Journal of Steroid Biochemistry, Vol. 11, pp. 1113 to 1119

0022-4731/79/0801-1113$02.00/0

IN VITRO AND *IN VIVO* STUDIES ON TWO ^{75}Se LABELLED CHOLESTEROL ANALOGUES: 19-METHYL-[^{75}Se]-SELENOCHOLESTEROL AND 6-[^{75}Se]-METHYL-SELENOMETHYL-19-NOR-CHOLEST-5(10)-EN-3β-OL

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(*Received* 31 *May* 1978)

SUMMARY

19-methyl-[^{75}Se]-selenocholesterol and 6-[^{75}Se]-methyl-selenomethyl-19-nor-cholest-5(10)-en-3β-ol have been suggested as possible adrenal scanning agents. This study compares these selenium-labelled cholesterol analogues and cholesterol in some of the metabolic transformations in which cholesterol is involved in mammalian systems. The activity of cholesterol 7α-hydroxylase, lecithin:cholesterol acyltransferase, microsomal cholesterol acyltransferase and the sterol side-chain cleavage enzyme systems to transform the mentioned cholesterol analogues has been investigated. The uptake and secretion in bile of 19-methyl-[^{75}Se]-selenocholesterol and 6-[^{75}Se]-methyl-selenomethyl-19-nor-cholest-5(10)-en-3β-ol has been evaluated. The tissue distribution of these sterol analogues has been studied in rats. The findings suggest that 6-[^{75}Se]-methyl-seleno-methyl-19-nor-cholest-5(10)en-3β-ol should be a useful agent for adrenal scanning.

INTRODUCTION

The use of [^{131}I]-19-iodocholesterol and 6β-[^{131}I]-iodomethyl-19-nor-cholest-5(10)-en-3β-ol for clinical studies as adrenal scanning agents is well known [1, 2]. However, the physical properties of the ^{131}I-label are not ideal for this purpose. The use of cholesterol analogues labelled with radioactive selenium has advantages over ^{131}-I-labelled sterols. Thus, such a labelled molecule would have a lower β absorbed dose and a higher usable photon yield. Furthermore radioactive selenium has a longer half-life and will therefore increase the shelf life of such a labelled compound and reduce production costs.

In a tissue distribution study conducted by Sarkar *et al.*[2] a higher adrenal uptake of 19-methyl-[^{75}Se]-selenocholesterol was obtained, compared with [^{131}I]-19-iodocholesterol. Furthermore these authors deduced that the adrenal medulla in dogs took up 19-methyl-[^{75}Se]-selenocholesterol. They related the uptake of these cholesterol analogues in the adrenal medulla to the chromaffin granules which are rich in lipids [3].

The present study has been undertaken to investigate the metabolic transformation of 19-methyl-[^{75}Se]-selenocholesterol (19-[^{75}Se]-cholesterol) and 6-[^{75}Se]-methyl-selenomethyl-19-nor-cholest-5(10)-en-3β-ol (6-[^{75}Se]-cholesterol) in mammalian systems. Cholesterol is well known to be the precursor of bile acids and steroid hormones. This investigation is designed to compare and contrast the metabolism of these cholesterol analogues (Fig. 1), with the metabolism of cholesterol in the tissues of the rat.

It is well known that bile acid biosynthesis appears to be initiated with the hydroxylation of cholesterol by a mixed function oxidase enzyme—the cholesterol 7α-hydroxylase. This oxygenase is present in the microsomal fraction of liver [4]. The cholesterol 7α-hydroxylase enzyme system is considered to be the rate limiting step in the formation of bile acids from cholesterol [5]. For this reason the metabolism of these seleno-cholesterol analogues was compared with the metabolism of cholesterol in a rat liver microsomal cholesterol 7α-hydroxylase enzyme system.

