existing at the boundary of sediment and supernatant liquid. The magnitude of the "membrane" potential is said to be equal and opposite to the suspension effect. According to Brönsted (*1*), "the membrane potential is measurable as the difference between the electromotive potentials of two standard electrodes (e.g., calomel electrodes) in contact with the solutions on the two sides of the membrane, the junction potential being eliminated." Following Loeb (*4*), the membrane potential is often calculated from the difference of the pH values observed potentiometrically between "inside" (sediment) and "outside" solution.

With numerous resin and clay systems we have verified the identity of the "membrane" potential and the Δ pH values (suspension effect). However, our explanation is different. According to our picture we have merely measured the same junction potential twice, once when measuring pH and once when measuring the "membrane" potential (Fig. 2, *a, b, d*).

It is important to note that in our systems

$$\begin{array}{l} \mathrm{KCl}\,|\,\text{K-cation exchanger} \\ \qquad\quad | \\ \mathrm{C_1}\,|\,\mathrm{KCl} \\ \quad Y \end{array}$$

no Donnan distribution across the interface, *Y*, could be observed. Even after long standing, the Cl^- concentration of solution removed by rapid filtration from the right-hand compartment was identical with that of the solution in the left-hand compartment. Likewise, if colloidal H-resin particles are added to one side of a two-compartment cell containing equal amounts of HCl on both sides, no redistribution of HCl takes place. This suggests that *Y* is not a phase boundary in the usual sense, and that the solution phase KCl (C_1) exists on both sides of *Y*. No membrane potential in the Donnan sense is expected to exist. Yet, by inserting two calomel electrodes with salt bridges, large emf values are obtained. These we attribute to the junction potential.

The following question still remains: If the boundary of supernatant liquid|sediment is not the chief seat of the potential difference observed between two calomel electrodes (Fig. 2 *d*), how is the absence of an emf between two glass electrodes (Fig. 2 *c*) to be explained? Two explanations come to mind. First, the activities of the H ions are identical in the supernatant liquid and in the sediment; this would imply that the H ions of the ion swarm of the particles have the same activity as those of the intermicellar liquid. Second, the adsorbed H ions (swarm) do not affect the glass electrode. Only the diffusable H ions of the intermicellar liquid are recorded by the electrode.

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Technical Papers

Chemical Composition of the Vitelline Membrane of *Ascaris lumbricoides* var. *suis*[1]

Richard W. Timm

Department of Biology,
The Catholic University of America, Washington, D. C.

A large number of living female *Ascaris lumbricoides* var. *suis* were slit open and the uteri dissected out. The edge of a glass slide was rubbed gently along the uteri to force the eggs out on a glass plate. The eggs at the distal end of the uteri were frequently checked under the microscope to make certain that they were fertilized. Immediately after collection the eggs were placed in a 0.2% solution of concentrated CP hydrochloric acid to dissolve the outer mucoid coat. The material was allowed to stand overnight at room temperature and was then centrifuged, and the supernatant fluid was stored in the refrigerator for further testing. The eggs were washed three times in distilled water and then exposed to artificial gastric juice at 37° C. This step was to eliminate any remnants of the mucoid layer. The supernatant fluid after this procedure showed no quantitative or qualitative differences from artificial gastric juice. This indicates that the previous hydrochloric acid extraction was complete.

The eggs were then rinsed and placed in a large test tube in a water bath and the temperature was held between 72° and 74° C in a warming oven. The vitelline membrane melted at this temperature and gathered in large globules, usually at one end of the egg. The eggs were allowed to cool and an acetone extraction for fats and fatty acids was made at room temperature. The supernatant fluid was filtered off, and the extraction was repeated three times. On chilling, this supernatant fluid showed a white flocculent precipitate. Later tests with heated beeswax in acetone showed a similar precipitate on chilling. The nature of the precipitate in our acetonic

[1] This work was carried out under the guidance of B. G. Chitwood.

extract of vitelline membrane has not as yet been investigated, but it seems probable that some of the vitelline substance was dissolved at the temperature of the extraction.

The eggs were then exposed to ethylene dibromide, the supernatant fluid from this procedure being thereafter drawn off and placed in a dish and evaporated to dryness. A waxlike substance remained on the bottom of the crucible. Neither the acetonic nor the ethylene dibromide extractions gave positive ninhydrin or biuret reactions for protein, whereas the eggs after the ethylene dibromide extraction gave strong positive reactions.

