Metabolism of Thiamine-S^{35} in the Rabbit.* (24091)

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McCarthy, Cerecedo, and Brown(1) showed that rats which had received an intramuscular injection of thiamine-S^{35} excreted about 64% of the radiosulfur in urine, and between 1 and 2% in feces, within 10 days after administration. Isolation of thiamine-S^{35} from urine by adsorption on synthetic zeolite Decalso showed that approximately 60% radiosulfur of the neutral sulfur fraction was contained in unchanged thiamine-S^{35}, and the remaining 40% was present in neutral sulfur compounds not adsorbed by Decalso. The present study was based on use of doses of radiovitamin that were in the physiological range, *i.e.*, from 50 to 200 γ administered by oral and parenteral routes, to ascertain whether different routes of administration would lead to gross differences in the metabolic pattern.

Materials and methods. Metabolic studies were carried out using Chinchilla or Dutch breed adult rabbits. These were housed in wire-bottom metabolism cages allowing complete separation of urine and feces, and were given Purina Rabbit Chow and water *ad lib.* with supplement of lettuce and carrots once or twice weekly except immediately prior to and during experimental periods. Each individual experiment was carried out with 2 rabbits, and in so far as possible, the same pair of rabbits was used in successive experiments so that, in effect, each rabbit was its own control. Urine samples were collected in 10 ml of glacial acetic acid as a preservative which brought the pH from range 9-10 to 4-5, in which thiamine-S^{35} and its metabolites would be more stable. Urine samples were filtered and diluted with distilled water. Feces were homogenized with 150 ml of water containing about 10 ml of concentrated hydrochloric acid. The homogenate stood several hours, was stirred occasionally, and finally centrifuged to remove the bulk of solid matter. The supernatant was filtered and diluted with distilled water. Radiosulfur of urine was determined by analysis of 3 sulfur fractions: inorganic sulfate, ethereal sulfate, and neutral sulfur. Fecal sulfur was partitioned into inorganic sulfate and neutral sulfur fractions. The method of Folin was utilized for isolation of inorganic sulfate, and total sulfate (inorganic plus ethereal) fractions, while the neutral sulfur fraction was obtained by subtracting total sulfate from total sulfur, as determined by the Denis modification of the method of Benedict (2a). All sulfate fractions were precipitated as barium sulfate, and filtered onto porous glass frits mounted in stainless steel holders. Precipitates were washed successively with water, methanol, and acetone, and dried at 110°C for one hour. Samples were counted in a Nuclear windowless or thin-window flow-gas Geiger-Mueller counter and scaler. All count rates were corrected for background, counter efficiency, decay of sulfur-35, and self-absorption. A sample of barium sulfate-S^{35} was prepared from an amount of thiamine-S^{35} identical to that administered to rabbits. This standard sample was counted each day that experimental samples were counted, and comparison of count rates of samples with this standard gave the percent recovery of the radiosulfur. In addition to sulfur analyses, urea excreted in urine was determined each day by the colorimetric method of Rosenthal, using diacetyl monoxime in arsenic acid(3). Under normal conditions of nutrition, daily excretion of urea parallels that of inorganic sulfate in urine(2b). Therefore urea : inorganic sulfate ratios were calculated daily to ascertain that no marked alterations occurred in nitrogen equilibrium. Preliminary experiments indicated that unchanged thiamine-S^{35} and its thiazole-S^{35} moiety were main metabolites of radiovitamin in rabbit urine, and procedures were devised to isolate these substances. A modification of the method of Herr(4) was used for isolation of thiamine-

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TABLE I. Percent Recovery of S^{35} in Urine and Feces of Rabbits after Administration of 100 γ of Thiamine-S^{35} by Stomach Tube.

24-hr excretion period	Fraction	Urine		Feces	
		Rabbit 1	Rabbit 2	Rabbit 1	Rabbit 2
1st	Inorganic sulfate	2.60	1.29	2.10	.47
	Ethereal "	.92	1.37		
	Neutral sulfur	37.85	43.22	7.39	5.92
	Total	41.37	45.88	9.49	6.39
2nd	Inorganic sulfate	.74	.42	.57	.90
	Ethereal "	.42	.00		
	Neutral sulfur	3.26	2.28	2.37	1.35
	Total	4.42	2.70	2.94	2.25
3rd	Inorganic sulfate	.59	.42		
	Ethereal "	.11	.00		
	Neutral sulfur	2.99	.96		
	Total	3.69	1.38	1.48	.90
4th	Inorganic sulfate	.11	.21		
	Neutral sulfur	.88	.26		
	Total	.99	.47	.73	.55
5th	Total	.67	.21		
	Total recovery	51.12	50.64	14.64	10.09

Total recovery, urine and feces: Rabbit 1, 65.76%; Rabbit 2, 60.73%.

