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Radioactive Compounds Excreted by Rats Treated with ³⁵S-labelled British Anti-Lewisite. Preliminary Studies

By BEVERLEY TAMBOLINE, A. T. MATHESON* AND S. H. ZBARSKY

Department of Biochemistry, University of British Columbia, Vancouver 8, B.C., Canada

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Although British Anti-Lewisite (BAL) has been used, since its discovery, for the treatment of cases of poisoning by heavy metals (Stocken & Thompson, 1949), and more recently as a chemotherapeutic reagent in neurological disorders (Cumings, 1951; Streifler & Feldman, 1953), little is known of its metabolism in the mammal. Stocken & Thompson (1946) reported that the urine of rats and rabbits which had been given BAL by injection showed a strong nitroprusside reaction and an increased iodine uptake during the first few hours after administration, indicating the possible presence of a thiol. Evidence was adduced by Spray, Stocken & Thompson (1947) to show that in rabbits injected with BAL a dithiol compound was excreted which was very similar to BAL but not identical with it. Simpson & Young (1950) were also unable to detect the presence of BAL in the urine of rats treated with BAL. In further experiments, Spray (1947) concluded that BAL is not conjugated with sulphuric acid and he obtained indirect evidence that BAL might be excreted in conjugation with glucuronic acid.

Studies with ³⁵S-labelled BAL have shown that, in rats, most of the ³⁵S is excreted in the neutral sulphur fraction of the urine (Peters *et al.* 1947; Simpson & Young, 1950). Similar results were obtained in the present work, which showed

further that the greatest excretion occurred during the first 6 hr. after administration of the BAL. Because the sulphur of the BAL appeared to be excreted in an organically bound form, it was felt that an investigation of this sulphur-containing material would provide information concerning the manner in which BAL is metabolized. It was first necessary to determine whether one or more compounds derived from BAL were excreted and this was done mainly by paper chromatography. A system was developed for the detection of BAL on paper chromatograms and this was applied to the urines of rats which had been injected with radioactive BAL. The chromatograms were scanned for radioactive areas. It appeared that at least two radioactive materials were excreted; although these have not yet been identified, it appeared that one of the compounds is the chief excretory product of BAL. The second compound behaved like inorganic sulphate. A third material, detected in acidified urine, appeared to be a derived product resulting from the action of acid on other radioactive substances in the urine. Free BAL was not detected.

METHODS

Determination of radioactivity. To determine the radioactivity in the total sulphur of urine or other materials containing organically bound sulphur, the material was oxidized in a Carius bomb (cf. Young, Edson & McCarter, 1949). After heating, the bomb was cooled and opened,

* Present address: Department of Biochemistry, University of Toronto, Toronto 5, Ontario, Canada.

and the contents transferred quantitatively to a 100 ml. beaker and evaporated to dryness under an infrared lamp. To the residue, in 5 ml. of water, was added enough non-radioactive Na_2SO_4 to bring the total sulphur in solution to approximately 2 mg. The sulphate was precipitated as the benzidine salt (Young *et al.* 1949) and the salt collected on a weighed filter paper disk, 2.56 cm. in diameter, using an apparatus similar to that described by Henriques, Kistia-kowsky, Margnetti & Schneider (1946), except that the filter paper was supported on a perforated brass disk instead of a sintered glass disk. This arrangement allowed the rapid filtration necessary for the formation of a smooth layer of benzidine sulphate on the filter paper. After washing with 95% ethanol and ether, the paper and adhering precipitate were removed, dried in air and weighed. These samples were counted with a Geiger-Mueller tube with a mica end-window of thickness 1.6 mg./cm.² or in a windowless methane flow counter (Type CFIS-2, purchased from Atomic Energy of Canada Ltd., Chalk River, Ontario). Corrections for decay were made by referring to a standard source of radioactive benzidine sulphate and for self-absorption using a curve in which counts obtained with samples of radioactive benzidine sulphate of varying thicknesses were expressed in terms of the count of a sample of thickness of 17.6/cm.² (2 mg. S). Where no carrier sulphate had been added, specific activities were calculated directly from the weight of the benzidine sulphate. When carrier sulphate was added, a quantitative determination of sulphur was made on another portion of the original material.

