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Distribution of Radioactivity after Administration of Taurine-^{35}S in Rats[1]

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1) Taurine-^{35}S was injected intravenously to rats and the radioactivity in tissues, bile, urine and feces was measured at various time intervals after the administration.

2) Taurine-^{35}S was rapidly eliminated from the blood stream almost regardless of its dosage levels examined. The largest accumulation of radioactivity in the earlier periods was found in the kidney, liver and small intestine. The spleen and lung followed them. The least uptake of ^{35}S was observed with the brain.

3) The radioactivity in the heart, diaphragm and muscle increased gradually, whereas that in the other organs decreased.

4) As for the intracellular localization, radioactivity was present in the soluble fraction of all the tissue homogenates tested.

5) ^{35}S readily appeared in the bile, wherein free taurine-^{35}S as well as its conjugates with bile acids was recognized.

6) A large, initial uptake of ^{35}S by the intestinal walls was found to be essentially independent of the reabsorption of radioactive biliary components.

7) No particular accumulation of taurine-^{35}S was noticed in a newly formed granuloma tissue within the limits of the present study.

It is well known that taurine is conjugated with bile acids in the liver of many animals.[3] However, a large amount of taurine is found in other organs, especially in those rich in the muscular tissue.[4] Recently, it has been reported that taurine is converted to isethionic acid in the dog heart,[5] and also in the rat heart and brain.[6] Although Welty and Read[7] have suggested a possible role of taurine on the potassium transport system of the cell membrane of dog heart, exact knowledge of physiological functions of taurine in the organs other than the liver has not been obtained.

The localization of taurine-^{35}S in rats is reported by several workers.[8–10] However, the studies are not thoroughgoing enough from the view point of estimating the distribution of the total radioactivity including those of metabolites as percentage of the intravenously administered dose.

This paper presents the results of studies on the quantitative distribution of radioactivity after the intravenous injection of taurine-^{35}S to rats from the above view-point, and of further

1) The 86th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, October, 1966.
2) Location: *a*) *Yayoi-cho, Chiba*; *b*) *Takada-3-chome, Toshimaku, Tokyo.*
3) G.A.D. Haslewood, "Bile Salts," ed. R. Peters, F.G. Young, Methuen & Co. Ltd., London, 1967, pp. 82—106.
4) D.N. Stern and E.M. Stim, *Proc. Soc. Exper. Biol. Med.*, **101**, 125 (1959).
5) W.O. Read and J.D. Welty, *J. Biol. Chem.*, **237**, 1521 (1962).
6) E.J. Peck and J. Awapara, *Biochim. Biophys. Acta*, **141**, 499 (1967).
7) J.D. Welty and W.O. Read, *J. Pharmacol. Exper. Therap.*, **144**, 110 (1964).
8) O.W. Portman and G.V. Mann, *J. Biol. Chem.*, **213**, 733 (1955).
9) J. Awapara, *J. Biol. Chem.*, **225**, 877 (1957).
10) P.L. Boquet and P. Fromageot, *Biochim. Biophys. Acta*, **97**, 222 (1965).

studies on other problems, *i. e.*, intracellular localization, effect of dosage–amount, relation of accumulation by intestinal walls to enterohepatic circulation, excretion of free taurine in bile, and uptake by granuloma tissue.

Materials and Methods

Taurine-^{35}S——This was obtained from the Radiochemical Centre, Amersham, England and had a specific radioactivity of about 10 mCi, per mmole. Paper chromatography of this compound using either *n*-BuOH–AcOH–H_2O (4:1:2) or pyridine–H_2O (6:1) showed a single radioactive area corresponding with that of authentic taurine as runned together on each strip and the radiochemical purity was more than 95%.

