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THE DISTRIBUTION OF ^{35}S-LABELLED SULPHURIC ACID ESTERS ADMINISTERED TO MICE AND RATS

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Abstract—The distribution of a number of ^{35}S-labelled sulphuric acid esters administered to mice and young rats was investigated by whole-body autoradiography which allows the visualization of radioactivity in various organs and tissues. The autoradiograms obtained demonstrated that no cellular accumulation of radioactivity occurred with esters which are not metabolized. In contrast, distribution patterns obtained with esters which undergo metabolic conversion *in vivo* were characterized by cellular accumulation of radioactivity. It was possible to make a number of detailed observations with these esters and areas of accumulation of radioactivity were identified.

THE APPEARANCE of sulphuric acid esters in mammalian urines is well documented and considerable information is available on the enzyme systems involved in their synthesis and degradation.[1, 2] In spite of the fact that a great deal is now known about the intracellular localization and mechanisms of action of the degradative enzymes their biological significance remains obscure. Attempts have been made to assign a functional role to the enzymes by studying the metabolic fate of sulphuric acid esters administered to animals. There is however a paucity of information on the behaviour of the esters *in vivo* and little is known of their ability to penetrate biological barriers and to enter various organs and tissues. A study was undertaken with a view to providing such information and in this preliminary investigation the technique of whole-body autoradiography was used to investigate the gross distribution of a number of ^{35}S-labelled sulphuric acid esters in rats and mice.

The technique of whole-body autoradiography is essentially a method of visualizing the distribution of radioactive materials administered to small animals. Data on distribution, relative accumulation in organs and tissues, routes of excretion and information concerning the ability of administered materials to cross various biological barriers is made available by this method. One of the aims of the investigation was to discover how distribution patterns could be interpreted in terms of the metabolic fate of the esters and their ability to penetrate biological barriers.

MATERIALS AND METHODS

Sulphuric acid esters. Esters employed in this study are not commercially available and were therefore prepared in the laboratory as follows: potassium phenyl ^{35}S-sulphate (sp. act., 10 μc/mg) by the method of Hawkins and Young[3]; potassium *p*-hydroxyphenylacetic acid ^{35}S-sulphate (10 μc/mg, Dodgson, Powell, Rose and Tudball[4]); dipotassium 2-hydroxy 5-nitrophenyl ^{35}S-sulphate (28 μc/mg, Flynn[5]); potassium L-serine *O* ^{35}S-sulphate (5 μc/mg, Tudball[6]); potassium L-serylglycine *O*

^{35}S-sulphate (17 μc/mg, Tudball, Noda and Dodgson[7]); potassium glycyl-L-serine *O* ^{35}S-sulphate (0·1 μc/mg, Tudball, Noda and Dodgson[8]); potassium L-threonine *O* ^{35}S-sulphate (11·6 μc/mg, Tudball[9]); cortisone 21 ^{35}S-sulphate (7·1 μc/mg, Lloyd[10]); potassium L-tyrosine *O* ^{35}S-sulphate (6 μc/mg, Dodgson *et al.*[4]); potassium L-tyrosylglycine *O* ^{35}S-sulphate (6·2 μc/mg, Dodgson *et al.*[4]).

The authenticity and purity of the esters was confirmed by paper and thin-layer chromatography using a number of solvents, paper electrophoresis, spectroscopy and determination of sulphate and potassium content.

Experimental animals. Animals used were mature white mice (20–40 g body wt.) and young MRC hooded rats (30–50 g body wt.). Each animal was injected i.p., with an aqueous solution (0·2–0·5 ml) of the appropriate sulphuric acid ester, while under light ether anaesthesia.

Mice and rats received the following ^{35}S-labelled sulphuric acid esters: potassium *p*-hydroxyphenylacetic acid sulphate, dipotassium 2-hydroxy 5-nitrophenyl sulphate, potassium phenyl sulphate and potassium L-tyrosine *O*-sulphate (1 mg/20 g body wt.), potassium L-serine *O*-sulphate (1 mg/30 g body wt.), potassium L-serylglycine *O*-sulphate (1·2 mg/20 g body wt.), cortisone 21-sulphate and potassium L-tyrosylglycine *O*-sulphate (1 mg/40 g body wt.). The ^{35}S-sulphuric acid esters of glycyl-L-serine (8 mg/30 g body wt.) and L-threonine (8 mg/50 g body wt.) were administered to rats only.

