MUCOSUBSTANCES IN PLAGIORCHOID AND MONOSTOMATE CERCARIAE (TREMATODA: DIGENEA)

FRANCIS J. KRUIDENIER AND KRISHNA N. MEHRA
University of Illinois, Urbana

INTRODUCTION

The development, distribution, and function of a series of glands, highly metachromatic to thionin and toluidine blue O, have been described from the cercariae of various species of digenetic trematodes. The widespread occurrence of the glands indicates their importance in the economy of the parasites. The glands appear in all groups of xiphidiocercariae studied (Kruidenier, 1951, 1953) and in the notocotylid (monostome) cercariae (Kruidenier, 1953a).

Variation in the shape, distribution, and metachromasia of the glands was reported. Glands of the xiphidiocercariae were variably compact to diffuse in closely related species of cercariae. They were ventrally located and highly chromophilic. Glands of the notocotylids were dorsal to dorsolateral and varied from slightly irregular in *Cercariae Nudacotyle novicia* Barker, 1916, to pleomorphic but generally stellate in *C. urbanensis* Cort, 1914 (see Kruidenier, 1953a). The metachromasia of the notocotylids was less stable in the presence of alcohol than was that of analogous glands in the xiphidiocercariae.

Preliminary attempts by the senior investigator to demonstrate metachromatic glands in the cercaria of *Macravestibulum eversum* Hsü (Pronocephalidae) were unsuccessful. Further study resulted in their discovery. The glands are apparently not described for any of the pronocephalid cercariae. Preliminary observations demonstrated that the glands differed from those of xiphidiocercariae and the notocotylid species.

ACKNOWLEDGMENTS

This investigation was supported in part by a research grant (E-1319) from the National Institute of Health, Public Health Service.

MATERIALS

Two species of virgulate cercariae, *C. geddesi* Ameel, 1939, from *Pomotiopsis lapidaria* (Say), and an undescribed species from *Goniobasis livescens* Menke included in the earlier study of virgulate cercariae were used in this investigation. One ornate species, *C. Macroderoides typicus* (Winfield, 1929) Van Cleave, 1932, from *Helisoma trivolvis* (Say), was used. An undescribed microcotylous species from *G. livescens*, also reported previously, was obtained. *C. urbanensis* Cort, 1914, from *Physa sayii* (Tappan), and *C. Macravestibulum eversum* Hsü, 1937, from *G.*

livescens Menke represented notocotylid and pronocephalid monostomes, respectively. All the above species were collected near Ann Arbor, Michigan. One armate species, tentatively identified as *C. Tetrapapillatrema concavocorpa* Sizemore, 1936, was obtained from *Helisoma trivolvis* (Say) collected in Urbana, Illinois.

Methods

Serial sections, of infected snails killed and fixed entire in Bouin's fluid, were used. Whole mounts were made of developing cercariae dissected from snails and fixed in corrosive sublimate. Serial sections and whole mounts were made of normally emerged cercariae fixed in corrosive sublimate and in alcohol-formalin. Bouin-fixed sections were occasionally mordanted in $HgCl_2$ for specific test purposes.

Metachromasia of glandular secretions was tested in 0.001 toluidine blue 0 (Allied Chemical and Dye Corporation, C.I. No. 925) buffered to pH values of from 1 to 6.4 with acetate buffer solutions. A model G Beckman pH meter was used. Sections were brought to water, placed in the appropriate buffered dye solutions for one hour, drained or blotted, and then rinsed for exactly five seconds in absolute ethyl alcohol and then cleared in xylol and mounted in H and R medium.

Metachromasia in alcohol was tested by the above procedure except that test solutions consisted of 0.001 toluidine blue 0 in 25%, 50%, 75% and absolute ethyl alcohol in distilled water at pH 6.5 to 7.

The Schiff reaction was performed on sections without prior oxidation (controls) and following oxidation with periodic (PAS) peracetic and chromic acids (Bauer) or with potassium permanganate (Casella). Sections were exposed to pyridine at 25°C. for 24 hours and to malt diastase at 40°C. for 1 hour before testing with periodic acid-Schiff procedures (PAS).

