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Studies on the Distribution of Radioisotopes by Whole Body Autoradiography. XXII.[1] Fate of ^{14}C-Fluocinolone Acetonide in Mice

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Fate of ^{14}C-fluocinolone acetonide (^{14}C-FL) administered subcutaneously and percutaneously was studied in mice. The radioactivity after subcutaneous injection was very high in the liver and to a lower extent in the myocardium, lung, kidney, pituitary, adrenal gland, skeletal muscles and lacrimal gland but scarcely detectable in the central nervous system. The radioactivity in the tissues decreased markedly 24 hr later, while that in the intestinal contents increased. The autoradiograms indicated also that the radioactive substance was excreted mostly through the bile duct. The percentage of ^{14}C-FL recovered in the liver was 9.5% of the radioactivity in liver 60 minutes after subcutaneous injection. The observation after dermal application of ^{14}C-FL cream revealed that the radioactivity was potent at the applied skin area and scarcely measurable in the liver, gall bladder and in the intestinal contents.

Dermal application of 0.2% ^{14}C-FL cream to mice revealed that about 7.5% of the applied amount was found in the skin at 60 min after application and about 65% of radioactivity in the skin was unchanged ^{14}C-FL. Eighty two% of the radioactivity after subcutaneous injection of ^{14}C-FL was excreted in 24 hr feces and 6.2% in 24 hr urine.

Corticosteroids are in extensive use for topical treatment of various skin diseases due to their potent anti-inflammatory action. It is shown that fluocinolone acetonide (FL) cream and ointment in concentration of 0.025% exert the most potent efficacy above all and further that a better therapeutic effect is expected with FL of a higher concentration of 0.2% in treatment of mycosis fungoides, epidermolysis bullosa or pretibial myxedema circumscripta.

A number of studies on percutaneous absorption of corticosteroids have been made. Scott, *et al.*[3] applied ^{14}C-labelled hydrocortisone ointment onto skin surface and recognized by means of micro autoradiography (micro ARG) that the corticoid penetrated through hair follicles. Kuwahara[4] and Kukita, *et al.*[5,6] traced the percutaneous penetration with ^{3}H-FL ointment and ^{14}C-FL cream respectively by means of micro ARG and found that the radioactive substance was distributed in the corneum. On the other hand, Shinkai[7] made a comparative study *in vitro* of percutaneous absorption of ^{14}C-hydrocortisone and its acetate and the result was that the acetate form was absorbed several times more than the free steroid. Malkinson[8] reported that ^{14}C-hydrocortisone when was applied to the normal skin surface was absorbed to a level of only 1%.

The present investigation was undertaken to elucidate the absorption, distribution, excretion and metabolism of ^{14}C-FL dy means of whole body ARG and thin-layer chromatography (TLC) of tissue extracts. Some of finding obtained in this experiments will be reported.

1) Part XXI: Fate of ^{14}C-Arginine in Mice; T. Takahashi, T. Kimura and Y. Sato, *Radioisotopes*, **19**, 353 (1970).
2) Location: *Kawagishi, Toda, Saitama.*
3) A. Scott and F. Kalz, *J. Invest. Dermat.*, **26**, 149 (1956).
4) K. Kuwahara, *Japan. J. Dermatol.*, **77**, 326 (1967).
5) T. Kukita and T. Matsuzawa, *Clinical Dermat. Urology.*, **19**, 341 (1965).
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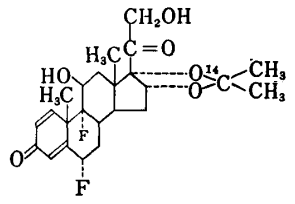
Experimental

Preparation of Material——The ^{14}C-FL was synthesized with ^{14}C in acetonide group in laboratory of Syntex Co., as shown in the following structural formula, and its specific radioactivity was 22.1 μCi/mg (10 mCi/m mole):



In preparing ^{14}C-FL cream, 5.43 mg of ^{14}C-FL was dissolved in 0.13 g propylene glycol and this solution was added to the cream base warmed up to 60° and stirred to be homogenized. This ^{14}C-FL cream contained ^{14}C-FL in the concentration of 0.2% had a specific radioactivity of 0.46 μCi/10 mg.