In the adrenal cortex, two mechanisms appear to regulate steroid hormone biosynthesis. In some species cholesterol is stored within the adrenal gland as cholesterol esters and there is evidence that when the adrenal gland is activated by ACTH the cholesterol ester hydrolase enzyme present in the cytosol of some adrenal cortical cells is activated and hence achieves the release of free or non-esterified cholesterol from the cholesterol ester stores. The free cholesterol in the adrenal gland is taken up rapidly by the mitochondria. Adrenal cortical mitochondria contains a mixed function oxidase associated with cytochrome P450, which, in the presence of oxygen and NADPH, effectively cleaves the side chain of cholesterol to yield pregnenolone. In the present study, the metabolism

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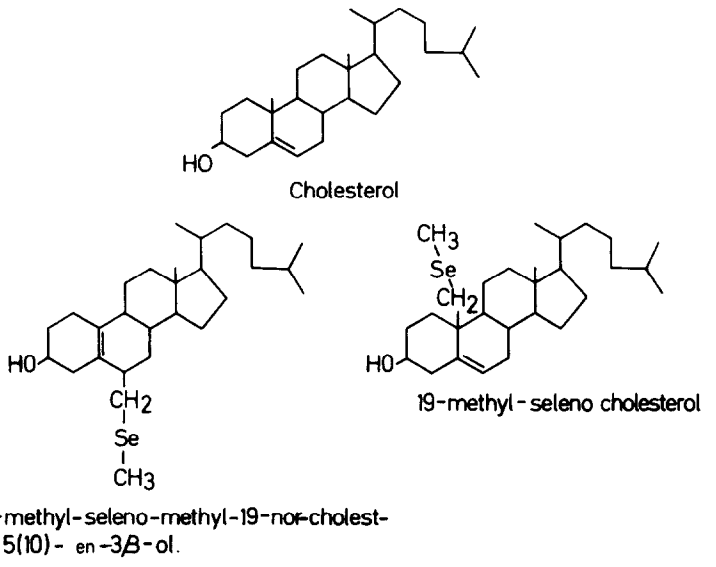

Fig. 1. Structure of cholesterol and the two analogues used in this study.

of the seleno-cholesterol analogues was compared with the metabolism of cholesterol in mitochondria obtained from bovine adrenal glands.

Cholesterol esters are present in quantity in blood plasma and it is known that they are formed in plasma by an enzyme system, the lecithin:cholesterol acyltransferase (LCAT) which transfers an acyl group from position 2 of lecithin to the 3β-hydroxyl of cholesterol, resulting in the production of lysolecithin and cholesterol esters [6]. In this communication the activity of the lecithin:cholesterol acyl transferase enzyme system to acylate the seleno-cholesterol analogues was compared with the ability of this enzyme to acylate cholesterol. If the selenocholesterol analogues are to be useful in *in vivo* studies in humans it is essential to investigate the tissue distribution of these analogues in animals. We studied the tissue distribution of the seleno analogues in rats.

The results obtained in this study allow a comparison of the metabolism of the seleno-cholesterol analogues with cholesterol in both *in vivo* and *in vitro* investigations.

MATERIALS AND METHODS

Materials. [4-^{14}C]-Cholesterol, (57.7 mCi/mmol), 19-methyl-[^{75}Se]-cholesterol (0.6 mCi/mg) and 6-[^{75}Se]-cholesterol (9 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, England. The Radiochemical purity was established using thin-layer chromatography on silica gel H. The solvent system was petroleum ether–diethyl ether–acetic acid (25:75:1, by vol.). Thin layer chromatography of the selenium-labelled compounds required the use of ascorbic acid on the TLC plate to minimise their oxidation.

For *in vivo* experiments, the seleno-labelled compounds were obtained from the Radiochemical Centre, Amersham, solubilised in polysorbate 80 containing 1% ascorbic acid as a stabiliser. ATP was purchased from Boehringer (Mannheim, Germany). Silica gel H from Merck (Darmstadt, Germany). Albumin and coenzyme A were purchased from Sigma Ltd (St. Louis, Missouri, U.S.A.).

