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# THE APPLICATION OF WHOLE-BODY AUTORADIOGRAPHY TO A STUDY OF THE DISTRIBUTION, METABOLISM AND MODE OF EXCRETION OF $^{35}$S-LABELLED ARYL SULPHATE ESTERS*

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**Abstract**—The technique of whole-body autoradiography has been used to study the distribution, metabolism and mode of excretion of potassium cyclohexylphenyl 4-$^{35}$S-sulphate, potassium cyclohexylphenyl 2-$^{35}$S-sulphate, potassium biphenylyl 4-$^{35}$S-sulphate, potassium naphthyl 2-$^{35}$S-sulphate and potassium 5,6,7,8-tetrahydronaphthyl 2-$^{35}$S-sulphate. The validity of the interpretations of whole-body autoradiograms have been assessed in the light of the results of conventional quantitative and qualitative investigations. The results clearly demonstrate that it is not only possible to obtain information relating to the site and extent of metabolic conversion but in some instances it is also possible to gain insight into the nature of certain metabolic conversions.

IT HAS recently been shown[1] that the technique of whole-body autoradiography can be usefully employed to investigate various aspects of the behaviour of aryl sulphate esters *in vivo*. Further, it has been suggested that whole-body autoradiograms can furnish preliminary evidence of metabolic breakdown, the site of metabolic breakdown and the route of excretion. In the preceding papers[2, 3] the mode of excretion and metabolic fate of a series of structurally related aryl sulphate esters has been extensively investigated. The ability of these esters to penetrate some biological barriers can be inferred from such studies but it is not possible to obtain precise information on which membranes are permeable to the esters. In order to obtain such additional information, the technique of whole-body autoradiography was utilized.

## MATERIALS AND METHODS

### *Aryl sulphate esters*

The $^{35}$S-labelled sulphate esters of 4-hydroxybiphenyl, 4-cyclohexylphenol, 2-cyclohexylphenol, 2-naphthol and 5,6,7,8-tetrahydro 2-naphthol were prepared as described by Hearse, Olavesen and Powell.[4] The specific radioactivities of the preparations were 26 $\mu$c/mg, 36 $\mu$c/mg, 37 $\mu$c/mg, 27 $\mu$c/mg and 41 $\mu$c/mg respectively.

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*Experimental animals*

Animals used were young MRC hooded rats of both sexes (40–70 g body wt.). Each animal was injected intraperitoneally with an aqueous solution of the appropriate aryl sulphate ester at a dose level of 1·0 mg/100 g body wt. while under light ether anaesthesia.

*Whole-body autoradiography*

The procedure described by Powell, Curtis and Dodgson[1] was used throughout. For each ester studied, animals were killed at intervals of 10 min, 20 min, 30 min, 60 min, 6 hr and 12 hr after administration of the $^{35}S$-labelled ester. Carcasses were used immediately for the preparation of autoradiograms.

## RESULTS

*Potassium biphenylyl 4-$^{35}S$-sulphate*

Autoradiograms obtained from animals killed 30 min after injection showed only trace amounts of radioactivity in the i.p. cavity. Thus peritoneal barriers are freely permeable to biphenylyl 4-$^{35}S$-sulphate. However, the ester was unable to penetrate the blood–brain barriers to any significant extent since radioactivity could not be detected in areas of autoradiograms corresponding to the central nervous system. Autoradiograms showed that radioactivity accumulated in kidney, urinary bladder and liver since in areas corresponding to these organs the level of radioactivity was greater than that associated with the blood supply.

The kidney showed the greatest concentration of radioactivity, the organ becoming heavily labelled 10 min after injection. Maximum radioactivity was recorded 1 hr after injection, following which time the level of radioactivity gradually declined and after 12 hr the kidneys contained only trace amounts of radioactivity. Cellular accumulation of radioactivity in the liver was observed in autoradiograms obtained from animals killed 10 min after injection. The concentration of radioactivity in the liver increased up to 1 hr and subsequently declined until after 12 hr only trace amounts remained. Radioactivity was associated with the contents of the gastrointestinal tract 60 min after injection of the $^{35}S$-labelled ester.

Only traces of radioactivity were detectable in autoradiograms obtained 12 hr after injection and, in these, the small amount of residual radioactivity was associated with the cartilage of long bones and the gastrointestinal wall.

These collective findings indicate that the administered radioactivity is eliminated primarily via the renal route. However, the accumulation of radioactivity in the liver and its subsequent appearance in the gut indicates that some biliary elimination occurs. The occurrence of cellular accumulation of radioactivity strongly suggests that biphenylyl 4-$^{35}S$-sulphate is metabolized *in vivo* and, further, that the liver and kidneys represent possible sites of metabolism.

