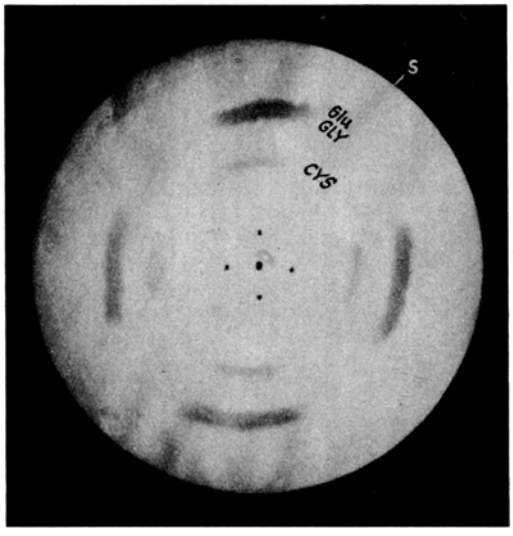
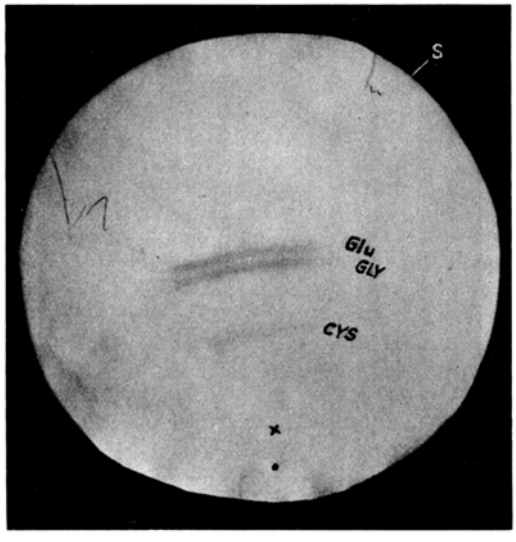
acetic acid-water (4:1:5) with R_f values of 0·31 and 0·38 respectively, can be easily achieved by the modified method, whereas this is not possible in the usual way.



3.—Conventional method (irrigated twice).

This method has been found useful in the identification of unknown substances by running the pure suspected amino acids simultaneously on either side of the unknown ones. Success has also been obtained in the separation of common sugars having close R_f values, e.g. lactose and maltose, R_f value 0·24 and 0·27 respectively, in butanol-acetic acid solvent as above at 22°C. Attempts are now being made to employ this method for the separation and identification of constituents of biological fluids.



4.—Modified method–n-Butanol-acetic acid-water. *CYS* = Cystine, *GLY* = Glycine, *GLU* = Glutamic acid, *S* = Solvent front, *X* = Position where the mixture of the amino acids is spotted. ● = Position of the wick.

R_f values for 24 amino acids and 13 common sugars have been calculated and, as expected, they were identical with those obtained by the conventional method. The maximum difference in a few cases was not more than 0·02.

It will thus be seen that with the modification suggested above, better separations are obtained with the 18 cm diameter filter paper disc by a single irrigation, thus saving time and material.

V. K. MOHAN RAO

Central Drug Research Institute, Lucknow, India, December 10, 1952.

Zusammenfassung

Bei horizontaler Papierchromatographie auf Rundfiltern wird das Trennungsvermögen grösser, wenn man die mobile Phase an einem Punkt in Peripherienähe zuführt und die zu chromatographierenden Substanzen auf einen um diesen Punkt gelegenen Kreisbogen aufträgt.

A New Nuclear Staining Method with a Vegetal Indicator Dye[1]: The "Sambucyanin"

In the course of investigations about some vegetal dyes, an extract from *Sambucus nigra* (L) berries has been used for the first time in histological technique. The plant is a very common *caprifoliacea* in Europe and Asia minor. A hydroalcoholic solution and a dry extract were prepared from bruised and retted berries, gathered in July and August, when fully ripened.

The hydroalcoholic solution (30% of alcohol) appears as a limpid liquid of deep red colour and contains, besides the dyestuff, traces of malic, tannic and tartaric acids, sugar and a colourless glucoside (Sambunigrin). The removal of these substances is not necessary for practical purposes, inasmuch as the solution itself is quite suitable for staining without further addition of mordants.

The solution, preserved at a low temperature, with the possible addition of some thymol crystals to avoid the development of mildews, keeps its own staining power unchanged for about two months.

After this period of time, the solution turns brown and greatly loses its staining power.

A dry extract can be prepared from hydroalcoholic extract, by means of low temperature vacuum evaporation suitable purification by repeated washing of the concentrate in ether-alcohol.

The dry extract appears as an amorphous hygroscopic blackish-red coloured powder which can be preserved active indefinitely at a low temperature. The hydroalcoholic solution can be prepared again from the dry extract. However, before using, it is advisable to allow this solution to "mature" for at least 48 h at room temperature in an uncovered container.