Radioactivity determinations were conducted in a scintillation counter (Packard-3320). The scintillation mixture used contained PPO 20g, POPOP 150 mg in 5 l. of toluene. For aqueous samples the same amount of scintillator was dissolved in 5 l. of toluene containing Triton X-100 (2:1, v/v). A thin-layer radiochromatogram scanner, Panax Ltd, was used to locate the radioactive spots on the thin-layer chromatograms.

In vitro *experiments*

Cholesterol 7α-hydroxylase assay. Male rats of the Wistar strain weighing 150–200 g were fed a diet containing 5% cholestyramine for at least 5 days before killing. The animals were anaesthetised with ether, the liver washed *in situ* with 0.154 M KCl, removed and homogenised in 0.154 M KCl (25% w/v), and microsomes were prepared in the usual way [5]. The cholesterol 7α-hydroxylase assays were conducted as previously described [5].

Lecithin:cholesterol acyltransferase assay (LCAT) (2.3.1.43). The LCAT assay was performed using rat serum according to the methods of Stokke *et al.*[7]. The incubation was stopped with methanol and after centrifugation the aqueous phase was extracted three times with chloroform. The extracts were combined and taken to dryness under nitrogen and analysed by thin-layer chromatography using silica gel H. The solvent system for this TLC analysis was petroleum ether–diethyl ether–acetic acid (70:30:1, by vol.). Principal radioactive peaks were scraped off the thin-

layer plate and directly transferred into scintillation phials and counted. The extent of esterification was calculated as a percentage of the radioactivity found for seleno analogues of cholesterol esters in the zone corresponding to sterol esters. The percentage conversion of non-esterified cholesterol to esterified cholesterol by this enzyme system could be calculated.

Cholesterol acyltransferase assay in rat adrenal microsomes (2.3.1.26). Female rats of the Wistar strain weighing 150–200 g were used. The adrenals were removed, freed of fat and homogenised in 0.25 M sucrose (20 mg/1 ml). The homogenate was centrifuged at 20,000 *g* for 20 min and the supernatant subsequently centrifuged at 85,000 *g* for 60 min. The microsomal pellet was resuspended in 0.1 M phosphate buffer pH 7.1 to give a suspension equal to the original volume. The protein content was measured according to Lowry *et al.*[8] and in general 2 mls of the microsomal suspension containing about 1 mg of protein was used for each incubation. Cofactors were added as follows: ATP 40 μmol, reduced glutathione 20 μmol, magnesium chloride 5 μmol and coenzyme A 0.2 μmol. The incubation volume was adjusted to 3 mls with 0.1 M phosphate buffer. The radioactive substrates were added in 5 μl of propylene-glycol, incubations were performed at 37°C for 90 mins. The incubation mixture was extracted with chloroform–methanol (2:1, v/v) and the extraction was repeated a further two times. This extraction was followed by an extraction using petroleum ether. The combined extracts were evaporated to dryness under nitrogen and analysed by thin-layer chromatography. The same procedure was used as for the LCAT assay to measure the transformation of the substrate sterol into the corresponding sterol ester.

Sterol side-chain cleavage assay using bovine adrenal cortex mitochondria. Bovine adrenal cortex mitochondria were obtained according to the methods as previously described [9]. The mitochondrial pellet was suspended in water and the suspension was freeze dried. The freeze dried powder was resuspended in 0.1 M phosphate buffer pH 7.4 and centrifuged at 105,000 *g* for 60 min. The supernatant fluid containing the cholesterol side-chain cleavage activity had a protein content of 8–12 mg/ml depending upon the preparation. The incubation mixture contained 1 ml of enzyme suspension, NADP 3.75 mg, glucose-6-phosphate 12.5 mg, glucose-6-phosphate dehydrogenase (1 U), magnesium sulphate 12.3 mg, the volume was adjusted to 5 ml with 0.1 M phosphate buffer pH 7.4. The incubation mixture was preincubated at 37°C for 5 min and incubated for 15 min after the addition of the substrate sterol. The radioactive substrates were added in 50 μl of acetone. The incubation was stopped with methanol and after centrifugation, the aqueous phase was extracted with chloroform. The precipitated proteins were washed with ethyl acetate. The organic phases were combined and taken to dryness under nitrogen. The extracts were analysed by TLC using silica gel H and petroleum ether–diisopropyl ether–acetic acid (30:70:2, by vol.). By the methods which have been described previously the sterol side-chain cleavage activity, that is the conversion of sterol to pregnenolone or a pregnenolone analogue was determined.