Animal Experiments——Unless otherwise indicated, animal experiments were conducted as follows. Male Wistar rats weighing 120—150 g were used. They were given water and cubic normal diet(CE-2, Nihon Rat Co.) *ad libitum* prior to experiments. Taurine-^{35}S(0.5 ml) dissolved in 0.2 ml of saline was injected in each animal through the femoral or caudal vein. The rats, three in one group, were sacrificed by exsanguination from the common carotid artery at various time intervals. Organs were removed and the radioactivity was measured by the routine methods described later. In the case of the short-term experiments, 3, 15 and 60 minutes, rats were fixed on boards and urine was pooled in the bladder by pre-ligation of the penis. Diet and water got rid of them during the entire experimental periods. For longer intervals, 1 and 5 days, rats were placed individually in glass metabolism cages allowing separate collection of urine and feces, and gave free access to water and the diet that had adequately ground to granule to limit its contamination with excreta. Bile duct-cannulation was carried out following the method of Ozawa *et al.*[11] The granuloma tissue was formed on the back of rats by subcutaneous injections of 25 ml of air and 1 ml of 1% croton oil into the interscapular space, according to the method of Selye.[12]

Preparation of Subcellular Fractions—All operations were performed in the cold (0—4°). Tissues were homogenized in 0.154 M KCl and adjusted to 5% homogenate, from which clots were removed by filtration through nylon cloth. The nuclear fraction (debris) was isolated by centrifugation at $600\times g$ for 10 minutes. The supernatant and washings from the debris were combined and centrifuged at $105000\times g$ for 60 minutes to separate the mitochondrial and microsomal particles (particle fraction). The last supernatant fluid was designated as the soluble fraction.

Radioactivity Measurement——In order to convert organic sulfur to sulfate, the method of Pirie[13] has been usually employed, but the method involves considerably dangerous and time-consuming processes. For the determination of taurine-^{35}S, Portman and Mann[8] digested tissues in the Benedict reagent[14a,b] after the pretreatment of samples with a HCl–HCO mixture.

We have also examined the use of the reagent for the oxidation of taurine-^{35}S in tissues, and have succeeded in conversion of taurine-^{35}S to $H_2{}^{35}SO_4$ with accuracy, safty and facility, by heating the tissue samples in a mixture of the Benedict reagent and conc. HNO_3 (1:1). Actual procedure is as follows: A tissue sample, less than 500 mg, was weighed and put in an oxidizing flask, to which was added 10 ml of the Benedict reagent–conc. HNO_3 mixture. The flask was heated in boiling water for 30 minutes, evaporated to dryness on a heater, and heated further over the free flame of the Bunsen burner until the entire residue turned to black. After cooling to room temperature, 10 ml of dilute HCl (1:10) was added to the flask, which was then warmed to dissolve the contents, and the clear solution was concentrated to 1 ml. This solution was added with 1 ml of 6.1% of Na_2SO_4 and 10 ml of water, warmed, cooled, neutralized roughly with 1N NaOH, and again acidified by addition of 1 ml of 2N HCl. Radioactive barium sulfate was precipitated by dropwise addition of 2.5 ml of 10% $BaCl_2$ to the flask under warming in a gently boiling water bath and allowed to cool overnight to room temperature. Using a glass filtrating device, the precipitate was collected on a filter paper (Toyo Roshi No. 5C) of known weight, washed with water and ethanol, and a disc-shaped sample (2 cm in diameter) of $Ba^{35}SO_4$ of uniform thickness was thus obtained. It was noted that the added taurine-^{35}S to various tissues was recovered 98% or more by this procedure. Radioacitivity was measured either with an end window Geiger-Müller counter or a low background gas-flow counter (Nihon Musen Co., LBC-20). The results were expressed in terms of percent of the administered dose localized in one gram of tissue (%(g)) or in whole organ (%(org)).

