, small droplets of glycogen were noted in the ovarian cells. No decrease in fat content was observed in *H. diminuta* from starved rats.

Various factors which may contribute to the distribution of these substances in tapeworms are discussed.

A comprehensive review of the distribution of certain organic substances within the bodies of parasitic protozoa and helminths is included as a guide to further studies of this sort.
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PLATES
A section through the scolex of *Hymenolepis diminuta* showing the pattern of glycogen distribution and particularly the heavy concentrations at the base of the radial musculature in the suckers. Periodic acid-Schiff method. 450x
a. Transverse section through the immature region of *Hymenolepis diminuta* showing the general pattern of glycogen distribution. Periodic acid-Schiff method. 100x

b. Transverse section through the immature region of *Raillietina cesticillus* showing the general pattern of glycogen distribution. Periodic acid-Schiff method. 100x
a. Transverse sections through a mature proglottid of *Hymenolepis diminuta* showing heavy concentrations of glycogen in the parenchyma and sperm, but none at all in the ovary and testes. Periodic acid-Schiff method. 35x

b. A transverse section through a mature proglottid of *Raillietina cesticillus* showing heavy glycogen storage in the parenchyma and none at all in the organs other than the vitellarium (center). Just dorsal to the vitellarium, the oviduct at the position of the shell gland can be seen. Periodic acid-Schiff method. 20x
a. A transverse section through the gravid region of *Hymenolepis diminuta* demonstrating the general parenchymal distribution of glycogen and its presence within the eggs. Period acid-Schiff method. 35x.

b. A transverse section through the gravid region of *Raillietina cesticillus* showing the heavy glycogen deposits in the parenchyma and in the eggs. Periodic acid-Schiff method. 20x.
a. A transverse section through the mature region of *Hymenolepis diminuta* from a rat starved 48 hrs. Note retention of glycogen by sperm and parenchymal muscles. Periodic acid-Schiff method. 100x.

b. A transverse section of the gravid region of *Hymenolepis diminuta* from a rat starved 216 hrs. Note large amount of glycogen in eggs although the parenchyma is nearly glycogen-free. Periodic acid-Schiff method. 100x.
PLATE 6.

a. A transverse section through a mature proglottid of *Hymenolepis diminuta* showing the medullary distribution of fatty substances. Sudan Black. 20x

b. A transverse section through the mature region of *Raillietina cesticillus* showing a cortical band of fat concentration. Sudan Black. 100x.
a. The distribution of fatty substances in the gravid region of *Hymenolepis diminuta* as seen in transverse section. Sudan Black. 100x.

b. Transverse section through gravid proglottid of *Haillietina cesticillus* showing ring of fat deposition in the cortical parenchyma. Sudan Black. 35x.
a. Sectioned eggs of *Hymenolepis diminuta* showing lipid deposits either within the embryo or immediately adjacent to it, and finely granular fatty material in the membrane just beneath the egg-shell. Sudan Black. 450x.

b. Transverse section through the immature region of *Raillietina cesticillus* showing the heavy subcuticular concentrations of phospholipid. Acid-hematin method. 100x.
PLATE 9.

a. A transverse section of the mature region of Hymenolepis diminuta showing globular deposits of phospholipid in the subcuticular cells and scattered deposits in the parenchyma. Also, phospholipid material in certain cells of the testes and the walls of the osmoregulatory canals shows well in the photograph. Acid-hematin method. 80x.

b. A transverse section of a mature proglottid of Raillietina cesticillus showing phospholipid concentrations in the subcuticular cells, certain cells of the testes, and the walls of the osmoregulatory canals. Acid-hematin method. 100x.
PLATE 10.

a. A transverse section of the gravid region of *Hymenolepis diminuta* showing phospholipid deposits (black) in the subcuticular layer and adjacent to the uterus. Sudan-positive material (neutral fats) shows as grey deposits. Acid-hematin method. 100x.

b. A transverse section through the gravid region of *Raillietina cesticillus* showing heavy subcuticular and medullary concentrations of phospholipid. Acid-hematin method. 100x.
Sectioned eggs of *Hymenolepis diminuta* showing the staining reaction (light gray) of the membrane beneath the eggshell. Acid-hematin method. 450x.
a. A transverse section through the immature region of *Hymenolepis diminuta* showing concentrations of alkaline glycerophosphatase activity in the cuticle and subcuticular cells. Modified Gomori method. 100x.

b. A transverse section through the immature region of *Raillietina cesticillus* showing evidence of alkaline glycerophosphatase activity in the cuticle, parenchyma, and calcareous corpuscles. Modified Gomori method. 100x.
a. A transverse section through a mature proglottid of *Hymenolepis diminuta* showing concentrations of alkaline glycerophosphatase activity in the cuticle, limiting membranes of the testes and, to a lesser extent, those of other organs, and in the walls of the dorsal osmoregulatory canals. Modified Gomori method. 60x.

b. A transverse section through the mature region of *Raillietina cesticillus* showing alkaline glycerophosphatase concentrations in the cuticle, calcareous corpuscles, and medullary parenchyma adjacent to the organ systems. Modified Gomori method. 100x.
a. A transverse section through a gravid proglottid of *Hymenolepis diminuta* showing alkaline glycerophosphatase activity concentrated in the cuticle, walls of the uterus and adjacent parenchyma, and in the egg-shell. Modified Gomori method. 20x.

b. A transverse section through the gravid region of *Raillietina cesticillus* showing concentrations of alkaline glycerophosphatase activity in the cuticle, subcuticular cells, and egg-shells. Modified Gomori method. 35x.
a. A transverse section through the immature region of *Hymenolepis diminuta* showing the localization of succinic dehydrogenase activity. A modification of the technique of Rutenburg et al. (1953). 100x.

b. A transverse section through the immature region of *Raillietina cesticillus* showing the localization of succinic dehydrogenase activity. A modification of the technique of Rutenburg et al. (1953). 100x.
a. A transverse section through a mature proglottid of *Hymenolepis diminuta* showing concentrations of succinic dehydrogenase activity, particularly in the cortical and subcuticular layers, the ovary, and occasional cells of the testes. A modification of the technique of Rutenburg et al. (1953). 20x.

b. A transverse section through a mature proglottid of *Raillietina cesticillus* showing concentrations of succinic dehydrogenase activity in the cortical and subcuticular layers, and in the testes. A modification of the technique of Rutenburg et al. (1953). 100x.
a. A transverse section through the gravid region of *Hymenolepis diminuta* showing concentrations of succinic dehydrogenase activity in the subcuticular layer and in the eggs. A modification of the technique of Rutenburg et al. (1953). 100x.

b. A transverse section through the gravid region of *Haillietina cesticillus* showing concentrations of succinic dehydrogenase activity in the subcuticular layer and in the eggs. A modification of the technique of Rutenburg et al. (1953). 100x.
Sectioned eggs of *Hymenolepis diminuta* showing concentrations of succinic dehydrogenase activity in the membrane just beneath the egg-shell. A modification of the technique of Rutenburg et al. (1953). 450x.