parts of the plants containing them, have shown that fluorescent stains may be obtained from a considerable variety of plant species. One of the first to be used was an extract of the roots of the greater celandine (*Chelidonium majus*), a European weed now naturalized in the eastern United States. We have used alcoholic extracts of the roots of this species and also ones prepared from several related native American papaveraceous plants, especially species of Argemone and the common blood-root (*Sanguinaria Canadensis*). All these act as powerful yellow fluorochromes, as does also the alkaloid, sanguinarine nitrate, kindly supplied to us in purified form by the S. B. Penick Company of New York City. The brilliant yellow bark of the Asiatic cork-tree (*Phellodendron amurense*) forms another particularly good stain, due possibly to the berberine that it contains, although the color is a deeper yellow than that produced by pure berberine or by an extract of some of our common horticultural forms of barberry, which stain by virtue of their considerable content of berberine. Phellodendron is a member of the natural family Rutaceae.

It appears thus that fluorescent alkaloids or other materials which act as fluorochromes, and consequently as efficient biological stains, occur in various groups of flowering plants. In some cases this would seem to be due to the rather wide distribution of a berberine type of alkaloid among members of several families of plants (*e.g.*, Ranunculaceae, Papaveraceae, Berberidaceae, Rutaceae). However, the vigorous staining properties of purified sanguinarine indicate that action of the blood-root extract can not depend entirely on its berberine content.

This case is particularly interesting, since it has been shown that sanguinarine, like colchicine, can induce polyploidy in certain plants (Antirrhinum).[3] Although brilliantly fluorescent in solution, colchicine does not act as a nuclear stain on any of the materials with which we have tested it.

A preliminary survey, including a considerable number of native plants, has disclosed only a few that may be expected to serve as satisfactory biological stains. Most notable among these are Sanguinaria and the gold-thread, *Coptis trifolia*. The great majority of extracts made from roots, stems or bark are brightly fluorescent, commonly blue, yellow or white, but the fluorescence of the solution is no indication that it will act as a fluorochrome. The same is true of numerous therapeutic alkaloids, most of which are fluorescent, often brilliantly so.

We have not yet been successful in finding any material that will act as a blue fluorochrome, although this color is so frequently encountered in plant extracts and alkaloids, and is characteristic of the natural fluorescence of some insect tissues, especially that of striated muscle.

Fluorescin and auramin O and, more lately, acridine orange, acridine yellow, acriflavine and rivanol (ethoxydiamine-acridine) have been recommended as fluorochromes, but in our experience, these have proved inferior to the alkaloids mentioned above. They do not give the brilliant differentiation of nuclei and other cellular structures that is characteristic of these fluorochrome-alkaloids.

The fluorochromes stain at very great dilutions in aqueous solution after immersion of the sections for half an hour or longer, and the preparations commonly retain their fluorescent properties for many days, provided of course that they are stored in a dim light.

Staining with a series of solutions buffered over a considerable range of hydrogen-ion concentration shows a great variation in brilliancy, but a neutral solution (near pH 7) seems to be quite universally the most satisfactory for a number of fluorochromes tested in this way.

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[3] T. M. Little, SCIENCE, 96: 188–189, 1942.

A CONCENTRATED BASAL MEDIUM FOR MICROBIOLOGICAL VITAMIN ASSAY

THE Snell and Strong microbiological assay for riboflavin[1] as improved by Strong and Carpenter[2] is used successfully in many laboratories and in general is accepted as a speedy, highly quantitative method for the determination of riboflavin in a wide variety of biological materials. Of equal success has been the application of the Snell-Wright microbiological assay for niacin[3] as modified by Krehl, Strong and Elvehjem.[4]

In stride with the increased importance and demand for the evaluation of biological materials in terms of their nutritional value, workers in this field of biochemistry have sought to standardize their methods in order to cooperate successfully in certain collaborative studies pertinent to their efforts. The development and improvement of the above methods have partially fulfilled the demand for such standardization. However, recent collaborative studies have revealed to us a wide difference in method of preparation and storage of the basal media used in the above assays.

[1] E. E. Snell and F. M. Strong, *Ind. and Eng. Chem.*, 11: 346, 1939.

[2] F. M. Strong and L. E. Carpenter, *Ind. and Eng. Chem.*, 14: 909, 1942.

[3] E. E. Snell and L. D. Wright, *Jour. Biol. Chem.*, 139: 675, 1941.

[4] W. A. Krehl, F. M. Strong and C. A. Elvehjem, *Ind. and Eng. Chem.*, 15: 471, 1943.

It occurred to us, therefore, that a basal medium of constant composition would be of great value in routine control assay work as well as in collaborative research. The preparation of a basal medium possessing the above qualities has been successfully accomplished in our laboratory and has prompted our describing it here.