Thin-Layer Chromatography——Bile (12—15 mg) was spotted on a silicagel plate (5×25 cm) and ascendingly developed in a Kazuno's solvent system[15a] with slight modification.[15b] Localization of radio-

11) H. Ozawa, K. Momose, M. Ozeki and T. Nozu, *Nippon Yakurigaku Zasshi*, **64**, 199 (1968).
12) H. Selye, *J. Am. Med. Assoc.*, **152**, 1207 (1953).
13) N.W. Pirie, *Biochem. J.*, **26**, 2041 (1932).
14) *a*) S.R. Benedict, *J. Biol. Chem.*, **6**, 363 (1909); *b*) $CuSO_4$...200 g, $NaClO_3$...50 g, distilled water to...1 liter
15) *a*) T. Kazuno, *Taisha* (Japan), **2**, 869 (1965); *b*) AcOEt–MeOH–AcOH–H_2O (6:2:1:1).

activities on the plate was scanned by means of a chromatoscanner (Nihon Musen Co., ASF-7). The radioactivity ratio of free taurine and conjugates of tri- and dihydroxycholanic acids with taurine was determined from the actinogram of thin-layer chromatogram of each sample.

Results and Discussion

The Distribution and Excretion of ^{35}S

As can be seen in Table I, ^{35}S was rapidly eliminated from the blood stream after the intravenous administration of taurine-^{35}S, so that a high concentration gradient was observed within the first 15 minutes between the blood and most of organs other than the brain and testis. Approximately fifteen percent of the total ^{35}S injected was immediately accumulated in the kidney but this decreased to about 6% after 1 hour, while no urinary excretion of ^{35}S corresponding with the reduced amount in kidney was noticed. In this connection, Gilbert, *et al.*[16] have described that taurine would be rapidly re-absorbed from the cell surface of renal tubles. The second highest concentration of ^{35}S was observed in the liver and small intestine (2.5—3.0%(g)), which were followed by the lung and spleen (1.0%(g)). The lowest radioactivity appeared in the brain during the earlier periods of time.

Table I. Distribution of Radioactivity after Intravenous Administration of Taurine-^{35}S in Rats

	3 min	15 min	1 hr	24 hr	5 day
(A) Tissue-concentration of ^{35}S (%(g))					
Blood	0.77±0.184	0.18±0.014	0.07±0.012	0.04±0.007	0.01±0.001
Kidney	10.8 ±0.17	10.5 ±0.12	5.4 ±0.25	0.74±0.033	0.30±0.026
Liver	2.9 ±0.19	3.5 ±0.43	3.6 ±0.83	0.54±0.212	0.25±0.028
Duodenum	2.9 ±0.09	3.0 ±0.33	2.9 ±0.35	1.5 ±0.18	0.53±0.032
Jejunum	1.7 ±0.09	3.2 ±0.32	2.9 ±0.22	1.5 ±0.21	0.56±0.033
Heart	0.81±0.024	0.55±0.078	0.56±0.020	0.92±0.105	1.2 ±0.13
Lung	1.4 ±0.41	1.1 ±0.14	1.5 ±0.15	1.2 ±0.14	0.43±0.049
Spleen	0.74±0.030	0.90±0.091	1.4 ±0.17	1.2 ±0.07	0.53±0.082
Diaphragm	0.58±0.025	0.46±0.041	0.46±0.087	0.75±0.040	0.81±0.072
Femoral muscle	0.36±0.044	0.21±0.015	0.21±0.010	0.44±0.012	0.52±0.019
Femoral bone	0.50±0.047	0.50±0.027	0.64±0.061	0.54±0.091	0.21±0.094
Abdominal skin	1.1 ±0.24	0.44±0.058	0.42±0.032	0.28±0.044	0.25±0.034
Pancreas	0.55±0.053	0.46±0.024	0.41±0.095	0.27±0.014	0.21±0.010
Prostate	0.53±0.037	0.51±0.015	0.62±0.069	0.28±0.088	0.02±0.010
Seminal vesicle	0.60±0.046	0.72±0.203	0.43±0.032	0.24±0.029	0.18±0.062
Testis	0.13±0.028	0.11±0.013	0.11±0.001	0.10±0.007	0.07±0.001
Eye	0.27±0.010	0.30±0.046	0.24±0.039	0.31±0.009	0.33±0.014
Brain	0.05±0.003	0.05±0.015	0.07±0.023	0.18±0.039	0.20±0.015
(B) Distribution of ^{35}S in whole organ (%(org))					
Blood[a]	5.1 ±0.73	1.2 ±0.12	0.52±0.15	0.32±0.043	0.08±0.008
Kidney	15 ±0.6	15 ±0.7	6.1 ±0.65	1.3 ±0.09	0.43±0.003
Liver	16 ±1.0	22 ±1.4	20 ±3.7	3.5 1.44	1.5 ±0.15
Heart	0.41±0.009	0.24±0.031	0.28±0.009	0.47±0.064	0.55±0.047
Lung	1.1 ±0.07	0.80±0.169	1.1 ±0.15	1.4 ±0.15	0.29±0.012
Spleen	0.36±0.038	0.46±0.043	0.59±0.038	0.52±0.058	0.20±0.022
Urine	1.2 ±1.07	2.3 ±1.54	3.8 ±1.23	27 ±5.0	45 ±3.7
Feces	—	—	—	1.5 ±0.06	8.6 ±1.31

