Further studies on nemic skeletoids and their significance in the chemical control of nemic pests. B. G. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.

Recently the writer (1936) described a series of chemical experiments on the external cuticle of *Ascaris lumbricoides,* finding it to be composed of several substances including a collagen, a fibroid, and a keratin. Solubility in alkalis precluded the possibility of chitin in the external cuticle. Further data regarding the external cuticle and other hard parts of the nemic body have been accumulated during the past two years. Limitations of material or time have prevented a more thorough study in many cases and interesting tests were found to be useful after much of the work had been completed. During the preliminary work the writer was working in the U. S. Bureau of Animal Industry. He also received considerable assistance from Mr. Leon Jacobs, then of the U. S. Bureau of Plant Industry.

1. *Toxocara canis.* The esophageal lining is not digested by artificial gastric juice; it dissolves in boiling 10 per cent KOH; is pale yellowish in iodine-1 per cent H_2SO_4; gives strong xanthoproteic and sulphide reactions. These reactions are presumptive evidence of keratin.

2. *Trichuris vulpis.* The spicule is digested by artificial gastric juice; softened but not dissolved by Fairchild's trypsin; dissolved by hot 10 per cent KOH; gives positive xanthoproteic and mercuric nitrite tests; gives negative or very faint sulphide test; and is colored deep orange by iodine-H_2SO_4. These tests eliminate chitin and keratin. Negative nitrite, xanthoproteic and iodine-sulphuric tests after Fairchild's trypsin indicate that the spicule is a mixture, possibly containing collagen and a glucoprotein. The external layer is physically different from the internal prismoid layer. The cloacal lining in the same tests behaves throughout as keratin.

3. *Spironoura affine.* Spicules (formalin fixed) are not digested by gastric juice or Fairchild's trypsin; dissolve in hot 10 per cent KOH; give positive xanthoproteic and nitrite reactions; become deep orange in iodine-H_2SO_4; and give a negative sulphide reaction. Solubility in KOH eliminates chitin; insolubility in gastric and tryptic solutions would supposedly indicate keratin but that substance is apparently precluded by the negative sulphide reaction. Since the xanthoproteic reaction became negative after exposure to trypsin, it seems possible that we are dealing here with a collagen-glucoprotein mixture, the collagen being somewhat protected from gastric digestion. Results dubious.

4. *Ascaris lumbricoides.* The shell of ascarid eggs (including *Ascaris lumbricoides, Parascaris equorum, Toxocara canis, T. cati,* etc.) has been studied by several workers including Fauré-Fremiet (1912-1913), Yoshida and Takano (1923), Zawadowsky (1914, 1928), Kosmin (1928), Schulze (1924) and Schmidt (1936).

It is now generally recognized that the so-called shell consists of 3 layers of different chemical composition, these being known as (1) the albuminous layer, (2) the shell proper (described as 3 layers by Zawadowsky) and (3) the fibrous layer (vitelline membrane).

(1) *Albuminous layer.* Apparently this layer has been studied only by Yoshida and Takano (1923) and Kosmin (1928). Present observations confirm and extend their results. This layer is dissolved by artificial gastric juice, Fairchild's trypsin, 0.2 per cent HCl, 1 per cent acetic acid, 1 per cent KOH, picric acid, and picric acid-alcohol at room temperature; is insoluble in water and does not coagulate upon heating in acidified solution. Upon the basis of these tests the albuminous layer is certainly not an albumin, collagen, fibroid or keratin. It is, however, almost certainly a protein. One would presume it to belong to the conjugated proteins such as mucoids, which form a similar covering of the egg in other animals (e.g. the "gelatinous envelope" of the frog egg is a glucoprotein). Ordinarily this layer is formed after the shell and all of the evidence indicates that it is a secretion product of the uterus; it is formed on both fertile and infertile eggs.

(2) *Shell proper (chitinous layers, refractive layers, birefringent layers).* Fauré-Fremiet obviously must have had chemical proof of the constitution of the egg shell since he described the mode of its origin from glycogen. However, the evidence has only been presented by Schulze and Schmidt (Zawadowsky's papers inadequately read by the present writer). Insolubility in hot KOH and optical characteristics were the only forms of evidence presented by these authors. Following the technic given by Campbell (1929) further evidence is presented by the present writer.

