Autoradiographic Study of Localization of Aurintricarboxylic Acid in Experimental Beryllium Poisoning.* (22475)

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The effectiveness of aurintricarboxylic acid (ATA) in the treatment of acute beryllium poisoning in experimental animals has been described(1,2). The therapeutic effect of this dye is ascribed to the fact that it combines readily with free beryllium ions in tissues to form a chelate and thence a stable lake which renders beryllium innocuous to tissues in which it is deposited. Histopathological studies of the principal target organs in acute beryllium poisoning have shown that ATA produces arrest of damage in liver, spleen and bone marrow followed by prompt repair of the lesions, thus allowing animals to survive a toxic dose of beryllium ($LD_{98/3\ days}$) for an indefinite period of time(3). Anatomical distribution of early lesions within an organ can be correlated with the distribution pattern of beryllium as described by Kaylor and Van Cleave, who visualized the deposition of tracer amounts of radio-beryllium in tissues of rats with the aid of autoradiograms(4). Studies of C^{14}-labeled ATA in the rat have shown a high and almost constant level of retention of this material in liver, spleen and kidney up to 3 months or more(5). The following experiment was undertaken in an attempt to visualize the simultaneous localization of beryllium and ATA in various organs of mice.

Methods. Beryllium was prepared for injection by dissolving carrier $BeSO_4$ and carrier-free Be^7Cl_2 to give an aqueous solution of pH 3.5 with a radioactivity level of about 4.3 mc/ml; the dose was 0.54 mg/kg (LD_{70}) and about 1 mc Be^7 per mouse. The ATA injection consisted of a 3% solution of ammonium salt of C^{14}-methyl-labeled ATA adjusted to pH 7 and containing approximately 0.25 mc C^{14}/ml; the dose was 200 mg/kg with 0.05 mc of C^{14} activity per mouse. Twelve young adult CF#1 female mice weighing 30 g each were divided into 3 groups of 4, receiving intravenously: (I) Be only; (II) Be, followed after one hour by ATA; (III) ATA only. Two animals of each group were sacrificed by cervical fracture at 12 and 48 hours after injection. Kidney, liver, lung and spleen were fixed in 10% neutral formalin and embedded in paraffin, and sections were cut at 15 μ. Autoradiograms of Be-containing tissues were prepared by a technic similar to that of Kaylor and Van Cleave(4), using type 506 Ansco X-ray film and 0.5 mil gold foil in contact with the sections at −10°C for 3-6 weeks. Autoradiograms of tissues containing ATA were prepared in the same manner except for the elimination of gold foil between film and section. For detection of ATA in tissues containing both Be and ATA the sections were stored until Be^7 was no longer detectable in control sections (4 half-lives) after which the sections were again autoradiographed. Finally they were stained with hematoxylin and eosin for histologic examination.

Results.† *Liver.* All of the beryllium autoradiograms showed a diffuse blackening with an occasional area of more intense activity; in no case was it possible to correlate radioactivity either with normal anatomic structures or with areas of liver damage. Failure to demonstrate a characteristic pattern in the deposition of beryllium in this organ was in contrast to the results reported by Kaylor and Van Cleave(4) who showed liver autograms with numerous punctate areas of concentration of radioactivity, corresponding with the peripheral parts of liver lobules. ATA pictures likewise failed to show a distinct pattern of localization in the liver; when ATA was given alone the liver showed a uniform degree

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† Illustrations are restricted to reproductions of C^{14}-ATA autograms since Be^7 autograms, although adequate for analysis, do not readily lend themselves for reproduction.