a) on the assumption that total blood volume equals 5% of the body weight

Values (mean ± standard error) are in percent of the administered dose (0.5 mg of taurine-^{35}S per rat) localized in one gram of fresh tissue (A) or in whole organ (B). Three rats per group were used.

16) J.B. Gilbert, L. Lorene, Y. Ku, L. Rogers and R.J. Williams, *J. Biol. Chem.*, **235**, 1055 (1960).

The 24-hour levels of ^{35}S in tissues were very low in most organs, of which only the small intestine, lung and spleen contained 1.2—1.5%(g) of the initially administered ^{35}S.

The average excretion of ^{35}S in 5 days was 45% in the urine and 8% in the feces. Consequently, forty-six percent of the initially administered ^{35}S was remained in the body at the end of 5 days. The 5-day levels of ^{35}S in tissues were as follows: heart (1.2%(g))>diaphragm (0.8%(g))>intestinal walls, spleen and muscle (0.5%(g))> other organs including kidney and liver (less than (0.3%(g)).

In general, a gradual increase of radioactivity was observed in the muscular tissues, whereas a rapid or gradual decrease occurred in the other tested tissues. These distribution patterns of total radioactivity after intravenous injection of taurine-^{35}S are in good accordance with those of Awapara's report,[9] in which relative concentrations of taurine-^{35}S in tissues have been compared. Moreover, a similarity was found between the relative tissue-concentrations of ^{35}S at the 5th day in this study and those of endogenous taurine described in the literature.[10,17,18] It was assumed, therefore, that the remainder of taurine-^{35}S in the body at the end of 5 days would be in the state of sufficient equilibrium with endogenous taurine. This assumption is also supported by the data of Boquet and Fromageot[10] that the specific radioactivity became almost equal in all the tissues including the blood in a week after the injection of taurine-^{35}S.

Effect of the Dosage-amount

To examine whether initial incorpration percents of ^{35}S were altered with amounts of the administered taurine-^{35}S or not, the first 15-minute levels of radioactivity in various tissues were compared at dosages of 0.05, 0.50 and 5.0 mg of taurine-^{35}S.

As shown in Fig. 1, the blood levels of ^{35}S at the two lower dosages were both 0.2% (g), which increased only 2 fold even when the largest dosage was employed. In spite of the large difference in dosage–amounts, quite slight changes were also observed in the initial rate of ^{35}S-uptake by each tissue.

These results, togather with the relative concentrations of endogenous taurine in tissues reported by the previous authors,[10,17,18] lead to a presumption that the large, initial accumulation of ^{35}S in the kidney, liver and small intestine are due to a rapid, net incorporation of administered taurine rather than a quick exchange of exogenous taurine for endogenous taurine.