In vivo *experiments*

Male rats of the Wistar strain weighing 150–200 g were used. The animals were anaesthetised with ether, a small vertical incision was made in the abdomen and the bile duct isolated and cannulated. The cannula was fixed in position and the radioactive compound under study was administered to the animal by direct intravenous injection into the portal vein. The incision was closed and the animal was transferred to a restraining cage. To replace salts lost due to biliary drainage, the animals were given a drinking solution containing 1% sodium chloride containing potassium chloride at a concentration of 5 mEq/l. In these experiments [4-^{14}C]-cholesterol was administered suspended in an albumin solution (10 mg albumin in 0.2 ml of 0.9% sodium chloride). Radioactive cholesterol (4.3 μCi) was dissolved in acetone, this solution was added under continuous stirring to the albumin solution and the acetone was removed under nitrogen. The selenium-labelled compounds (20 μCi) were administered in a polysorbate solution. The injection volume was less than 0.25 ml. Bile was collected and hydrolysed with 4 M KOH, hydrolysis was conducted in nickel crucibles at 120°C under pressure for 6 h. After hydrolysis the alkaline aqueous bile was extracted with ether three times and subsequently the solution was acidified with concentrated hydrochloric acid to pH 2–3 and re-extracted with ether. The acidic fraction was analysed by liquid–liquid partition chromatography as previously described [10, 11]. After chromatography, the fractions were taken to dryness and counted. The eluted material was also analysed by thin layer chromatography using silica gel H plates; methylene chloride–acetone–acetic acid (70:20:10 by vol.) was used as the solvent system for dihydroxy bile acids; chloroform–methanol–acetic acid (80:12:3 by vol.) was used for trihydroxy bile acids.

For the tissue distribution study of 19-[^{75}Se]-cholesterol and 6-[^{75}Se]-cholesterol, rats were given the radioactive sterol intravenously through the femoral vein, under ether anaesthesia. The animals were killed 48 h later and various tissues including adrenals, liver, spleen, muscle, kidney, brain and gut, were removed. The tissues were weighed and digested in 5 M KOH, the suspension was neutralised and extracted with chloroform–methanol (2:1, v/v.). The lipid phase was taken to dryness and counted.

RESULTS

In vitro *experiments*

The capability of several systems involved in cholesterol metabolism, to transform 19-[^{75}Se]-choles-

Table 1. Conversion of [^{14}C]-cholesterol and [^{75}Se]-cholesterol analogues by various enzyme systems*

Sterol	Sterol 7α-hydroxylase activity	LCAT activity	Microsomal cholesterol acyltransferase activity	Sterol side-chain cleavage activity
[^{14}C]-cholesterol	12.5 (1.2†)	30.4 ± 4.6 (<1†)	13.0 ± 1.7 (<1†)	53.4 ± 4.4 (2.1†)
19-[^{75}Se]-cholesterol	4.2 (2.8†)	17.8 ± 0.8 (<1†)	2.0 ± 0.1 (0.7†)	13.2 ± 2.5 (11.5†)
6-[^{75}Se]-cholesterol	9.5 (10.4†)	0.8 ± 0.1 (0.21†)	15.9 ± 2.3 (0.4†)	29.5 ± 3.2 (20.4†)