Preparation of ^{35}S -labelled BAL. The ^{35}S used was obtained from Atomic Energy of Canada Ltd. as a dilute solution of $\text{H}_2^{35}\text{SO}_4$ in aqueous HCl. A small amount of carrier SO_4^{2-} was added to this and $\text{Ba}^{35}\text{SO}_4$ precipitated from the solution. This was reduced to Ba^{35}S , which in turn was converted into radioactive NaHS (cf. Peters *et al.* 1947). Labelled BAL was then prepared from the NaHS and 2:3-dibromopropanol (Simpson & Young, 1950). In a typical preparation, using 3 g. of 2:3-dibromopropanol, the yield of BAL was 40%. (Found: SH, 52.8, 52.9% by iodine titration. Calc. for $\text{C}_3\text{H}_5\text{OS}_2$: SH, 53.2%.) This BAL had a specific activity of 3000 counts/min./mg. of S. In order to test the radioactive purity of the BAL, a portion was converted into the crystalline 2-phenyl-4-hydroxymethyl-1:3-dithiolane, m.p. 67–68°, as described by Simpson & Young (1950). (Found: S, 30.0, 30.2%; Calc. for $\text{C}_{10}\text{H}_{12}\text{OS}_4$: S, 30.2%.) The dithiolane had a specific activity of 3012 counts/min./mg. of S, in excellent agreement with the BAL used as starting material.

Determination of sulphur and ^{35}S in the urine fractions. In the experiments on the rats of groups 1 and 2 only total sulphur and total sulphate of urine and the radioactivity in these fractions were determined (cf. Simpson & Young, 1950). With this method, however, it could not be certain that true values for total sulphate could be obtained. Further, the precipitates of benzidine sulphate in the total sulphate determinations did not pack to smooth layers on filtration, and it was difficult to obtain agreement between counts on duplicate samples. Accordingly, in later experiments a procedure based on the work of Owen (1936) and McKittrick & Schmidt (1945) was used to determine total and inorganic sulphate. To 1 ml. of urine, or 1 ml. of urine plus carrier sulphate, was added 0.5 ml. of 3N-HCl and the acid solution was evaporated almost to dryness. Bromophenol blue indicator solution was added and dilute NaOH

was added dropwise, with stirring, until the bromophenol blue end-point was reached. Dilute HCl was then added to bring the solution just to the acid side of the indicator. At this pH, approximately 2.8, benzidine sulphate has its minimum solubility. The salt was precipitated and 24 hr. later it was filtered off, weighed and counted. Inorganic sulphate was determined on another sample of urine which was not hydrolysed with HCl. Neutral sulphur was then calculated from the total sulphur and total sulphate.

In many instances, in order to conserve as much of the urine as possible for other studies, determinations were carried out on samples of the order of 0.1 ml. or less. This was done by suitable adaptation of the colorimetric method described by Dodgson & Spencer (1953), using a Cenco Photometer, Clinical Type C-5. Total and inorganic sulphate were determined on 5–30 μl . portions and total sulphur on 5 μl . samples of the urine. Radioactivity was measured on separate samples to which carrier sulphate was added.

Detection of BAL by paper chromatography. Descending chromatograms of the urine or other solutions were run on strips of Whatman no. 1 filter paper, using as a solvent *tert*.-butanol–water (75:25 by vol.). For chromatography, the jar was flushed out with nitrogen and sealed. After 24 hr. the paper was removed and dipped, while still wet, into a 1% (w/v) solution of nickelous chloride. The presence of BAL on the paper was indicated by the formation of a brown spot with an R_F value 0.90–0.95. By this technique it was possible to detect 10–20 μg . of BAL, applied as a known solution in urine, water or ethanol. Cysteine, in amounts of the order of 10–20 μg ., gave a green spot, R_F = 0.20, which appeared after the paper had dried for a few hours after dipping. Other solvent systems, such as phenol–water (80:20, v/v), *n*-butanol saturated with water, and *tert*.-butanol–water (50:30, v/v) were also tried with some success, but the best-defined BAL spots were obtained with the *tert*.-butanol–water mixture.