Every attempt was made to standardize the timing of the steps of the Schiff procedure. Exposure to leucofuchsin reagent was 15 minutes. Fresh solutions of oxidizer, iodide-thiosulfate reducing bath, and sulfite wash were used for each test. Sections were rinsed in the sulfite wash and then passed through a minimum of three successive changes for a total of six minutes. Critical test sections were washed thoroughly in running tap water before rapid dehydration. Fresh alcohols were used to prevent the leucofuchsin, which leaches into the solutions, from producing false reactions. Haematoxylin, fast green and, occasionally, orthochromatic methylene blue or toluidine blue (in absolute alcohol) were used as counter stains.

Whole mounts were prepared with dilute, aqueous thionin at approximate neutrality or by means of PAS procedure.

Mercury was removed with iodine solutions where necessary.

Observations

Virgulate xiphidiocercariae: Metachromasia in *C. geddesi* (Fig. 1) has not been described previously. It is similar to that of the other virgulates reported (Kruidenier, 1951). Present studies thus are supplementary.

Six pairs of metachromatic glands are visible in the developing, intraradial cercariae as the tail stem, oral sucker and ventral sucker differentiate. Glands reach maximal development when cercariae attain maturity within the rediae. Secretions are discrete droplets in young glands but coalesce into a single mass as they accumulate. The glands are aligned ventrally, on either side of the mid-line with three pairs anterior to the acetabulum, one pair at that level, and two pairs posterior to it.

Ducts from the anterior four glands have been traced to the level of the buccal cavity and from the third pair to the posterior border of the oral sucker. The fourth pair of ducts was traced mid-way between the suckers, and those from the posterior glands discharge on either side of the tail stem.

All glands discharge as cercariae reach maturity within the rediae. The virgula forms concurrently in the oral sucker and the periphery of the cercarial bodies become strongly metachromatic. Their tails are not metachromatic at any time. Small remnants of secretion remain in the glands and are visible in normally emerged cercariae (Fig. 1). Metachromatic layers are heaviest in areas adjacent to the pores of discharge.

The virgula resembles one of those previously described (Kruidenier, 1951:681, Fig. 20), appearing as swollen tubes coiled around the buccal cavity. Paired ducts open into the buccal cavity mid-way between the oral aperture and the esophagus. Strands of secretion commonly extend from the pores, convoluting extensively beyond the mouth. The adhesion of debris demonstrates the sticky nature of the strands. Appreciable loss in size during migration within the snails and after emergence attests to the constant discharge of virgular contents, but virgulae never discharge completely from free-living cercariae. Virgulae were not observed during cercarial penetration of second intermediate hosts.

A distinct cuticula is present prior to the discharge of metachromatic glands and the concurrent appearance of peripheral metachromasia.

The large ventral glands and the virgula show strong gamma metachromasy after treatment with toluidine blue at pH 1, and peripheral metachromasia is identical as it appears. Metachromasy increases to maximal violet to blue-black intensity with increased basicity of dye solutions. No other structures within the cercariae or rediae are metachromatic at the test levels and, at pH 1, only mucosubstances are chromophilic; all other tissues, cercarial and snail, remain unstained. Metachromasia is strong in 75% alcohol solutions of toluidine blue and roughly parallels that observed at high acid levels. Epithelial mucoid of the snail is metachromatic at these levels also and affords constant controls for many of the studies. Chromophilia of parasites and snail tissues increases with the basicity of the dye solutions. It becomes intense at pH 6.4 and in the absence of alcohol.

A very faint pink reticulum is variably present in the metachromatic glands and virgulae of some specimens treated with leucofuchsin after PAS, Bauer, and Casella procedures. The erratic reaction does

not intensify with 45 minutes of exposure to the leucofuchsin reagent. The counterstains used obscure all traces of the reaction. Hematoxylins stain the mucosubstances deeply.

Malt diastase or pyridine digestion do not affect the reactions. The activity of virgulae and peripheries remains identical after the emergence of the cercariae from snail tissues.