In previous investigations[5] in which whole-body autoradiograms were prepared following the administration of inorganic $^{35}S$-sulphate to young rats, accumulation of radioactivity was observed in cartilage and gastrointestinal mucosa. Similar observations following the administration of biphenylyl 4-$^{35}S$-sulphate provides evidence of the release of inorganic $^{36}S$-sulphate from the ester *in vivo*.

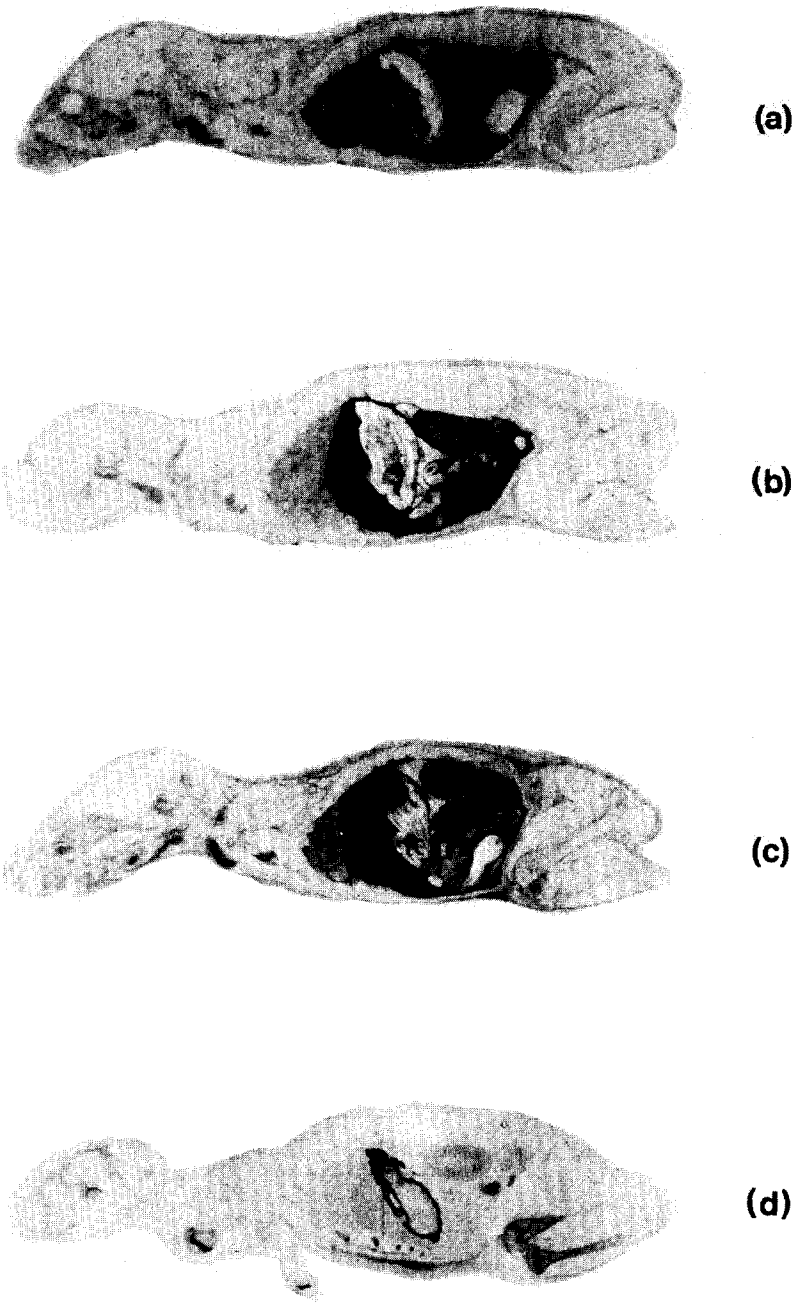


FIG. 1. Whole-body autoradiograms obtained from young rats receiving (1 mg/100 g body wt.) potassium cyclohexylphenyl 4-$^{35}$S-sulphate. Animals were killed (a), 10 min; (b), 30 min; (c), 60 min and (d), 12 hr after administration of the ester.