Radioactive chromatograms (radiochromatograms). Urine was collected for specified periods from groups of rats which had been given ^{35}S -labelled BAL by intraperitoneal injection. Chromatograms were prepared as described above, on paper strips 15 cm. wide, 4 urine spots being placed on one strip. When the strip was removed from the jar, it was allowed to dry in air. From the wide strip a narrower strip, 2.8 cm. wide, was cut, containing the path followed by the urine spot to be studied. This strip was divided into transverse sections 1 cm. in width, starting from the section just before the origin of the chromatogram and extending to the solvent front. Each small section was then counted. The observed count of each section, after being corrected for background, was then plotted against the corresponding R_F value to give a diagram as shown in Fig. 1. No corrections were made for self-absorption in these experiments.

Animal experiments

In the first experiment, 6 male Wistar rats were used, in two groups of 3. Rats of group 1, 737 g. and those of group 2, 796 g. Each rat was given, in a single intraperitoneal injection, 20.3 mg. of BAL dissolved in 0.5 ml. of propylene glycol. The BAL had a specific activity of 9100 counts/min./mg., so that each group received a total of 5.54×10^5 counts/min. of ^{35}S . Each group of rats was placed in a metabolism cage which permitted the collection

of urine separately from faeces. The animals were allowed water *ad lib.*, while in the cage, but for feeding purposes they were transferred to a different cage for 2 hr. intervals every 12 hr. and the urine excreted during these intervals was added to the main collection. Urine was collected for the periods 0-3, 3-6, 6-12, 12-24 and 24-48 hr. In these experiments sufficient dilute HCl was added to the collecting vessels to maintain the urine at about pH 1, in order to minimize the loss of any BAL which may have been excreted. It was shown by Simpson & Young (1950), and confirmed here, that the loss of BAL in urine exposed to the air is reduced if the urine is acidified. In this experiment only total sulphur and total sulphate were determined, so that it was possible to calculate values only for the neutral sulphur fraction. In the experiments involving groups 3-6, BAL of a much higher specific activity was used. Two dose levels of BAL were administered, in order to determine whether the amount of BAL had any effect on the partition of the excreted ^{35}S among the urinary sulphur fractions or on the distribution of radioactive areas detected on the radiochromatograms. The effect of collecting the urine in acid was also studied.

Each of groups 3-6 consisted of 3 male rats. For periods of 4 or 5 days prior to injection of BAL, each group was kept in a metabolism cage equipped with a glass separating device of the type described by Lazarow (1954), and was allowed the stock diet of pellets of U.B.C. Ration 1054 and water *ad lib.* Urine was collected for each 24 hr. period and determinations were made of the sulphur fractions. The rats were then injected with radioactive BAL as follows. The rats in group 3 (combined weight 625 g.) each received 5.3 mg. of BAL in 0.5 ml. of propylene glycol in a single intraperitoneal injection. The BAL had a specific activity of 1.16×10^6 counts/min./mg. BAL so that the group received a total of 1.85×10^6 counts/min. of ^{35}S . Each rat in group 4 (combined weight 630 g.) received 5.25 mg. of the BAL, or a total of 1.83×10^6 counts/min. for the group. The urine of group 3 was collected in acid as in Expt. 1; no acid was added to the urine of group 4. The urines of both groups were collected for the intervals 0-6, 6-12, 12-24 and 24-48 hr. after the injection, since insufficient urine was

excreted during the 0-3 hr. period to allow any sulphur analyses to be made. Apparently the amount of BAL administered to these two groups was too small to have the slight diuretic effect observed with larger doses.

Into each rat in groups 5 and 6, which had combined weights of 625 and 656 g. respectively, was injected 14.5 mg. of the radioactive BAL in 0.5 ml. of propylene glycol, approximately 3 times the amount of BAL administered to the animals in groups 3 and 4. Each group received a total of 5.06×10^6 counts/min. of ^{35}S . Urines were collected for the periods 0-3, 3-6, 6-12, 12-24 and 24-48 hr. after injection, no acid being added to the urine of group 6.

When possible, determinations of sulphur and radioactivity on the urines were started immediately after collection and the radiochromatograms prepared at the same time. Otherwise, the urine was frozen quickly and stored in that condition until used.

