RATE OF EXCRETION OF RADIOACTIVE SULFUR AND ITS CONCENTRATION IN SOME TISSUES OF THE RAT AFTER INTRAPERITONEAL ADMINISTRATION OF LABELED SODIUM SULFATE

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A study of the distribution of S$^{35}$ in the rat, 14 to 16 hours after the intraperitoneal administration of sodium sulfate, labeled with S$^{35}$, has been reported by Singher and Marinelli (1). Of particular interest is their observation that the highest concentration of the radioactive sulfur was found in the bone marrow. This observation stimulated us to determine the change with time in the concentration of S$^{35}$, given as labeled sodium sulfate, in the bone marrow of the rat and to relate this changing concentration with the concentration in other tissues, particularly the blood.

Only a brief report (2) was found in the literature on the excretion in urine of S$^{35}$ ingested as sodium sulfate. The rate of excretion of S$^{35}$ in the urine and feces of rats after intraperitoneal injection of labeled sodium sulfate was, therefore, also determined.

EXPERIMENTAL

Adult rats, 180 to 330 gm. in weight, from the colony of Professor E. V. McCollum, were each given 1 mg. of sodium sulfate (in 1 ml. of distilled water), labeled with radioactive sulfur (S$^{35}$), by intraperitoneal injection. They were then placed in individual metabolism cages, with food and water, to allow for the separate collection of urine and feces. The cages had been so designed by Professor McCollum and his coworkers that there was a minimum of food spillage, and therefore contamination, of urine and feces with food. The rats were sacrificed at intervals of time, as follows: A rat was anesthetized in an ether jar. The heart was exposed and as much blood as possible was withdrawn directly from the heart.

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1 The S$^{35}$ used in this investigation was supplied by the Clinton Laboratories, Monsanto Chemical Company, and obtained on allocation from the United States Atomic Energy Commission.
EXCRETION OF $^{35}$S

with a hypodermic needle and syringe. A portion of the liver and the brain were then removed and placed in small vials. Weighed samples of these tissues, the feces collected in the time the rat sojourned in the metabolism cage, and 1 ml. of the blood were placed in separate nickel crucibles, each of which contained 2 ml. of 10 per cent sodium hydroxide and 4 gm. of anhydrous sodium carbonate. The crucibles were placed in a drying oven at 110–120° until dry. The dry material in each crucible was oxidized with sodium peroxide according to Bailey (3).

The humeri, femurs, and tibiae were freed of muscle and periosteum. The epiphysis of each bone was cut off at its junction with the diaphysis. The diaphysis of each bone was then freed of its marrow by pushing the latter out with a stainless steel wire. This marrow was placed immediately in a small screw cap vial and weighed. The residual marrow in the bone was wiped out by pushing through a small plug of moist cotton repeatedly until no further marrow was in evidence. As a check, each bone was split and carefully examined. It was further cleaned, if necessary, by wiping with moist cotton. The bone shafts were combined and powdered in a stainless steel mortar with a stainless steel pestle. A weighed portion of the powdered bone was oxidized with Benedict-Denis reagent (4).

The weighed bone marrow was extracted 3 times with at least 10 times its weight of 5 per cent trichloroacetic acid in a centrifuge tube. The combined extracts and the residue were each neutralized with 10 per cent sodium hydroxide, with phenolphthalein as an indicator, and then oxidized with Benedict-Denis reagent (4).

The urine excreted by each rat was combined with the washings of the metabolism cage and diluted to a volume of 100 ml. The total sulfur in aliquots of the urine was oxidized to sulfate according to Denis (4).

Before precipitation of sulfate from any sample as barium sulfate, 5 ml. of 0.05 N sodium sulfate solution were added to each sample so as to bring the final weight of barium sulfate to about 30 mg. Precipitation of barium sulfate was allowed to proceed for 16 to 20 hours at room temperature.

All barium sulfate samples were isolated by centrifugation and, after washing with distilled water in the centrifuge tubes, transferred to counting cups as slurries in 70 per cent ethanol as previously described (5).

The activity of each sample was determined with mica end window (2.8 mg. per sq. cm.) Geiger-Müller tube (Victoreen) and a Cyclotron Specialties scaler. At least two separate determinations of the activity of each sample were made for a sufficiently long period of time to obtain a precision of about 2 per cent. All values were corrected for decay and self-absorption.
RESULTS AND DISCUSSION

Of the twenty-seven rats used, fourteen were males and thirteen were females. The points given in Figs. 1 to 3 are average values, calculated from results on four animals at 4 hours, three animals at 8 hours, four animals at 16 hours, eight animals at 24 hours, and two animals each at 48, 72, 96, and 120 hours.

Fig. 1 shows the rate at which the $S^{35}$ was found to be excreted in the urine and feces of the rats. Approximately 67 per cent of the activity injected was excreted in the urine by the end of the 24th hour. Borsook et al. (2) could account for only 47 per cent at the end of 24 hours in the urine of a man. The subject studied by Borsook et al. ingested the labeled sodium sulfate by mouth. The rats studied by us received the sodium sulfate containing $S^{35}$ by intraperitoneal injection. This difference in the route of administration may account for the difference in the fraction recovered at the end of 24 hours. On calculating the intake by the human subject and by the rats, assuming that the human subject weighed somewhere within the limits of 45 to 90 kilos, one
Arrives at an intake of approximately 9.8 to 19.7 mg. of sodium sulfate per kilo as compared to approximately 4 mg. per kilo by the rats. This difference in intake might also be considered as a possible explanation for the difference in amount of S\textsuperscript{35} found excreted in the urine within 24 hours. One should, however, consider in addition a possible species difference as regards rate of sulfate excretion.