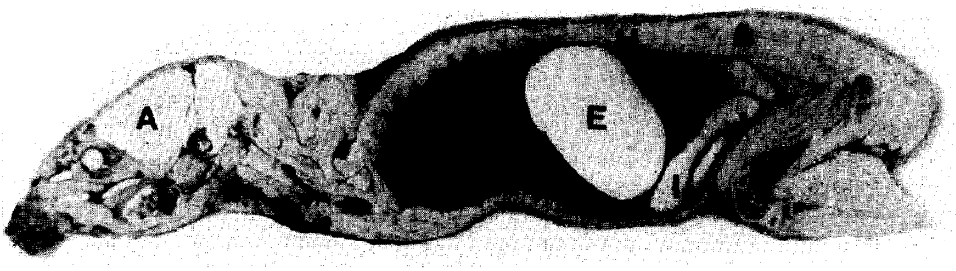


FIG. 2. Whole-body autoradiogram obtained from a young rat killed 10 min after the injection of 5,6,7,8-tetrahydronaphthyl 2-$^{35}$S-sulphate (1·0 mg/100 g body wt.). A = Brain; B = Lung; C = Heart; D = Liver; E = Stomach; F = Kidney; G = Pancreas; H = Spleen; I = Gastrointestinal tract; J = Femur.

*Potassium cyclohexylphenyl* 4-$^{35}$S-sulphate

In autoradiograms obtained from animals killed 1 hr after injection (see Fig. 1(c)) only traces of radioactivity remained in the i.p. cavity. The most striking feature of the autoradiograms was the accumulation of radioisotope in areas corresponding to the liver. Thus, 10 min after injection (see Fig. 1(a)) the level of radioactivity in hepatic tissue was greatly in excess of that associated with the blood supply; only traces of radioactivity could be detected in areas corresponding to the major blood vessels. The level of radioactivity associated with the liver was maximal 30 min after injection (see Fig. 1(b)) and then steadily declined. As the amount of radioactivity in the liver declined, increasing amounts of isotope appeared in the gastrointestinal tract. Cellular accumulation of radioactivity was also observed in the kidney; maximum levels were recorded 10 min after injection (see Fig. 1(a)) after which time the radioactive content declined steadily.

In autoradiograms obtained from rats killed 12 hr after injection (see Fig. 1(d)) radioactivity was associated with cartilage and gastric mucosa. This observation indicates that inorganic $^{35}$S-sulphate is released during the metabolism of cyclohexylphenyl 4-$^{35}$S-sulphate. The heavy accumulation of radioactivity in the liver indicates considerable metabolism of the ester and visual assessment of the quantities of radioactivity associated with the contents of the gastrointestinal tract indicates an extensive biliary circulation.

*Potassium cyclohexylphenyl* 2-$^{35}$*S-sulphate*

As in experiments with cyclohexylphenyl 4-$^{35}$S-sulphate, autoradiograms showed that virtually all the radioactive dose had disappeared from the intraperitoneal cavity 1 hr after injection. After 12 hr only traces of radioactivity remained in the carcasses. Cellular accumulation of radioactivity was evident in kidney and liver only. Areas of autoradiograms corresponding to kidney were significantly labelled after 10 min and maximum radioactivity was recorded 30 min after injection. The level of radioactivity in the liver was greater than that of the blood supply 10 min after injection and reached a maximum after 30 min. The subsequent gradual reduction in the level of hepatic radioactivity was associated with the appearance of radioisotope in areas corresponding to the lumen of the gastrointestinal tract.

The distribution patterns show that the administered radioactivity is eliminated via both urinary and biliary routes. They further suggest metabolism of cyclohexylphenyl 2-$^{35}$S-sulphate in the liver and/or kidney. There is no evidence of release of inorganic $^{35}$S-sulphate.

*Potassium naphthyl* 2-$^{35}$*S-sulphate and potassium* 5,6,7,8-*tetrahydronaphthyl* 2-$^{35}$*S-sulphate*

The distribution patterns obtained following the injection of either of these two esters were virtually identical. High concentrations of radioactivity were recorded in the blood stream 10 min after injection (see Fig. 2) and only traces of radioisotope remained at the site of injection after 1 hr. Accumulation of radioactivity was recorded only in renal tissue. The observations suggest that following the administration of naphthyl 2-$^{35}$S-sulphate or 5,6,7,8-tetrahydronaphthyl 2-$^{35}$S-sulphate, the radioactive dose is eliminated via the urine. Renal clearance of radioactivity from the blood stream

is evidenced by the presence of substantial amounts of radioactivity in areas of autoradiograms corresponding to renal pelvis. The presence of cellular radioactivity in regions of the kidney, tentatively identified as the sub-cortex, was recorded in studies with 5,6,7,8-tetrahydronaphthyl 2-$^{35}$S-sulphate. It is possible that such accumulation might indicate metabolic conversion of the ester within the kidney.