Whole-body autoradiography

The method employed was essentially that of Martin, Harrison and Bates.[11] At suitable time intervals after injection (ranging from 5 min to 48 hr) the animals were anaesthetized and rapidly cooled by immersion in an acetone—solid carbon dioxide mixture at −78°. Each carcass was embedded in an aqueous solution (5 per cent, w/v) of acacia gum at −78° and machined down to a suitable level using a 2 in. Surform drum cutter attached to an electric drill. The animal surface was thoroughly cleaned and placed in close contact with X-ray film (Ilford Industrial B). The whole was maintained at low temperatures in contact with solid carbon dioxide for periods of between 1 and 2 weeks. The film was then developed (Ilford contrast FF developer). A coloured photograph of the animal surface (exposed by machining) was taken and by studying the photograph together with the autoradiogram, the distribution and localization of the administered isotope with reference to various parts of the animal surface was readily visualized.

RESULTS AND DISCUSSION

Information concerning the absorption of the esters from the intraperitoneal cavity was readily obtained in both species. Thus it was shown that intraperitoneal barriers are freely permeable to the esters since radioactivity could be detected in the blood within 5 min after injection in all experiments. Further, no radioactivity could be detected in the intraperitoneal cavity 60 min after injection. By contrast, Embery[12] has shown that sulphated mucopolysaccharides pass into the blood from the intraperitoneal cavity of mice much more slowly.

The sulphuric acid esters used in this investigation did not penetrate the blood-brain barrier since it was not possible to demonstrate the presence of radioactivity in the central nervous system following their administration to either rats or mice.

The overall speed of excretion of radioactivity was readily determined by examining areas of autoradiograms, corresponding to kidney and urinary bladder, at various time intervals after injection. In addition, the appearance of radioactivity at these sites identified the excretory route as urinary. Those esters which are excreted unchanged were rapidly eliminated (within 2 hr) while those esters which are metabolized were excreted more slowly (within 24 hr). Biliary excretion of radioactivity (recorded only in experiments with cortisone 21 ^{35}S-sulphate) was evidenced by the appearance of radioactivity in gall bladder (in mouse) and gastrointestinal tract.

The autoradiograms demonstrated that certain sulphuric acid esters are able to pass into and accumulate in various organs and tissues of rats and mice. It was possible to assess the relative affinities of tissues for the radioactive materials since the densities of areas of autoradiograms could be assessed visually and related to appropriate areas of the animal surfaces.

Thus autoradiograms of animal surfaces prepared after administration of L-tyrosine *O* ^{35}S-sulphate to rats showed accumulation in a well-defined area in the kidney while those obtained after administration of L-tyrosylglycine ^{35}S-sulphate to rats showed accumulation in liver and kidney. Distribution patterns obtained with cortisone 21 ^{35}S-sulphate were consistent with a predominantly biliary excretion and showed high concentrations of radioactivity in gall bladder (in the mouse), intestinal tract and liver. Distribution patterns obtained after administration of L-serine *O* ^{35}S-sulphate (rats and mice), L-serylglycine *O* ^{35}S-sulphate (rats and mice), glycyl-L-serine *O* ^{35}S-sulphate (rats) and L-threonine *O* ^{35}S-sulphate (rats) demonstrated accumulation of radioactivity in a number of tissues (e.g. liver, spleen, lung, salivary glands). However, the main area of accumulation corresponded to brown adipose tissue. This observation demonstrates the usefulness of the technique in detecting uptake of radioactivity in tissues, which might well be overlooked when using more conventional methods.

It was of special interest to relate the distribution pattern obtained with any one ester with the metabolic fate of that ester. Thus, in addition to making observations on the speed and route of excretion and sites of accumulation it was possible to arrive at a number of general conclusions.