The formation and function of mucosubstances in the undescribed virgulate cercaria from *Goniobasis livescens* used in these studies have been detailed (Kruidenier, 1951: 680, Figs. 1-11; and 682, Figs. 28-32). The metachromatic and Schiff reactivity of ventral glands, virgulae and peripheral substances parallels that of the analogues of *C. geddesi* (*v. supra*).

NON-VIRGULATE XIPHIDIOCERCARIAE: *Armate.*—Metachromatic substances have not previously been described in the cercariae of *Tetrapapillatrema concavocorpa* Sizemore, 1936 (Fig. 4). This species generally resembles *C. isocotylea* (Cort, 1914), studied less intensively by Kruidenier (1953).

Six large glands develop on either side of the mid-ventral line and distribute as described for the analogues in *C. geddesi* (Figs. 2, 3). Glands first appear as diffuse metachromatic areas and attain maturity as cercariae reach maximal intraredial development. They do not branch profusely but do develop lateral processes which terminate in small temporary lateral reservoirs (Fig. 3). The ducts appear similar to those of *C. isocotylea* (Kruidenier, 1953). All of the glands discharge prior to cercarial emergence from rediae. Concurrently, caudal pockets enlarge and fill with secretions; the peripheries of the cercariae become highly metachromatic (*v. infra*). A cuticula is distinct around the cercariae before the glands discharge and peripheral metachromasia is acquired. The supracuticular layer is distinctly thicker at the extremities of the body. It is heaviest within the caudal pocket, although large deposits obscure this. Tails do not acquire surface metachromasia.

Ventral glands, caudal pockets and peripheries of respective cercariae are metachromatic (gamma) at pH 1 and above. Further, metachromatic and Schiff reactivity tests are also identical to those described herein for *C. geddesi.* Hematoxylin reacts strongly with the mucosubstances.

Ornate.—The gross metachromasy, glandular complex, caudal pocket and peripheral metachromasia (gamma) of the cercaria of *Macroderoides typicus* have been reported by Kruidenier (1953). In the present differential tests the metachromatic substances show strong gamma metachromasia at the pH 1 to 6.4 levels tested and in concentrations of alcohol to 75%. Metachromatic substances are negative to PAS, Bauer, and Casella tests and are not affected by exposure to malt diastase and pyridine. Hematoxylin reactions are strong.

Microcotylous.— An undescribed species of microcotylous cercariae was discussed by Kruidenier (1953: 386, Figs. 23-24). Further studies demonstrate that the ventral metachromatic glands and peripheral substances develop increasingly strong gamma metachromasia at pH levels from 1 to 6.4 at alcohol concentra-