The waxlike substance produced by the ethylene dibromide extraction is the material which formed the vitelline membrane in the original living egg. This substance was tested for the presence of an ester (R_1COOR_2) in the following manner. A drop of the ethyl ethereal solution of the vitelline membrane was placed in a microcrucible. To this were added a drop of an alcoholic solution of hydroxylamine hydrochloride and a drop of alcoholic sodium hydroxide. The crucible was heated over a flame until there was a slight effervescence. The solution was acidified with 0.5*N* hydrochloric acid and a drop of 1% ferric chloride was added. Reddish-yellow lumps formed. Upon evaporation pyramidal crystals (with a square base) were formed. Testing under a microscope with polarized light showed that these crystals were nonbirefringent, but on their surface a few minute, elongate, birefringent crystals were observed. We are not entirely satisfied with this test.

A control of the above test was run with a known ester, myricyl palmitate (beeswax), and an identical reaction was obtained. Crystal formation was not observed, but the presence of impurities was shown by the low melting point (65° C). U.S.P. beeswax (chiefly myricyl palmitate) was obtained for comparison and further refined by dissolving in warm acetone, chilling, and centrifuging. The melting point of the product was found to be 66.2° C. The melting point of pure myricyl palmitate is given as 72° C. The vitelline membrane wax produced by ethylene dibromide extraction was tested for melting point; it began to melt at 69° C and ran slowly at 72° C. In the living egg the vitelline membrane does not begin to melt until the temperature 70° C is reached and it melts completely at or below 73° C. Hence our product is not as pure as that produced by the nematode. Equal amounts of the vitelline membrane wax and the refined beeswax were mixed together, the mixture was fused at 75° C and cooled to 58° C, and thereafter the temperature was raised 1° C at 5-min intervals. This mixture began to melt at 68° C and ran slowly at 70° C. Such evidence is usually accepted as conclusive proof of the identity of the two principal ingredients of the mixture. If the principal ingredients had not been identical, the mixture should have had a melting point below either of the original ingredients. Instead, the melting point was higher than one ingredient and lower than the other. One would judge from these facts that the refined commercial U.S.P. beeswax contained impurities not present in the nemic vitelline membrane wax. Upon the basis of all of this evidence, we feel justified in concluding that the vitelline membrane in the living egg of *Ascaris lumbricoides* var. *suis* is myricyl palmitate.

The myricyl palmitate as produced by our ethylene dibromide extraction was softer than commercial beeswax and took longer to harden. There may be some stratification on cooling. The latter point will require further investigation. The myricyl palmitate produced by precipitating the hot acetonic extract of either the commercial grade or the U.S.P. grade of beeswax is of a higher degree of purity than the original material. It seems possible that one might be able to produce a completely pure compound by repeating this process an adequate number of times.

This is the first paper presenting proof of the nature of the vitelline membrane in the Nematoda. Since the melting point, solubility, and other characteristics of the vitelline membrane as previously determined in *Meloidogyne hapla, M. javanica, Parascaris equorum, Rhabditis strongyloides, Ditylenchus dipsaci,* and *Strongyloides canis* do not differ materially, it seems probable that the vitelline membrane in these organisms is the same or a similar compound. This may be the first paper presenting evidence of a wax in unsegmented animals.

Effect of Trace Minerals on Growth and Fattening of Swine [1]

Earle W. Klosterman, W. E. Dinusson, Earl L. Lasley, and M. L. Buchanan

Department of Animal Husbandry, North Dakota Agricultural College, Fargo

The beneficial effects of cobalt for ruminants in areas of cobalt deficiency have been demonstrated. Knowledge is limited as to the value of trace minerals, particularly cobalt, for swine. Willman and Noland (*5*) reported increased weight gains for swine receiving four minerals—cobalt, copper, iron, and manganese—when added to a corn-soybean oil meal ration in dry lot.

Rickes *et al.* (*4*) showed that vitamin B_{12} contains cobalt. Recently, Abelson and Darby (*1*) demonstrated that cobalt is used in the synthesis of vitamin B_{12} by rumen bacteria. Becker, Smith, and Loosli (*2*) reported, however, that on the basis of preliminary observations, administration of vitamin B_{12} did not altogether relieve symptoms of cobalt deficiency in sheep, and that cobalt was probably required for other body processes in the ruminant.

For the initial study of the effects of cobaltized and trace-mineralized salt on pigs, a ration was formulated using ground yellow corn, ground barley, linseed oil meal, tankage, ground alfalfa, steamed bone meal, and salt. These pigs were kept in concrete pens, which were washed daily. Treatments and results are presented in Table 1.

Two more experiments were conducted to study the effect of cobalt on the growth and fattening of swine

[1] North Dakota Agricultural Experiment Station Progress Report Project R&M 10, published with approval of director.