In the preparation of the riboflavin basal medium alkali-treated photolyzed peptone, l-(-)-cystine and a yeast supplement, prepared and combined in the generally accepted manner,[1,2] were dissolved in a minimum quantity of distilled water (approximately 5.0 per cent. solids) and adjusted to pH 6.6–6.8 with 5N sodium hydroxide using brom thymol blue as an outside indicator. The solution was then dried from the frozen state for a period of thirty-six hours after which the dehydrated residue was removed and stored over phosphorus pentoxide at room temperature. A 2.25 per cent. solution of the desiccated product was used for the riboflavin basal medium to which was added anhydrous glucose at a level of 2.0 per cent. Results with this basal medium over a period of twelve weeks are summarized in Table 1.

TABLE 1

THE RESPONSE OF LACTOBACILLUS CASEI TO RIBOFLAVIN ON A 2.25 PER CENT. SOLUTION OF A CONCENTRATED BASAL MEDIUM STORED AT 75° F. OVER PHOSPHORUS PENTOXIDE FOR A PERIOD OF TWELVE WEEKS

	Lactic acid production (cc N/10 NaOH)									
Micrograms riboflavin	Trial No. 1	Trial No. 2	Trial No. 3	Trial No. 4	Trial No. 5	Trial No. 6	Trial No. 7	Trial No. 8	Mean	Mean per 0.05 microgram increments of riboflavin
0.00	1.15	1.50	1.95	1.65	2.30	2.10	1.40	1.50	1.69	...
0.05	3.20	3.50	4.60	3.40	4.25	4.05	3.60	3.70	3.78	2.09
0.10	5.55	5.85	6.65	5.60	6.55	6.30	6.35	6.00	6.11	2.33
0.15	7.50	8.20	8.30	7.45	8.65	8.75	8.40	8.15	8.18	2.07
0.20	8.90	9.30	9.65	8.40	9.70	10.00	9.80	9.20	9.37	1.19
0.25	9.75	10.30	10.50	9.00	10.05	10.55	10.25	9.60	10.00	0.63
0.30	10.25	10.55	10.80	10.10	10.75	11.05	10.55	10.65	10.59	0.59

A dehydrated basal medium for the assay of niacin has been obtained in a similar manner. Ingredients of the medium were prepared and combined in the proportions described by Krehl and co-workers.[4] In addition, a "folic acid" concentrate was added. The desiccated product was stored over phosphorus pentoxide at room temperature as in the case of the riboflavin basal medium. A 2.0 per cent. solution of this product was used for assay purposes. Anhydrous glucose and sodium acetate were added at a level of 4.0 per cent. Preliminary data obtained with this basal medium over a storage period of three weeks indicate results as successful as were obtained with the riboflavin basal.

Results obtained on the above described concentrated basal media are encouraging from the standpoint of standardization of microbiological assay methods. Many variables affect reproducibility of standard curves in the average control laboratory. Practical variations in incubation time, use of different incubators whose temperature controls vary in precision, different operators and the use of several lots of only approximately standardized alkali contribute to variations in standard curves. It has been impossible in this preliminary work to attempt rigid control of these various factors. We believe, therefore, since the basal used throughout this study was from a homogenous lot and since there is no apparent trend in variability, that this medium has maintained its original characteristics for a period of twelve weeks.

In view of these conclusions, together with the economy of time involved, it seems feasible that this type of product is justified for general use in microbiological assay.

Investigations are underway on the preparation of such basal media for pantothenic acid and "folic acid" as well as for media used in the microbiological assay of the amino acids. A complete manuscript describing details of the procedure involved will appear later.

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BOOKS RECEIVED

BLACKEN, KENNETH D. and LOUIS K. DIAMOND. *Atlas of the Blood in Children.* Illustrated. Pp. xiv + 320. The Commonwealth Fund. $12.00. 1944.

FRAPRIE, F. R. and FRANKLIN I. JORDAN. *The American Annual of Photography, 1945.* Illustrated. Pp. 200. American Photographic Publishing Co. $1.50, paper cover. $2.25, cloth cover. 1944.

MORGAN, BANNER BILL. *Bovine Trichomoniasis.* Pp. iii + 150. Burgess Publishing Company. $3.25. 1944.

NICHOLS, J. E. *Livestock Improvement.* Illustrated. Pp. vi + 208. Oliver-Boyd, Ltd., 10/6 net. 1944.

SMITH, MAY. *Handbook of Industrial Psychology.* Pp. 304. Philosophical Library. $5.00.

WOLF, FREDERICK TAYLOR. *The Aquatic Oomycetes of Wisconsin.* Part I. Illustrated. Pp. 64. The University of Wisconsin Press. $1.50. 1944.