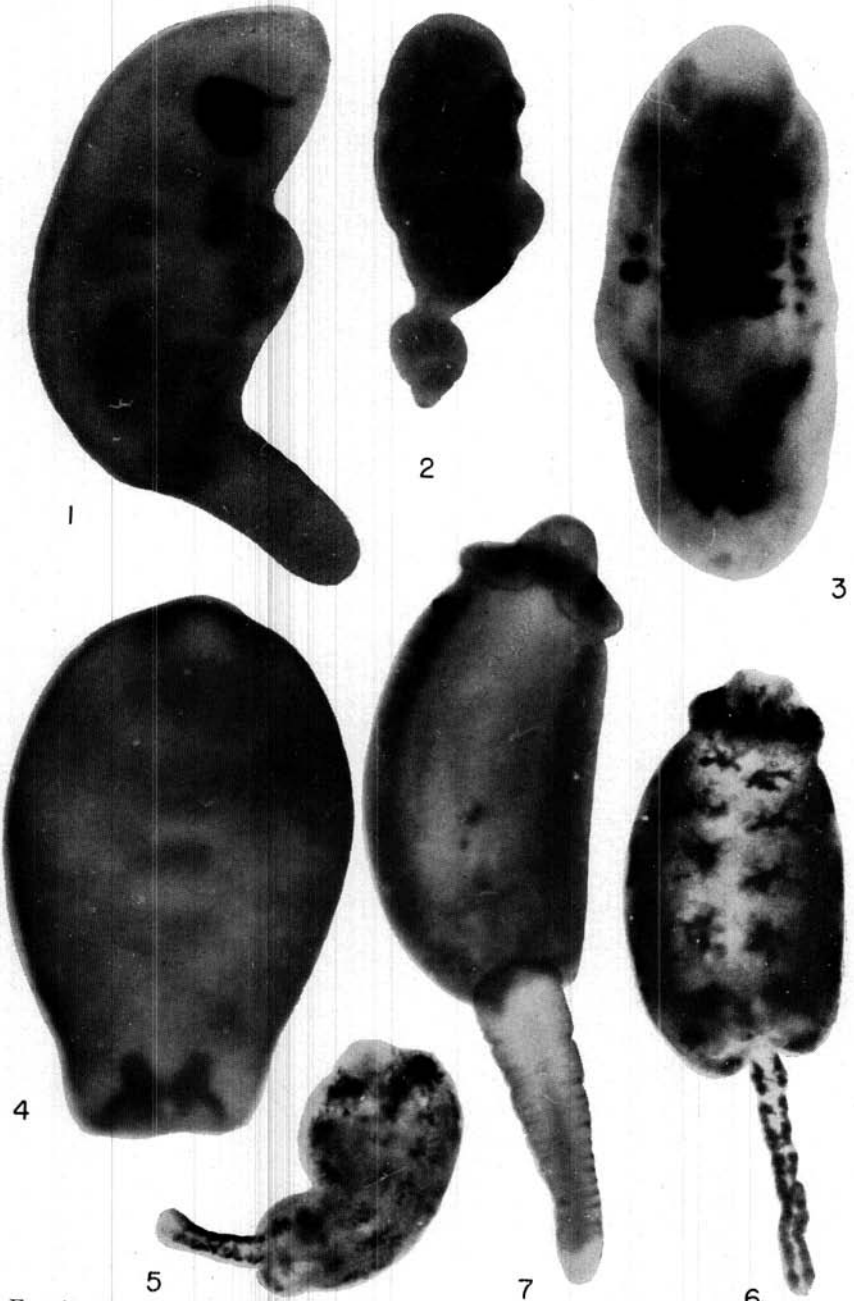

FIG. 1.—*Cercaria geddesi*, emerged, $HgCl_2$ thionin, X500 (approx.); Figs. 2-4.—*C. Tetrapapillatrema concavocorpa*, developing (2, 3) and emerged (4), $HgCl_2$, thionin, X 250 (approx.); Figs. 5-7.—*C. Macravestibulum eversum*, developing (5, 6) and mature (7), $HgCl_2$, PAS, X150 (approx.) Mucosubstances (Figs. 1-7) and eyespots (Figs. 5-7) are black.

tions to 75%. PAS, Bauer, and Casella tests are negative and reactions are not affected by malt diastase or pyridine extraction.

MONOSTOME CERCARIAE: *Notocotylidae.*—Details of the development, morphology, and fate of the metachromatic glands of *C. urbanensis* Cort, 1914, have been reported (Kruidenier, 1953a). Glands are included within the oral suckers, along the dorsa of the cercariae, associated with the locomotor organs, and in the tails. They stain deeply with hematoxylin.

The metachromatic reaction of the stellate body glands of *C. urbanensis* contains a considerable blue-black element which produces a net result of red-purple to violet. Secretions are coarsely granular but a homogeneous substrate is also apparent. Granules develop beta metachromasy while the substrate shows clear red or gamma metachromasia. The secretions of the glands within the tails are homogeneous.

Stellate and tail glands of *C. urbanensis* are orthochromatic at pH 1.5 and metachromatic at pH 2.5 to 3 and above. Granular contents of the stellate glands are beta metachromatic, substrates are gamma. Metachromasia develops in 0 to 50% alcohol. The glands within the oral suckers are faintly metachromatic at pH 3.8 and above. Glands of the locomotor organs are metachromatic at pH 3 and above. Peripheral metachromasia appears as stellate body glands discharge and parallels the acid and alcohol levels of these glands; it is restricted to dorsal and dorso-lateral surfaces. The granules (beta metachromatic) persist as distinct entities after cercarial emergence. Extraction with pyridine and digestion with malt diastase do not change the metachromatic levels of any of the glands. Prior treatment with $HgCl_2$ does not affect the metachromatic reactions.

