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Radioautographic Localization of Hydralazine-1-C_{14} in Arterial Walls.* (31195)

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Hydralazine, a potent antihypertensive agent first described in 1950(1), decreases peripheral resistance and thereby reduces arterial pressure, despite increased cardiac output(2). In contrast, most other currently available antihypertensive agents produce at least part of their effect by decreasing cardiac output. While the precise mechanism of its action is not known, there is evidence to suggest that hydralazine acts peripherally(3). Activity has been shown to depend on a pyridazine ring with an attached hydrazine side chain(4). The fate of hydralazine has been studied by using isotopically labeled compound with a radiocarbon atom between the pair of nitrogen atoms in the pyridazine ring and the pair in the hydrazine side chain. Two hours after receiving hydralazine-1-C_{14} by intramuscular injection, normal mice had higher levels of radiocarbon in aorta than in any other tissue tested, and the rate of disappearance of radioactivity was slower from aorta than from other tissues(5). The work reported here further emphasizes the unusual affinity of hydralazine for vascular tissue by demonstrating the striking radioautographic localization of radiocarbon from hydralazine-1-C_{14} in arteries throughout the body.

Methods. Adult female Tumblebrook mice weighing between 15 and 20 g were given single intramuscular injections of 0.4 mg of hydralazine-1-C_{14} containing 4.8 μc of C_{14}. The animals were sacrificed by a blow on the head from 2 to 240 hours later. Sections from tissues fixed in formalin for 24 hours and frozen sections from unfixed tissues were cut to thicknesses of 7-10 μ. Sections from brain, lung, heart, spleen, liver, kidney, and thigh muscle were placed upon glass slides which were dipped into 1% celloidin in alcohol-ether and allowed to dry. In a darkroom, the edge of a cover slip was dipped into Kodak Nuclear Track Emulsion (NTB3), which had been warmed to 40°C, and lightly drawn over each slide. The slides were placed in plastic light-tight boxes and exposed at 4°C for from 15 to 240 days. After exposure, the slides were developed in Kodak D19 developer, washed, fixed, and rewashed.

To determine whether fixation in formalin leached radiocarbon from tissue, the fixing solution was tested and found to have accumulated as much as 15% of the radioactivity from tissues of animals sacrificed 2 hours after injection of hydralazine-1-C_{14} but less than 3% from tissues of animals sacrificed 24 hours after injection. At all intervals after hydralazine injection, radioautographs of fixed tissues looked both quantitatively and qualitatively similar to those obtained from frozen unfixed tissues. Underlying structures