RESULTS

Urinary excretion of ^{35}S

The urinary excretion of ^{35}S by the injected rats is shown in Table 1. The results agree with those reported by previous investigators (Peters *et al.* 1947; Simpson & Young, 1950). It is of interest that the excretion of ^{35}S was extremely rapid, the highest rate occurring during the first 6 hr. after injection and sometimes during the first 3 hr. The rapid metabolic oxidation of BAL was indicated by the early excretion of radioactive inorganic and ethereal sulphate. The amount of BAL administered did not appear to affect the percentage of the injected ^{35}S which was excreted.

Excretion of neutral sulphur and inorganic sulphate

The excretion of most of the ^{35}S of the injected ^{35}S -BAL in the neutral sulphur fraction of the urine agreed with the finding that the administration

Table 1. Percentage of injected radioactivity excreted by rats given ^{35}S -BAL intraperitoneally

Group	^{35}S -BAL administered	Fraction	Period of collection, hours after injection					Total % excreted
			0-3	3-6	6-12	12-24	24-48	
1	20.3 mg.; 5.54×10^6 counts/min.	Neutral S	23.8	19.1	9.7	9.7	3.5	65.8
2	20.3 mg.; 5.54×10^6 counts/min.	Neutral S	24.2	28.6	12.0	11.0	1.5	77.3
3	5.3 mg.; 1.86×10^6 counts/min.	Neutral S	31.0		18.1	5.7	1.4	56.2
		Inorg. SO_4^{2-}	1.2		1.2	0.8	0.3	3.5
		Ethereal SO_4^{2-}	0.05		0.5	0.1	0.0	0.65
4	5.25 mg.; 1.83×10^6 counts/min.	Neutral S	23.1		15.3	*	1.0	—
		Inorg. SO_4^{2-}	1.0		0.8	*	0.1	—
		Ethereal SO_4^{2-}	0.2		0.2	*	0.0	—
5	14.5 mg.; 5.06×10^6 counts/min.	Neutral S	26.0	14.3	6.5	3.0	0.9	50.7
		Inorg. SO_4^{2-}	1.3	1.2	0.5	0.6	0.2	3.8
		Ethereal SO_4^{2-}	0.1	0.1	0.1	0.1	0.05	0.45
6	14.5 mg.; 5.06×10^6 counts/min.	Neutral S	19.0	29.4	12.3	3.4	1.1	65.2
		Inorg. SO_4^{2-}	1.4	1.3	0.8	0.6	0.3	4.4
		Ethereal SO_4^{2-}	0.1	0.0	0.1	0.1	0.0	0.3

* This urine sample was lost before determinations were completed.

of BAL led to an increased excretion of total neutral sulphur during the 24 hr. period following the injection, in agreement with the earlier observations of Spray (1947). Spray also reported a marked rise in the excretion of inorganic sulphate. No such rise was observed in the experiments described herein. Spray (1947) attributed the increased excretion of inorganic sulphate to tissue damage resulting from the injection (cf. Cuthbertson, 1930, 1931). In his work the BAL was injected intramuscularly or subcutaneously, whereas in the experiments which have been described herein the BAL was administered to the rats intraperitoneally in smaller amounts than those used by Spray, and it is possible that tissue damage suffered by the rats may not have been sufficiently great to lead to an increased sulphate excretion.

Paper chromatography of the collected urines

Radiochromatograms were prepared from each collection of urine. In several instances, a second chromatogram was prepared which was treated with the nickelous chloride solution to test for the presence of free BAL. None was detected in any of the collected urines, either by the use of the colour reagent, or radioactive scanning.

collected 24–48 hr. after injection because in these the observed counts were very close to background and they were of doubtful significance.

A typical diagram of observed radioactivity versus R_f value is shown in Fig. 1, which represents the radiochromatograms obtained from the urine of the rats of group 5.

The R_f values of the peaks shown in Table 2 were not constant, shifting in several cases to higher values. The reasons for the shift were not investigated. It should be pointed out, however, that in these experiments the chromatograms were prepared from the urine as collected. Some of the urines were diluted during collection by the drinking water in the metabolism cages, with the result that the urines differed in concentration of salts and of other substances. The former, especially, may have affected the R_f values of the radioactive materials.