* The conversion is expressed as percentage of the added sterol.
† Data obtained in control assays with boiled enzymes.

terol and 6-[^{75}Se]-cholesterol was investigated, under the conditions described in "Materials and Methods". It must be emphasised at this point that all the tissue and body fluids used in the following enzyme studies contain cholesterol as an integral component. The addition of [^{14}C]-cholesterol to these biological samples is likely to equilibrate with the endogenous cholesterol more readily than will the addition of a seleno analogue of this sterol. For this reason, the following results which have been obtained comparing and contrasting the results obtained using cholesterol and seleno cholesterol analogues should be treated as semi-quantitative studies in that it is virtually impossible to calculate or derive precise substrate concentrations in any of these studies. The results should therefore be treated as comparative enzymatic conversions rather than absolute enzymic activities. The selenium labelled analogues were easily oxidised. Consequently, during the work-up of the extracts and the thin-layer chromatography of the radioactive products, control incubation assays using boiled tissue were always included. The results obtained are shown in Table 1. Cholesterol, under the conditions of the assay, was transformed into 7α-hydroxycholesterol to an extent of 12.5% of the added sterol. Due to the high formation of various oxidation products, it is difficult to state whether or not the selenium labelled compounds are transformed into the corresponding 7α-hydroxy sterol analogues. In these *in vitro* incubation conditions, no selective attack at the 7α-hydroxy position of the selenocholesterol analogues seems to occur.

The activity of the lecithin:cholesterol acyltransferase to acylate cholesterol, 19-[^{75}Se]-cholesterol and 6-[75Se]-cholesterol is also reported in Table 1. It can be seen that the LCAT enzyme is apparently less active towards the selenium labelled sterols than towards cholesterol. Nevertheless the 19-[^{75}Se]-cholesterol appears to be quite a good substrate for this enzyme. On the other hand the 6-[^{75}Se]-cholesterol undergoes a much slower transformation to ester by this enzyme system in rat plasma.

Interestingly the situation is different in the adrenal cortex; when the esterification of the selenium labelled analogues was evaluated in adrenal microsomes, cholesterol, 6-[^{75}Se]-cholesterol and 19-[^{75}Se]-cholesterol were transformed into the corresponding esters at rates in the ratio of about 13:15:2 respectively.

The results obtained in studies on the activity of the sterol side-chain cleavage enzyme system to yield pregnenolone and pregnenolone analogues, indicated no detectable cleavage of 19-[^{75}Se]-cholesterol to the corresponding pregnenolone analogue. In fact the amount of radioactivity associated with possible side-chain cleavage products was almost identical in assays with boiled and native enzyme systems. Despite the presence of considerable other oxidation products, the formation of a pregnenolone analogue of 6-[^{75}Se]-cholesterol was significant.

In vivo *experiments*

4-[^{14}C]-Cholesterol, 19-[^{75}Se]-cholesterol and 6-[^{75}Se]-cholesterol were administered i.v. to rats, as described in "Materials and Methods", in order to evaluate and compare the uptake and secretion of these sterols in bile in rats subjected to total biliary drainage. Bile collected from animals receiving the labelled sterols, was hydrolysed, extracted and the extract analysed as described. The analysis of the "aci-

Table 2. Bile uptake of [^{14}C]-cholesterol and [^{75}Se]-cholesterol analogues in rats

	Time after administration	% Of radioactivity in bile	% Of radioactivity*		Neutral/acidic ratio
			Neutral fraction	Acidic fraction	
[^{14}C]-Cholesterol	48 h	13.9	1.2	8.4	0.14
	6 days	18.7	1.6	10.9	0.15
19-[^{75}Se]-Cholesterol	48 h	7.3	2.8	1.9	1.5
6-[^{75}Se]-Cholesterol	48 h	8.2	3.8	2.3	1.6
	4 days + 17 h	12.9	6.3	4.4	1.4

* The percentage is calculated on the administered radioactivity.

dic fraction" of bile was carried out for evidence of metabolism of the selenium labelled compounds into bile acid analogues. The results, expressed as percentage of the administered dose of radioactivity, are shown in Table 2. 4-[^{14}C]-cholesterol secreted into bile was 14% at 2 days and almost 19% 6 days after administration of the labelled sterol.

