METABOLISM OF $^{14}$C-LABELLED ALPHAXALONE IN MAN

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SUMMARY
The metabolism of $^{14}$C-labelled alphaxalone, dispensed as Althesin, was studied in normal patients, patients with obstructive jaundice and patients with chronic renal disease and anuria. The radioactive label was removed rapidly from the plasma following i.v. administration. The major portion of the label was excreted in the urine. In patients with normal renal function $^{14}$C-labelled alphaxalone is probably taken up by the liver, metabolized to a more polar compound and excreted in the urine; a small amount is excreted in the bile. In the patient with anuria, hepatic uptake appears to be relatively normal and the length of action of Althesin is not prolonged. It is assumed that in such patients the eventual route of excretion is via the bile and faeces.

Alphaxalone is the major active constituent of the steroid induction agent Althesin (0.9% alphaxalone, 0.3% alphadalone acetate, 20% polyoxyethylated castor oil, 0.25% NaCl in water). Studies in the rat (Card, McCulloch and Pratt, 1972; Child et al., 1972) showed that the plasma half-life of $^{14}$C-alphaxalone was 6–8 min, and the liver was the main site of metabolism. There was no redistribution in fat and approximately 70% of the radioactivity was excreted in the bile during the first 3 h after administration. Further studies of excretion over 5 days showed that 60–70% of the radioactivity appeared in the faeces and only 20–30% in the urine.

This paper reports a study of the metabolism in man of alphaxalone (3α-hydroxy-5β-pregnane-11,20-dione), labelled with carbon-14 in the 21 position (fig. 1), made up as Althesin with a specific activity of approximately 5 μCi/ml. The material was better than 98% pure (Ayres, Newall and Phillips, 1975).

PATIENTS AND METHODS

Patients
The nature of the study was explained to the patients, who gave informed consent for the insertion of a central venous cannula and the administration of $^{14}$C-alphaxalone as part of their anaesthetic. In addition, the study was approved by the Ethics Sub-Committee, King's College Hospital and the Medical Research Council's Isotope Advisory Panel.

Three groups of patients were studied:

1. Five patients with normal hepatic and renal function undergoing minor surgery.
2. Five patients undergoing cholecystectomy and exploration of the biliary tree, in whom a T-tube was inserted to the common bile duct as part of the operative procedure, and allowing sampling of bile.
3. Five patients with chronic renal failure undergoing surgery. These patients were all anuric and on long-term haemodialysis.

Anaesthesia
A central venous cannula (14 gauge, 70 cm, Intramedic) was inserted under local anaesthesia via the basilic vein. For induction of anaesthesia, 10–20 μCi of $^{14}$C-alphaxalone (2–4 ml) was administered via a Butterfly needle inserted into the hand opposite to the central venous cannula (to prevent

![Fig. 1. $^{14}$C-alphaxalone labelled in the 21 position*]
contamination of the subsequent rapid blood samples) of patients in groups 1 and 3. Central venous blood samples were taken at frequent intervals after this induction dose. Anaesthesia was maintained by a standard regime for the particular patients and operative procedure.

The patients in group 2 were treated differently from the other groups. As the certainty of inserting a T-tube could not be established until after an operative cholangiogram had been performed, unnecessary administration of $^{14}$C-alphaxalone was avoided by the following anaesthetic procedure. Anaesthesia was induced with thiopentone, the trachea was intubated after pancuronium and anaesthesia was maintained with nitrous oxide, oxygen, increments of pethidine and intermittent positive pressure ventilation. A central venous catheter was inserted and kept patent by a slow infusion of Hartmann's solution. As soon as the T-tube had been inserted and a free flow of bile established, $^{14}$C-alphaxalone 2 ml (10 µCi) was injected through a Butterfly needle in the back of a hand and central venous blood samples were taken at frequent intervals.

**Sampling of blood, urine and bile**

Blood was sampled as follows: before $^{14}$C-alphaxalone was injected (control), at 1-min intervals after injection for the first 10 min, at 15 min, followed by 15-min samples until the end of surgery and then hourly for the next 3 h (in some patients, samples were taken at 2-min intervals for the first 6 min). A further blood sample was taken 24 h after the injection of $^{14}$C-alphaxalone. The patients with renal disease in group 4 were not studied so intensively, as most were outpatients.

Urine samples were collected for 5 days. Some of the patients in group 2 had a urethral catheter inserted (this is routine at King's College Hospital in jaundiced patients), and urine was collected from these patients at 15-min intervals for the first hour, and hourly thereafter for approximately 5 days (at this time the radioactive counts per minute (c.p.m.) were not significantly greater than the background count). The individual urine volumes were measured and recorded.