By the end of the 120th hour after the administration of the labeled sodium sulfate, the activity of S\textsuperscript{35} recovered in the urine accounted for approximately 85 per cent of the activity given. Approximately 95 per cent of the S\textsuperscript{35} is accounted for at the end of 120 hours if the activities found in the urine and feces are added.

In Fig. 2 are presented curves showing the change in concentration of S\textsuperscript{35} with time in the blood, liver, and brain. From these curves it would seem that sulfate sulfur as such, when it enters the circulation or the liver in a normal rat, is rapidly eliminated from these tissues. Whether the brain is similar in this respect or whether it slowly accumulates S\textsuperscript{35} presented to it as sulfate sulfur is difficult to say without extending the number of observations reported here. In any case, the concentration of S\textsuperscript{35} in the liver and blood appeared to have reached a similar concentration at about the 48th hour after the injection of the

![Fig. 2. Retention of S\textsuperscript{35} in the blood, liver, and brain by adult rats after intraperitoneal injection of labeled sodium sulfate.](http://www.jbc.org/)

1 mg. Na\textsubscript{2}S\textsuperscript{35}O\textsubscript{4} (15.4 x 10\textsuperscript{5} counts/min.) injected intraperitoneally

--- Blood
**Liver**
• Brain
Fig. 3. Retention of $^{35}S$ in the bone and bone marrow by adult rats after intraperitoneal injection of labeled sodium sulfate.

**Table I**

**Extent of $^{35}S$ Removal from Rat Bone Marrow by 3-Fold Extraction with 5 Per Cent Solution of Trichloroacetic Acid**

A male rat, 355 gm. in weight, was used 24 hours after the intraperitoneal administration of 1 mg. of sodium sulfate, containing $^{35}S$ ($15.4 \times 10^6$ counts per minute). The values for activity are corrected for radioactive decay and self-absorption.

<table>
<thead>
<tr>
<th>Sample</th>
<th>counts per min. per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st three trichloroacetic acid extracts combined</td>
<td>4960</td>
</tr>
<tr>
<td>4th trichloroacetic acid extract</td>
<td>0-10</td>
</tr>
<tr>
<td>Three alcohol-ether (1:3) extracts combined</td>
<td>64</td>
</tr>
<tr>
<td>Residue</td>
<td>793</td>
</tr>
</tbody>
</table>

labeled sulfate. A similarity in the concentration of $^{35}S$ in the brain and blood, from the observations thus far made, was more slowly attained; a similar concentration was reached at about the 72nd hour.

In contrast to the rapid fall of the $^{35}S$ concentration in blood and
liver, after intraperitoneal injection of labeled sodium sulfate, is the pronounced rise in the concentration of \( ^{35}S \) found in bone and bone marrow (Fig. 3). The highest concentration in the bone was observed at about the 8th hour, that in the bone marrow at about the 24th hour after injection of the labeled sodium sulfate. The subsequent drop in the \( ^{35}S \) concentration in these tissues is also less rapid, particularly in the bone marrow. Even at the end of 120 hours, on a weight basis, the \( ^{35}S \) concentration is about 2 times as high in the bone and nearly 12 times as high in the bone marrow as in the blood. The observations confirm the report of Singher and Marinelli (1) that the concentration of \( ^{35}S \), after administration of labeled sodium sulfate, is higher in bone marrow than that found in most of the other tissues of the rat. The major portion of the activity found in the bone marrow is in a compound or compounds which are soluble in a 5 per cent solution of trichloroacetic acid. That a 3-fold extraction of bone marrow with a 5 per cent solution of trichloroacetic acid, as employed, was effective in removing all or nearly all of the \( ^{35}S \)-containing material, which was soluble in this solution, is indicated in Table I. Further work on the characterization of the materials containing \( ^{35}S \) in the various fractions listed in Table I is contemplated.

**SUMMARY**

The excretion by rats in urine and feces of \( ^{35}S \) given in the form of sodium sulfate appears to be rapid. Excretion by these routes accounts for the major portion of the \( ^{35}S \) given. By the end of the 120th hour approximately 95 per cent was found to have been eliminated by these routes.

In the period of 120 hours the concentration of \( ^{35}S \) in the blood, liver, and brain was found to have fallen to relatively low levels. A similar concentration of \( ^{35}S \) in the blood and liver was attained by the 48th hour, in the blood and brain by the 72nd hour.

In contrast to the rapid fall observed in the liver and blood, the concentration of the \( ^{35}S \) was found to increase until about the 8th hour in bone and until about the 24th hour in bone marrow. The subsequent fall in the \( ^{35}S \) concentration of the bone and bone marrow was also slower than that in blood, liver, and brain.

**BIBLIOGRAPHY**

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