The dyestuff of *Sambucus nigra* is insoluble in absolute alcohol, ether, acetone, chloroform. It shows a characteristically strong red colour change in the presence of acids and a blue colour change in the presence of alkalis.

The potentiometer colour change showed the breakdowns as follows:

[1] F. B. MALLORY: "It is doubtful, if the artificial dyes ever entirely replace the natural stains because the latter have certain valuable qualities of their own."

Colour	pH
red	1·00–2·50
slightly violet red	2·50–5·00
violet rose . . .	6·10–7·00
violet	7·20
blue	7·80–9·00
deep blue . . .	10

Hence the stuff shows the properties of a sensitive indicator and can be used as such instead of common litmus.

If a diluted acid solution of the dye is submitted to pyridine-free amylic alcohol treatment, according to WILSTATTER's and EVEREST's technic for the identification of anthocyans, the dye runs only partially into amylic alcohol and can be removed therefrom by stirring the alcoholic layer with fresh acid. Therefore the pigment shows the features of monoglucosidical anthocyanins.

The designation "Sambucyanin" is temporarily suggested as a name for this dyestuff.

As a histological stain, a natural hydroalcoholic solution of "Sambucyanin" can be used, which–as already stated–colours tissues, either directly without the addition of mordants or with a potash alum mordanted solution, and is provided with a greater staining power.

The following formula gave me the best results:

hydroalcoholic solution (30%) of "Sambucyanin" 5% . . . . . cm^3 100
potash alum . . . . . g 6

The alum is dissolved hot slowly in hydroalcoholic solution without reaching boiling point.

The addition of alum is also indicated for the reconditioning of the staining power of unmordanted poorly active old solutions.

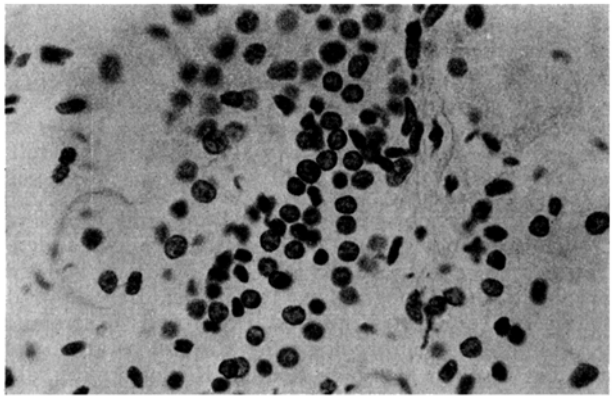


Fig. 1.—Kidney of a man: staining of nuclei with "Sambucyanin".

The "Sambucyanin" stains the cellular nuclei selectively and provides a peculiar image sharpnes and detail without tending to superstaining, thus being, from this point of view, preferable to carmalum and even to hematoxylin.

Staining method.

(1) Stain neutral formalin or alcohol fixed tissues in the solution of "Sambucyanin" 5–10 min or even longer.
(2) Wash in distilled water.
(3) Dehydrate in absolute alcohol.
(4) Clear in xylol and mount in neutral balsam.

Results. Nuclear chromatin of red or violaceous red colour of various tonalities according to tissues. Sometimes a light blue staining of the non-chromatinic nuclear part. Besides nuclei, the cartilage can be stained, which appears either rose or light blue. Occasional deposits of calcium salts or other alkaline salts assume a blue-violet colour, sometimes very strong. The other structures do not appear to be stained. The stain is therefore of particular interest because it preserves a certain colour change also in preparation, which could be referred to a different pH of cellular elements. Actually, in previous observations, I was able to ascertain a different behaviour of normal and pathological tissues as regards staining. However it must be borne in mind that the possibility of an exact tissue pH determination with this method may be impaired by the occurrence of electrolytes, and especially by the combination with proteinic substances of tissues as well as by the action of alcohol. These factors can all produce errors which are not easily remedied.

Finally it is emphasized that a dye, analogous to the "Sambucyanin" described, can be also obtained from the berries of another variety of "Sambucus": the "*Sambucus ebulus*". However the solutions of this dye do not colour without the addition of mordants. The aluminated solutions, used according to the above technique, colour the nuclei violet-blue as does common hematoxylin, only less strongly. On the whole, the first results obtained with this second dye appeared to be less outstanding and interesting than those seen with original "Sambucyanin".

A. NOVELLI

Department of General Pathology and Bacteriology, University of Genova, December 20, 1952.

Zusammenfassung

Ein neuer, aus *Sambucus-nigra*-Beeren extrahierter Farbstoff wird in die Histologie eingeführt und *Sambuzianin* benannt. Sambuzianin zeigt die Kennzeichen eines guten Kernfarbstoffs sowie eines empfindlichen Indikators. Die Farblösung ergibt einen pH-abhängigen Farbumschlag von Rot zu Blau. Die mit Sambuzianin gefärbten mikroskopischen Präparate weisen Unterschiede im Farbton auf, was für einen histochemischen Indikator eines zellulären pH-Unterschiedes zu sprechen scheint.