In patients in group 2 bile samples were collected at the following intervals: 30 min after the injection of $^{14}$C-alphaxalone, 60 min after injection, and then hourly for the next 3 days or until c.p.m. was not significantly greater than background. The individual bile volumes were measured and recorded.

**Analysis of samples**

**Blood samples.** Central venous blood samples were taken into lithium heparin tubes (Searle). The samples were centrifuged and the resultant plasma separated within 3 h of sampling (plasma samples which could not be processed immediately were stored, frozen, overnight). Plasma samples were diluted 1:10 with water (because the colour of undiluted plasma tended to quench the resultant counts) and 1 ml of this dilution was taken up into 10 ml of Unisolve 1-phosphor (Koch-Light Laboratories Ltd). This resultant solution was counted for 10 min in a Packard Tri-Carb Scintillation Counter using glass counting vials. An internal standard of 50 µl of 0.5 µCi $^{14}$C-hexadecane (Radiochemicals Ltd) was used to calculate quench values.

**Urine samples.** One ml of urine was taken up into 10 ml of Unisolve 1-phosphor and counted in the same manner as for the plasma samples; quenching was corrected using the same internal standard technique as for plasma. If the urine colour was very dark, a 1:10 dilution with water was made and 1 ml of this resultant dilution counted. The urine was counted as soon as possible after collection.

**Bile samples.** The bile was diluted 1:20 with water and 1 ml of this resultant solution taken up into 10 ml of Unisolve 1-phosphor. The counting vials were allowed to stand in daylight for 12 h to bleach out the bilirubin and then stored in the dark for 12 h before counting. Quenching was corrected as in the plasma and urine samples.

The radioactivity present in all samples was expressed as disintegrations per minute (d.p.m.).

**RESULTS**

**Group 1—normal patients**

The uptake of $^{14}$C-alphaxalone from the plasma was very rapid (fig. 2), with the clearance curve exhibiting three distinct phases. Rapid clearance for the first 2 min was followed by a plateau effect lasting between 2 and 100 min and then a slow clearance between 2 and up to 17 h.

Significant radioactivity was detected in the urine within 30 min (fig. 3). Fifty-nine per cent of the dose administered was excreted in the urine in the first 24 h and approximately 80% was excreted over a period of 5 days.

**Group 2—patients with T-tubes inserted**

Although the initial rapid clearance of $^{14}$C-alphaxalone was slightly slower (fig. 4) than that of the control group (0–8 min), a similar plateau effect
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was observed lasting approximately 100 min, followed by a slow decrease over the next 15 h.

Urine clearance of the $^{14}$C-label was less than in group 1 (fig. 5). Thirty-eight per cent of the dose administered was excreted in the first 24 h and approximately 56% was excreted over a period of 5 days.

The $^{14}$C-label appeared in the bile within 10 min (fig. 6). The peak of excretion was approximately 200 min and decreased gradually thereafter. Bile clearance had ceased effectively after 2 days. Approximately 15% of the $^{14}$C-label was excreted in the bile, with a mean 24 h excretion rate of 13%. However, in one patient there was virtually no biliary excretion.

Addition of mean biliary excretion to mean urinary excretion of $^{14}$C-label shows that 53% was excreted in the first 24 h in the patients in group 2.

**Group 3—patients with renal disease**

The plasma clearance in these patients was slower than that of the other two groups, being between
Fig. 5. Patients in group 2. Cumulative excretion of $^{14}$C-alphaxalone in the urine. (Urine not available from fifth patient.)

Fig. 6. Patients in group 2. Excretion of $^{14}$C-alphaxalone in the bile.

Fig. 7. Patients in group 3. Uptake of $^{14}$C-alphaxalone from the plasma.
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1 and 10 min (fig. 7). However, a similar plateau effect was seen as in the other groups, but in the two patients in whom a 17-h sample was obtained significant radioactivity remained.

**DISCUSSION**

A major problem in the study of metabolism of steroids is that quantitative analysis in body fluids is often very difficult. Therefore it is common practice to use radio-isotope-labelled compounds, since quantitative assay of the label is easier. Of course, such assays identify only the radioactive marker and it is essential to establish the stability of the "steroid-label" complex, otherwise confusion may result. For example, if the radioactive label splits off, this may suggest an apparent metabolic pathway for the whole complex which is in fact followed only by the "label". In the present study, alphaxalone was labelled with carbon-14 in the 21 position. Ideally, a steroid compound should be labelled within its nucleus, but in the case of alphaxalone this is very difficult technically. Therefore, labelling in the 21 position, which is considered very stable in this type of steroid, was accepted. Alphaxalone was labelled in preference to alphadalone, since the former is the major active constituent of Althesin. It should be appreciated, therefore, that the results of the present study indicate the metabolism and excretion of $^{14}$C-alphaxalone, made up as Althesin, and the assumption has been made that the carbon-14 label is stable.

