pressure cooker costing $15.00 as with the larger standard autoclave. Hot air sterilization can be efficiently and effectively completed in an ordinary baking oven with ordinary bunsen burners as the heat source. Fractional sterilization equal to the Arnold can be secured in a large-sized double boiler, by perforating the bottom of the enclosed utensil. Many of our fancy electrical set-ups can be efficiently made from radio equipment. Rheostats, potentiometers and meters are available at small cost. They must be calibrated, but then so should the more costly material from the supply houses.

The care of small animals is generally a very costly and inefficient process. In many cases the larger the animal the more the wastage. Small rodent colonies are useful for many types of work, but frequently are too costly to maintain on an adequate scale for continuous work. The cost of an attendant where the colony is of large size is no small item. If a part-time assistant can be used for this work, a larger saving can be effected. If a full-time assistant is available, part of his time can be used for other things demanding time without interruption.

As a standard stock feed for rats and mice, the dog and fox chow marketed by the Purina Company has many advantages over the ones usually used. It can be purchased in reasonable quantities, is easily stored in galvanized iron cans, keeps until used. The animals do not tire of it and it seems to be a complete diet under our laboratory conditions. This food is cheaper than any special baking brand which we have so far secured. It is supplemented with lettuce (3 times per week) and with stale bread, both of which can be obtained cheaply. Many complex drinking bottles are available, most of them costly. We use a 16 ounce Whitall-Tatum bottle with an L tube which leads to the cage interior. The L tube is fitted to the bottle with one inch of pressure tubing used instead of the usual perforated rubber stopper. The bottle is fastened to the top or side of the cage with a brass spring, preventing dislodgement and breakage. Cleanliness of an animal room is one of the most completely neglected of the arts. If all metal cages are used and supported 18 inches above the catch pans, which are covered with sawdust, the cages keep much cleaner than is the case where they are directly on the sawdust base. We use single plane sheets of aluminum for the catch pan. This can be scraped and washed in a few minutes. Our routine calls for complete cleaning operations three times each week. This is a minimum for a reliable rodent colony.

The wastage usually is more excessive in the operating room than in the animal colony. Ether for anesthesia is wasteful of this reagent as well as of animals under the methods generally employed. Narcosis is seldom satisfactory, and often the work must be hurried because of the animal's condition. Sodium amytal (Lilly) is an efficient, reliable and cheap method of settling the expense of narcosis. Antiseptic solutions often serve as irritants or astringents as used in animal work. Sterile 5 per cent. borax solution or chlorozene is just as effective as the more costly proprietary solutions. Clips so necessary for the suturing of skin incisions are usually the baby-skin clips used in human surgery. A metal stapling plier, H-54, distributed by the Hotchkiss Sales Company (Stamford) can readily be adapted as a skin suturing device by sawing 5 mm from the fixed jaw on the lower side and removing the lower part of the cover of the upper jaw.

The above are just a few of the economies which can be used to reduce the costs on most colonies from 40 to 60 per cent., without impairment of efficiency.

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SPECIAL ARTICLES

THE STORAGE SAC FOR CAPSULE REINFORCEMENT IN NERITIDAE

ONE hundred years ago, when Qouy and Gaimard included in their multitudinous findings of the voyage of the *Astrolabe* (1830–1933) the discovery that the snail, Nerita, is not hermaphrodite, they figured and described two problematical organs of the female—the one a sac accessory to the uterus, containing white limey objects thought to be eggs; and the other a pyriform organ containing elongated filaments. Speaking of their illustrations, they recommended those who desire to understand the usage of organs to give special pains to the verification of what is represented of the reproductive organs.

In 1857, a student of Johannes Mueller, Eduard Claparede, having made a detailed study of the anatomy and development of the fresh-water snail *Neritina fluviatilis* near Berlin, was not able to clear up either of the above two problematic organs, but upon dissecting specimens of *Nerita peleronta* L., *N. atrata* Chemnitz, and *N. albicilla* L., found in the females that the above uterus accessory sac contained, not eggs, but lime concretions of 100 μ to 500 μ diameter, white and spheroid in the first two species, but red brown and discoid in the last, from the Red Sea.

While he described in detail the eggs of *N. fluviatilis* as laid in capsules often covered over with shells of diatoms, he surmised that in Nerita limey capsules

might be made by the accessory sac in the breeding season, but that the sac at other times might deposit its excess of lime as these remarkable concretions. Still the organ was problematical. "Die Bedeutung des Organs ist hoechst räthselhaft."