Stellate body glands and tail glands are variably PAS positive. The reaction is strong in younger cercariae but it weakens and becomes restricted to a faint reticulum in nearly mature specimens. Granular secretions are not visible in the PAS preparations. A concentration of PAS positive material against the cell membrane of the glands may be a fixation artifact. Bauer and Casella reactions parallel the PAS reaction but appear generally weaker. The peracetic-Schiff reaction is negative. Malt diastase or pyridine do not the metachromatic reactions.

Glands within the oral sucker and those of the locomotor organs are strongly PAS, Bauer, and Casella positive throughout their history. They are peracetic-Schiff negative and their reactions are not changed by pyridine or malt diastase.

Pronocephalidae.—The trioculate cercaria of *Macravestibulm eversum* possesses 11 pairs of pleomorphic body glands whose numerous stellate processes branch irregularly (Figs. 5, 6). The glands grossly resemble their analogues in *C. urbanensis* but remain smaller.

Six pairs of glands distribute symmetrically on either side of the mid-dorsal line. The anterior pair is obscured by the dorsal, pigmented eye spots. Branches from the posterior pair extend almost to the posterior limits of the body. Eight glands align bilaterally along the dorso-lateral body margins from the

level of the eye spots to the posterolateral body curvatures. A single stellate gland is closely associated with each locomotor organ. The tail of the cercaria contains 10 pairs of irregular glands. The secretions of all the glands appear homogeneous.

The glands appear after the differentiation of the tail, oral sucker, and lateral eye spots (Fig. 5). *C. eversum* develops considerably after discharge from rediae into snail tissues (see also Hsü, 1937). The glands of the cercariae develop largely during extra-redial phases of embryology (Figs. 5, 6). Glands discharge completely as the cercariae finally mature (Fig. 7) and a fine granular film of their secretion distributes over the entire periphery of the cercariae during their later development. A distinct cuticula is readily visible prior to the appearance of secretions at the periphery. The granules lose their identity as the film becomes a homogeneous layer.

Body and tail glands are hematoxylin-positive and metachromatic at pH 3.8-4. Peripheral metachromasy reacts identically. Metachromasia tends towards the violet (beta) range. Tail and locomotor glands appear distinctly redder. The description of the former glands as beta metachromatic, however, may overemphasize the slight differential reaction.

All glands and the peripheral substances are uniformly and strongly PAS positive. Bauer and Casella tests are weaker. Peracetic-Schiff tests are negative. Malt diastase and pyridine do not visibly affect the reactions. The cuticula is thick and it is weak to negative after the PAS reaction.

Discussion

The full significance of the phenomenon of metachromasia remains unclear despite the numerous competent investigations since its discovery by Jurgens (1875). It is not possible to review the investigations here but several pertinent facts should be presented.

Pischinger (1925) associated the pH level of metachromasia with the isoelectric points of the different compounds. Bank and Bungenberg DeJong (1939) believed the reaction dependent on the charges of colloid ions and the type of ion grouping produced by reactants. They found sulfate, phosphate, and carboxyl colloids to be metachromatic. Dempsey and Singer (1946) considered that treatment with mercuric salts destroyed or suppressed the metachromatic reactivity of carboxyl radicals.

Lison (1936) presented evidence that metachromasy, obtained under conditions of rigorous alcoholic dehydration, identifies high molecular weight sulfuric esters of mucosubstances. Freyrter (1936) demonstrated metachromasia in myelin sheaths; Meyer (1946) noted a lytic action by hyaluronidase on ganglia of tendon sheaths, and Altschuler and Angevine (1949) reversed myelin metachromasia with hyaluronidase. Pearse (1954) felt that sheath metachromasia is possibly due to phosphate moieties.