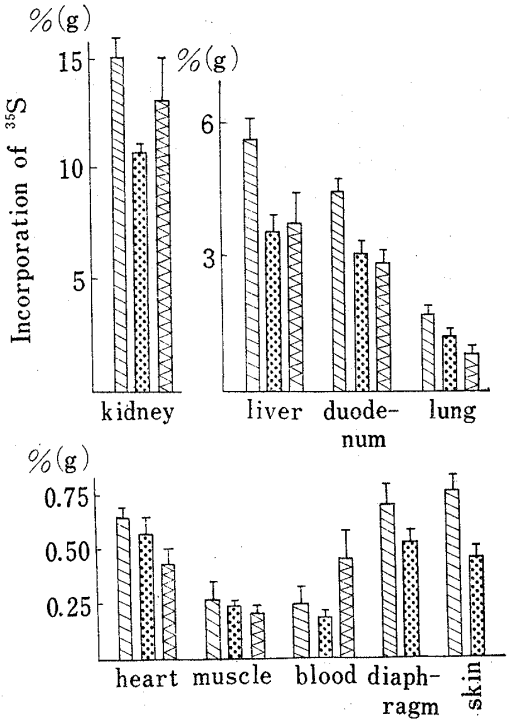

Fig. 1. Effect of the Dosage–Amount on Tissue–Distribution of ^{35}S

Rats were sacrificed at 15 minutes after the intravenous injection of taurine-^{35}S at the dosage–amounts of 0.05, 0.50 and 5.0 mg, respectively.

Each bar indicates standard error of the mean obtained from three experiments.

The values for 0.5 mg–administered group are based on the data presented in Table I.

▨ : 0.05 ▩ : 0.5 ▨ : 5.0 (mg)

The Biliary Excretion and Tissue-concentration of ^{35}S in Bile Duct-cannulated Rats

Four male rats weighing about 200 g were given intravenously 0.75 mg of taurine-^{35}S immediately after bile duct–cannulation. Bile

17) C. Wu, *J. Biol. Chem.*, **207**, 775 (1954).
18) J. Awapara, *J. Biol. Chem.*, **218**, 571 (1956).

was collected at 15-minute intervals and tissues were removed at the end of 60 minutes for radioactivity measurement. The data are presented in Table II.

Table II. Biliary Excretion and Tissue Distribution of Radioactivity after Intravenous Administration of Taurine-^{35}S in Bile Fistula Rats

(A) Biliary excretion of ^{35}S

	Time in minutes			
	0—15	15—30	30—45	45—60
Excretion (%)	1.1±0.50	2.8±1.03	2.7±1.23	1.7±0.47
Bile weight (mg)	261±60.0	298±66.1	359±96.4	353±87.6

(B) 1-Hour concentrations of ^{35}S in tissues

	Duodenum	Jejunum			Ileum	Kidney	Liver	Lung	Heart
		Upper	Middle	Lower					
%(g)	2.4±0.18	2.0±0.03	1.9±0.24	1.5±0.12	1.4±0.07	5.8±0.92	1.6±0.36	0.9±0.05	0.3±0.03

Experimental conditions are described in the text.

^{35}S appeared readily in the bile and approximately 9% of the dose was recovered in 1 hour. The livers showed a significant decrease in radioactivity as compared with those of intact animals (Table I). This is probably attributed to continuous removal of radioactive biliary components from bile fistula rats. On the other hand, the small intestines accumulated ^{35}S as much as in the case of intact rats. This fact indicates that the intestinal walls have an ability to take up taurine directly from the blood stream independent of radioactive bile. In addition, it was also noted that ^{35}S was distributed in different portions of the small intestine according to their degrees of peristaltic movement or nutrient absorption.

