driasis was primarily a peripheral anticholinergic response since both the tertiary and quaternary derivatives of atropine and scopolamine were potent inducers of mydriasis. It also was concluded that inhibition of physostigmine-induced lethality was primarily a measure of central anticholinergic activity since the quaternary derivatives were much less potent inhibitors of physostigmine-induced lethality than their respective tertiary forms.

A careful evaluation of a drug's relative activity in these two procedures in the same species, the induction of mydriasis and the inhibition of physostigmine lethality, should predict its relative activity as a central and a peripheral anticholinergic in humans.

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[x] To whom inquiries should be directed. Present address: Department of Pharmacology, ICI United States, Inc., Wilmington, DE 19899

Tissue Distribution of *N*-^{14}C-Azure C (Methylthionine) in the Rat

R. ENG, S. LINDBERG, K. WHITE, and L. A. SPITZNAGLE [x]

Abstract □ The distribution of radioactivity at 5-, 10-, and 15-min intervals following the intravenous administration of *N*-^{14}C-azure C was determined. The concentrations of radioactivity observed indicated that radioactive derivatives of azure C would not be useful pancreas or parathyroid scanning agents.

Keyphrases □ Azure C, radiolabeled—tissue distribution, considerations related to use as pancreas or parathyroid scanning agent □ Methylthionine, radiolabeled—tissue distribution, considerations related to use as pancreas or parathyroid scanning agent □ Scanning agents—tissue distribution of radiolabeled azure C

The report (1) that the intravenous administration of the phenothiazine dye tolonium chloride (toluidine blue O) caused a blue coloration of the pancreas and parathyroid glands but not of surrounding organs generated much interest and has been verified in experimental animals (2–4) and humans (5–7). Toluidine blue O has been used for the identification of the parathyroid gland at surgery (5–7) and as an aid in the diagnosis of small oral cancers (8), and it has been suggested as a potential parathyroid and pancreas scanning agent if labeled with a suitable radionuclide (2–4, 6, 7). Recent studies with an iodinated analog of toluidine blue O labeled with ^{131}I showed it to be useful for imaging parathyroid adenomas (9).

DISCUSSION

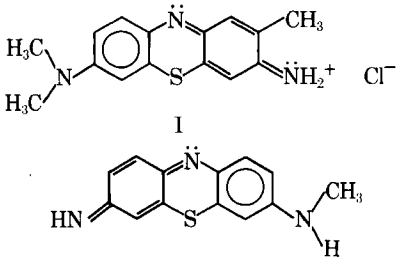
Kang and DiGiulio (3) found the highest concentrations of toluidine blue O in the parathyroids, heart, pancreas, kidneys, stomach, lungs, thyroid, muscles, liver, and blood, in that order. They extracted the excised tissues with ethanol and measured the dye concentrations colorimetrically. Mortenson and McRae (2) reported similar results using a similar assay. In both studies, rather low concentrations were observed in the organs surrounding the pancreas, suggesting the development of a pancreas scanning agent provided a suitable labeled derivative could be prepared.

Larose *et al.* (10) studied the distribution in rats of an iodinated derivative of toluidine blue O labeled with ^{125}I. Using an assay method that allowed the measurement of tissue radioactivity without extraction, they found the concentrations of radioactivity in the pancreas, liver, and kidneys to be similar for up to 60 min after intravenous administration. Likewise the parathyroid was observed to have concentrations of radioactivity similar to those found in the thyroid gland. They concluded that iodinated toluidine blue O was an unsatisfactory scanning agent for the pancreas and parathyroid glands.

Archer *et al.* (11) also studied the tissue distribution of a ^{125}I-labeled derivative of toluidine blue O. However, they extracted the tissues with ethanol and measured the radioactivity of the extracts. Their results differed from those of Larose *et al.* (10) in that the parathyroid extract contained approximately three times the concentration of extractable radioactivity as did the thyroid. The pancreas extract contained only a slightly higher concentration of radioactivity than the liver extract, indicating that iodinated toluidine blue O was an unsatisfactory scanning agent for the pancreas. However, these investigators observed that the percentage of the injected dose taken up by the pancreas was much greater when unlabeled toluidine blue O was given instead of iodinated toluidine blue O.

Preliminary work in this laboratory confirmed the results of Klopper and Moe (1); 30 min after the intravenous administration of toluidine blue O, extracts of the pancreas had 10 times the concentration of dye compared to extracts of the liver when measured colorimetrically (12). In a preliminary investigation of the distribution of ^{35}S-labeled toluidine blue O (12), concentrations of radioactivity in the pancreas, liver, and kidneys were found to be similar. These results seemed to confirm the conclusions of Larose *et al.* (10) and Archer *et al.* (11) that labeled derivatives of toluidine blue O were unsatisfactory pancreas scanning agents.

One possible explanation of the blue coloration of the pancreas consistent with these results is that the dye molecule may be



I

II

"decolorized" by the liver and kidneys but not by the pancreas.