Whole Body Autoradiography——0.06 ml of a 0.2% propylene glycol solution of ^{14}C-FL was given by subcutaneous injection in ddY strain male mice weighing about 20 g. The animals were anesthetized with ether and were killed at different intervals (30 min to 24 hr) after admin is tration by immersion in a mixture (−70°) of solid carbon dioxide and acetone. A number of sagittal sections of 30 μ thickness were prepared by a microtome in a cryostat kept at −15° and dried at the same temperature. These sections were placed in contact with X-ray film Type-N (SAKURA) and after one-month exposure in a cool room they were developed and fixed.

For the autoradiographic observation after percutaneous absorption, the dorsal skin of male mice was depilated with EBA cream and cleansed with warm water and 30 mg of ^{14}C-FL cream containing 1.38 μCi was uniformally applied to the area of about 12 cm^2. At 60 min and 6 hr after application the remaining cream was wiped off with a 70% methanol solution and the animals were put into a polyethylene bag and sacrificed by freezing. The subsequent operations were carried out in the same manner as those of the subcutaneous injection.

Measurement of Radioactivity in Tissues——Mice received a subcutaneous injection of 2.7 μCi of ^{14}C-FL solution on the back and were sacrificed by bleeding from the carotid artery at 30, 60 min, 3, 6 and 24 hr after injection. The liver, kidney, brain, lung, salivary gland, myocardium, skeletal muscle, skin, spleen, pancreas, testis, gastro-intestinal wall and blood were homogenized with ten volumes of 70% methanol. After centrifugation a part of the supernatant fluid was added to the 50% toluene–alcohol (v/v%) scintillator and the radioactivity was measured with a liquid scintillation counter (Aloka, LSC-502).

Radioactivity in the urine was measured using a part of the urine and that in the faeces using the supernatant fluid of the extract with 70% methanol.

To determine the excretion into bile, a canula was inserted into the bile duct of male Wistar rats weighing about 230 g under urethane anesthesia and after subcutaneous injection of 2.7 μCi of ^{14}C-FL solution to femoral skin, the bile was collected to determine the radioactivity excreted at different intervals. The expired $^{14}CO_2$ from mice given ^{14}C-FL was traped with 20% NaOH solution and a portion of the solution was determined for ^{14}C radioactivity.

Determination of Radioactivity after Dermal Application——20 mg of ^{14}C-FL cream was applied to the dorsal skin of mice by the same method as for the whole body ARG. The measurement of radioactivity in the cream remaining on the skin cannot indicate the correct amount of absorption because it may involve significant errors due to insufficient wiping of unabsorbed FL. Therefore, the remaining ^{14}C-FL cream on the skin was wiped off with 70% methanol and centrifuged to obtain the extract. The radioactivity of extract was measured and the radioactive excretion in the bile was measured at different time intervals after dermal application of 60 mg of ^{14}C-FL cream on the chest of rats.

Identification of ^{14}C-FL in Tissues and Urine——The liver of mice that had received subcutaneous injection of ^{14}C-FL was homogenized with 10 volumes of 70% methanol and the supernatant was concentrated to dryness under reduced pressure. The residue was suspended in 10 ml of distilled water and FL and fat soluble metabolites were extracted three times with 10 ml of chloroform. The chloroform extracts were concentrated and spotted on the thin-layer plate (15 × 50 cm) prepared with Kieselgel GF_{254} and developed with a solvent system of *n*-butyl acetate–chloroform–acetone (4:4:2). The residual aqueous solution was incubated with 2000 units of β-glucuronidase in 0.2M acetate buffer (pH 5.0) at 37° for 24 hr. The incubation mixture was then extracted with chloroform, and subjected to TLC.

