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.

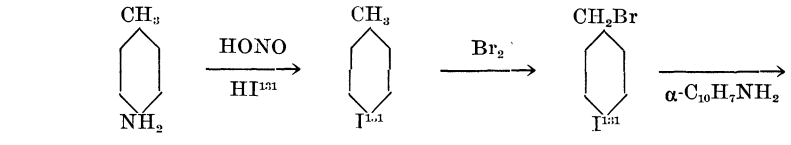
Preparation of a Radioactive Oxazine Dye[1]

Henry A. Sloviter

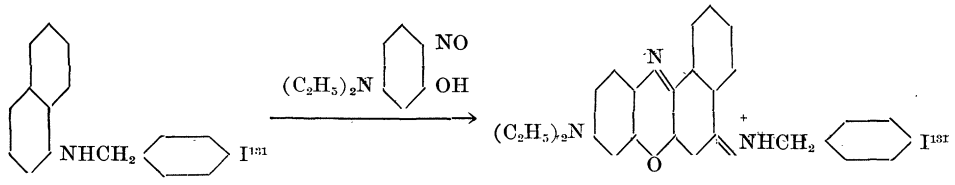
Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania and *the Department of Neurosurgery, Hospital of the University of Pennsylvania, Philadelphia*

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

[1] Aided in part by a grant to Dr. Margaret R. Lewis from the National Cancer Institute.

[2] Obtained from Oak Ridge National Laboratory.

accurately weighed sample of the dye was dissolved in ethanol (about 0.1 g per 100 ml) and a 0.50-ml aliquot was placed on a 1-in. square of filter paper. As soon as the ethanol had evaporated, the paper square was placed between gummed paper labels. This was placed in direct contact with the Geiger-Müller tube with a tight rubber band. The Geiger-Müller tube was calibrated by counting in identical fashion an aliquot of the original I^{131} solution which had been assayed at the Oak Ridge National Laboratory. The activity of the dye, measured in this manner, was 0.55 mc/g.[3]

This radioactive dye has been administered to tumor-bearing experimental animals and to a small number of human subjects. Results appear elsewhere (*4*).

References

1. Lewis, M. R., Sloviter, H. A., and Goland, P. P. *Anat. Rec.*, 1946, **95**, 89.
2. Lewis, M. R., Goland, P. P., and Sloviter, H. A. *Anat. Rec.*, 1946, **96**, 201.
3. Sloviter, H. A. *J. Amer. chem. Soc.*, in press.
4. ———. *Cancer Research*, 1949, **9**, 677.
5. Society of Dyers and Colourists. *Colour Index*. Great Britain, 1924.

Occurrence and Life Histories of Commercial Shrimp

Martin D. Burkenroad[1]

Institute of Marine Science,
University of Texas, Austin

Grooved shrimp (*Penaeus*, Division II) are unusually prominent in the North Carolina catch. Study of the species has been impeded not only by lack of means to distinguish them from each other before adolescence (or to distinguish their eggs and larvae from those of the ungrooved *P. setiferus*, with which it seems possible that Pearson (*3*) has confused them), but by lack of means for rapid field identification of adults. A simple diagnostic test for the commercial sizes of the two grooved species common in continental North America is that *Penaeus duorarum* Burkenroad, the spotted shrimp, bears a reddish brown midlateral patch at the juncture of third with fourth pleonic segment. This pigmented spot, first called to my attention by Captain O. Purifoy, is larger and denser than the one at the midlateral hinges that lock the other pleonic somites together, or the one at the equivalent site in *P. aztecus* Ives, the brown shrimp. Field confirmation of the rapid diagnosis is seen in the difference between the common North American forms of these two species in width of groove alongside the mid-dorsal carina of sixth pleonic segment (*1*).

No special fishery for spotted shrimp has previously been described. A 10-year-old one in the shallow Straits between Core Sound and Beaufort Inlet, North Carolina, employs some seventy channel nets, like boardless, long-winged otter trawls, which are staked or anchored in strong tide-currents during night ebb in May, June, and July. These stationary tide traps intercept shrimp swimming off the bottom (presumably migrating to sea). A catch of over a ton by one 75-ft net on one ebb has been reported, but the annual production of the whole fishery is probably around 100 tons. The catch, especially prized for hardness, sometimes contains a proportion of brown shrimp but is chiefly immature *P. duorarum*. Since grooved shrimp in the North Carolina commercial trawl catch are mostly *P. aztecus*, the tide traps evidently select the spotted species from an inshore and inside grooved shrimp population which is as a whole preponderantly of brown shrimp. There is evidence, however, that young spotted shrimp are in the majority on the shallowest bottoms of the saltier sounds.

During the last decade, the proportion of grooved shrimp in the annual North Carolina catch has probably fluctuated between a third and a half. Brown shrimp have always been prominent in the catch of Carteret County, but testimony from fishermen suggests that since 1936 these shrimp have become absolutely, as well as relatively, more abundant not only there but on the grounds farther south around Cape Fear, which before 1936 supplied a larger share of the state's catch, including a higher proportion of the gray shrimp, *P. setiferus*. Since there are indications of unusual abundance of *P. aztecus* during the same period along its whole Atlantic Coast range, it seems possible that the population of this species has undergone a recent increase. Such an increase may have contributed to the development since 1936 of an extensive new fishery in Pamlico Sound. This, the most northern American market fishery for peneid shrimp, sometimes supplies more than a third of North Carolina's production, chiefly from a summer run of relatively large brown shrimp.

Brown shrimp in North Carolina shoal-water catches are often nearer maturity than is usual along the more brackish shores of Louisiana, but the spawning grounds of the North Carolina population have not yet been found. Sporadic offshore catches of jumbo shrimp by winter fish trawlers in the Hatteras-Lookout area are chiefly of gray and of spotted shrimp; and a reliable and widely experienced North Carolina fleet owner, Mr. L. Hardee, who has encountered the abundant, though scattered, orange-colored, yellow-roed breeding population of *P. aztecus* present off Louisiana in 15–70 fathoms the year round (*1*), does not know of a North Carolina equivalent.

Although this apparent scarcity of adult brown shrimp off North Carolina may simply reflect the inadequacy of exploration, it is not necessarily inconsistent with the high density of the state's inshore immature population. Half-grown brown shrimp appearing in New Jersey in midsummer are evidently postlarval immigrants (*1*). It is therefore conceivable that North Carolina brown shrimp, in contrast to the endemic spotted kind, chiefly originate farther south.

It should be observed that, as long as the Atlantic habitat of mature brown shrimp remains unknown, the belief that there is no extensive Atlantic deepwater population of gray shrimp (*4*) cannot be regarded as

[1] Private laboratory: Ecos, Bogue Sound Road, Newport, North Carolina.

[3] Miss Ruth E. Brown assisted with the radioactivity measurements.