About 30 pounds of fossiliferous slabs and fragments were selected and carried to the U. S. National Museum. Specimens of Catopteridae and Ptycholepis were particularly abundant. One large and very interesting impression was later classified as *Diplurus*. Small nodules, possibly coprolites of Triassic reptiles replaced by a phosphoric, carbonaceous material, were much in evidence (*2*).

The following approximate measurements of the exposures on Licking Run were taken under water, beginning with typical Manassas sandstone and extending into Bull Run shale:

8 ft—Pink and yellow shale with a few sandstones.
3 ft—Compact shale, alternating with thinly laminated siltstone.
1½ ft—Dark, carbonaceous, very fissile shale. } Fossil beds
½ ft—Dark, carbonaceous, coarsely bedded semicrystalline limestone. } Fossil beds
3½ ft—Thinly laminated siltstone and compact shale.
10 ft—Buff shale and sandstone.
..... —Typical red shale and sandstone.

This sequence is repeated with some variation in thickness at an exposure on the south branch of the south fork of Broad Run. However, in other exposures along a roadway near Antioch, along Chestnut Lick, in the Millbrook Quarry, in a small stream near by, and in the excavation for a bridge abutment along U. S. Highway 15 at Catharpin Run (Fig. 2), the basal sedimentary strata are concealed and the carbonaceous fossil beds have greater thicknesses (5 ft or more), grading upward into the typical red shale and sandstone. In the Millbrook Quarry, where considerable core drilling has been done, the dark shales grade downward into a dark, arkosic conglomerate having a known thickness of 180 ft. The two easternmost exposures on Licking Run and on Broad Run indicate a thinning-out of the dark shales, and farther eastward the chances of encountering the fossil beds seem small.

Wherever scales occur *in situ*, it is probable that more or less complete preservations of fish exist near by. Therefore, it seems safe to assume that fossil fish may be found in a belt at least 18 miles long and approximately 2½ miles wide near the western Triassic border adjacent to the Bull Run Mountains, where scales are found in numerous exposures at well-spaced intervals. The discoveries outlined in this paper, not to mention the additional collecting possibilities in the entire fossiliferous belt, are significant if only because no ganoid fossils other than isolated scales have, to the knowledge of the authors, been recorded heretofore from the Triassic basins of northern Virginia. Furthermore, the character of the occurrence in siltstone near Thoroughfare Gap appears to be unique in the history of the Newark System.

References

1. ROBERTS, J. K. *Va. Geol. Survey Bull. 29*, 1928, Plate 1.
2. ROGERS, W. B. *Geology of the Virginias*, New York: D. Appleton, 1884. Pp. 478, 767.

Colchicine Poisoning in Relation to Hemerocallis and Some Other Plants

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Colchicum species contain appreciable amounts of colchicine (*4, 5*). According to Blakeslee (*1*) and Levan (*3*), *Colchicum* species they tested were not subject to colchicine poisoning under the conditions of their experiments.

On the basis of these reports, it would seem logical to assume that plants, other than *Colchicum* species, which also contain appreciable amounts of colchicine, might likewise be immune to colchicine poisoning. However, this hypothesis apparently has not as yet been tested. According to Klein and Pollauf (*2*), the presence of colchicine in *Hemerocallis fulva* L. has been demonstrated microchemically; but extensive experiments by the present writer have shown that *Hemerocallis* species, including *H. fulva* L., and various hybrid clones tested, are very sensitive to colchicine, and concentrations in aqueous solution in the range from 0.025% to 0.1%, tested with appropriate application techniques, proved to be quite effective in inducing polyploidy. Concentrations much above 0.1% usually led to the eventual death of the treated plants.

A number of colchicine-induced *Hemerocallis* polyploids have flowered. Their polyploid nature was established on the basis of chromosome counts, and pollen grain and stomate size. One polyploid, Tetra Starzynski, $n = 22$, a tetraploid of the clone Mayor Starzynski, $n = 11$, has been named. Its flowers are larger and finer-colored than those of the diploid form. This removes all doubt about the effectiveness of this compound as a mutagen in this genus. The object of the present note is not to elaborate here on these results, which will be reported in detail elsewhere, but rather to consider very briefly their implication, particularly with reference to the validity of the microchemical method used for determining colchicine.

Klein & Pollauf (*2*) used a microchemical procedure for the determination of colchicine in *Hemerocallis fulva* L. In view of the marked sensitivity to this compound of *Hemerocallis* species, including *H. fulva* L. and hybrid clones, their results require verification. If their report can be verified, then it would appear, in this instance at least, that plants which contain relatively smaller amounts of colchicine could be affected by the application of relatively larger amounts of the compound. It is also desirable to check the reported colchicine content of species in other genera. In this connection, it is of interest to note that *Gloriosa rothschildiana* O'Brien (a plant closely related to *G. superba* L., which is reported to contain colchicine) is subject to colchicine poisoning in the range tested, 0.05%–0.2% concentrations, particularly in the case of small seedling tubers.