The amount of unchanged ^{14}C-FL in urine and in the contents of gastro-intestinal tracts after subcutaneous injection and in the skin after dermal application was determined as follows. ^{14}C-FL on the thin-layer chromatogram was detectable by fluorescence with Manaslu light of a wave length of 253 mμ. The area corresponded to ^{14}C-FL was extracted with chloroform and isotope dilution method was applied to the extracts.

Result

Whole Body Autoradiography

The distribution of radioactivity at various times after subcutaneous injection of ^{14}C-FL is shown in Fig. 1. The systemic distribution of radioactivity reached its peak at 60 min

and was the highest in the liver and moderate in the myocardium and intestine. The radioactivity was noted to a lower degree in blood and skeletal muscle, and scarcely in the central nervous system. The distribution in individual organs and tissues at different intervals are described below.

Liver——A very high concentration of radioactivity in the liver was observed at 30 and 60 min after administration. It decreased at 6 hr and become far less after 24 hr.

Lung——The radioactivity level in the lung was of a moderate height but higher than that in blood within one hour after injection. No special localization was observed in the cartilage trachealis and bronchia. The radioactivity in the lung was reduced markedly after 3 hr.

Kidney——The radioactivity in the kidney was of a high level at 60 min, decreased thereafter and it was scarcely observed at 24 hr.

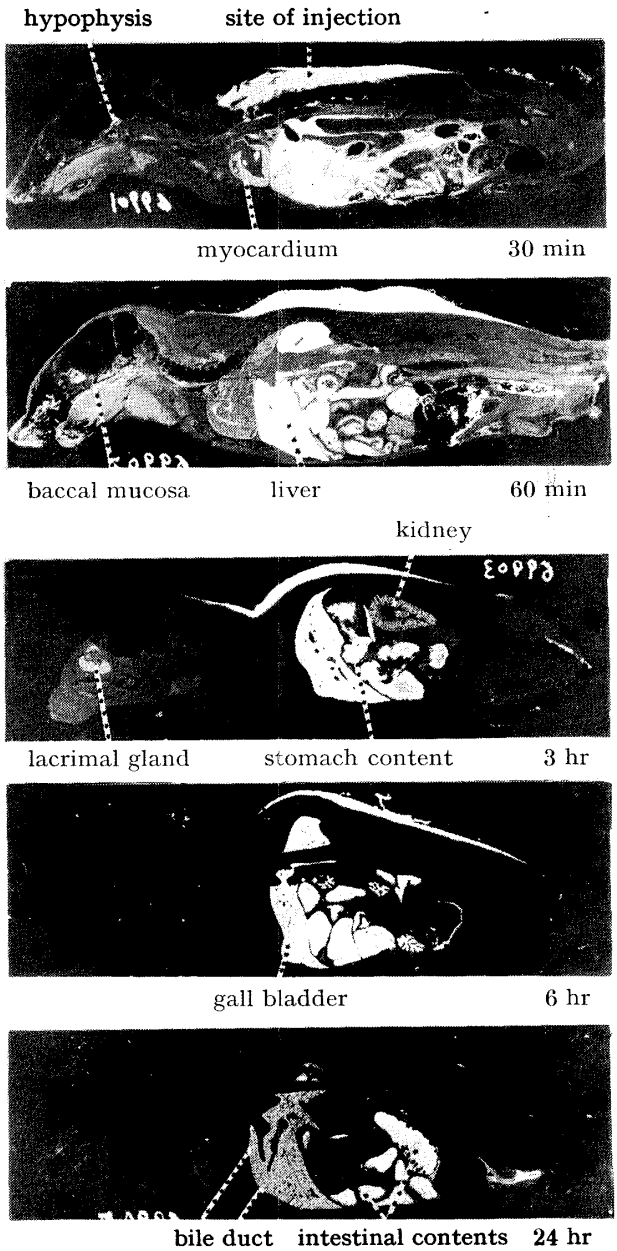


Fig. 1. Autoradiogram showing the Distribution of Radioactivity in Mice Various Hours after *s.c.* Injection of ^{14}C-Fluocinolone Acetonide