The distribution of radioactivity in the neutral and acidic fractions of bile gives a neutral:acidic ratio of 1:7 indicating a high proportion of radioactive acidic material. Liquid–liquid column chromatography performed on the acidic fraction and thin-layer chromatography of the eluted material, give evidence of the presence of monohydroxy, dihydroxy and trihydroxy bile acids. According to a recent study by Siegfried *et al.*[12] where the formation of bile acids in bile fistula rats given [^{14}C]-cholesterol was quantitated, monohydroxy, dihydroxy and trihydroxy cholanic acids were present in the acidic fraction of bile in the proportion of 0.012:1:3.

19-[^{75}Se]-Cholesterol and 6-[^{75}Se]-cholesterol secretion into bile is less than cholesterol; 48 h after administration it represented 7.3% and 8.2% of the injected dose respectively. The neutral:acidic fraction ratio of these compounds is 3:2 and indicates that a higher percentage of the radioactivity found in the bile is in the neutral (presumably unchanged) sterol fraction. Partition column chromatography performed on the acidic fraction of bile from rats given 19-[^{75}Se]-cholesterol, indicated the presence of radioactive material with chromatographic behaviour similar to mono, di- and trihydroxylated bile acids. The distribution of the radioactivity in the three classes is in the proportion ot 1.2:2.2:1. Thin-layer chromatography of the material eluted from the column confirms the presence of compounds having chromatographic characteristics suggestive of a structure similar to mono and dihydroxy acids. The results obtained in the chromatographic analysis of the acidic fraction of bile from rats given 6-[^{75}Se]-cholesterol, were unsatisfactory. We were not able to assess the nature of the acidic products lilely to be analogues of bile acids. Further study on the nature of this radioactive material will be needed.

Table 3 shows the concentration of the radioactivity in the tissues from rats receiving 19-[^{75}Se]-cholesterol and 6-[^{75}Se]-cholesterol. The results are expressed as:

$$\frac{\text{c.p.m. in organ/g wet tissue}}{\text{c.p.m. administered dose}}.$$

The distribution of the radioactivity of the selenium labelled compounds is almost identical in most of the tissues. However, a higher adrenal uptake of 6-[^{75}Se]-cholesterol was obtained compared with 19-[^{75}Se]-cholesterol. The turnover of sterols in brain is very low. It is interesting to note than despite the high concentration of free cholesterol in brain these seleno analogues of cholesterol were not taken up by this tissue during the 48 h period of study.

DISCUSSION

Biological and clinical investigators would like to be able to localise or visualise cholesterol in the animal organism during life. This could be most effectively accomplished by the incorporation into the molecule of an atom with gamma emitting characteristics. It would then be possible to scan the living organism and detect or quantitate possible localisation of the sterol molecule. Unfortunately there are no gamma emitting isotopes of suitable half life of carbon, hydrogen or oxygen so that this cannot be accomplished with the cholesterol molecule. There has therefore been a need to devise analogues of cholesterol which will behave like cholesterol physiologically and biochemically but can nevertheless be detected by an external scanning arrangement. In this study the metabolism of certain analogues of cholesterol containing selenium-75 has been investigated. There are of course distinct chemical and physical differences between cholesterol and these 6 and 19-seleno analogues of cholesterol. The study was undertaken to compare and contrast the metabolism of cholesterol, 6-seleno and 19-seleno cholesterol in a series of *in vitro* and *in vivo* investigations.