The egg shell may be characterized as follows: It is not digested by artificial gastric or pancreatic juices, is insoluble in acetic acid in all concentrations and temperatures, insoluble in dilute mineral acids, soluble in 5 per cent NaOCl at room temperature, and birefringent. However, all of these descriptions might well be applied to keratin. Keratin is often soluble with difficulty in alkalis and may easily resist boiling in saturated KOH where dilute KOH will cause it to swell and rapidly dissolve. However, keratin cannot withstand superheating in KOH. This was done by placing material in a vial, adding saturated KOH and a small glass rod to avoid boiling over; a rubber nipple with a fine cut at the tip was attached to the end of the flanged test tube forming a bunsen valve. The test tube was placed in a glycerin bath and heated at 160 to 170° C. under pressure for 1 hour. Thereafter water was added, and the substance washed by centrifuging several times. Only the hard egg shell remained after such treatment. It retains its appearance but its chemical properties are altered. It is immediately soluble in 3 per cent acetic acid, reprecipitated by 1 per cent H_2SO_4 as chitosan sulphate, turns brown in iodine-potassium iodide and purple in 1 per cent H_2SO_4. Shells may be dissolved in 75 per cent H_2SO_4 and chitosan sulphate reprecipitated through imbibition of water in a moist chamber (24 hours). Chitosan sulphate so formed is minutely sphaerocrystallin, said sphaerocrystals staining in 0.1 per cent Rose Bengal. These tests, involving the transformation first to chitosan then to chitosan sulphate, are supposedly conclusive proof of chitin.

(3) *Vitelline membrane (fibrous membrane, lipoid layers).* Fauré-Fremiet and Zawadowsky have identified the internal layer or semi-permeable membrane, as lipoid. It is apparently soluble in absolute alcohol, ether, chloroform, etc. Fauré-Fremiet first called it coprosterol but later named the substance "ascarylique acid" giving the formula $C_{20}H_{40}O_3$. Flury (1912) named the insaponifiable extract of ascarids ascaryl alcohol with the formula $C_{32}H_{64}O_4$. Differences in the melting point and staining properties of "ascarylique" as present in the ovum permit the suggestion that the extracts studied by Fauré-Fremiet and Flury were possibly of 2 coexisting interrelated substances. The membrane itself seems to behave as a sterol.

As observed by Fauré-Fremiet, both the true egg shell and the vitelline membrane are apparently formed by the ovum itself in the ectoplasm of the egg as differentiations when stimulated by entrance of a sperm.

5. *Dioctophyma renale.* The eggs of this species have been previously studied by Lukasiak (1930) who found that the shell withstands strong H_2SO_4, HNO_3, NaOH and KOH, while in 5 per cent KOCl the outer layers are dissolved first, finally leaving a thin inner membrane. He termed the shell pseudochitin. The eggs used in the following tests were from a formol preserved specimen some 20 years old. For that reason negative tests may be considered as questionable.

According to the writer's observations there are at least 4 distinct compounds forming the "shell" of *Dioctophyma,* namely: (1) the operculae or terminal plugs; (2) the exterior rugose or cortical layer comprising the bulk of the "shell"; (3) the internal refractive layer or shell proper; and (4) the vitelline membrane. None of the 4 substances are digested in artificial gastric juice or Fairchild's trypsin.

(1) *Operculae.* These structures are readily soluble in 10 per cent KOH, 10 per cent H_2SO_4, 5 per cent NaOCl and conc. HNO_3, but are not soluble in

10 per cent acetic acid even on boiling for extended periods. The solubility precluded microchemical color tests for proteins. Failure to digest in gastric juice or to dissolve in acetic acid may be due to the formation of a formate. If this is true, the operculae are probably mucoid.

(2) *Cortical layer.* This layer is extremely resistant to both acids and alkalis and may withstand boiling in KOH. However, it is dissolved by superheating in saturated KOH (see Campbell technic under *Ascaris*) or by autoclaving 4 hours at 20 pounds pressure in the same solution. It is also slowly soluble in NaOCl, boiling conc. HNO_3, and boiling 75 per cent H_2SO_4 (incompletely dissolved in 10 per cent H_2SO_4 on boiling). Xanthoproteic and nitrite reactions (latter sometimes dubious) are positive while the sulphide reaction is negative; reaction of vaseline heated, ninhydrin presoaked cortical layer is positive. [The colored product of the ninhydrin reaction is water soluble. In order to use this reaction as a microchemical test for protein (free carboxyl and free amino group) the tissue may be soaked in 0.2 per cent solution of ninhydrin; then the solution drawn off until the tissue becomes "just dry" under a binocular. Covered with a drop of vaseline and a cover slip, the object is then warmed. A blue color is obtained. Prior washing in absolute alcohol or heating may be desirable to permit entrance of ninhydrin through a thermolabile (sterol) membrane]. The cortical layer swells tremendously before dissolving in acids. Solubility in NaOCl cannot be regarded as evidence of chitin since the writer found that the following substances are acted upon and wholly or partially dissolved in this substance at room temperature in 18 hours: Hair (quickly), finger nail (slowly), wing of cockroach (slowly), tendon collagen (incomplete), ligament elastin (incompete).