Firstly, it was apparent that the sulphuric acid esters of phenol, *p*-hydroxyphenylacetic acid and 4-nitrocatechol which are excreted largely unchanged by the rat[3, 4, 13] showed similar distribution patterns in the rat and also in the mouse. The main features of such patterns were little or no cellular uptake of radioactivity, the radioactivity in the tissues did not exceed that of the blood except in kidney and urinary bladder where the accumulation reflected urinary excretion. This type of distribution pattern (see Fig. 1 for an example) is almost identical with that recorded with D-glucose 6-*O* ^{35}S-sulphate[14] and L-tyrosine *O* ^{35}S-sulphate[15] which are also excreted unchanged in the urine following their administration to mice. The second general conclusion emerged in relation to autoradiograms produced with sulphuric acid esters which are known to be degraded *in vivo* (see Fig. 2 for an example). Thus L-serine *O* ^{35}S-sulphate,[6] L-serylglycine *O* ^{35}S-sulphate,[7] glycyl-L-serine *O* ^{35}S-sulphate,[8] L-threonine *O* ^{35}S-sulphate,[9] cortisone 21 ^{35}S-sulphate,[16] L-tyrosine *O* ^{35}S-sulphate[4] and L-tyrosylglycine *O* ^{35}S-sulphate[17] all of which are known to undergo metabolic conversions in the rat gave rise to distribution patterns clearly distinguishable from

those obtained with sulphuric acid esters which are excreted unchanged. Although the autoradiograms obtained within this group varied from one ester to another they possessed certain common features. They showed cellular uptake and accumulation of radioactivity in tissues with the result that the level of radioactivity at these sites exceeded that of the blood.

Thus in autoradiograms of animal surfaces prepared after administration of L-serine *O* ^{35}S-sulphate (rats and mice), L-serylglycine *O* ^{35}S-sulphate (rats and mice), glycyl-L-serine *O* ^{35}S-sulphate (rats) and L-threonine *O* ^{35}S-sulphate (rats), the main area of accumulation corresponded to brown adipose tissue but radioactivity was also accumulated in a number of other tissues (e.g. liver, spleen, lung, salivary glands). Distribution patterns obtained when L-tyrosine *O* ^{35}S-sulphate was given to rats showed accumulation in a well-defined area in the kidney while those obtained with L-tyrosylglycine ^{35}S-sulphate in rats showed accumulation in liver and kidney. Distribution patterns obtained with cortisone 21 ^{35}S-sulphate were consistent with a predominantly biliary excretion and showed high concentrations of radioactivity in gall bladder (in the mouse), intestinal tract and liver.

These collective findings and considerations are compatible with the general principle that when cellular uptake is recorded for any one ester then that ester undergoes metabolic conversion *in vivo*. If this principle is accepted in general terms then the technique can be regarded as being extremely useful in forecasting the degradation of any sulphuric acid ester. In this connection it has also been found that the technique can be gainfully employed in explaining the metabolic fate of certain esters. One interesting example of this became evident in experiments with 2-hydroxy-5-nitrophenyl ^{35}S-sulphate. This ester is excreted largely unchanged by the rat although desulphating enzymes capable of hydrolyzing it are known to be abundantly present in rat liver. These conflicting observations were explicable by the present investigation since gross distribution patterns showed that the ester was rapidly excreted and moreover that significant amounts of radioactivity did not enter the liver. These findings have been supported by the demonstration that the ester is rapidly eliminated via the urine by a secretory process.[18]

It has been stated that evidence for cellular accumulation is obtained when the concentration of radioactivity in the tissues is higher than that of the blood. In some autoradiograms differentiation of areas corresponding to blood and tissue is not possible. This is particularly so in the liver and it is certainly possible that penetration and slight accumulation of radioactivity in tissues may occur in spite of the fact that the level of radioactivity in the tissues is lower than that of the blood. In order to detect such a small degree of uptake and accumulation it is necessary to prepare microautoradiograms with nuclear emulsions. The whole-body technique is, however, of immense value in pin-pointing those organs and tissues which merit further investigation.

It should be emphasized that autoradiograms obtained with compounds which are degraded *in vivo* represent composite distribution patterns for they reflect not only the distribution of the injected material but also that of its degradation products. Nevertheless the autoradiograms obtained in this investigation demonstrate quite clearly that distribution patterns of sulphuric acid esters are not completely dominated by the sulphate moiety. They also indicate the dangers of considering the esters as a homogeneous group in relation to their behaviour *in vivo*.