The data in Table 2 show that the peak with R_f range of 0.53–0.68, attributed to compound 2, was detected in the urine of all the groups of rats. This material was present in considerable amount and appears to be the chief radioactive excretory product of the injected ^{35}S -BAL. In the urines of those rats which received the larger doses of BAL

Table 2. *Radioactive areas on filter-paper radiochromatograms of urine excreted by rats given ^{35}S -BAL intraperitoneally*

Group	Period of collection of urine (hr.)	R_f at most active part of radioactive area			Counts/min. at most active part		
		1	2	3	1	2	3
3 (15.9 mg. BAL)	0–6	—	0.62	0.75	—	1014	700
	6–12	—	0.66	0.79	—	255	153
	12–24	—	0.62	0.75	—	52	48
4 (15.75 mg. BAL)	0–6	0.07	0.59	—	109	943	—
	6–12	—	0.68	—	—	622	—
	12–24	—	0.73	—	—	305	—
5 (43.5 mg. BAL)	0–3	0.02	0.53	0.62	301	1981	1469
	3–6	0.02	0.53	0.66	91	612	760
	6–12	0.02	0.60	0.74	36	420	436
	12–24	0.03	0.59	0.70	21	48	36
6 (43.5 mg. BAL)	0–3	0.03	0.61	—	742	242	—
	3–6	0.03	0.66	—	214	754	—
	6–12	0.03	0.66	—	38	436	—
	12–24	0.03	0.66	—	23	82	—

The radiochromatograms of the urines of the rats of groups 1 and 2 showed several radioactive areas. In several instances, however, little significance could be attached to the observed counts since they were only slightly above background. The rats of groups 3–6 received BAL of much higher specific activity, and the radiochromatograms of the urines from these animals showed radioactive areas with considerable radioactivity. The radioactivities and R_f values of the peaks of these areas are shown in Table 2. No values are shown for the urines

(groups 5 and 6) a peak was observed close to the origin, $R_f = 0.02$ – 0.03 . This material has been designated compound 1 and may be responsible for the peak of $R_f = 0.07$ found for the 0–6 hr. urine of group 4. It is possible that some of the material contributing to this peak may be inorganic sulphate. Radiochromatograms prepared from solutions of $\text{Na}_2^{35}\text{SO}_4$ showed single radioactive areas with the same R_f value. Other evidence, however, indicated that the first peak may not have been due entirely to inorganic sulphate. Calculations

showed that the amount of radioactivity present as compound 1 in several of the chromatograms constituted a greater percentage of the total radioactivity on the radiochromatogram than could be accounted for by the inorganic sulphate in the urine. One possibility is that, since this material occurred so close to the origin of the chromatograms, it may have included radioactive material which did not migrate as the solvent descended on the paper.

two peaks corresponding to compounds 2 and 3. In another experiment, an acidic urine sample which showed peaks for both 2 and 3 was made more strongly acid and another radiochromatogram prepared. It was found that the height of peak 2 had decreased, while that of peak 3 had increased. These results suggest that compound 3 is not an excretory product, but arises after excretion through the action of acid on other material in the urine.

Stability of ^{35}S -BAL in rat urine at pH 1

The possibility was considered that BAL may have been excreted in urine by the injected animals and converted after excretion into compounds which were responsible for the observed radioactive areas on the radiochromatograms. To test this possibility, urine was collected from a group of three untreated rats and the pH adjusted to 1. A known amount of ^{35}S -labelled BAL, having a specific activity of 9100 counts/min./mg., was dissolved in this urine to give a solution with an activity of 20 000 counts/min./ml. of urine. Two sets of paper chromatograms of this solution were prepared after it had been allowed to remain at room temperature for periods of 0, 3, 6, 9, 12, 24 and 48 hr. One set was scanned for radioactive areas, and each radiochromatogram was found to show only one radioactive peak at $R_f = 0.89-0.93$, characteristic of BAL, for the solvent used. The second set of chromatograms was treated with the nickel chloride solution and the brown spot due to BAL, $R_f = 0.91$, was observed in all the chromatograms except those prepared from the urine solution after it had been allowed to stand for 24 and 48 hr. It was of interest that the use of the counting technique showed the presence of BAL when the amount remaining was apparently too small to be detected by the colour reagent.

It was concluded from the above results that the observed peaks in the urines of injected animals were not due to compounds derived from excreted BAL. The results also provide added evidence that little, if any, BAL was excreted by the injected rats.