Comparative study of several Neritina and Nerita organs and egg capsules had made it evident that the pyriform organ is concerned with the holding and dissolution of large, remarkable spermatophores, while the sac accessory to the uterus has, as surmised by Claparede, a use in connection with the egg capsule but only as a reservoir for such hard mineral objects as are to be incorporated into the roof of the capsule. The spermatophore disposal will be considered elsewhere. The evidence that the accessory sac of the uterus is used as storage for reinforcements to the capsule is briefly presented here.

Dissecting one hundred *Neritina reclivata* from Florida, all the 61 females had an accessory sac homologous with that first made known by Quoy and Gaimard in Nerita, but not seen by Claparede in *Neritina fluviatilis,* and in very many cases this sac was stuffed full of minute clear sharp fragments of silica sand, which ran out on puncture of the sac wall, as sand runs in an hour-glass. In June some of these snails laid eggs in many capsules, the top surface of which was thickly strewn with sand agreeing with that in the accessory sac; moreover, the sand found in the rectum along with other indigested material was just like that in the sac.

Apparently, sand taken in with food was selected out and stored in the sac accessory to the uterus. In this species, the rectal reproductive complex has two terminations externally; the one that of the free, presumably protrusible, so-called vagina connected internally with the spermatophore storage and utilization pouch; the other a shallow cloaca-like opening in which discharge not only the uterus or oviduct and its accessory sac, but also the anal end of the rectum. Thus sand may be sorted out from the feces and stored in the sac accessory to the uterus and later passed from this sac to the capsule, which presumably is made in the uterus, that being supplied with remarkable glands producing vesiculated material similar to that of which the capsule is made.

Several other species of Neritinas have the same sac and store up sand which appears upon the roof of the capsules, which differ in different species and in different localities both in shape, size and in amount of reinforcement used.

Dissection of the old alcoholic specimens of *Nerita versicola* from Jamaica showed that the storage sac accessory to the uterus was full of the carbonate of lime concretions known from the work of Quoy and Gaimard and of Claparede, but that there was no evidence of these being made in that sac; on the contrary, almost identical spherulites were found in the ultimate lobules of the liver as if originating there and also scattered in liver ducts. Inferentially these mineral particles originating in the liver are taken out of the rectum and stored in the accessory storage sac to be used at proper season in the reinforcement of the egg capsules. Confirmation of such use was first had on July 13 at South Nigril Point, Jamaica, B. W. I., where just above the breaking surf on the etched limestone of the elevated coral reef *Nerita peleronta* (the bleeding tooth shell) was found on the rocks.

One large specimen in minute pool, 3×5 inches, had apparently long resided in this home depression, in water one inch deep. Near the snail were some 6–9 large 4 mm flat white capsules, which showed, with the pocket lens, the expected lime spherulites all over the surface of each capsule.

Later at White House sands, east of Montego Bay, *Nerita tesselata* was taken along with capsules that proved to be specifically distinct and covered over with pearl-like spherulites.

While *Neritina fluviatilis* was found by Claparede to have generally diatom shells over its capsules, and while several Neritinas show siliceous sand with, seldom, a few diatoms or sponge spicules as reinforcement to the top of the capsule, the above Neritas reinforce the egg capsule with lime spherulites that come from the storage sac as does the sand in the other snails. But in the small *Nerita alticola* Pilsbry, from the rapid waters of the Great River and its branch, the capsules are reinforced not with lime spherulites, but with fine siliceous sand. Is it that in passing from an assumed salt water ancestry, this snail has taken up the collecting of solid particles from its food in place of the hepatic manufacture of reinforcements for the egg capsules? Moreover, Neritinas both in salt and in fresh water are found to use not only silica but limestone fragments, or even a foram shell as reinforcement of the capsule roof.