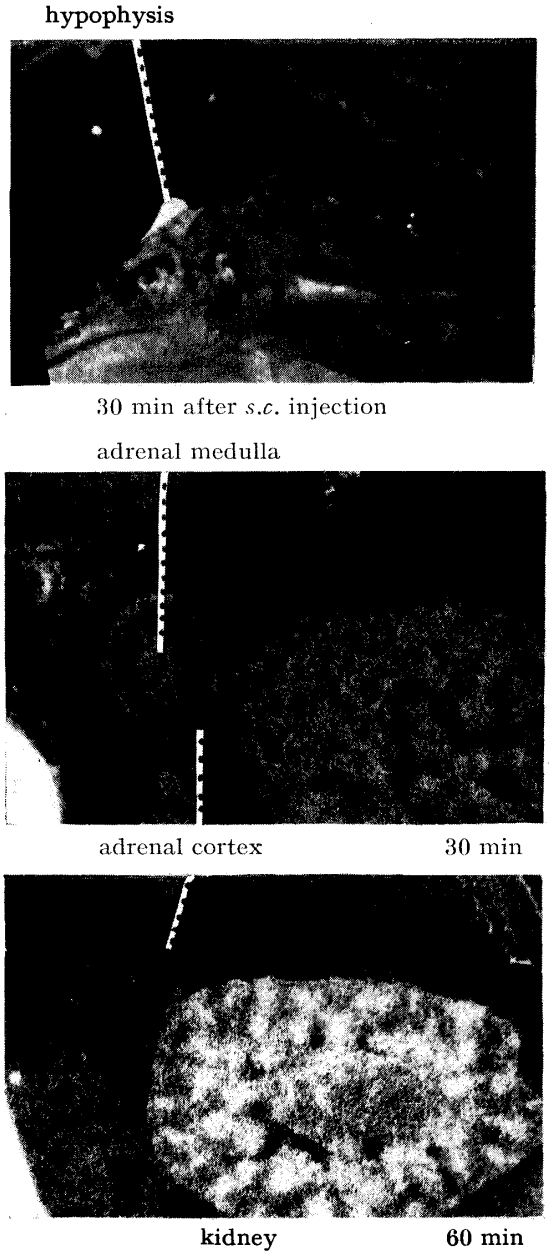


Fig. 2. Detail of Autoradiogram showing the Distriubtion of Radioactivity in Adrenal Gland and Hypophsis

Muscles——The radioactivity in skeltal muscle at 30 min and 60 min after injection was higher than that in blood. It was higher in the myocardium than in skeletal muscle as shown in autoradiogram (ARGM) at 30 min, and decreased after 6 hr.

Central Nervous System——The radioactivity was scarcely noted in the cerebrum, cerebellum and spinal cord at any intervals of observation.

Endocrine System——Moderate radioactivity was noted in the hypophysis as shown in the enlarged ARGM of Fig. 2. The radioactivity level in adrenal gland was lower than that in hypophysis and nearly equal to the lung. The uptake was observed both in adrenal cortex and medulla at 30 min as shown in the ARGM (Fig. 2) but not in the intermediate zone. At 60 min the radioactivity remained only in the cortex and disappeared from the medulla.

The radioactivity levels in the thyroid gland, thymus and testis were far lower than that in the hypophysis or adrenal gland. No accumulation of radioactivity was visible in those endocrine organs after 6 hr.