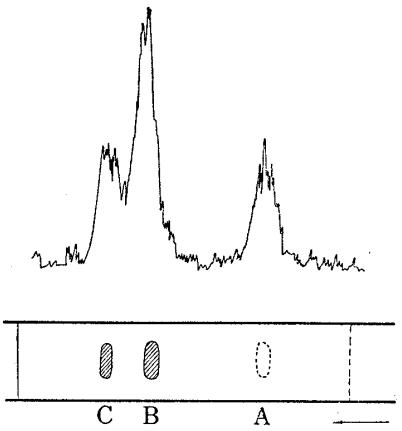

Fig. 2. Actinogram of Thin-Layer Chromatogram of the First 15-Minute Bile

TLC was carried out in the solvent system (AcOEt–AcOH–MeOH–H_2O, 6:2:1:1) on Kieselgel G nach Stahl 0.25 mm in thickness.
Radioactivity was measured with a chromatoscanner (slit 1.5×30 mm, speed 150 mm/hr).
Detections of the conjugates with bile acids, tri- (B) and di- (C) hydroxycholanic acids, and free taurine (A, authentic sample) were performed by spraying with 10% phosphomolibdic acid and 1% ninhydrin, respectively.

Bile was then submitted to thin-layer chromatography and three radioactive zones (A, B and C) were separated in all the samples tested. Typical chromatogram and actinogram were shown in Fig. 2.

Radioactive zone A corresponded exactly with the ninhydrin-positive spot of authentic taurine, which was runned togather in two kinds of the solvent systems with various compositions (iso-AmOH–AcOH–H_2O and *n*-BuOH–AcOH–H_2O). The other two zones regarded chromatographically as conjugates of taurine-^{35}S with bile acids, which were composed mainly of trihydroxycholanates (B) as represented with cholic acid, and minorly of dihydroxycholanates (C) as chenodeoxycholic acid, respectively.

Table III showes the ratio of three radioactive components in each bile sample of this experiment. The results obtained from the similar experiments at different dosages of taurine-^{35}S were also given in the table.

Free taurine-^{35}S was found to be excreted in the bile, especially with a greater proportion in the first

Table III. Ratio of Three Radioactive Components in Bile after Intravenous Adiministration of Taurine-^{35}S in Rats

Time intervals (min)	Free taurine (%)	Bile acid–conjugates (%)	C/B[a)]
0.25 mg-group			
0—15	19.4	80.6	0.263
15—30	4.3	95.7	0.234
30—45	3.1	96.9	0.218
45—60	2.5	97.5	0.245
0.75 mg-group			
0—15	23.4	76.6	0.379
15—30	5.7	94.3	0.339
30—45	5.8	94.2	0.292
45—60	2.9	97.1	0.350
1.0 mg-group			
0—15	26.6	73.4	0.270
15—30	9.1	90.9	0.277
30—45	5.3	94.7	0.228
45—60	5.5	94.5	0.211

a) Taurodihydroxycholanic acids/taurotrihydroxycholanic acids values are average of four experiments.
Experimental conditions are shown in the legend of Fig. 2.

15 minute samples, and its relative excretion was enhanced with increase in dosage-amounts. On the other hand, the radioactivity ratio between B and C was consistently 0.25—0.3 in all samples. This value for the ratio of tri- and dihydroxycholanates is no conflict with the previous authors' reports,[19,20)] which deal with the amounts of these acids in the rat bile. In detail, it was frequently noted that the width of radioactinogram for zone B was slightly broader than its green color band developed by spraying with 10% phosphomolibdic acid in ethanol solution. This suggests a possibility that there is another conjugate of taurine unresponsive to the color reaction with this reagent, other than taurocholate. Further works on this problem are now in progress.

The Intracellular Distribution of ^{35}S

As can be seen in Table IV, ^{35}S was found exclusively in the soluble fraction of all the tissue-homogenates obtained at 1 hour and 5 days after the injection of taurine-^{35}S. Thus,

Table IV. Intracellular Localization of Radioactivity 1 Hour and 5 Days after Intravenous Administration of Taurine-^{35}S in Rats

Fraction	Liver	Kidney	Lung	Spleen	Heart	Intestine	Muscle
1 Hour							
Soluble	96.7	98.2	98.2	95.2	95.0	98.9	97.6
Particle	1.2	0.8	0.8	3.0	1.1	0.2	0.0
Debris	2.1	1.0	1.0	1.8	3.9	0.8	2.4
5 Day							
Soluble	93.5	95.9	95.4	88.5	97.5	98.8	97.3
Particle	2.1	2.3	1.7	3.7	0.5	0.5	1.1
Debris	4.4	1.8	2.9	7.8	2.0	0.9	1.6

Values are in per cent. Experimental conditions are described in the text.