An observation in this laboratory that toluidine blue O can easily be *N*-demethylated (13) suggested that the data might be explained by *N*-demethylation of toluidine blue O in the liver and kidneys but not in the pancreas.

If both *N*-methyl groups were removed from toluidine blue O (I) in the liver and the kidneys but not in the pancreas, then the resulting dye (2-methylthionine) would probably not be visible when the viscera was observed, nor could the concentration of the dye be measured accurately at 625 nm. Therefore, the distribution of a dye molecule labeled in the *N*-methyl group, rather than in the phenothiazine ring, was of interest. If such a dye were rapidly *N*-demethylated by the liver and kidneys but not the pancreas, it might be possible to produce a pancreas scanning agent by labeling such a dye with an ^{11}C-*N*-methyl group.

Since toluidine blue O contains two amino groups that are not symmetrical, it would be difficult to label the *N*-methyl groups. Therefore, the distribution of azure C (*N*-methylthionine, II), an aminophenothiazine dye with similar chemical properties to toluidine blue O which can easily be labeled with ^{14}C in the *N*-methyl group, was studied.

The purpose of the present study was to determine if the concentration of radioactivity in the pancreas and parathyroid glands following the administration of an aminophenothiazine dye labeled in the *N*-methyl group would be sufficient to warrant further efforts to develop pancreas and parathyroid scanning agents from such dyes.

EXPERIMENTAL

Chemicals—The dyes[1] were labeled 80–90% pure. All other chemicals were reagent grade and were used as received.

^{14}C-Azure C was prepared as previously reported (14), and TLC indicated the radiochemical purity to be greater than 89%. The dye was dissolved in water prior to injection.

Animals—The animals used were Sprague–Dawley rats, 200–250 g, and they were maintained on rat chow and water *ad libitum* prior to use.

Tissue Distribution—The animals were anesthetized with pentobarbital sodium, and 5 μCi of ^{14}C-azure C was injected *via* an exposed femoral vein. The animals were sacrificed after 5, 10, or 15 min. Thyroid, parathyroids, heart, pancreas, adrenals, liver, kidneys, spleen, stomach, and a sample of blood were removed as rapidly as possible. The organs were frozen immediately in dry ice to prevent dehydration. The tissue samples were weighed while still frozen.

Tissue samples too large to be dissolved directly were minced with a razor blade and then homogenized[2] in distilled water equal to the tissue weight. The tissues and aliquots of the tissue homogenates were dissolved by heating at 50–60° in liquid scintillation counting vials containing 1 ml of a solubilizer[3]. The dissolved samples were decolorized by slowly adding 30% H_2O_2. The dissolved and decolorized samples were then counted[4] at 5°. The sample counts were corrected for quenching by the external standard ratio method, which had been shown to give a linear relationship between external standard ratio and counting efficiency over the range of counting efficiencies obtained by this method of sample preparation.

[1] National Aniline and Eastman Kodak.

[2] Polytron homogenizer, Brinkmann Instruments, Westbury, N.Y.

[3] Protosol, Packard Instrumental Co., Des Plaines, Ill.

[4] In a Beckman LS-230 liquid scintillation counter, using Aquasol (New England Nuclear, Boston, MA 62118) as the counting solution.

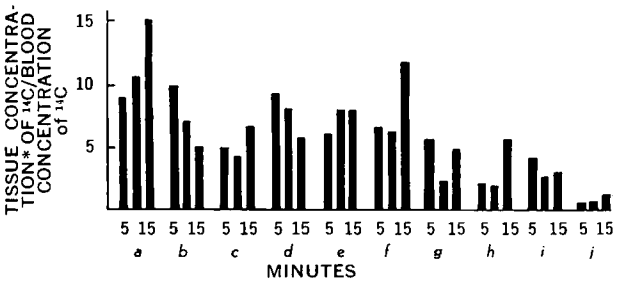


Figure 1—*Tissue distribution of ^{14}C activity at 5, 10, and 15 min after the intravenous administration of ^{14}C-azure C. (*Average from six animals at each time.)* Key: a, *adrenal;* b, *parathyroids;* c, *thyroid;* d, *lung;* e, *liver;* f, *kidney;* g, *heart;* h, *stomach;* i, *pancreas; and* j, *muscle.*

RESULTS

The ratios of tissue–blood radioactivity 5, 10, and 15 min after the intravenous administration of ^{14}C-azure C are shown in Fig. 1. The distribution of radioactivity is similar to that found after the administration of ^{35}S-toluidine blue O (12) or an ^{125}I-labeled analog of toluidine blue O (10). These results indicate that the blue coloration of the pancreas observed after administration of azure C is probably not due to rapid demethylation of the dye in the liver and kidneys. These results, when considered with the previous data obtained with toluidine blue (2, 3, 10–12), suggest that the pancreas turns blue after the dye administration because of its thickness and translucent appearance in comparison with surrounding organs. The results also indicate that ^{11}C-labeled azure C would not be a good parathyroid or pancreas scanning agent.

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[x] To whom inquiries should be directed.