DISCUSSION

As has been shown by other investigators (Peters *et al.* 1947; Simpson & Young, 1950), the administration of ^{35}S -labelled BAL to rats was followed by the rapid excretion of most of the ^{35}S in the neutral sulphur fraction of the urine. In the present work, the BAL was injected intraperitoneally, and 50–77% of the radioactivity was found in the urinary neutral sulphur during the 48 hr. following injection; 3.4–4.4% was excreted as inorganic sulphate and 0.3–0.6% as ethereal sulphate. The highest excretion of ^{35}S occurred during the first

Fig. 1. Radiochromatograms of urine excreted by rats of group 5. Each rat received by intraperitoneal injection 14.5 mg. of ^{35}S -BAL in 0.5 ml. propylene glycol and urine was collected in acid for the indicated periods after injection. Total radioactivity administered, 5.06×10^6 counts/min.

The third peak, corresponding to compound 3, was observed only in those urines collected in acid, the pH of which was approximately 1. It was thought that this material may have arisen as a result of the action of acid on other radioactive material in the urine. To test this possibility, an experiment was carried out in which urine giving a peak for compound 2 only was acidified and a radiochromatogram of the acidified urine was prepared. It was found that the urine now gave

6 hr. and in some experiments during the first 3 hr. after injection. The amount of BAL administered did not appear to influence the general excretion pattern. A rise in the total neutral sulphur was observed during the 24 hr. period following the injection of BAL. No effect was found on the excretion of total inorganic sulphate, although Spray (1947) had found a marked rise in the excretion of inorganic sulphate by rats given BAL subcutaneously or intramuscularly.

Filter-paper radiochromatograms of the urine samples showed radioactive areas of R_f ranging from 0.53 to 0.68 in all the samples. The radioactive material appearing in this area of the filter paper has been designated compound 2 and appears to be the main excretory product of BAL. A study of the identity of this material is now under way. Another radioactive peak, corresponding to compound 1, was found in the urine excreted by the rats which received the larger amounts of BAL. It has been assumed that the amount of this material excreted by those rats receiving the smaller doses of BAL, 5.25 and 5.3 mg. each, was too small to be detected by counting. The peak appeared close to the origin, $R_f = 0.02-0.03$, and there is evidence to suggest that some of the material contributing to the peak may be radioactive inorganic sulphate. The possibility is not excluded, however, that part of the radioactivity present in this area arose from other material which did not descend on the filter paper.

A third radioactive area, corresponding to compound 3, with an R_f ranging from 0.62 to 0.79, was observed only in those urines which had been collected in acid, and it was concluded on the basis of other evidence which has been cited that this material was not an excretory product but was produced in the urine, after excretion, through the action of acid.

Although no other radioactive peaks were observed, it is possible that other radioactive products may have been present and escaped detection. The observed radioactive areas on the chromatograms sometimes extended over a considerable distance on the filter paper and, especially where relatively large amounts of activity were present; these may have masked smaller peaks. Further, the method of scanning the radiochromatograms may not have given fine enough resolution, where high levels of radioactivity were encountered.

The presence of unchanged BAL was not indicated on any of the radiochromatograms, even on those of the urines collected during the first 3 hr. after the injection of the labelled BAL. The use of nickelous chloride as a colour reagent also failed to show the presence in the urine of BAL or of other free thiol compounds.

SUMMARY

1. Following the intraperitoneal injection of ^{35}S -labelled BAL into rats, there was an immediate excretion of the ^{35}S in the urine, most being excreted within the first 6 hr.

2. During the 48 hr. period after injection, 50–77 % of the injected radioactivity was found in the neutral sulphur fraction of the urine, 3.4–4.4 % in the inorganic sulphate fraction and 0.3–0.6 % in the ethereal sulphate fraction.

3. The injection of BAL led to a rise in the total neutral sulphur excretion. No effect was observed on the excretion of inorganic sulphate.

4. A method has been described for the detection of BAL by filter-paper chromatography, using *tert.*-butanol–water as solvent and a 1 % solution of nickelous chloride as the colour reagent. A brown spot, $R_f = 0.92$, indicates the presence of BAL in amounts as low as 10–20 μg .