19) S. Bergström and J. Sjövall, *Acta Chem. Scand.*, **8**, 611 (1954).
20) S. Eriksson, *Proc. Soc. Exper. Biol. Med.*, **94**, 578 (1957).

it may be inferred that taurine is intracellularly in a soluble or loosely bound form as suggested by Rixon and Stevenson.[21]

Uptake of ^{35}S by the Granuloma Tissue

Taurine is known to be formed in large quantity from inorganic sulfate in the chick embryo[22,23] and it has been also reported that certain tumor cells markedly accumulated taurine from the surrounding medium *in vitro*.[24,25] On the basis of these facts, an attempt was made to examine whether taurine-^{35}S might be incorporated into an experimentally induced granuloma tissue as a model for a newly growing tissue. The experimental designs were indicated in the legend of Table V.

Table V. Concentrations of Radioactivity in Granuloma and Other Tissues after Administration of Taurine-^{35}S to Rats

	Single, subcutaneous[a]		Single, intraperitoneal[a]	Repeated, subcutaneous[b]
	Male	Female	Male	Male
Heart	0.95±0.043	1.29±0.039	0.79±0.089	0.76±0.070
Diaphragm	0.67±0.043	0.95±0.019	0.78±0.045	0.60±0.009
Abdominal muscle	0.50±0.108	0.94±0.176	0.63±0.078	0.79±0.064
Granuloma	0.48±0.029	0.81±0.024	0.49±0.033	0.54±0.020
Femoral muscle	0.38±0.025	0.45±0.015	0.40±0.048	0.36±0.024
Skin	0.24±0.024	0.45±0.048	0.27±0.004	0.28±0.043
Kidney	0.64±0.075	0.97±0.062	0.73±0.095	0.70±0.016
Liver	0.29±0.042	0.43±0.133	0.23±0.106	0.62±0.043
Blood	0.04±0.012	0.06±0.001	0.04±0.001	0.04±0.005
Final body weight (g)	196±7.6	163±5.2	202±2.0	195±6.3

a) Administration of 0.1 mg of taurine-^{35}S was made on the 7th day after croton oil-treatment and the animals were killed at 50 hours later.
b) Twenty-five micrograms of taurine-^{35}S was repeatedly injected 3 times per day for 3 days from the 4th day after croton oil-treatment and the rats were killed at 24 hours after the last dosing.
Values (mean ± standard error of 4 rats) are in per cent of the total dose per gram of tissue (%(g)).

After a single administration of 1.0 mg of taurine-^{35}S by subcutaneous route, the levels of ^{35}S found in the granuloma and muscular tissues of both sexes are in the following descending order; heart, diaphragm, abdominal muscle, granuloma, femoral muscle and skin. This order of radioactivity concentration in these tissues was similarly obtained by the other methods of administration. Any particular uptake of taurine by the granuloma tissue could not be noticed in this experiment. However, the concentration of ^{35}S was higher than that observed in another connective tissue, skin. Barcheri and Fromageot[26] have found larger amounts of taurine in such organs showing higher activity of metabolism. From the result of the subcutaneous experiment, it was noted that the tissue levels of ^{35}S were higher in the females than in the males, as already described by Awapara.[9]

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21) R.H. Rixon and J.A.F. Stevenson, *Am. J. Physiol.*, **194**, 363 (1958).
22) J.P. Lowe and E. Roberts, *J. Biol. Chem.*, **212**, 477 (1957).
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26) M.C. Barcheri and P. Fromageot, *C.R. Soc. Biol.*, **156**, 1231 (1962).