Cholesterol in the animal organism is principally synthesised in the liver, gut and various other tissues. Much of the cholesterol synthesised in the liver is discharged as lipoproteins into plasma. Within the plasma compartment cholesterol exchanges between various plasma lipoprotiens and some of the sterol is esterified. The plasma cholesterol, held in lipoprotein-macromolecules is picked up by various extrahepatic tissues for metabolism.

In the liver, cholesterol is metabolised to bile acids and there is evidence to suggest that the rate limiting step in the conversion of cholesterol to bile acids is the 7α-hydroxylation of the sterol. In this study we have therefore compared the 7α-hydroxylation of cholesterol and the similar hydroxylation of the selenocholesterol analogues. We have used an established *in vitro* liver microsomal cholesterol 7α-hydroxylase assay. When cholesterol is used as the substrate the

Table 3. Distribution of the [^{75}Se]-cholesterol analogues in rats 48 h after administration*

Sternol	Liver	Adrenal	Kidney	Gut	Spleen	Brain	Muscle	Plasma
19-[^{75}Se]-Cholesterol	0.25	3.16	0.16	0.24	0.40	0.03	0.07	0.13
6-[^{75}Se]-Cholesterol	0.20	10.7	0.19	0.23	0.38	0.04	0.12	0.09

* The results are expressed as (c.p.m. in organ/g wet tissue)/c.p.m. in administered dose.

ratio of 7α-hydroxylation to autoxidation is about 10:1. When the 19-seleno-analogue was used in this assay the ratio was about 4:3, while when the 6-seleno-analogue was used the ratio was about 1:1. Thus these seleno cholesterol analogues are not good substrates for the liver microsomal 7α-hydroxylase.

We have also investigated in the rat the *in vivo* conversion of cholesterol to bile acids and the subsequent excretion of these bile acids in bile. In these studies rats were prepared with a cannula in the bile duct and after the intravenous administration of cholesterol or the seleno-analogues of cholesterol, the excretion of neutral sterols or bile acids metabolites was studied. As shown in Table 2 the 6- and 19-seleno-analogues of cholesterol were secreted in bile to a lesser extent than was cholesterol. The biliary neutral sterol:acid metabolite ratio was 1:7 for cholesterol and 3:2 for both the 6-seleno and 19-seleno analogues.

When cholesterol is secreted by liver as plasma lipoproteins the sterol undergoes esterification by the lecithin:cholesterol acyltransferase reaction (LCAT). Accordingly we compared cholesterol and the seleno-analogues of cholesterol in a standardised LCAT assay. The 19-seleno-analogue of cholesterol was only about half as rapidly esterified as was cholesterol while the 6-seleno-analogue was barely esterified.

Another well known fate of cholesterol in mammals is the uptake of cholesterol as lipoproteins by the adrenal cortex and the subsequent esterification of cholesterol to cholesterol esters in that tissue. Sterol esters in the adrenal cortex cells appear as lipid laden droplets. We have investigated the esterification of cholesterol in the adrenal microsomal preparation and compared the esterification of cholesterol with the esterification of the seleno analogues of cholesterol. In this assay the extent of esterification of cholesterol and the 6-seleno-analogue was of the same order, while the 19-seleno-analogue was barely esterified under these conditions.

In the adrenal cortex, free cholesterol is taken up by the adrenal cortex mitochondria, and by an oxygenase reaction the sterol is converted to pregnenolone. This is the so-called cholesterol side-chain cleavage reaction. The cleavage of the side-chain of cholesterol and the side-chain of the seleno-analogues of cholesterol was studied using adrenal cortex mitochondria. The 19-seleno-cholesterol analogue was metabolised to an insignificant extent to the corresponding pregnenolone analogue. However, the 6-seleno-analogue of cholesterol was converted to a side chain cleavage product to a significant extent.

The effective use of a cholesterol analogue in animals or in man necessitates a study on the distribution of the analogues. In the rat the tissue distribution of the seleno-analogues of cholesterol 48 h after the sterols had been administered intravenously to the animals showed a high uptake of the 6-seleno cholesterol by the adrenal glands.

