

the department of zoology of the University of Pennsylvania, has joined the same institution as professor of zoology. Walter D. Kline, Ph.D. (Yale, '23) becomes professor of chemistry.

PROFESSOR E. STRÖMGREN, of the University of Copenhagen, has been called to a professorship of theoretical astronomy at the University of Berlin.

DR. G. HERGOLTZ, of the University of Leipzig, has been called to a professorship of mathematics at the University of Munich.

DISCUSSION AND CORRESPONDENCE

HAEMATOXYLIN

HAEMATOXYLIN is a natural dye found in logwood, and requires only to be extracted with ether and water and then crystallized. There are, of course, details of manufacture requiring attention but the method is simple and there is no difficulty in obtaining a pure product. In view of this fact it is surprising that there should have been so much trouble in getting from American sources a haematoxylin comparable with the one commonly in use at the time the foreign supply was cut off by the war. The miserable black logwood extracts that were then sold as haematoxylin were an abomination to microscopists. Failing to secure any decent material on the market, I undertook to make haematoxylin from the logwood chips and found no difficulty in doing so. Later, there appeared for sale by a number of dealers a product called "white crystals" and this has proved to be generally satisfactory. It now appears that the source of practically all this supply is the firm of McAndrews & Forbes Company, of Camden, N. J. A description of the method employed by them for the preparation of haematoxylin from logwood extract appeared in the *Journal of Industrial and Engineering Chemistry* of February, 1920.

Complaints having come to the Committee on the Standardization of Biological Stains that solutions of the white crystals did not keep well, the chairman, Dr. Conn, asked me to investigate the matter. Accordingly, with the help of Dr. Carothers, I undertook a study of a series of products which had been submitted for trial. Later I got directly in touch with the manufacturers and from them received samples of the material at different stages of manufacture and under different treatment.

As a result of all this it was learned that the "white crystals" produced to meet the demand for something different from the black crystals (?) previously on the market, owe their absence of color to the use of sulphur dioxide. In some way this agent renders the haematoxylin less stable and solutions made up with heat become very dark and stain a rusty brown with-

out selectivity. Apparently also it is responsible for the poor keeping quality of solutions. The manufacturers, upon learning these facts, discontinued the use of sulphur and now market their product as crystals of a light brown color, similar to those of the imported substance. It is now possible, therefore, to secure in this country an entirely satisfactory haematoxylin, which can be used for the finest cytological work by the iron-haematoxylin method. This has been compared with recently imported haematoxylin and found in every way as satisfactory.

In the investigation of the various haematoxylin submitted a number of facts appeared which are of value in cytological technique. It was found, for example, that the color of a given sample might be entirely satisfactory and yet its selectivity be almost entirely lacking. Again one sample might produce a hard, sharp, vigorous coloration while another was weak and indefinite. Of course, it is possible to modify, or even reverse, the staining reaction, as I have previously announced, by incomplete removal of Flemming's fluid, but in the present series of experiments this factor did not enter.

Just what is the cause of these variations in staining reaction is not clear, but having noted certain peculiarities in the operation of the cruder samples and having observed the somewhat turbid character of their solutions, I added a small quantity of lead acetate to them. The result was to greatly improve the vigor and selectivity of the stain. I am inclined, therefore, to recommend the following procedure in cases where satisfactory results are not obtained by the iron-haematoxylin method: To 100 cc of one half per cent. haematoxylin add about three drops of a saturated solution of lead acetate and shake. The solution will become very dark but upon standing for a number of hours a fine black precipitate will form. When this is filtered out a bright clear liquid will remain. This should then give a satisfactory stain.

A good object upon which to try out the stain is a root tip section. Here it is desirable to have the chromatin stain a good vigorous black and yet without such density as to obscure the finer internal structure of prophase and telophase stages. The nucleolus at the same time should stain like the chromatin and should not, on the one hand bleach out entirely or on the other remain a dense black. All outlines should be clear and sharp in the nuclear structures without gradation into the ground substance. The cytoplasm should be light gray in color and clear and transparent. These are conditions which manifest themselves in *Podophyllum* root tips after fixation in Flemming's strong fluid.

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