Insolubility in water following Na_2S seems to exclude keratin since the following comparable results were obtained: Cockroach wing and elastin not affected, collagen very little affected, boiled white of egg, hair and finger nail completely dissolved.

Conclusion dubious; possibly due to formalin fixation.

(3) *Shell proper.* This layer apparently dissolves in HNO_3 and H_2SO_4 more readily than does the cortical layer. It is not dissolved on superheating in KOH, is not affected by Na_2S and is less readily soluble in NaOCl than is the cortical layer, but it eventually dissolves. Iodine-potassium iodide on superheated KOH material gives a deep purple coloration and such shells are also dissolved by 3 per cent acetic acid. Xanthoproteic and nitrite reactions are uniformly negative. Shells dissolved in 3 per cent acetic acid give no sulphide reaction. Material prepared by autoclaving in saturated KOH instead of using the Campbell technic, gives the same results except that the violet color in 1 per cent H_2SO_4 following iodine-potassium iodide rapidly disappears and in such material a thin membrane representing the *external* surface of the cortical layer often persists, this membrane being shrunken up against the shell proper as though a matrix had dissolved.

Autoclaved shells dissolved by heating in 50 per cent HNO_3, recrystallize as minute sphaerocrystals and true crystals insoluble in water and selective to Rose Bengal. Similar sphaerocrystals were also obtained by recrystallizing in 75 per cent H_2SO_4. All of the evidence seems to indicate that the true egg shell is chitin.

(4) *Vitelline membrane.* This membrane (probably the inner membrane referred to by Lukasiak) is insoluble in NaOCl; it is absent in KOH heated material.

6. *Strongylus equinus.* (1) External cuticle. Bondouy (1910) published a statement relative to the skeletoids of the external cuticle of *Strongylus.* He found it to be soluble in KOH, to be digested by trypsin (not by gastric juice), and to have the following reactions: Biuret, positive; xanthoproteic, positive; Adamkiewicz, positive.

Observations by the present writer are very incomplete owing to lack of material but appear to be substantially similar to those previously obtained

(Chitwood 1936) for *Ascaris*. The possibility of the presence of a sterol was not thought of at the time the work was done. The cuticle is subdivisible into layers and Bondouy's observations apply to the cortical layer. This latter is soluble in hot 10 per cent NaOH, is not digested by gastric juice, and gives very strong positive xanthoproteic and sulphide reactions. It is apparently a keratin. The fiber layers are digested by gastric juice and, therefore, are presumably related to ascarocollagen which forms the corresponding layers in *Ascaris*.

(2) *Stomatal lining and esophagus.* Immink (1924) characterized the stomatal and esophageal linings of *Strongylus* as a chitinoid containing protein, the mixture having the following reactions: Millon's, positive; xanthoproteic, positive; Biuret, positive; iodine-H_2SO_4, negative; insoluble in KOH.

The writer has found that boiling in 10 per cent NaOH first dissolves the musculature and body wall of *S. equinus* then that part of the stoma between the internal corona radiata and the base, excluding the dorsal gutter and esophageal lining. Further vigorous boiling with the addition of 10 per cent NaOH at intervals appears eventually to dissolve the entire esophageal and stomatal lining. As previously shown (Chitwood 1936), keratin in nematodes may be very resistant to alkalis and its hydrolysis may be incomplete or slow if the alkali is either too strong or too weak. Solubility in alkali under any condition eliminates chitin from consideration.