S^{35}, in which it was removed from urine by adsorption on column of strong cation exchange resin Dowex-50-X8 with subsequent elution by concentrated hydrochloric acid. Recovery experiments indicated that between 80 and 90% of thiamine-S^{35} could be removed from urine by this procedure. The hydrochloric acid eluate was evaporated to dryness over soda lime and phosphorus pentoxide in vacuum desiccator; the residue was taken up in about 10 ml of anhydrous methanol, the resulting solution filtered, and the filtrate evaporated to dryness. The final residue was dissolved in 5 to 8 ml of anhydrous methanol. and an aliquot of this solution was used for determination of the radiosulfur content. Thiazole-S^{35} was extracted from urine with ether. Prior to extraction, about 25 mg of non-radioactive thiazole were added to urine to act as carrier, the urine adjusted to pH 10 by addition of sodium hydroxide, then extracted with ether in a continuous extractor for 12 to 16 hours. The ethereal extract was dried over anhydrous magnesium sulfate, and ether distilled off. The thiazole-S^{35} residue was dissolved in 5 to 8 ml of anhydrous ethanol, and an aliquot used for determination of radiosulfur content. The presence of these metabolites in the extracts was confirmed by paper chromatography on strips of Whatman #1 paper (1.5 x 22 inches) in the solvent system n-butanol-acetic acid-water (4 : 1 : 1). Ascending chromatography was used. The chromatograms were developed 8 to 10 hours in the solvent system, then dried at room temperature. Spots were identified by inspecting the chromatograms in ultraviolet light and by scanning the strips in a windowless flow-gas Geiger-Mueller counter designed for this purpose (Anderson Scannergram). Thiamine-S^{35} bromide hydrobromide was prepared from thiourea-S^{35} (5).†

Results. Oral administration. In experiments in which thiamine-S^{35} was administered to rabbits by oral route, 3 dose levels in the physiological range were used: 50, 100, and 200 γ. The thiamine-S^{35} (about 25,000 counts/min/100 γ) was dissolved in 1 ml of water and administered to rabbits by stomach tube. The excretion pattern was the same for all 3 levels; however, total recovery of radiosulfur in urine varied from 50 to 90% in 4 to six 24-hour periods following administration of radiovitamin. In all cases, more than 50%

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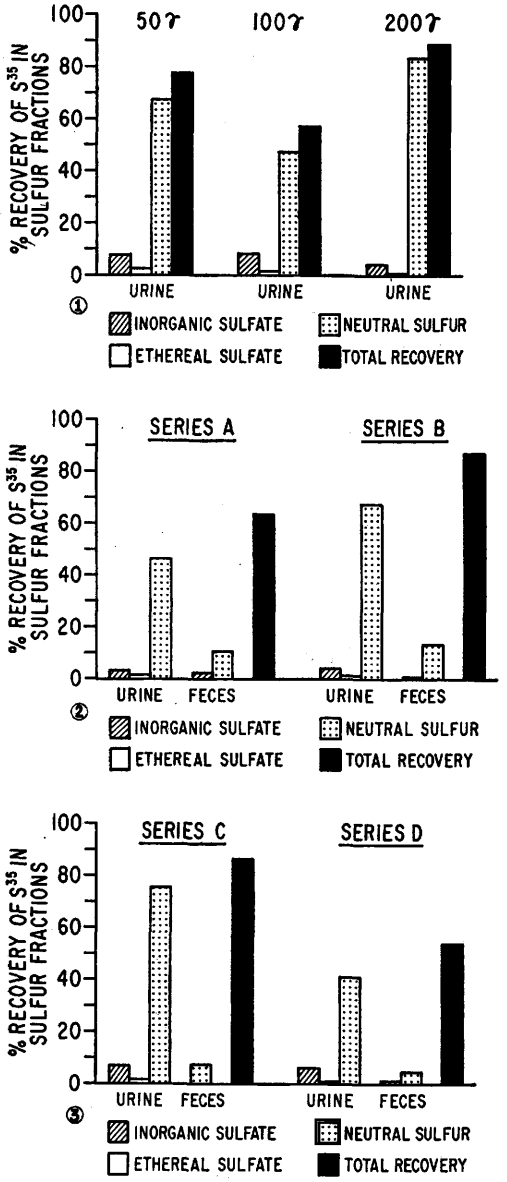

FIG. 1. Oral administration of thiamine-S^{35} to rabbits. Recovery of radiosulfur in urinary sulfur fractions (five 24-hr periods) after administration of 50, 100 and 200 γ of thiamine-S^{35} by stomach tube.