It is obvious that there are distinct structural differences between the seleno-analogues of cholesterol and the physiological metabolite cholesterol. The incorporation of selenium into the sterol molecule presents certain difficulties with regard to the chemical work-up of tissue preparations containing these seleno-compounds. The seleno-analogues are more sensitive to oxidation and for this reason reducing agent such as ascorbic acid must be included in the various work-up procedures. Although sensitive to oxidation it is possible to work with these compounds with care without too much loss due to oxidation. On the other hand there are certain advantages of the seleno-compounds in that the isotope, selenium-75 is a convenient gamma emitter and as such use of these analogues is convenient in *in vivo* studies coupled to the application of external detection equipment to allow the localisation of the sterols in specific sites in the animal organism during life. It is for example possible to measure the localisation of the seleno-analogues of cholesterol in the adrenal cortex. These studies may provide some of the background to the observations that 6-seleno-cholesterol is an excellent adrenal scanning agent in man [13]. This labelled sterol has obvious advantages for possible clinical use for detection of certain abnormalities in the adrenal gland including adrenal tumours.

In this study there are certain unsolved problems such as the exact nature of the acidic material from the seleno-analogues found in bile. We have not in this study fully characterised the acidic material from bile. Some of the seleno-analogue of the bile acids behave like their corresponding physiological cholic acids and chenodeoxy-cholic acids but they also exhibit certain differences. Work will be required to fully characterise these acidic materials which arise from the metabolism of seleno-cholesterol analogues in the liver. It will be noted in this study that the seleno-analogues of cholesterol while they are not very good substrate for the cholesterol-7α-hydroxylase enzyme system in the liver endoplasmic reticulum, appear nevertheless to be converted to acidic materials in the bile. The present view in sterol metabolism is that the sterol 7α-hydroxylase is the primary initial rate-limiting event in the conversion of cholesterol to bile acids. In this study we have shown that the seleno-cholesterol analogues is converted to acidic material *in vivo* but the 7α-hydroxylation of the analogues *in vitro* is much less than the corresponding 7α-hydroxylation of cholesterol. This apparent anomaly will be further investigated.

These seleno cholesterol analogues behave like cholesterol in certain respects. The analogues are actively taken up by the adrenal cortex from plasma, esterified in the glands and the free seleno anlogues are converted to a limited extent to compounds like the C_{21} steroid hormone intermediates. Although Sarkar *et al.*[2] deduced that 19-methyl-[^{75}Se]-seleno-cholesterol was taken up by adrenal medulla chromaffin granules, in our study the sterol in the adrenal was shown to be esterified. This suggests that most of the

sterol uptake is into the adrenal cortex as the sterol in chromaffin granules is in the membranes as free sterol. It is possible that the observations of Sarkar *et al.*[2] could also be explained on the basis of invagination of adrenal cortical tissue into the medullary tissue. Such invagination would complicate the interpretation of autoradiographic experiments.

The *in vitro* studies reported in this paper show that these seleno-cholesterol analogues are esterified by the adrenal cortex microsomal system to the corresponding ester derivatives. The 6-seleno analogue is a very good substrate for this enzymic reaction as judged by the incorporation of the sterol into the corresponding ester in this *in vitro* assay. This 6-seleno derivative seems to be taken up in part by the adrenal cortex as shown by the *in vitro* and the *in vivo* studies in this paper and also by the studies of Merrick [13] in human subjects.

As a result of these animal experiments and also the consequence of *in vivo* studies in man, we conclude that some of these seleno-labelled cholesterol analogues are suitable as adrenal localising agents. Other aspects of the metabolism of these analogues remain to be explored.

Acknowledgements—This work was in part supported by a programmed grant from the Medical Research Council and by the Radiochemical Centre, Amersham. E.B. was in receipt of a scholarship from Ministero della Pubblica Istruzione, Italy.

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