to simply have the lever cut the current as soon as the ring tore itself from the liquid. This device proved to be extremely satisfactory. The only thing to do now to obtain an excellent measurement is to press a button and take a reading.

The motor is connected to the knob through a double worm reducing gear, the ratio of which is 1/1.600. The vernier is driven at a rate which can be varied from twenty to forty dynes a minute. The motor stops almost instantaneously, by itself, on account of the considerable amount of friction, which may be increased by a small brake on the main shaft. The error introduced by the momentum of the armature amounts to about 0.02 dynes, always in the same direction, of course, and is therefore negligible. However, we have reduced it to zero by a simple and powerful brake which is automatically and electrically set as soon as the current is cut off.

LECOMTE DU NOÜY

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SPECIAL ARTICLES

INTRANUCLEAR INCLUSIONS IN YELLOW FEVER[1]

TORRES[2] has recently described intranuclear inclusions in the liver cells of monkeys infected with the virus of yellow fever. He states that these inclusions are acidophilic and of "the same nature as those discovered in herpes simplex, symptomatic herpes, varicella and in virus III disease of the rabbit." The inclusions are, according to him, found in hepatic lesions like those described by Stokes, Bauer and Hudson[3] in West African yellow fever, which is interesting because apparently he himself investigated only the action of Brazilian viruses. His technique consists of staining with hematoxylin and eosin. He does not mention any particular fixative, but intimates that the inclusions may also be identified in frozen sections.

We wish briefly to report the discovery of intranuclear inclusions, which bear a striking resemblance to those of Torres, in the liver cells of eight *Macacus rhesus* monkeys infected with the West African virus of yellow fever. They also occur, though less abundantly, in the suprarenal gland. We confirm Torres in his contention that the inclusions are definitely associated with the specific lesions. In common with Torres, we have failed to find the inclusions in monkeys dying from causes other than yellow fever, or in normal control animals.

Morphologically the inclusions are made up of clusters, or colony-like clumps of particles. The particles themselves are very small in size, and though roughly spherical, are rather irregular in shape. Their contours are not smooth and evenly rounded. They are often contiguous but seldom confluent. The inclusions may be separated from the nuclear membrane by a layer of optically clear nucleoplasm, or they may be in contact with it. The affected nuclei, with their contained inclusions, pass through a series of changes which we hope to describe in detail subsequently. The large basophilic nucleolus remains intact until this process is far advanced. Side by side, or imbedded in these clumps of particles, we have detected in many cells a single, much larger, spherical, acidophilic mass, often limited by a distinct halo.

In regard to the staining reactions, we find, with Torres, that the inclusions are colored pink by eosin after application of the hematoxylin and eosin technique. The vestiges of basophilic chromatin remaining are stained blue. We have observed in addition, after preservation in Zenker's fluid, that the inclusions are colored pink by Giemsa's stain; but the best technique for their demonstration is to apply phloxin red and to counterstain with methylene blue, as in Mallory's method. The red coloration thus obtained is more brilliant and more specific than that secured with eosin. With these yellow fever inclusions, as in the case of those of chicken pox, herpes and virus III, the colors can be easily reversed. That is to say, they can be stained green instead of red, and the basophilic chromatin red in place of blue by using the well-known safranin-light green combination.

That the inclusions are present as such in the living animal and do not in any sense represent the coagulating action of the fixative is shown by the ease with which we have been able to study them in liver cells quickly removed from a chloroformed animal and examined in physiological salt solution. The addition of a supravital stain is not even necessary, since the refractive index of the individual particles is sufficiently different from that of the surrounding nuclear substance to render them easily visible with both direct and oblique illumination, when good lenses are employed; but they do not reflect or refract light to any great extent. Thus far we have not seen any trace of pigmentation. We have not used a polariscope. The particles become tinged when a trace of eosin is added to the salt solution, and are colored more intensely than any other elements in the cell when a little phloxin red is applied in the same way. In such supravital preparations they may be studied

[1] Conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

[2] *C. Rend. Soc. Biol.*, 1928, 99: 1344, 1655, 1660, 1669 and 1671.