FIG. 2. Oral administration of thiamine-S^{35} to rabbits. Recovery of radiosulfur in urine and feces: Series A, 100 γ of thiamine-S^{35} by stomach tube; Series B, 100 γ of thiamine-S^{35} by stomach tube, followed 6 hr later by intramusc. inj. of 2 mg of non-radioactive thiamine. (Urine: five 24-hr periods; feces: four 24-hr periods.)

FIG. 3. Parenteral administration of thiamine-S^{35} to rabbits. Recovery of radiosulfur in urine and feces: Series C, 100 γ of thiamine-S^{35} by intramusc. inj.; Series D, 100 γ of thiamine-S^{35} by intrav. inj. (Urine: six 24-hr periods; feces: four 24-hr periods.)

of the recovered radiosulfur was in the neutral sulfur fraction of the urine, and the greater portion of this was recovered in urine excreted in the first 24-hour period. The second 24-hour period usually contained half, or less than half, of the amount of radiosulfur recovered in the first period, and again this was contained primarily in the neutral sulfur fraction. Radiosulfur was found in continuously diminishing amounts in succeeding excretion periods. Inorganic sulfate and ethereal sulfate fractions of urine contained very small amounts of radioactivity. Feces contained 8 to 14% of administered radiosulfur, present primarily in neutral sulfur compounds. Table I contains data from typical experiments in which 100 γ of thiamine-S^{35} were administered to rabbits, and radiosulfur content of urinary and fecal sulfur fractions determined. Average recoveries of radiosulfur, obtained in urine fractions when the 3 dose levels were administered, are presented in Fig. 1.

In further experiments involving oral administration of thiamine-S^{35}, the oral dose of 100 γ of radiovitamin was followed 6 hours later by intramuscular injection of 2 mg of non-radioactive thiamine. This was done to determine if injection of the 20-fold excess of non-radioactive thiamine would cause increased excretion of radiosulfur from labeled vitamin. This increased excretion of radiosulfur, due to flushing out effect of excess non-radioactive thiamine injected was, in fact, realized, and an additional amount (average 17%) of administered radiosulfur was recovered. Practically all of this additional radiosulfur excreted was found in the neutral sulfur fraction of urine in the first 24-hour excretion period. Urinary and fecal recoveries of radiosulfur are shown in Fig. 2. For comparison, data are also presented from experiments in which 100 γ of thiamine-S^{35} were administered orally to rabbits without follow-up injection of non-radioactive thiamine, and urinary and fecal recoveries of radiosulfur determined for the same time. In contrast to the marked difference in excretion of radiosulfur in the neutral sulfur fraction of urine in these 2 series of experiments, inorganic sulfate and ethereal sulfate fractions of urine,

and fecal sulfur fractions show no significant differences in radiosulfur recovery.

In oral administration shown in Fig. 2, the main metabolites of thiamine-S^{35} were isolated from urine collected in the first 24-hour excretion period. It was found that thiamine-S^{35} accounted for approximately 60 to 65%, and thiazole-S^{35} accounted for approximately 34% of neutral sulfur. These results are similar to those obtained by McCarthy *et al.*(1), and by Iacono and Johnson(6), who found that when rats were given intraperitoneal injection of thiazole-2-C^{14}-thiamine, unchanged radiothiamine accounted for about 60% of radiocarbon of urine.

Parenteral administration. The radiovitamin was administered to rabbits by intramuscular injection, and by intravenous injection. In both series, thiamine-S^{35} was dissolved in normal saline and pH of solution adjusted to 7.0. Injections of 100 γ were given in the musculature of hind leg, and in marginal ear vein, respectively. The results are presented in Fig. 3.

When radiovitamin was administered by intramuscular injection, average total recovery of radiosulfur in urine and feces was 86% for six 24-hour periods. Radiosulfur recovered in neutral sulfur accounted for 75% of administered dose. Average total recovery of radiosulfur, for the same time, when radiovitamin was administered by intravenous injection, was 55%, of which about 41% was in the neutral sulfur fraction of urine. For both parenteral routes, as with oral administration, inorganic and ethereal sulfate fractions of urine, and fecal sulfur fractions are minor excretory pathways for radiovitamin and its metabolites.

The results of our intramuscular injection experiments are similar to those obtained by other workers who studied the fate of intramuscularly injected thiamine-S^{35} in other species. McCarthy and coworkers(1) obtained essentially the same results after intramuscular injection of 50 γ of thiamine-S^{35} to rats. Borsook and his associates(7), though working with pharmacological doses of radiovitamin, obtained similar results with a human subject. Six days after the last of 4 daily injections of thiamine-S^{35} (16 mg), 61% of radiosulfur had been recovered in urine, and 11% in feces.

