on intestinal digestion appeared under the names of Charles H. Williams and G. M. Garland, respectively.

Although Dr. Bowditch's laboratory was called a laboratory of physiology, it is obvious that it was hospitable to work on problems of pharmacology as well, even during the first years, 1871–76.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

GRASSHOPPER EGGS AND THE PARAFFIN METHOD

PETRUNKEVITCH,[1] in a recent issue of SCIENCE, has published formulae for several new fixatives, containing—among other ingredients—phenol or one of its derivatives. Phenol, he claims, gives "a peculiar elastic texture to the tissues, unlike anything produced by commonly used fixing fluids." It occurred to the present authors that such a fixative might be utilized in making sections of grasshopper eggs, a material which, hitherto, has proved extremely refractory to a paraffin technique. Following the ordinary methods of fixation, embedding, etc., the yolk is found to be hard and gritty, and sectioning becomes an impossibility. Freshly laid grasshopper eggs were, accordingly, fixed in the cupric-phenol solution (No. 1), as directed by Petrunkevitch. When eggs so treated were embedded in paraffin no difficulty was found in securing smooth, clean sections, providing that the surface of the block was wiped with a bit of damp filter paper immediately before cutting each section.

In order to test the value of such a solution for cytological purposes a grasshopper testis and a young grasshopper embryo were fixed with it. The results, however, were extremely unsatisfactory. Cytoplasmic details were badly distorted and the chromosomes were almost unrecognizable.

The idea then suggested itself that it might still be possible to secure the benefits of the new cupric-phenol mixture by allowing it to act after a fixative already known to be of value in chromosome studies had been employed. To this end grasshopper eggs were fixed over night in Bouin's solution. After a thorough washing in 70 per cent. alcohol these were placed in the Petrunkevitch mixture and allowed to remain there for approximately 24 hours. Eggs treated in this way were found to section in an entirely satisfactory manner.

In order to discover whether the second fixative could have any possible effect on cytological details, bits of grasshopper testis were fixed in Bouin's solution, then, after this had been washed out, treated with Petrunkevitch's mixture for periods varying from 2 hours to 4 days, sectioned and stained with Heidenhain's iron-haematoxylin. No difference could be detected between the chromosomes in such material and that prepared in the ordinary way.

As the next step Petrunkevitch's "Stock solution B" (consisting of 100 cc of 80 per cent. alcohol, 4 gm of phenol and 6 cc of ether) was used alone and found to be quite as efficacious as the entire mixture. It was likewise found that matters could be still further simplified by omitting the ether.

The procedure recommended at present, then, is as follows: Grasshopper eggs of the desired age are fixed in Carnoy-Lebrun, as suggested by McNabb.[2] These are washed in iodized alcohol, cut in half and the micropyle halves stored in 70 to 80 per cent. alcohol until needed. (Eggs which had been kept in alcohol for three months were found to be still amenable to the phenol treatment.) Exposure for 24 hours to 4 per cent. phenol in 80 per cent. alcohol is followed by dehydration in 95 per cent. alcohol. The eggs are then cleared in carbol-xylol, infiltrated with paraffin and each one blocked with the cut end out. The paraffin is trimmed away from the face of the block until the yolk is just exposed and the whole is then soaked in water for 24 to 48 hours. This last eliminates the tedious and time-consuming process of moistening each section separately and permits the egg to be cut as rapidly and as easily as any ordinary material.

As an alternative method the eggs, after fixation and exposure to the phenol solution, are dehydrated in 95 per cent. alcohol, cleared in anilin oil, washed in chloroform and embedded in paraffin, after which they are placed in water as before.

Preparations of various stages in the maturation and early cleavage of the eggs of *Melanophus differentialis* and *Chortophaga viridifasciata* have already been successfully obtained with the technique outlined above. Finally, it might be well to mention that Feulgen's stain, since it does not color the yolk, has been found an important aid in the study of such material.

The essential features of this process which differentiate it from those commonly employed consist of (1) treatment with phenol and (2) soaking in water. Either of these steps alone has been found insufficient; but the two combined give a perfect ribbon. It is not unlikely that other cytologists or embryologists dealing with objects which are difficult or impossible to handle with routine methods may find here a solution to their problems.

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[1] A. Petrunkevitch, SCIENCE, 77: 117, 1933.

[2] J. W. McNabb, *Jour. Morph. and Physiol.*, 45: 47, 1928.