References

1. BLAKESLEE, A. F. *Amer. J. Bot.*, 1939, **26**, 163.
2. KLEIN, G. and POLLAUF, G. *Oesterr. Botan. Z.*, 1929, **78**, 251.
3. LEVAN, A. *Hereditas*, 1940, **26**, 262.
4. SEKA, R. In Klein, *Handb. d. Pflanzenanal.*, 1933, **IV**, 507, 754.
5. WEHMER, C. and HADDERS, M. In Klein, *Handb. d. Pflanzenanal.*, 1933, **IV**, 763.

Preparation of a Radioactive Oxazine Dye[1]

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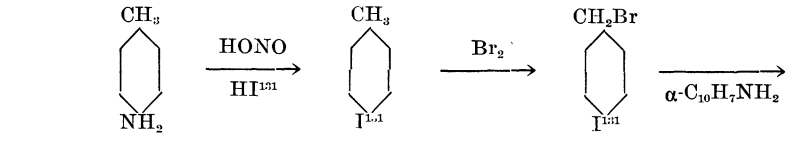
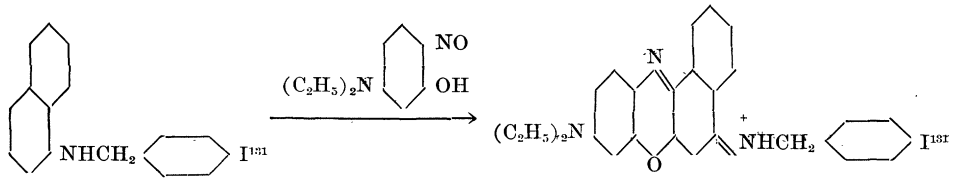
The discovery (*2*) that the oxazine dye Nile blue A stains and retards the growth of malignant tumors in mice stimulated the investigation of related compounds. Of the oxazine dyes tested on mouse tumors (*1*), the one known as Nile blue 2B, Colour Index No. 914 (*5*), showed the greatest affinity for tumor tissue. The halogen derivatives of Nile blue 2B have been prepared (*3*) with the intent of using one of them as a "carrier" of radioactivity to the tumor tissue when radioactive halogen is present in the molecule. It is the purpose of the present paper to describe the preparation of the radioactive iodine derivative of Nile blue 2B.

The synthesis of this radioiodo dye was carried out (without isolation of intermediate compounds) by the following series of reactions:



p-toluidine *p*-radioiodotoluene *p*-radioiodobenzylbromide



N-(*p*-radioiodobenzyl)-α-naphthylamine

5-(*p*-radioiodobenzylamino)-9-diethylaminobenzo[a]phenoxazine

A solution of 3.3 g of *p*-toluidine in 35 ml of water and 3.6 ml of concentrated sulfuric acid was carefully diazotized at 5° C with 2.1 g of sodium nitrite in 20 ml of water. To 18.5 mc of carrier-free I^{131} [2] in 15 ml of water, was added 5 g of potassium iodide and this solution was slowly added to the cold diazotized solution. A small quantity of sodium sulfite was added to reduce any iodine. The mixture was kept in an ice bath for 30 min, then at room temperature for 1 hr, and then heated gently on a steam bath for 1 hr. The resulting mixture was then steam-distilled until 250 ml of distillate was obtained. The distillate was made decidedly alkaline with sodium hydroxide solution, a small amount of sodium sulfite was added to it, and then it was extracted with 150 ml of carbon tetrachloride.

This solution of *p*-radioiodotoluene in carbon tetrachloride was washed three times with water and then evaporated to a volume of about 50 ml. To this solution was added 1.55 ml of bromine in 25 ml of carbon tetrachloride, and the reaction flask was attached to an efficient all-glass reflux condenser. The flask was illuminated by two 200-w clear Mazda lamps, and heated at gentle reflux for 3 hr, at which time the evolution of hydrogen bromide had ceased. The mixture was then cooled to room temperature and 5 g of potassium iodide in 25 ml of water was added. Sodium thiosulfate solution (approx. *N*/10) was then added till all iodine was reduced. The carbon tetrachloride layer was separated and washed with water. The carbon tetrachloride was distilled off on a steam bath.

The resulting *p*-radioiodobenzyl bromide was dissolved in 50 ml of ethanol and to this was added a solution of 12 g of α-naphthylamine in 50 ml of ethanol. The mixture was heated in a water bath under reflux condenser for 2 hr. The ethanol was distilled off and the residue extracted three times with 100-ml portions of 1: 50 hydrochloric acid to remove excess α-naphthylamine.

The resulting N-(*p*-radioiodobenzyl)-α-naphthylamine (containing a small quantity of α-naphthylamine) was dissolved in 75 ml of ethanol and 9 ml of concentrated hydrochloric acid. To this was added a solution of 10 g of 2-nitroso-5-diethylaminophenol in 25 ml of ethanol. The mixture was gently boiled under reflux condenser for 3 hr and then allowed to stand overnight. After cooling in an ice bath for 1 hr, the product was filtered and washed with cold ethanol and then with ether. The weight of product obtained was 8 g.

Radioactivity of the dye was measured, using a thick-window, cylindrical, gamma-ray Geiger-Müller tube. An

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[2] Obtained from Oak Ridge National Laboratory.