perature was extended to the first 24 hours of incubation, there was a 170 per cent. rise in the frequency of survival of homozygous Creeper embryos as compared with the control group. This difference is clearly significant. When the time of incubation at reduced temperature was increased to the first 48 hours the advantage to the homozygous Creeper embryos, as reflected in survival, disappeared.

The fact that a lowered rate of development for the first 24 hours favors increased survival of homozygous Creeper embryos, but that the same did not hold when the reduced temperature lasted for as much as 48 hours, requires explanation. The reasons can only be surmised. When the temperature of incubation was brought down to 93° F. for as long as seven days, the majority of embryos died during the second week of development and hatchability was reduced to zero. Even after as short a period as four days at 93° F. only 37 per cent. of the fertile eggs hatched, as compared with 56 per cent. of the controls (eggs from Creeper matings). It is evident, then, that prolonged exposure to a reduced developmental rate is harmful. Although such a harmful effect can not be discerned after 48 hours of incubation at 96° F., as judged by hatching results, it seems reasonable to assume that the beneficial effect which a reduced developmenal rate has on survival of homozygous Creeper embryos is vitiated by harmful agencies which come into play if the duration of lowered developmental rate is extended beyond an optimum.

The fact that more homozygous Creeper embryos survive to late stages when the first 24 hours of their development proceed at a reduced developmental rate fixes the activity of the homozygous Creeper condition at a considerably earlier embryonic period than it has been possible to demonstrate by morphological means. It may also be concluded from these observations that the improved chances of survival of homozygous Creeper embryos under the conditions of our experiment indicate that the production or utilization of a critical substance or the occurrence of some chemical transformation has been aided.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

FLUORESCENT STAINING OF INSECT TISSUES

THE staining of plant and animal tissues by means of fluorescent substances has come into use during recent years and numerous materials have been found suitable for this purpose. Known as fluorochromes, these act specifically to stain certain cellular structures, in a way comparable to the more commonly used microscopical stains like haematoxylin and various aniline dyes. While the latter are ordinarily viewed by transmitted white light or by certain other bands of the visible spectrum, fluorochromes show practically no color until irradiated by ultra-violet light. Thus illuminated, the stained structures fluoresce and appear as a brilliantly luminous pattern whose color (most commonly yellow) is that characteristic of the specific stain used. Fluorescence is usually most strongly activated by the longer wave-lengths of ultra-violet, especially those near λ3650 which are particularly strong in the light of mercury-vapor lamps. These, then, form a satisfactory source of illumination. The insertion of a "black" Corning glass filter (such as Wratten No. 18) between the lamp and the microscope removes the visible light (except a little red) and all the ultra-violet below about λ3100. Thus the light is perfectly safe for visual work, especially if a light yellow protective filter (such as Wratten No. 2a) is interposed between the microscope and the eyes.

The dyes earlier used by Haitinger and his associates[1] included a few plant extracts, alkaloids and dyes of various sorts whose staining properties these workers investigated briefly. More recently a number of other substances have been found to be satisfactory fluorochromes for certain purposes, for example, the use of auramin as a stain for bacteria, especially in the diagnosis of tuberculosis.

Following a simple procedure of fixing in 5 per cent. formalin followed by the ordinary methods of dehydration in alcohol, clearing in xylol, imbedding and cutting in paraffin, sections may be stained in aqueous solutions of the fluorochromes and examined under the microscope after mounting in glycerin. The latter is not fluorescent and must replace the ordinary balsam or other media which are highly fluorescent and "fog the picture," as will also the use of oil-immersion objectives, due to the natural blue fluorescence of immersion oils.

The application of several of these dyes to the staining of insects' tissues has been reported on by Metcalf and Patton,[2] who have used berberine extensively, finding it to be a powerful stain which differentiates nuclei very clearly as it causes the chromatin to fluoresce a brilliant light yellow.

A number of specific alkaloids, or these in mixture as obtained by extracting the roots, bark or other

[1] *Bot. Centralbl., Beihefte,* 50: pp. 432–444, 1933; *ibid.,* 53, pp. 378–396, 1935; *ibidem,* pp. 387–397; Abderhalden's Handb. d. Biol. Arbeitsmeth., Abt. II, Physik. Abt., Teil 3, Heft 5, Lief, 433, pp. 3307–3337, 1934.

[2] *Stain Technology,* 19: 11–27, 1944.

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.