The plan of these capsules in the Neritidae thus far studied is that of a closed-up box, with thin floor closely applied to the substrate and thus commonly flat but sometimes curved, from which floor there rises up all about an elliptical continuation as a low wall supporting a very large roof more or less convex. *Neritilia succinea,* however, has less specialized spheroidal capsules, very small and simple. The material is a dense secretion at first evidently very soft and sticky but later becoming hard and leather-like. It burns with charring and evolution of fumes like burnt hair and does not dissolve in acid or in

boiling potash. While the floor may show rather large vesiculate structure and the walls and roof very fine vesiculations, it is only the roof that is generally reinforced by many imbedded solid particles. The roof also is joined to the top of the walls with a preformed joint that finally allows the roof to rise up and fall off, leaving the wall to stand as rounded conspicuous white line about the nearly invisible floor for months' duration, after the young have escaped. The roof also may be prolonged laterally as more or less extensive rim or eaves just above the breaking joint; the top of the wall may also be somewhat prolonged laterally under the above named eaves of the roof.

The capsules of different species present differences in size and in outline, but especially in character of the reinforcement of the roof as well in extent or absence of the rim. Moreover, in any one species the capsules vary in size and form according to size of the females making them and differ in nature and size of solid objects used for roof reinforcement according to character of substrate on which the animal feeds in different localities; or even in the same locality, whether on bottom or on floating objects.

However, these capsules of the Neritidae, which are comparable to eggshells of birds and reptiles as being secretions placed about an albuminous mass in which one or more ova have been placed, may aid in classification.

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RESPIRATORY INFECTION IN EQUINE ENCEPHALOMYELITIS

EQUINE encephalomyelitis has been transmitted to two horses by single instillations of 50 cc of Berkefeld filtered virus into the nostril. The stock strain of virus used was originally obtained from the brain of a horse which was killed when prostrate with encephalomyelitis during the epidemic in Western Nevada in September, 1931. This strain of virus has been maintained by routine passage through guinea-pigs and horses for the past eighteen months.

For inoculation of the horses, a one per cent. Locke's solution emulsion was prepared with the brain and liver virus from three guinea-pigs. This was allowed to extract at 5° C. for three hours, with occasional agitation. The virus emulsion was then centrifuged and filtered through Berkefeld N filters (7 to 8 lb. test) and the filtrates pooled. Aerobic and anaerobic cultures made from the filtrates did not show any bacterial growth during ten days' incubation. The pH of the pooled filtrate was 7.2.

Fifty cubic centimeters of the Berkefeld filtered virus was instilled into one nostril of a horse. Seven days later, this horse showed a temperature of 105° F. and marked congestion of the conjunctiva. Definite symptoms of encephalomyelitis were present on the tenth day; namely—depression, incoordination and receding fever. Motor paralysis and prostration occurred on the eleventh day, when the animal was sacrificed for humane reasons. The virus was subsequently demonstrated in the brain of this horse by inoculation of guinea-pigs.

The second horse was inoculated with the same strain of virus recaptured from the first horse by guinea-pig passage, guinea-pig brain virus being used. The same experimental conditions were duplicated, namely, the method of preparing the virus emulsion and filtration.

Horse number two received 50 cc of Berkefeld filtered virus in one nostril. Six days later this horse had a temperature of 104° F. On the seventh day, the temperature was 105° F. and accompanied by profuse nasal discharge and congestion of the conjunctiva. On the eighth day depression and preparalytic symptoms appeared, the temperature receding to 103.8°. Motor paralysis, amaurosis and prostration occurred on the ninth day, when the subject was destroyed. Virus was recaptured from the brain and cord by guinea-pig passage.

As controls on each of the two intranasal infection experiments, guinea-pigs and another horse were injected intracranially under anesthesia with the same Berkefeld filtered virus. All the control guinea-pigs developed characteristic symptoms of encephalomyelitis and either had died or were destroyed by the fifth day.

The two control horses injected intracranially with virus developed typical symptoms of encephalomyelitis on the sixth day after injection. One was prostrate on the eighth day and was destroyed. The other lingered until the twelfth day, became prostrate and was sacrificed. This individual, being an old horse, presumably had some natural resistance.

The incubation period of the disease in horses which received virus in the nostril was two and three days longer than that following intracranial injection.

Postmortem examination of the two horses which received virus in the nostril showed hemorrhagic infiltration at the base of the brain tending to follow the olfactory tract and extending posteriorly to the piriform lobes. The spinal fluid in one instance was very turbid and had a large mononuclear count of 680 per cmm. Polyneuclear leucocytes were not observed. Test for sugar was negative.

It appears that inanimate objects, such as feed racks and watering troughs, are not vectors of infection. During the past year one corral has been