Studies on fate of intravenousliy injected radioactive thiamine have not been reported. As data in Fig. 3 indicate, recovery of radiosulfur is considerably lower than that obtained by either of the other routes used. A possible explanation is that, since radiovitamin is introduced directly into the bloodstream, it may be removed from the blood and stored by liver and other organs to a larger extent than when administered orally or intramuscularly, and is therefore retained and excreted over longer periods of time.

Summary. The fate of thiamine, labeled with sulfur-35 in the thiazole moiety, has been studied in rabbits after oral and parenteral administration of doses in the physiological range. Analyses of the urinary and fecal sulfur fractions for 4 to six 24-hour periods following administration of radiovitamin, gave average total recovery of radiosulfur of 77% after administration by stomach tube, 86% after intramuscular injection, and 54% after intravenous injection. When oral dose of radiovitamin was followed 6 hours later by intramuscular injection of a 20-fold larger dose of non-radioactive thiamine, an additional 17% of radiosulfur was recovered in urine. In all cases, the neutral sulfur fraction of urine contained more than 50% of the recovered radiosulfur, and the greater portion of this was excreted in the first 24-hour period following administration of radiovitamin. Isolation of main metabolites following oral administration indicates that unchanged thiamine-S^{35} and thiazole S^{35} moiety together account for approximately 95% of radiosulfur of the neutral sulfur fraction in the first 24-hour period, and are excreted in a ratio of 2 : 1, respectively.

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Immunological Characteristics of N. Y. Strains of Influenza A Virus From the 1957 Pandemic. (24092)

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A close antigenic relationship among influenza A virus strains isolated in various parts of the world during the 1957 pandemic has been demonstrated(1), and the large differences between these strains and earlier strains have been emphasized(2,3). However, evidence that there is slight antigenic relationship between strains prevalent in 1957 and older strains has been steadily accumulating. Mulder(4) first reported the presence of antibody to the new strains, in persons over 70 years of age, and this was confirmed by Davenport(5). Patients with influenza in various locations have occasionally shown rises in antibody titer to older strains(1,2). Monovalent Asian vaccine has produced antibody response to older prototype strains(6), and polyvalent vaccine not containing an Asian strain has produced a rise in titer to Asian strains in patients over 70(7). One of the problems in detecting infection and in evaluating antibody response with Asian strains has been the low hemagglutination-inhibiting titers obtained with Japan/305/57 strain. The 1957 influenza pandemic reached its peak in N. Y. City in October. From September to November 6 strains of influenza A virus were isolated from patients at the Hospital of the Rockefeller Institute. In the present communication the immunological characteristics of these strains are described. Evidence is presented that the new strains contain an antigenic component that has been present in previous influenza A virus strains. The reactivity of viruses from the 1957 pandemic with antibody and non-specific inhibitors of hemagglutination is described. One of the new isolates is a highly sensitive indicator of the presence of hemagglutination-inhibiting and neutralizing antibodies in human serum. This property has made the Rockefeller Institute/5/57 strain especially useful in diagnosing infection with recently prevalent influenza viruses, in demonstrating antibody against them in human sera obtained before the 1957 epidemic, and in detecting an immunological response to monovalent Asian influenza vaccine.

Materials and methods. Viruses. The 1957 New York isolates were recovered in embryonated eggs by combined amniotic and allantoic inoculation of throat washings. The new strains were designated in order of isolation A/Rockefeller Institute/1/57 to A/Rockefeller Institute/6/57. The viruses were used after 2 to 4 passages in the allantoic cavity. The Far East isolates Japan/305/57 and Formosa/313/57(2) were kindly provided by Miss M. L. Miesse of the Walter Reed Army Institute of Research. The strain A/New York/1/53 was isolated at the Rockefeller Institute. The strains Swine/15, PR8, FM1, and Cuppett are well known prototype strains. *Rabbit sera.* Rabbit antisera against the 1957 isolates and Swine/15 were prepared by intravenous injection of 10 ml of virus infected allantoic fluid, followed, in 2 weeks, by intraperitoneal injection of 10 ml of the same material. The rabbits were bled 2 weeks after the second injection. Antisera against PR8, FM1, Cuppett and New York/1/53 had been previously prepared in this laboratory, and stored at 4°C. In each case a pool of sera from 2 rabbits was employed. *Patients' sera.* Acute sera and convalescent sera collected 14 to 21 days from onset of illness were obtained

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