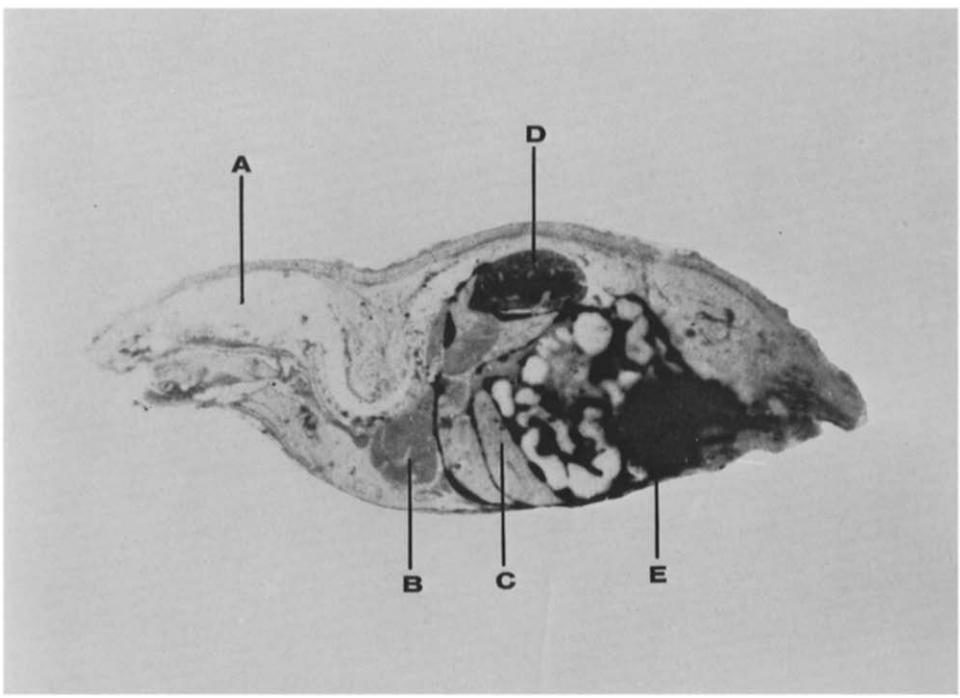

FIG. 1. Autoradiogram showing the distribution of radioactivity in a mouse, 8 min after i.p. injection of L-tyrosine *O* ^{35}S-sulphate; A—brain; B—heart; C—liver; D—kidney; E—bladder.

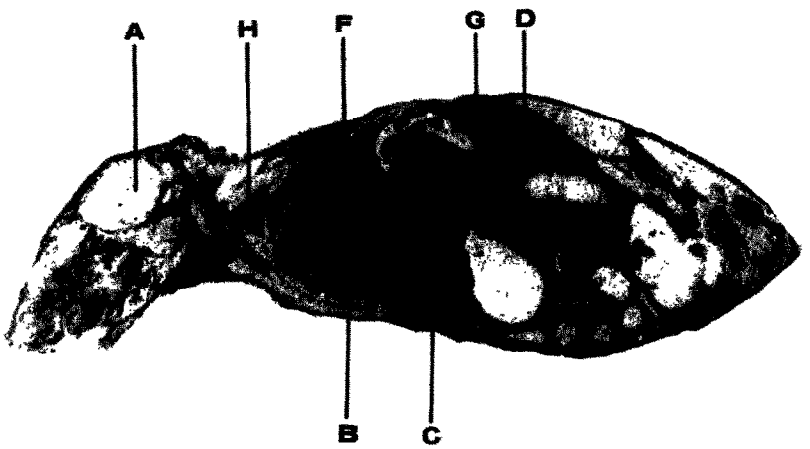

FIG. 2. Autoradiogram showing the distribution of radioactivity in a young rat, 10 min after i.p. injection of L-serylglycine *O* ^{35}S-sulphate; A—brain; B—heart; C—liver; D—kidney; F—lung; G—spleen; H—brown adipose tissue.

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