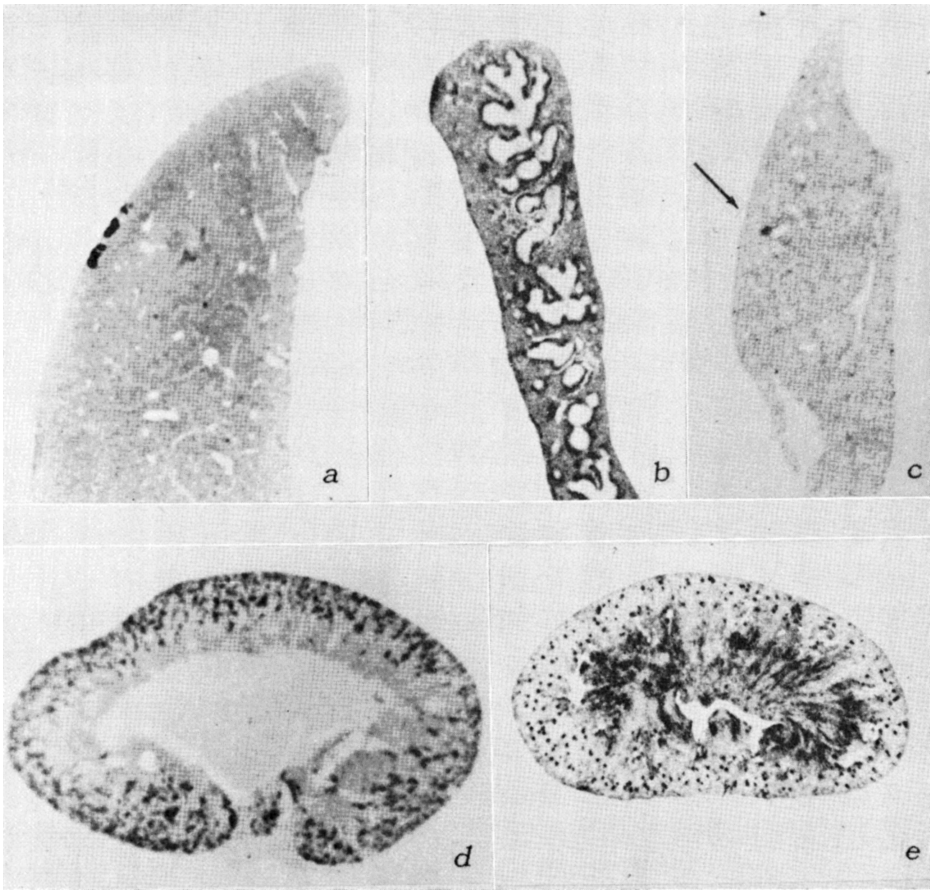

FIG. 1. Autoradiograms showing distribution of C^{14}-ATA in tissues of beryllium-poisoned mice. All magnifications 5.5 ×. (a) Liver; generally uniform distribution of ATA. Discrete areas of high concentration correspond to focal necroses of liver cells. (b) Spleen; concentration exclusively in red pulp, especially in marginal sinuses. (c) Lung; focus of intense radioactivity associated with thrombus in branch of pulmonary artery. (d) Kidney; ATA given alone; concentration in tubules in outer cortex and absence of radioactivity from medulla. (e) Kidney; animal given Be and ATA; concentration of ATA primarily in damaged glomeruli and tubules.

of blackening of the film. In contrast, 2 animals with beryllium-induced liver damage showed in addition a striking concentration of C^{14} activity as small punctate dots and streaks (Fig. 1a); these corresponded to areas of focal necrosis of liver cells.

Spleen. The beryllium and ATA autoradiograms in all groups showed an identical pattern of localization of the 2 substances in this organ; the radioactivity was confined to the red pulp, leaving the lymphatic nodules as sharply outlined pale areas. The perifollicular or marginal sinuses of the red pulp showed the greatest concentration of Be and ATA, and as a consequence they appeared as intensely dark rings (Fig. 1b).

Lung. Beryllium was demonstrable in all lungs at both intervals but it showed no specific pattern of distribution. ATA pictures showed a mild degree of diffuse darkening, sparing the lumina of bronchi, blood vessels and alveoli. In all animals and at all intervals there were many discrete small foci and thin streaks of higher radioactivity; these were associated with intrapulmonary lymphatic nodules and lymph channels. One autoradiogram, obtained from an animal 48 hours after injection of beryllium and ATA, showed an intensely dark spot of radioactivity which was found to correspond to a fresh

thrombus in a pulmonary artery (Fig. 1c). The beryllium autogram showed no corresponding increased activity in this region.

Kidney. In animals injected only with beryllium, radioactive material was deposited throughout the renal cortex but was absent in the medulla. The cortical deposition appeared in two distinct compartments of different concentration: an outer zone, showing a generally mild degree of blackening of the film, and a narrow inner zone along the cortico-medullary junction, which showed more intense blackening. When both beryllium and ATA were given, there was a shift in the beryllium radioactivity from the inner zone toward the outer zone; and instead of being evenly distributed, beryllium was concentrated in large and small streaks, some of which extended throughout the cortex. These streaks corresponded to tubular injury in the inner and outer zone of the cortex. Animals injected only with ATA showed a very characteristic concentration of radioactivity in the cortex. It appeared as little clumps and as discontinuous and slightly tortuous bands; the activity was greater in the outer than in the inner zone and corresponded to kidney tubules. There was no radioactivity in the glomeruli or in the medulla (Fig. 1d). The ATA autoradiograms of beryllium-poisoned animals showed changes in distribution with a concentration of ATA primarily in damaged tubules, including those in the medulla. Of particular interest was the kidney of an animal sacrificed at 12 hours which showed numerous punctate foci of radioactivity throughout the cortex and heavy streaks in cortex and medulla (Fig. 1e). Each punctate focus corresponded to a severely damaged glomerulus, whereas the coarse streaks represented radioactivity in injured kidney tubules with necrotic epithelium and casts in the lumina.

Comparison of overall densities of autoradiograms obtained from the three groups of mice revealed the following: In the kidney and lung of Be-injected, ATA-treated mice there was generally a higher uptake at 12 and 48 hours of both Be and ATA than in the animals receiving either alone. In the liver and spleen there appeared to be a loss of Be at the 12-hour interval as a result of ATA treatment.