[3] *Am. J. Trop. Med.*, 1928, 8: 103.

with ease and the details of their morphology and topography can be distinguished rather better than in fixed and stained preparations, particularly if a binocular microscope giving perspective is employed. It is readily seen by focusing up and down through entire nuclei that clumps of particles which in thin sections appear to be isolated, are, in reality, often in contact with one another. Frequently there is a central mass, from which clumps of particles stretch out like arms. As yet we have been unsuccessful in our attempts with fresh, unstained cells to ascertain how the particles are formed. Whether this takes place in single or multiple foci within the nucleus remains to be determined. No indications have been observed of independent motility or of multiplication by division, nor have we detected any increase in size of the particles through accretion or condensation of further materials on their surfaces. The uniformity in size of the particles is noteworthy. They do not grade down past the border line of visibility, nor are there any specially large ones. It should be possible to follow their behavior by implanting groups of affected cells, or even single cells, in pure line tissue cultures of rhesus liver.

For a detailed comparison of these yellow fever inclusions with those produced by other viruses we must await the results of further experimentation. It is evident, however, that they resemble somewhat the inclusions of herpes, as we have studied them in the brain, and as Goodpasture and Teague[4] have very briefly reported them in the rabbit's liver. If it proves possible to maneuver the herpetic virus into the monkey's liver, a direct comparison can be made, which will be helpful, for it is our ambition to learn to know the viruses by their deeds. The inclusions produced by virus III in the testicles of rabbits are, in our preparations, much more dense and compact, but what they would look like in the liver, if virus III were capable of attacking liver cells, we have no means of knowing without experimentation. Caution is necessary, for we have found that the inclusions caused by the submaxillary virus in the brain are rather different morphologically from those which it provokes in salivary glands. The large, spherical inclusions, already referred to as occurring in association with the typical clumps of fine particles, are not unlike the acidophilic inclusions of Borna disease, as the latter exist in nerve cells.

Through the kindness of Dr. Oscar Klotz, we have been able to examine tissues from human cases of yellow fever contracted in widely separate localities as follows: 4 cases from New Orleans, 4 from Brazil, 2 from Ecuador, 3 from San Salvador, 7 from Lagos, 2 from Accra, 3 from Dakar and 2 from Senegal. Most of the tissues had been preserved in formalin and for this reason it was difficult to color them with acid dyes. Nevertheless, intranuclear inclusions like those seen in the monkey tissues were found in 22 of the total of 25 cases. In some of the cases, the large forms previously referred to were very abundant, sometimes several in a single nucleus. It was impossible to distinguish between the intranuclear inclusions in the African and American cases. One of those negative for inclusions seemed to be without doubt a case of true yellow fever; another was recorded as questionable; while the third was said to be some form of poisoning, not yellow fever.

[4] *J. Med. Res.*, 1923, 44: 121.

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WILL THE ADULT FIREFLY LUMINESCE IF ITS LARVAL ORGANS ARE ENTIRELY REMOVED?

IT is well known that the adult luminous organ of the firefly is entirely formed anew during the pupal stage and that the larval organ persists and glows in the pupa and is only absorbed at the time the adult emerges. Therefore, we should expect an adult light organ to form even if the larval organ is removed, but the interesting point concerns the luminescence of the reconstituted adult organ. If luminescence is a fundamental characteristic of the photogenic cells due to the chemical production of a luminescent material, there is no doubt but that the reconstituted adult organ would luminesce even if the larval organs had been removed. On the other hand, if the luminescence is due to symbiotic luminous bacteria, as Pierantoni,[1] Büchner[2] and some others believe, removal of the larval organ should remove completely the bacteria, as no other region of the larva is luminescent, and we should expect no luminescence of the adult.

Experiments carried out by one of us (R. T. H.) have demonstrated that the adult luminous organ of the firefly will develop perfectly from larvae both of whose light organs have been removed. The statement is based on two surviving animals of many operated on. One had its light organs removed with iridectomy scissors as a full-grown larva on October 24, 1927. It was kept in moist earth and leaves in a refrigerator at 3° C. during the winter, and when examined from time to time at room temperature showed no trace of luminescence, although the controls

[1] U. Pierantoni, *Rend. Ac. Sc. Napoli*, 20: 15. 1914.

[2] P. Büchner, "Tier und Pflanze in introzellularer Symbiose," Berlin, 1921. p. 344.