## DISCUSSION

The collective results demonstrate that peritoneal barriers are freely permeable to each of the aryl sulphate esters employed in this study, since 10 min after injection considerable amounts of radioisotope were present in the blood. However, the distribution patterns clearly indicated a limited ability with respect to the penetration of certain other biological barriers. For example, it was not possible to detect any radioactivity in association with the central nervous system, thus demonstrating the inability of the esters to cross the blood–brain barrier. This generalization is in accord with previous observations made with other sulphate esters.[1] Further, the only organs in which the level of radioactivity was greater than that of the blood supply, following the administration of any one of the esters, were kidney and urinary bladder. Following the injection of some of the esters, accumulation of radioactivity was also recorded in liver, gastrointestinal tract and cartilaginous tissue. The appearance of radioactivity in urinary bladder and renal pelvis provides uneqibocal evidence of urinary elimination and the extensive localization of radioisotope at these sites following the injection of naphthyl 2-$^{35}$S-sulphate, 5,6,7,8-tetrahydronaphthyl 2-$^{35}$S-sulphate and biphenylyl 4-$^{35}$S-sulphate indicates the predominance of a renal excretory route.

Autoradiograms obtained from animals receiving cyclohexylphenyl 4-$^{35}$S-sulphate, cyclohexylphenyl 2-$^{35}$S-sulphate and biphenylyl 4-$^{35}$S-sulphate showed hepatic accumulation to various degrees; intense accumulation occurring following cyclohexylphenyl 4-$^{35}$S-sulphate administration. With cyclohexylphenyl 2-$^{35}$S-sulphate, the extent of accumulation was less marked and only slight in the case of biphenylyl 4-$^{35}$S-sulphate. These observations suggested the possibility of a biliary circulation of radioactivity and this was confirmed by the subsequent appearance of corresponding amounts of radioisotope in the gastrointestinal tract. The validity of these interpretations with respect to routes of excretion and the relative amounts of urinary and biliary elimination have been wholly substantiated by quantitative metabolic investigations.[2]

It has been proposed[1] that autoradiograms obtained following the injection of $^{35}$S-labelled sulphate esters can be divided into two groups. Those exhibiting no cellular accumulation of radioactivity were obtained in experiments in which the esters were not metabolized. In contrast, distribution patterns obtained with esters which undergo metabolic conversion *in vivo* were characterized by cellular accumulation of radioactivity. This generalization is borne out by the present investigation; naphthyl 2-$^{35}$S-sulphate, belonging to group I and the remaining esters to group II. Thus autoradiograms obtained following the injection of naphthyl 2-$^{35}$S-sulphate show no cellular accumulation and indicate elimination of the unchanged ester via the urine. However, with 5,6,7,8,tetrahydronaphthyl 2-$^{35}$S-sulphate the autoradiograms were very similar but, in addition to the presence of radioactivity in the renal pelvis as recorded with naphthyl 2-$^{35}$S-sulphate, there was an accumulation of radioactivity in certain regions of the kidney tissue. Thus as well as showing renal elimination of the

5,6,7,8-tetrahydronaphthyl 2-$^{35}$S-sulphate the autoradiograms indicate some metabolic conversion of the ester within the kidney.

The cellular accumulation of radioactivity in liver tissue following the injection of either cyclohexylphenyl 4-$^{35}$S-sulphate, cyclohexylphenyl 2-$^{35}$S-sulphate or biphenylyl 4-$^{35}$S-sulphate provides evidence for the metabolic conversion of these esters in the liver and the degree to which such accumulation occurs also provides an indication of the extent of metabolism. This interpretation has been validated by metabolic studies. However, it is not only possible to obtain information relating to the site and extent of metabolic conversion but in some instances it is possible to gain insight into the nature of certain metabolic conversions. Thus the localization of radioactivity in cartilaginous tissue and gastric mucosa (following the administration of cyclohexylphenyl 4-$^{35}$S-sulphate or biphenylyl 4-$^{35}$S-sulphate) is characteristic of the distribution obtained following the injection of inorganic $^{35}$S-sulphate[5] and provides evidence of the release of inorganic $^{35}$S-sulphate during the metabolism of biphenylyl 4-$^{35}$S-sulphate and cyclohexylphenyl 4-$^{35}$S-sulphate. This has been confirmed by qualitative and quantitative metabolic investigations.[2, 3]

These studies illustrate the correlation between the results obtained by whole-body autoradiography and those obtained in conventional metabolic investigations. They emphasize the value of whole-body autoradiography as a powerful preliminary investigative technique.

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