Discussion. The autoradiographic visualization of the distribution of ATA and beryllium in tissue confirms earlier chemical findings and previous observations on the therapeutic effect of ATA on beryllium-induced tissue damage. In general, ATA has been found localized in areas that contained beryllium and exhibited beryllium damage. An identical pattern of localization was noted especially in the spleen, where the patterns of beryllium and of ATA distribution in the red pulp corresponded closely with areas of tissue damage noted in morphological studies(3) and with earlier histochemical observations of Aldridge *et al.*(6). In the liver and lung, where no specific areas of high concentration of either ATA or beryllium were found, it may be assumed from the uniform distribution of the two throughout these organs that there was opportunity for contact between ATA and beryllium. The linear and small focal aggregates of ATA that were correlated with lymphatics and lymphatic nodules in the lung are perhaps best explained as representing ATA that was drained from the lung by the lymphatic system, having been deposited initially in the phagocytic cells of the lung in a manner identical with the removal from the circulation of ATA by the phagocytic cells in the spleen and liver. The kidney was the only organ showing minor differences in the localization of Be and ATA when given separately. While both substances were restricted to the renal cortex, beryllium was found mainly in the inner zone while ATA was concentrated primarily in the outer zone of the cortex. When both substances were given together there was a change in the distribution of both substances and this could be readily correlated with damaged areas in the kidney. The increased concentration of ATA in the damaged tissue in kidney and liver probably was the result of aggregation of formerly evenly distributed material in the collapsed necrotic parenchyma. Potentiation of beryllium-induced kidney damage by a dose of 400 mg/kg of ATA has been described earlier(3), but it is uncertain whether and how the shifts

in localization of Be and of ATA may be related to this phenomenon. A similar potentiation of kidney damage has been observed in cadmium-poisoned rabbits treated with BAL(7). The critical importance of renal damage in animals undergoing treatment which promotes redistribution of toxic metals in the tissues warrants further investigation of the complex situation which prevails in the kidneys of animals undergoing such treatment.

Summary. The distribution of Be^7 and of C^{14}-labeled aurintricarboxylic acid (ATA) was studied autoradiographically over a period of 48 hours in the spleen, liver, lung and kidney of mice. It was found that in general beryllium and ATA were deposited in similar tissue loci. The highest simultaneous concentrations and identical patterns of deposition were seen in the red pulp of the spleen. Other organs showed reasonably good correlations in deposition of the two substances, confirming earlier therapeutic studies. An increased concentration of C^{14}-ATA was observed in areas of tissue damage produced by beryllium in the liver, kidneys and lung.

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1. White, M. R., Finkel, A. J., and Schubert, J., *J. Pharmacol. Exp. Therap.*, 1951, v102, 88.

2. Lindenbaum, A., White, M. R., and Schubert, J., *Arch. Biochem. Biophys.*, 1954, v52, 110.

3. Lisco, H., and White, M. R., *Brit. J. Exp. Path.*, 1955, v36, 27.

4. Kaylor, C. T., and Van Cleave, C. D., *Anat. Rec.*, 1953, v117, 467.

5. Lindenbaum, A., Schubert, J., and White, M. R., *Arch. Biochem. Biophys.*, 1954, v52, 143.

6. Aldrige, W. N., Barnes, J. M., and Denz, F. A., *Brit. J. Exp. Path.*, 1949, v30, 375.

7. Gilman, A., Philips, F. S., Allen, R. P., and Koelle, E. S., *J. Pharmacol. Exp. Therap.*, 1946, v87, 85. Supplement.

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Histochemistry XLIV. Use of Density Gradient for Isolation of Mast Cells.* (22476)

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Padawer and Gordon(1) described a differential centrifugation technic for the rapid separation of mast cells from peritoneal fluid of the rat. They flushed the peritoneal cavity with 2 or 3 ml of 0.9% NaCl, layered the cell suspension over a sucrose-gelatin-NaCl solution and centrifuged the mast cells down into the latter medium while retaining the less dense cells above the interface between the layers. Thus they were able to obtain suspensions containing more than 75% mast cells.

Effective use of density gradients for the separation of cellular particulates(*e.g.* 2-7) suggested that this principle might offer advantages in the separation of cells. The first study undertaken in this connection was the isolation of mast cells which were required for other investigations in this laboratory. Significant improvements in the method for isolation have been effected, and suspensions which were 100% pure with respect to cell type have been obtained with yields as high as 87%.

Modified procedure. Padawer and Gordon exsanguinated the rat under ether anesthesia before opening the abdomen by a mid-ventral incision. In an attempt to minimize further the contamination of the peritoneal fluid with blood, and the associated clotting which entraps many mast cells, other procedures were tested in the present study. The most effec-

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