

to Feldman's "Biomathematics," students of biology are finding themselves to-day in need of mathematics for certain types of investigations. As few of those biologists now in middle life have any considerable knowledge of mathematics and as it is not even to-day widely recognized by those collegians who plan to specialize in biological or medical sciences that they will need mathematics, and as it is by no means certain that, even with a realization of the need, the desired parts of mathematics could be found in the collegiate courses offered by departments of mathematics, it is evident that there is at present and probably will for many years remain a place in biological literature for books that expound those principles and algorithms of mathematics which are of greatest importance for such students and elaborate the exposition with a larger variety of worked examples from these fields of science. Feldman's "Biomathematics" is one of the first books directed to meet these special desiderata and seems to be likely to succeed in meeting them.

The titles of the 21 chapters are: "Introductory," "Simplified methods in arithmetic," "A few points in algebra," "A few points in elementary trigonometry," "A few points in elementary mensuration," "Series," "The simple and compound interest laws in nature," "Functions, variables and constants," "Differentials and differential coefficients," "Maxima and minima," "Successive differentiations," "Integral calculus," "Biochemical applications of integration," "Thermodynamic considerations and their biological applications," "The use of integral calculus in animal mechanics," "Use of integral calculus for determining areas, lengths, volumes and moments of inertia," "Special methods of integration," "Fourier's theorem," "Differential equations," "Mathematical analysis applied to the coordination of experimental results," "Biometrics."

Whether the author might not have omitted some topics is a serious question. Fourier's series is presented without biological illustrations. And what are the occasions on which biologists or others must differentiate  $x^n$ ? One may expect mathematicians to take an interest in the cute tricks of their trade and may excuse them for inserting in their texts artificial examples and methods suited to their solution, but in a book especially written for some class of non-mathematicians it would seem to be better pedagogy to eschew all methods which were not used in illustrative material of interest to the readers. In places Feldman's is too much a text on mathematics with illustrations from biology instead of an exposition of quantitative problems of biology with an explanation of their mathematical treatment (it is of course

far easier to write the mathematical text). Still I know of nothing better for its intended clientele.

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## LABORATORY APPARATUS AND METHODS

### CERTIFIED SAFRANIN

IN accordance with the plan announced in *SCIENCE* for July 20, 1923 (Vol. 58, p. 41), the Commission on Standardization of Biological Stains has been extending its plan of certification of stains, the latest addition to the list of those certified being safranin. This certification, as stated in the earlier article, is issued only for the batch of which a sample has been tested and found satisfactory.

The procedure followed by the commission when asking for the submission of samples to be tested for certification is to furnish the companies with specifications, provided preliminary work has been done to show what these specifications should be; but otherwise to ask the companies to submit the samples which they think will be most satisfactory, assuring them that certification will be based on performance of the samples rather than upon their chemical composition. This second course was followed in the case of safranin, and upon the basis of those samples submitted which proved satisfactory, specifications have now been drawn up which will be used in the future as the basis for accepting samples for certification. The batches of safranin which are now being certified fulfill all these specifications in regard to performance. One of them, however, is of lower dye content than the commission will recognize in the future. The lower dye content of this sample, however, does not seem to make it less satisfactory as a stain.

The specifications that are now drawn up for safranin on the basis of the samples which have been found to be satisfactory and which will be applied to any sample hereafter submitted to the commission are as follows:

(1) Samples of safranin O must be of the type represented by Schultz No. 679 and on spectrometric analysis should have an absorption curve maximum at approximately  $515\mu$  as determined in a one cm layer by a spectrophotometer. Other dyes must not be present.

(2) Safranin samples to be certified by the commission must contain at least 75 per cent. total color as determined when reduced by titanous chloride in an atmosphere of carbon dioxide. One gram of the dye must consume at least 4.195 cc normal titanous chloride solution.