Michaelis and Granick (1945) suggested the formation of thiazine dye polymers with varying substrates. Thus, an alpha monomer (blue), a beta dimer (violet) and a gamma polymer (red) are possible. Michae-

lis (1947) found that highly polymerized carbohydrates (carboxyl groups) alone can induce metachromasia.

Pearse (1954) believed that water must be present to produce the reversal of metachromasia to orthochromasia noted above.

Thus, numerous compounds may produce metachromasia under different conditions but it seems most probable, at present, that gamma metachromasia at high alcohol or acid levels differentiates acid mucopolysaccharides containing sulfuric esters. Lesser metachromasia indicates phosphate esters, hyaluronic acids, and/or neutral mucopolysaccharides.

In the present tests metachromasia was graded under rigidly controlled schedules and at varying acid and alcohol levels, but it is obviously not possible to identify specific substrates from this. Gamma metachromasy at levels below pH 4 indicates acid mucopolysaccharides and at pH 2 or below almost certainly indicates sulfuric esters. High resistance to alcoholic reversal most probably confirms the latter. Metachromasy bordering the beta type at pH 4 or slightly less would characterize more weakly acid compounds such as the neutral mucopolysaccharides or mucoproteins. It might also indicate lower molecular weights of substrate complexes or, simply, "smaller" compounds.

It seems to us that beta metachromasy might also indicate carbohydrate-protein ratios. Protein complexes are orthochromatic. Variable combination with blue monomers of the thiazines would proportionally dilute the gamma metachromasy attributable to given sulfuric esters of hexose compounds.

The Schiff reaction usually demonstrates 1:2 glycol groups when oxidation is effected with periodic or chromic acids or with permanganate. Compounds which can produce the reaction include glycogen, mucopolysaccharides, muco- and glycoproteins, glycolipids, phospholipids, and even unsaturated lipids (—C=C—). It is essential to control the reaction with pyridine extraction (removal of known lipids, malt diastase (glycogen), and peracetic oxidation (unsaturated lipids).

Bauer and Casella reactions further characterize Schiff positive substances. In the present studies the Casella reactions were occasionally inconsistent but less so when fresh solutions were used. Removal of excess permanganate appears to be the major problem.

Conclusions

The metachromatic level of the glands of *C. eversum* indicates the presence of relatively less acid mucoproteins but does not clearly demonstrate the neutrality of the compounds. Darker metachromasia may well indicate a higher protein content of the substrates as well as more weakly acidifying esters (*e. g.*, phosphate). The homogeneous secretions possess oxidizable 1:2 glycols.

The metachromasia of the glands of *C. urbanensis* is intermediate between those of the xiphidiocercariae and of *C. eversum* in acid-base and alcohol stainability levels. The closer relationship to *C. eversum* reactions is emphasized by the metachromasy of the monostomates at lower test levels. The tests may roughly in-

dicate relative protein content and are a measure of the relative molecular acidity of the metachromatic complexes. Their reaction cannot be attributed to carboxyls because it was not affected by prior exposure to mercuric salts ($HgCl_2$) which destroy the metachromatic reactivity of such groups (Demsey and Singer, 1946).

The glands within the oral sucker and those associated with the locomotor organs of *C. urbanensis* differ from the stellate body glands and the tail glands of that species. Persistent oxidizable 1:2 glycols are not present in the final, definitive secretions of stellate and tail glands (PAS negative). They are lightly but distinctly orthochromatic at pH 1.5 whereas sucker and locomotor glands are not chromophilic at this level. The lower acid dissociation level of the latter appears to be limited to approximately pH 3. They thus approach the weakly acid to neutral mucopolysaccharide or mucoprotein reactions (+pH 4).

Sulfate ions dissociate at values far below pH 4 and are therefore indicated by the chromophilia of the stellate and tail glands. Their orthochromasia at pH 1.5 may indicate a relatively high molecular protein component. This is more certain because compounds with relatively high carbohydrate-sulfuric moieties (*e. g.,* chondroitins and mucoitins) would show the gamma metachromasy observed in the epithelial mucoids (snail) present in the sections. Metachromasia at pH 2.5-3 indicates sulfuric esters. The secretions of matured glands therefore appear to be highly saturated hexose sulfuric esters of large protein complexes.