5. Filter-paper chromatograms of the urine excreted by the injected rats were scanned for radioactive areas. A radioactive area, with R_f of 0.53–0.68, was observed in all the urines. This material appeared to be the major radioactive excretory product of ^{35}S -labelled BAL. A second peak of activity, R_f 0.02–0.03, was detected in the urine of the animals receiving the larger doses of BAL and may be due, in part at least, to inorganic sulphate. A third radioactive area, R_f 0.62–0.79, was observed only in urines collected in acid. It was concluded that this material was formed through the action of acid on other radioactive compounds in the urine.

6. Unchanged BAL was not detected in the urine of the injected rats.

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Cholesterol Metabolism

4. THE NEUTRAL FRACTION OF THE FAECAL LIPID OF RATS FED DIETS WITH LOW AND HIGH CHOLESTEROL CONTENTS*

By CHRISTINE RIDDELL AND R. P. COOK

Biochemistry Department, Queen's College, Dundee (University of St Andrews)

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In this communication an account is given of the neutral fraction or unsaponifiable matter (UM) of the extract (ether- and ethanol-soluble materials) of the faeces of rats fed diets with and without added fat (16.6%), containing either small amounts of sterols (*C* series) or with added cholesterol (1.6%; *S* series). Emphasis has been placed on the products of faecal excretion in the intact animal as an indication of the pathways of sterol degradation.

The excreted material represents the final result of a number of processes. There is the contribution by the unabsorbed lipid component of the diet—for example carotenoids and vegetable sterols—and in the case of the diets containing added cholesterol a considerable part is unabsorbed. The secretions of the intestine and the microbial population of the gut also contribute to the lipid component. A discussion of the factors is given by Cook (1952).

In calculating the effect due to the administered cholesterol it has been taken that the subtraction of the faecal material of the *C* series from that of the *S* series shows the gross changes undergone by the sterol in the entire animal. Here a number of processes again are concerned and the products include the compounds formed after absorption and excreted either via the bile or by the intestinal mucosa, those compounds metabolized by the intestine itself and those derived from microbial action. It is impossible to separate these processes, but the work is exploratory and is intended to devise methods suitable for studying metabolic degradation products of sterols. Apart from the major sterols (cholesterol and coprostanol) there is little information on the chemical nature of the neutral fraction of faeces. It has not been possible to isolate all the compounds present, but by the use of chemical and physical methods and by paper

partition chromatography good evidence has been obtained for the presence in small amounts of a number of compounds. The main methods used have been adsorption chromatography on alumina and recrystallization, together with some chemical separations. The cholesterol used in these experiments, and in former work, contains small amounts of companion sterols such as cholestanol (see Fieser, 1953*b* and Discussion, p. 669).

The feeding experiments and preparation of initial UM and acidic fractions were done in collaboration with Dr D. C. Edwards and Dr R. O. Thomson. A preliminary account has been published (Cook & Edwards, 1952).

Nomenclature. The Ciba Foundation Rules (see *J. chem. Soc.* 1951, p. 3526) are followed generally, but some trivial names are used. Cholesterol is cholest-5-en-3 β -ol, lathosterol cholest-7-en-3 β -ol; 7-dehydrocholesterol is cholesta-5:7 dien-3 β -ol and coprostanol coprostan-3 β -ol (5 β -cholestan-3 β -ol). The name 'stenol' is used to designate unsaturated sterols, with the prefix Δ^5 - or Δ^7 - to indicate double bond position; 'stanol' is used for saturated sterols. The prefix 3 α - or 3 β - is used to indicate those sterols that are not and those that are precipitated as digitonides by an ethanolic digitonin solution. Some abbreviations are used to describe diols, e.g. '7 β -diol' is cholest-5-ene-3 β :7 β -diol and '24- or 25-diols' is cholest-5-ene-3 β :24- (or 25) diol. The term 'phytosterols' is used generically to indicate all sterols of vegetable origin.

METHODS AND MATERIALS

Animals. The same strain of rats, method of housing, feeding and collection of excreta as used previously were employed (Cook, Polgar & Thomson, 1950). Six male rats were used in each group in each experiment. The faeces were collected daily and dried *in vacuo* at 100°.

* Part 3: Cook & Thomson (1951*a*).