(3) The sample should prove satisfactory for histological use. No exact method for determining this can be given, but the sample must be submitted to

one or two experts in histological technic in order to get their judgment. Their judgment must be based to a considerable extent upon the behavior of the stain in the Flemming triple staining technic, in which it is used together with orange G and gentian violet. In other words, the stain must be of such a shade as to contrast well with both of these two other dyes.

(4) It must be understood that these standards refer to samples to be used for general histological staining. Special standards for safranin to be used for certain special purposes will undoubtedly be necessary. These standards, however, have not yet been determined.

At the present time permission to use the commission's label on the batches of safranin submitted has been given to three companies. Work is still pending on samples submitted by one or two other companies, so that the omission of some concern from this list does not prove that their safranin is necessarily unsatisfactory. The three samples so far approved fulfil the above specifications in every respect except total dye content; but the three samples vary somewhat in the amount of actual dye present. These three samples with their total dye content are as follows:

National Anilin and Chemical Company.....	90 per cent.
Empire Biochemical Company.....	87 per cent.
Providence Chemical Laboratories.....	55 per cent.

That one of those three samples which is below 75 per cent. in total dye content has given perfectly satisfactory results in the hands of the investigators who tested it. Nevertheless, purchasers must take its lower concentration into account. For this reason the commission is requiring that certified samples of safranin be labeled as to their total dye content. This will enable the purchasers to take the matter of concentration into account in making up solutions, and will enable them to make a fair comparison between the products of the different concerns.

Attention of biologists is again called to the fact that these stains do not have to be ordered from the companies listed above. Nearly all the regular dealers in biological supplies are planning to carry the certified stains as rapidly as the certification is extended to cover new products. It is possible, therefore, to order these stains from any laboratory supply house by specifying that certified stains are desired or by even specifying the company whose product is wished for if the purchaser has any preference.

H. J. CONN, *Chairman,*  
*Commission on Standardization of*  
*Biological Stains*

GENEVA, N. Y.,  
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## STAINING WOODY TISSUES WITH SAFRANIN AND Picro-ANILIN BLUE

THE use of safranin in combination with picric acid and anilin blue in the staining of woody tissues has been developed at the Forest Products Laboratory, and some excellent results have been obtained with certain kinds of woods. The triple stain herein described calls for safranin as a first stain, followed by a single solution containing picric acid and anilin blue.

Safranin is one of the most important of the coal-tar dye stains because of its selective properties, brilliancy and permanence. Picric acid, which is less well known in microscopy, is a yellow crystalline compound obtained variously, as by the action of nitric acid on phenol. Anilin blue is a basic derivative of the base rosanilin. Best results follow the use of pure chemicals from reliable manufacturers.

Stains are generally divided into two types, general and selective. The former acts on all the elements of a specimen while the latter takes effect on, and makes prominent, only some or parts of them. It is of value to the worker in microscopic anatomy to obtain this differentiation, as he is thereby able to trace and follow structural differences and relationships.

If the material to be sectioned is green, it usually requires no further treatment. If dry wood blocks are used, they should be prepared for the microtome by boiling for about thirty minutes or longer in water. Extremely refractory and hard woods should be immersed in commercial hydrofluoric acid (30-40 per cent.) for a few days and again boiled in several changes of water to wash out the contained acid, previous to cutting sections. The writer secured best results with sections 10-15 micromillimeters in thickness.

The safranin stain is prepared by mixing a saturated water solution of water-soluble safranin, and a saturated alcoholic solution of alcohol-soluble safranin. Equal amounts of these two safranin solutions are mixed. The safranin may be used several times.

The picro-anilin stain is prepared as follows: Make a saturated solution of picric acid and one of anilin blue, each in 95 per cent. alcohol. From these two make a single alcoholic solution containing 78 per cent. of the picric acid and 22 per cent. of the anilin blue solutions.

The staining process is as follows:

- (1) Rinse sections with 50 per cent. alcohol.
- (2) Flood sections with safranin and leave two hours.
- (3) Wash off excess safranin with 50 per cent. alcohol leaving sections light pink in color. If the sections remain red, bleach with 70 per cent. alcohol to which a few drops of acetic acid have been added.