The stomatal and esophageal linings of dissected specimens have the following characteristics: (a) Corona, teeth, dorsal gutter and esophageal lining turn rich orange-brown in iodine-1 per cent H_2SO_4; give strong xanthoproteic and mercuric nitrite reactions (indicating a benzene ring and tyrosine); give a very strong sulphide reaction (indicating cystine); are not digested by artificial gastric juice or Fairchild's trypsin; and are insoluble in boiling 10 per cent acetic acid. These tests all indicate keratin, possibly infiltrated with a mucoid. (b) The chief part of the stomatal lining of dissected specimens, i.e., that between the internal corona radiata and the basal teeth, exclusive of the dorsal gutter, has the following characteristics: Colors rich orange-brown in iodine-1 per cent H_2SO_4; gives strong xanthoproteic and mercuric nitrite reactions; is digested in artificial gastric juice but not in Fairchild's trypsin; gives negative sulphide test; dissolves slowly in boiling 10 per cent acetic acid. These characteristics positively eliminate keratin and fibroids. They leave two possibilities: a mucoid or a mucoid-collagen mixture. Before being digested in gastric juice the stomatal lining first becomes soft, retaining its gross structure. At this time xanthoproteic and iodine-1 per cent H_2SO_4 tests are weak or negative. This is regarded as evidence that the wall itself is collagen from which mucoids are dissolved before actual digestion takes place.

7. *Miscellaneous observations.* (1) The esophageal lining of fresh *Agamermis decaudata* is not digested by gastric juice but is soluble in hot 10 per cent KOH and gives a positive xanthoproteic reaction.

(2) The spicules of *Theristus setosus* give positive xanthoproteic and ninhydrin reactions, a negative iodine-H_2SO_4 reaction and are not digested by gastric juice.

(3) The external cuticle of *Rhabditis strongyloides* and of *Oncholaimium oxyuris* behaves like that of *Ditylenchus dipsaci* (see below) in gastric juice and trypsin and also in sodium hypochlorite followed by alcohol. There seem to be 4 types of compounds involved: (1) A "lipoid" or sterol (thermolabile membrane), (2) a keratoid (cortical layer), (3) a fibroid (matrix) and (4) a collagen (fiber=basal layers).

8. *Heterodera marioni.* The eggs of *H. marioni* are deposited in a "gelatinous" substance. In common with other eggs, they have a vitelline membrane and an egg shell.

(1) *"Gelatinous" substance.* This material is minutely fibrous, gives positive ninhydrin and xanthoproteic reactions, indicating protein; it is insoluble in a saturated solution of picric acid, alcohol-picric acid and acid mercuric

nitrate. It is rapidly soluble in 5 per cent NaOCl and in 30 per cent Scott's reagent (Na_2CO_3-CaOCl); is slowly and decreasingly soluble in 10 per cent NaOH, 10 per cent acetic acid, 2 per cent NaOH, 0.1 per cent HCl, sat. $Ca(OH)_2$ and 0.2 per cent NaOH in the order named. It is apparently insoluble in 0.2 per cent HCl and sat. Na_2S. It is digested partially, freeing many eggs after 24 hours at 38° C. in either artificial gastric juice or Fairchild's trypsin. The extract of egg masses formed by boiling in 10 per cent acetic acid gives a strong Molisch test but it is not proven that this carbohydrate test actually came from the "gelatinous" mass. However, on the basis of circumstantial evidence it is presumed to be a mucoid. The xanthoproteic and ninhydrin tests together show it to be a protein containing a benzene ring, a free amino group and a free carboxyl group. The very slow solubility in dilute alkalis or saturated lime water differentiates it from the more common mucoids. The function of this jelly seems to be protective in the following manner: Carbon disulphide, being relatively insoluble in water, is prevented from reaching the vitelline membrane; similarly sodium sulphide reaches the vitelline membrane and embryo much more slowly when the eggs are enclosed in jelly.

(2) *Shell proper.* The egg shell of this species like those of other nematodes withstands autoclaving in 10 per cent NaOH or heating to 160° C. for 15 minutes in sat. KOH. After the former treatment the egg shell turns lavender to violet in zinc-chlor-iodide. After the latter treatment it is soluble in 3 per cent acetic acid and gives positive iodine-1 per cent H_2SO_4 tests. It is fairly rapidly (30 minutes) dissolved in 5 per cent NaOCl and eventually dissolves in 30 per cent Scott's reagent. Evidently it is chitin.