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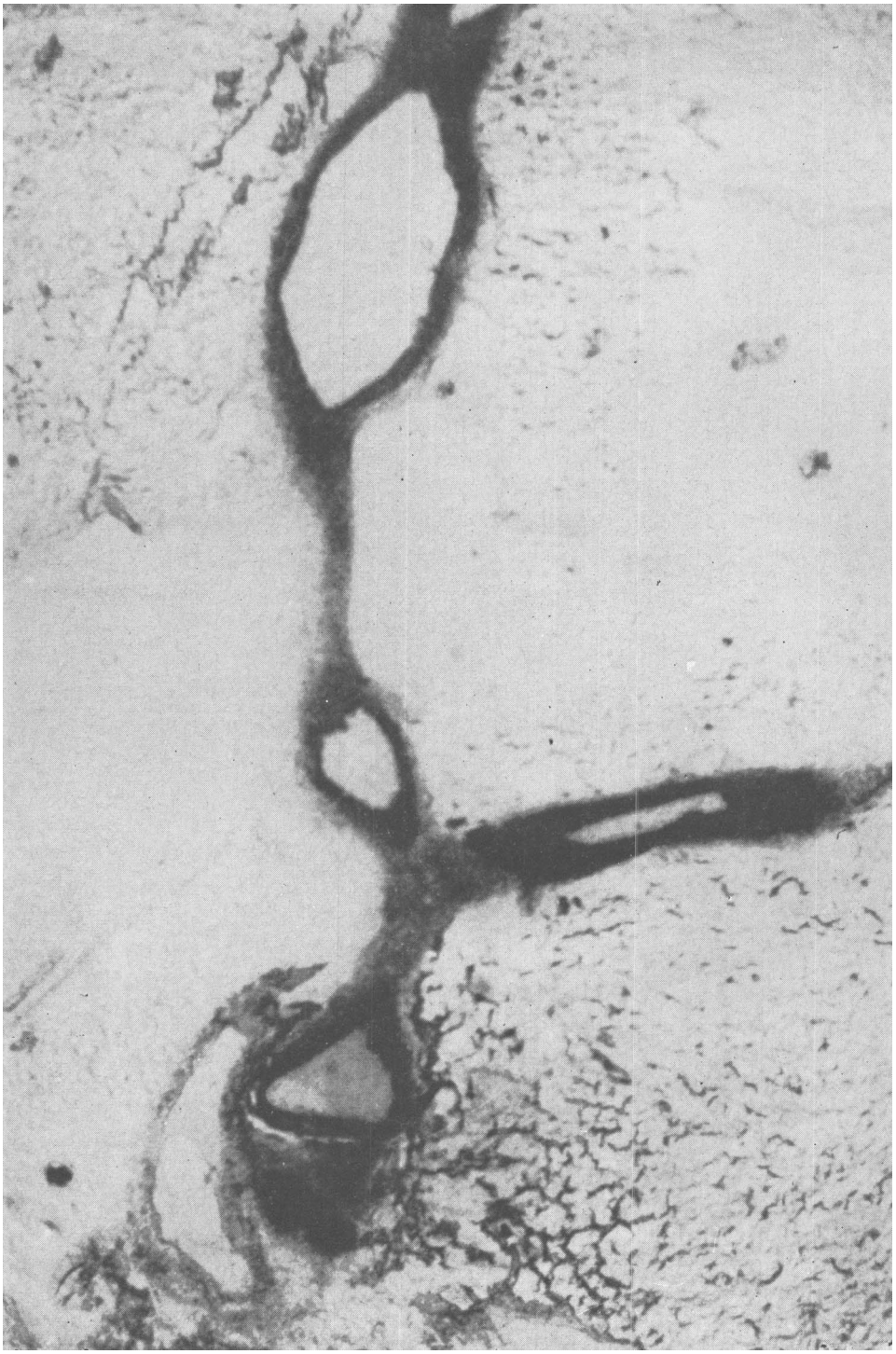

FIG. 1. Kidney: mouse sacrificed 2 hr after injection of hydralazine-l-C_{14}, frozen tissue, magnification × 40.