The orthochromasia of the granules within the stellate cells of *C. urbanensis* at pH 1.5, and the failure of their homogeneous substrate to stain at this level, indicate that these are chemically different substances. This is confirmed by the beta metachromasia of the granules and the gamma metachromasia of the substrates in identical glands at the same pH levels (2.5 and above). The use of both substances in the formation of the metachromatic peripheral film indicates that they are end products of the glandular secretory activities.

The change during the development of the glands from a strong to weak or negative PAS reactivity is confined to the substrate. Both end products are negative. It is reasonable to assume that the early, PAS positive substances contribute to the formation of the granular and the homogeneous secretions. Synthesis in the glands thus appears to include esterification of the glycol radicals. The unchanged metachromasia of the substrates, in the tests performed, indicates the maintenance of roughly the same carbohydrate - ester - protein ratios in their molecular constitution. Changed metachromasia in the granular secretions, and their increased orthochromatic chromophilia, indicate appreciable increments of protein complexes. Thus, the synthesis of the secretions appears to involve the protein esterification of polysaccharide materials.

The reactions are remarkably uniform within the xiphidiocercariae. It does not seem probable that the metachromatic complexes studied in

the different species of these cercariae are chemically identical. It does seem probable that their physical characteristics are similar (Kruidenier, 1951, 1953) and that certain fundamental chemical features are common to them. The mucosubstances are almost certainly sulfuric ester mucopolysaccharides. They lack 1:2 glycols, an indication of the high saturation of their carbohydrate moieties. Difficulties with the permanganate reagent of the Casella test may indicate particular or considerable combining characteristics under specific conditions not clearly understood. Basic chemical and physical similarities imply similar uses of the secretions. Further study is needed to explore this possibility.

The metachromatic and Schiff techniques demonstrate a decided difference between the mucosubstances of plagiorchoid and monostomate cercariae. The latter are measurably less resistant to the repressant effects of acidity and alcohol. They thus appear to be less highly acid and nearer the level of neutral mucopolysaccharides or mucoproteins. It is interesting that the variation is grossly group consistent and accompanies a phylogenetic differentiation which also includes marked life history differences. The plagiorchoids use insects (naiads for known virgulate species) and tadpoles (*C. typicus* and *C. concavocorpa*) as intermediate hosts. The monostomates encyst on detritus (*C. urbanensis*) or on specific surfaces such as snail opercula (*C. eversum*). Minor differences also accompany the variation between monostomates. Lesser metachromasia and greater Schiff reactivity appear to correlate with the specific use of snail opercula (*C. eversum*). However, it would be unjustified to generalize widely in the absence of more complete data.

A cuticula can be distinguished prior to the discharge of mucosubstances in all of the species of cercariae studied. It is differentiated as a distinct layer, less reactant (or negative) to the differential techniques used, and immediately beneath the sheath of mucosubstances which forms around mature cercariae. No structural separation of the two layers is visible. It is possible that the metachromatic or the PAS positive substances combine chemically with the cuticula at the interface. Individual layers thus would retain their chemical identity, as observed, and, presumably, their physical characteristics.

The mechanics of the actual distribution of the peripheral substances have been discussed by Kruidenier in previous reports concerning various groups of cercaria. The differential distribution on the cercariae studied here appears to correlate superficially with the loci of glandular discharge. Thus, mucosubstances are more abundant ventrally and over the anterior and posterior surfaces of xiphidiocercariae. The caudal pockets of armate and ornate species are lined by the heaviest permanent layers of metachromatic materials, although this is more difficult to determine because of the masses of the secretions that are stored within the pockets. The ventral surface of *C. urbanensis* is non-metachromatic.