(3) *Vitelline membrane.* This structure may best be studied by removing the gelatinous mass and egg shell in NaOCl. At room temperature it is insoluble in this solution and slowly permeable to it. It is dissolved immediately by absolute alcohol, acetone, or glacial acetic acid; is more slowly dissolved by sat. Na_2S; is insoluble in 10 per cent NaOH, sat. KOH, 10 per cent HCl, and 10 per cent acetic acid but is eventually (24 hours) penetrated by these substances. Artificial gastric juice and Fairchild's trypsin do not penetrate nor dissolve the membrane. Raising the temperature to 48° C. makes the membrane permeable to sodium hypochlorite causing the egg contents to be dissolved. The membrane melts at approximately 70° C. (73+, 65—, 70±). It is not stained by osmic acid or Sudan III, and gives a negative ninhydrin reaction. When dealing with whole eggs (with shell) gentian violet is useful as a criterion of permeability and membrane presence. Ordinarily eggs cannot be stained with gentian violet but after heating to 70° C. or washing in absolute alcohol the stain readily penetrates. This membrane is presumably a sterol such as cholesterol.

9. *Ditylenchus dipsaci.* (1) *Egg shell.* The egg shell of *D. dipsaci* is apparently devoid of mucoids, there being 2 layers,—the shell proper and the vitelline membrane.

a. *Egg shell.* This structure is soluble in sodium hypochlorite; is insoluble at room temperature in alcohol, acetone, 10 per cent or concentrated glacial acetic acid, 10 per cent HCl, 10 per cent NaOH and sat. KOH; is insoluble in boiling 10 per cent NaOH, superheated (160° C.) sat. KOH, and boiling acetic acid. Other solvents were not tested. Presumably it is chitin but eggs in sufficient quantity for establishing this statement have not been obtained.

b. *Vitelline membrane.* This membrane is soluble in alcohol, acetone and glacial acetic acid; is insoluble in 10 per cent acetic acid, 5 per cent sodium hypochlorite, 10 per cent NaOH and 10 per cent HCl; is melted by heat (the exact temperature not as yet determined); and is not stained by osmic acid or Sudan III. This substance is apparently a sterol.

(2) *External cuticle.* The cuticle of preadults is quite resistant to reagents and impermeable as evidenced by lack of penetration by 0.25 per cent gentian violet (the stain enters very slowly at the stoma, amphidial and excretory pores). Investigations come under 2 headings: (a) A study of cut specimens wherein

all the various layers are exposed and (b) a study of uncut specimens, which in reality amounts to a study of the superficial layer. No notable difference between the cuticle of adults and preadults has been observed but the open, functional, normal body openings of adults (i.e., anus, vulva) make them much less resistant to chemical reagents.

a. *Cut specimens.* The cuticle is not digested in gastric juice, Fairchild's trypsin, papain, or ficin but is split into 2 distinct (thick) layers in Fairchild's trypsin. It is soluble in 10 per cent NaOH (external layer slowly); soluble (24 hours) in 1 per cent NaOH; and vaseline heated presoaked ninhydrin specimens give a positive ninhydrin reaction; "dry" xanthoproteic reaction is positive; and the sulphide reaction is apparently negative (? Cuticle dissolves too readily). Sodium hypochlorite dissolves all except the outer part of the exterior layer; the latter is an exceedingly delicate membrane which dissolves in acetone, alcohol, and 10 per cent NaOH (24 hours) but does not stain in osmic acid, scharlach R or Nile blue sulphate. It is absent after specimens have been heated to 65° C. The evidence, as it stands, indicates that the external cuticle is a complex made up of several layers as follows: The exterior *thermolabile membrane,* possibly a wax or sterol (but differing from the vitelline membrane in being soluble in 10 per cent NaOH); the chief exterior layer (cortical layer), apparently a keratoid (insoluble in gastric juice; soluble in water after prolonged exposure to Na_2S); and, underneath, a fibroid matrix layer (soluble in trypsin) and collagenous fiber layers (insoluble in trypsin).

b. *Uncut specimens.* Preadults exposed to papain for 7.5 hours at 40° C. revived but did not revive after 24 hours' exposure; similar specimens revived after 24 hours' exposure to ficin at 40° C.; specimens exposed to NaOCl may survive 30 minutes or more but eventually the solution enters at the normal body openings and thereafter the specimen is immediately killed. Living specimens after being placed in water at 50° C. for 10 minutes revive and are not stained by gentian violet but if the stain is put in the heating solution it enters the specimen and kills it; if such specimens are afterwards treated with NaOCl one finds the *thermolabile membrane* has persisted. Specimens heated to 65° C. for 10 minutes are killed and rendered permeable to gentian violet; when such specimens are treated in NaOCl one finds that the thermolabile membrane has been destroyed. From this evidence it seems assured that ordinarily the thermolabile membrane governs permeability. Heating to a sufficiently high temperature or dissolving in alcohol, acetone, or acetic acid will remove this membrane and render the organism permeable; at such a time it is dead. Heating to lower temperatures (i.e., 50° C.) increases the permeability of the membrane temporarily, and during such a temporarily permeable period if a substance which otherwise could not enter (i.e., a non-sterol permeable substance) is present in solution it may enter and cause death.