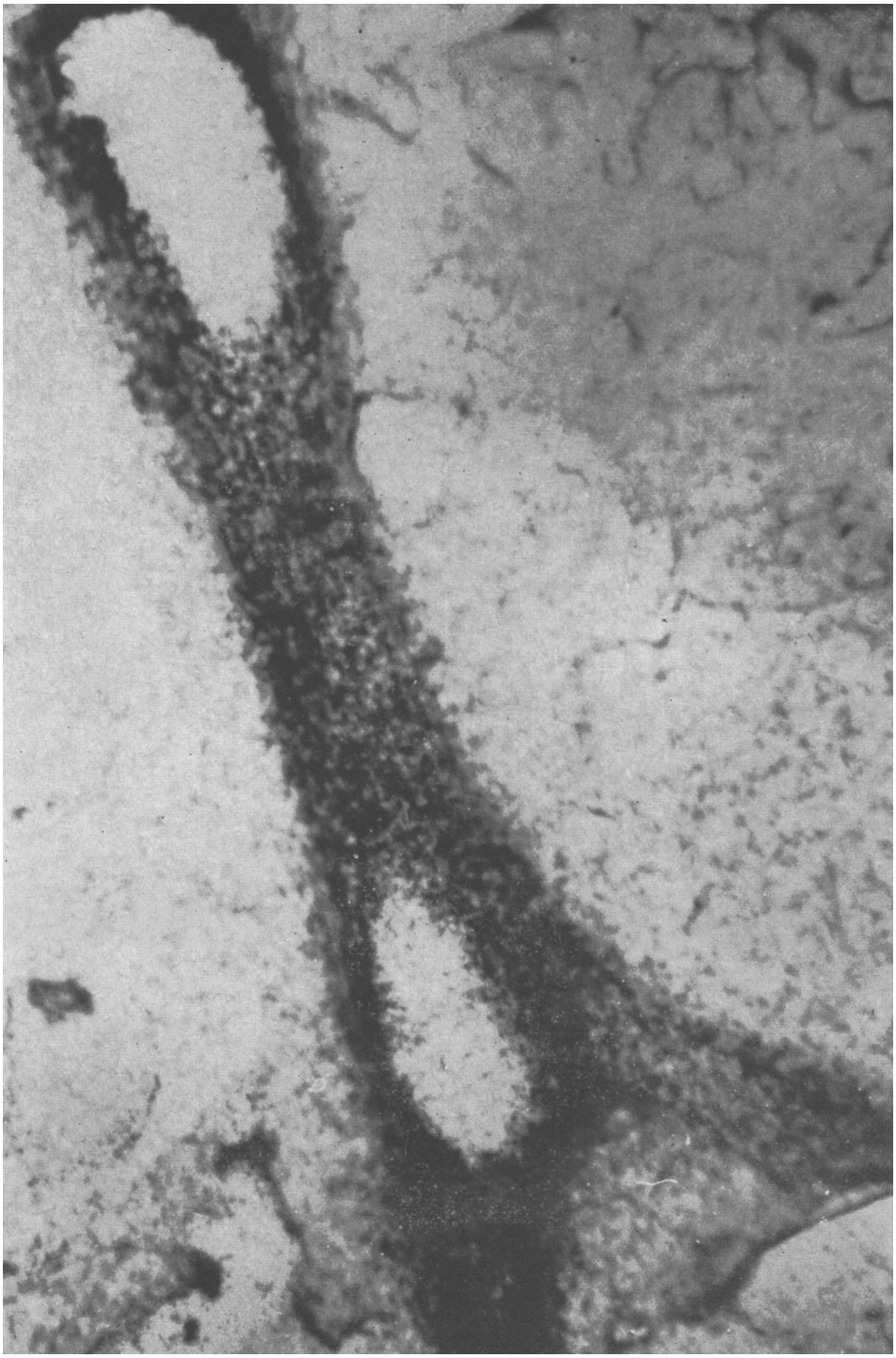

FIG. 2. Kidney: mouse sacrificed 24 hr after injection, fixed tissue, magnification × 300.

were more easily distinguished in fixed than in frozen tissues.

Results. In radioautographs of tissues from mice sacrificed at 2, 24, 48, and to a lesser extent at 120 hours after injection of hydralazine-1-C_{14}, muscular arteries throughout the body were strikingly darkened, indicating considerable fixation of radiocarbon. The radioactivity was not quantitated, but it appeared to be most marked in the interlobar, arcuate, and interlobular arteries of the kidneys (Fig. 1 & 2); however, it was qualitatively similar in arteries of similar size in all tissues examined, including the lungs. Although it was difficult to recognize structures in unstained tissues through the developed emulsion, smaller amounts of radiocarbon were found over what appeared to be large pulmonary veins. The relationships of some minimal linear aggregations of radiocarbon to small arteries suggested arterioles, but no underlying structures could be distinguished. The radiocarbon seemed to be widely disseminated throughout the arterial wall and could not be localized to any one region of it. Although no particular structure could be implicated, a linear arrangement was sometimes suggested (Fig. 2). Radiocarbon was not obviously concentrated in any histologically recognized, non-vascular structures; the mass of cardiac muscle fixed no more than other tissues.

There were no obvious radioautographic differences, either quantitative or qualitative, between muscular arteries in tissues obtained 2 hours after injection, when prior work indicated that only one-eighth of the radiocarbon had been excreted, and tissues obtained 48 hours after injection, when excretion of the radiocarbon was more than half complete and when its tissue distribution had changed markedly(5). Muscular arteries could still be distinguished radioautographically 120 hours after injection, albeit with difficulty; but after 240 hours they were indistinguishable from background radioactivity.

Discussion. The localization described here of radiocarbon from hydralazine-1-C_{14} in muscular arteries recalls the binding of the same radiocarbon in aorta previously observed with the Geiger-Müller counter(5). The persistence of radioactivity in muscular arteries for more than 48 hours suggests that hydralazine might have more persistent pharmacologic effects than has been supposed. Such delayed effects have recently been demonstrated by finding altered reactivity of aortic strips to pressor agents in animals which received single injections of hydralazine 24 hours before sacrifice(6). The marked affinity of hydralazine for vascular tissue may also be related to the toxic effects of this compound which has the unique ability to induce the clinical picture, and presumably the vascular lesions, of lupus erythematosis(7,8).

Summary. By radioautography, radiocarbon from hydralazine-1-C_{14} has been shown to be concentrated in the walls of muscular arteries in kidney, liver, spleen, heart, lung, brain, and muscle. It remains there in high concentrations for more than 2 days and in lower concentrations for more than 5 days.

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