The results show that $^{14}$C-alphaxalone is rapidly removed from the plasma in normal and anuric patients. Thereafter, although some is excreted in the bile, the majority of the injected amount was recovered from the urine. A further possible pathway of excretion of carbon-14 is through the respiratory tract but, as the majority of the radioactivity injected was detected in the urine, $^{14}$C-carbon dioxide excretion was not measured. In addition, the molecular configuration of $^{14}$C-alphaxalone suggests that breakdown to $^{14}$C-carbon dioxide is unlikely. In the anuric patients the duration of action of Althesin was not significantly different from the normal patients despite greater radioactivity in the plasma. Although the 21 position of the radioactive label is thought to be stable, alphaxalone must undergo metabolism in order to be excreted in the bile and urine; alphaxalone itself is not water soluble and must be converted to a more polar compound, possibly by conjugation, before it can be excreted. The most likely site for such metabolism is the liver, although it is possible that the kidney may contribute. It was not possible in this study to determine the exact nature of the steroid/radioactive complex in the plasma, bile or urine samples because of the small amount of radioactivity used. In addition, there is no accurate method available for chemical analysis of small quantities of alphaxalone in body fluids (Dubois, Allison and Geddes, 1975). Therefore it was not possible to determine the relationship between the radioactive plasma values and depth of anaesthesia. However, it is of interest that in all patients studied the plasma concentration of $^{14}$C-alphaxalone had decreased to very low values within a few minutes, suggesting either that plasma concentrations do not necessarily correlate with brain concentrations, or that Althesin is an extremely potent agent at small concentrations, since, in the patients studied, anaesthesia continued in the presence of very small concentrations.

Child and others (1972) showed that in the rat, 76% of an administered dose of $^{14}$C-alphaxalone was excreted in the bile. In the present study it can be seen that, in man, the major route of excretion is via the urine. This would seem to represent a species difference. In the patients in group 2 the metabolism and excretion of Althesin were comparable to those in group 1, and Althesin would seem to be an acceptable anaesthetic for such patients. The observation that summation of biliary and urinary excretion of radioactivity in the patients in group 2 is comparable with the total urinary excretion in the patients in group 1 suggests that entero-hepatic recirculation of metabolites of Althesin may occur. Although the patients in group 2 had a degree of disturbance of liver function, the action of Althesin was not prolonged. Ward, Adu-Gyamfi and Strunin (1975) have shown that Althesin is a suitable drug for patients with chronic liver disease and has no deleterious effects. However, since the liver is probably the main site for Althesin metabolism, reduced doses may be necessary in patients with severe liver damage.

In the anuric patients, although the plasma concentrations were greater than in the two other groups, the anaesthetic action of Althesin did not seem to be prolonged. It is interesting to speculate on the nature of the residual radioactive label present at 17 h in the plasma of the anuric patients, which did not occur in the two other groups. It may represent either a metabolite or metabolites which cannot be excreted by the kidney in these patients, or it may represent unchanged alphaxalone. As there...
was no urine output it was assumed that the eventual route of excretion of the $^{14}$C-alphaxalone was via the bile and faeces. Clinical experience shows that Althesin is a suitable drug for patients with impaired renal function, but, as in the case of patients with liver disease, the dose may have to be reduced.

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REFERENCES

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RESUME
Le métabolisme de l'alphaxalone marqué $^{14}$C, livré sous le nom d'Althesine a été étudié sur des patients normaux, des malades souffrant d'ictère par rétention ainsi que sur des malades souffrant de maladies chroniques des reins et d'anurie. Le marquage radio-actif a été rapidement enlevé du plasma après l'administration intraveineuse. La plus grande partie du marquage a été expulsée dans l'urine. Sur les malades ayant des fonctions rénales normales, l'alphaxalone marqué $^{14}$C est probablement absorbé par le foie, métabolisé en un composé plus polaire et expulsé dans l'urine; une petite quantité étant expulsée dans la bile. Chez le malade souffrant d'anurie, l'absorption hépatique semble être relativement normale et la durée de l'action de l'Althesine n'est pas prolongée. On suppose que sur ce genre de malades le chemin d'excrétion suivi est la bile et les matières fæcales.