The absence of a peripheral layer

from the tail surfaces of the xiphidiocercariae may indicate that mucosubstances do not reach the surfaces of the tails. This is in accord with the absence of glands from the tails, and with the presence of such glands as well as the deposition of a surface coating in the monostomate species. It is especially difficult to understand how mucosubstances fail to reach any part of the tail of *C. concavocorpa* when the base of the tail in this species attaches to its body literally within the caudal pocket itself. The formation of the caudal pocket late in the development of the cercariae (Hussey, 1941) and its final enlargement during the discharge of the posterior mucoid glands of the cercariae (Kruidenier, 1953) may be an important factor in this phenomenon. Investigations in progress may explain more fully the relation of peripheral mucosubstances to the cuticula of cercariae. Certainly present data are insufficient.

Summary

Brief descriptions are presented of the development and fate of previously unreported mucoid glands in *Cercaria geddesi* and *C. concavocorpa* (virgulate and armate xiphidiocercariae, respectively) and *C. eversum* (pronocephalid monostome). The glands of these species and of a second, undescribed virgulate cercariae, an undescribed microcotylous species (xiphidiocercaria), *C. typicus* (ornate xiphidiocercariae) and *C. urbanensis* (notocotylid monostome) are studied. These investigations amplify our knowledge of the distribution of the mucoid glands in xiphidiocercariae and extend it to the family Pronocephalidae of the monostomes.

The metachromatic and Schiff reactivity of the glands of the above species are analysed under controlled but varied conditions. The detailed analyses provide information pertaining to a more precise chemical nature of the mucosubstances studied and suggest certain sequences in the syntheses of the compounds. The tests make it possible to compare fundamental differences in the secretions produced by the various species of cercariae.

Factors in the relation of the discharged mucosubstances to the cuticula of the cercariae are suggested but demand further clarification.

Literature Cited

Altschuler, C. H., and D. M. Angevine. 1949. Histochemical studies on the pathogenesis of fibrinoid. Amer. Jour. Path., 25:1061-1077.

Bank, O., and H. Bungenberg Dejong. 1939. Untersuchungen über Metachromasia. Protoplasma, 32:490-516.

Dempsey, E. W., and M. Singer. 1946. Observations on the chemical cytology of the thyroid gland at different functional stages. Endocr., 38:270-295.

Freyrter, F. 1935. Über ein sehr einfaches Verfahren der Markscheidenfarbung zugleich eine neue Art der Färberol. Virch. Arch., 296:645-654.

Hsü, D. Y. 1937. The life history and morphology of *Macravestibulum eversum* sp. nov. (Pronocephalidae: Trematoda). Trans. Amer. Micro. Soc., 56:478-504.

Hussey, K. L. 1941. Comparative embryological development of the excretory system in digenetic trematodes. Trans. Amer. Micro. Soc., 60:171-210.

Jurgens, R. 1875. Eine neue Reaction auf Amyloidkörper. Virch. Arch., 65: 159-211.

Kruidenier, F. J. 1951. The formation and function of mucoids in virgulate cercariae, including a study of the virgula organ. Amer. Midl. Nat., 46: 660-683.

1953. Studies on the formation and function of mucoids in cercariae: non-virgulate xiphidiocercariae. Amer. Midl. Nat., 50:382-396.

1953a. The formation and function of mucoids in cercariae: monostome cercariae. Trans. Amer. Micro. Soc., 72: 57-67.

LISON, L. 1936. "Histochimie Animale." Paris, Gauthier-Villars, 320 pp.

MEYER, K. 1946. Mucolytic enzymes. Currents in biochemical research. New York, Academic Press, pp. 277-290.

MICHAELIS, L., AND S. GRANICK. 1945. Metachromasy of basic dyestuffs. Jour. Amer. Chem. Soc., 67:1212-1219.

MICHAELIS, L. 1947. The nature of the interaction of nucleic acids and nuclei with basic dyestuffs. Cold Spr. Harbor Symp. Quant. Biol., 12:131-142.

PEARSE, A. G. E. 1954. Histochemistry. Boston, Little, Brown and Co. (repr.), 530 pp.

PISCHINGER, A. 1925. Diffusibilität und Despersität von Farbstoffen und ihre Beziehung zur Färbung bei verscheidenen H-ionen Konzentristionen. Zeitschr f. Zellen u. Gewebelehre, 3:169-197.