c. *Specialized cuticular structures.* The spicules, stylet and esophageal lining all come under this general heading. These structures, for the most part, give identical reactions, staining deeply in Nile blue sulphate, or gentian violet, becoming orange-brown in iodine-1 per cent H_2SO_4, and giving strong ninhydrin and xanthoproteic reactions. The outer part of the stylet shaft and the knobs (mesorhabdions-telorhabdions) are digested by gastric juice and pancreatic juice while the other structures are not so digested. Since the corresponding morphological regions in *Rhabditis* are not digested, we must regard the knobs of the *Ditylenchus* stylet as specializations (a fibroid?) for the attachment of muscles and not as the original telorhabdions. The remaining structures are probably keratoid with possible mucoid infiltration.

General Considerations. On the basis of the assembled data it appears that there is little if any important difference in the nemic skeletoids in different types of nematodes. In so far as protection from environmental conditions is concerned, the functional membrane of egg, larva, or adult is apparently a sterol or related substance. Zawadowsky (1928) brought attention to the fact that such a "lipoidal" substance controlled permeability in ascarid eggs. This

is also true for eggs of *Heterodera* and *Ditylenchus* and *a similar substance* forms a thermolabile membrane on the surface of both adults and larvae of *Ditylenchus* and *Rhabditis*.

The thermolabile membrane was discovered after its existence was predicted through the effect of reagents on *Ditylenchus*. (Data, to be published elsewhere, include the effect of varied concentrations of chlorinated hydrocarbons, organic acids, alcohols, aldehydes and sulphides). The relative lack of penetration of nonfat solvent substances at room temperature, the increased lethality of the same concentrations of substances at higher temperatures, and the chief action of fat solvents within the effective range of their dissolving concentrations all pointed to some protection of the larva other than protein. The resistance of preadults or third-stage larvae of other nematodes can be explained since the partial closing of normal body openings requires substances to enter through this membrane. In conformity with these points it has been found that the preadult of *Ditylenchus dipsaci* (so-called resistant stage) is not the stage most resistant to chemicals. The egg is the most resistant stage since it is completely covered by the vitelline membrane whereas the thermolabile membrane covering the larva is broken at the mouth where substances such as NaOCl may slowly penetrate.

Furthermore, it may be noted that except in cases where a nematocide is supposed to be taken by the nema *per orem* or through other normal body openings, successful nematocides are fat solvents or react with fats (NaOH, etc.). The assumption of a sterol membrane would explain this. It would also explain the well known impermeability of the nemic cuticle.

If, as we believe, such a membrane may be general in nemas, then the study of nematocides essentially involves the permeability of this membrane. Soil nematocides effective against *Heterodera marioni* and *Ditylenchus dipsaci* should be just as successful in the sterilization of manure or fox runs for nemic eggs and larvae.

In the study of nematocides of plant parasitic nematodes where eggs and preadults coexist in the host (such as *Ditylenchus dipsaci*) effort should be made to study the egg as well as the larva since it is the egg that is most completely protected. By dissolving the egg shell in NaOCl the effect of reagents on the vitelline membrane can be easily demonstrated. Stains such as gentian violet also provide a useful index to permeability.

SUMMARY

The observations described in this paper demonstrate the following points:

(1) There is little, if any, difference in the chemical reactions of the same morphologic layer in different nemas or their eggs.

(2) The only truly chitinous structure in nemas is the egg shell proper. Other hard parts (supporting or skeletal in function) are scleroproteins or mixtures of scleroproteins and mucoids.

(3) Probably the function of the scleroproteins and chitin is chiefly or wholly supportive. Regulation of the environmental contact with the nema or nemic embryo is apparently governed by a "lipoidal" membrane which behaves as a sterol. This membrane takes the form of a vitelline membrane in the egg and a thermolabile membrane in the larva or adult.

(4) Nematocides, unless designed to enter the nema *per orem*, should be soluble in, dissolve, or be dissolved by lipoids.

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