Digestive Tract——The radioactivity in the salivary gland was a little higher than that in muscles and the same as in the gastro-intestinal wall. It was extremely high in the pylorus and upper cavity of the small intestine at 30 min. This highly concentrated radioactivity moved gradually from the small intestine to the large intestine. The excretion into bile was shown by the high radioactivity in the gall bladder and bile duct as shown by ARGM after 6 and 24 hr in Fig. 1.

Other Tissues——In the lacrimal gland and mucous membranes of the oral and nasal cavities, radioactivity was of a moderate level. Radioactivity in the skin and bone marrow was of the same level as in the muscle.

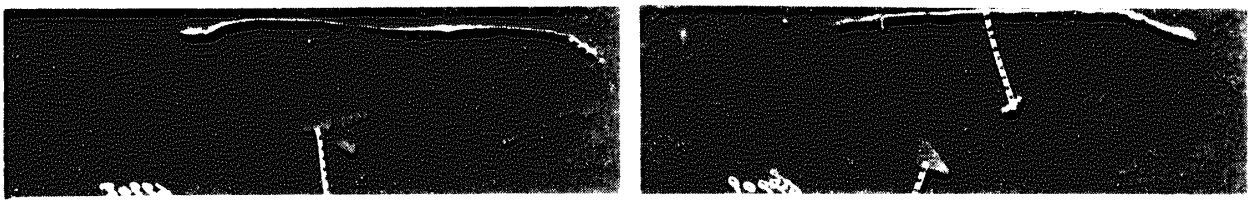


Fig. 3. Autoradiogram showing the Distribution of Radioactivity in Mice after Application on the Skin of ^{14}C-Fluocinolone Acetonide Cream

The whole body ARGM after dermal application of ^{14}C-FL cream is shown in Fig. 3. The radioactivity was high in the applied skin and surface of the skin, and the uptake of radoiactivity in the various organs was far less than in the case of subcutaneous injection. Only a low level of radioactivity was noted in the liver, gall bladder and intestines. The radioactivity level in the applied skin area was high even at 6 hr.

Distribution of Radioactivity in the Tissues after Subcutaneous Injection of ^{14}C-FL

Table I showes the distribution of radioactivity in various tissues of mice after subcutaneous injection. The values in the Table I show the radioactivity per gram of wet weight of the tissues or ml of blood in the percentage of the dose. The ^{14}C level was highest (9.5%) in the liver 30 min after injection. The levels were high in the gastrointestinal wall, kidney and myocardium and very low in the brain and testis. The level in the liver reached its peak after 60 min and decreased thereafter to about one-fifteenth of the maximum after 24 hr.

Distribution of Radioactivity after Dermal Application of ^{14}C-FL Cream

The radioactivity level in blood and tissues after dermal application of ^{14}C-FL cream was extremely low in contrast with the level after subcutaneous injection. As shown in Fig. 4,

Table I. Distribution of Radioactivity in Mice Tissues after Subcutaneous Injection of ^{14}C-Fluocinolone Acetonide into Mice

Tissues	Percentage of dose recovered (%/g or ml wet wt.)				
	30 min	60 min	3 hr	6 hr	24 hr
Liver	9.5	10.2	3.4	2.7	0.63
Kidney	2.6	2.0	0.61	0.43	0.10
Muscle	0.39	0.36	0.24	0.14	0.08
Brain	0.14	0.14	0.14	0.14	0.02
Myocardium	0.85	0.72	0.30	0.16	0.04
Lung	0.48	0.53	0.21	0.39	0.05
Salivary gland	0.62	0.67	0.36	0.18	0.08
Spleen	0.43	0.48	0.28	0.34	0.11
Pancreas	0.71	0.84	0.46	0.37	0.04
Testis	0.11	0.21	0.17	0.16	0.03
Skin	0.40	0.45	0.19	0.14	0.03
Gastrointestinal wall	3.60	3.80	5.30	3.80	2.10
Blood	0.64	0.52	0.30	0.16	0.02

(Each value indicates the mean of 3—4 animals.)

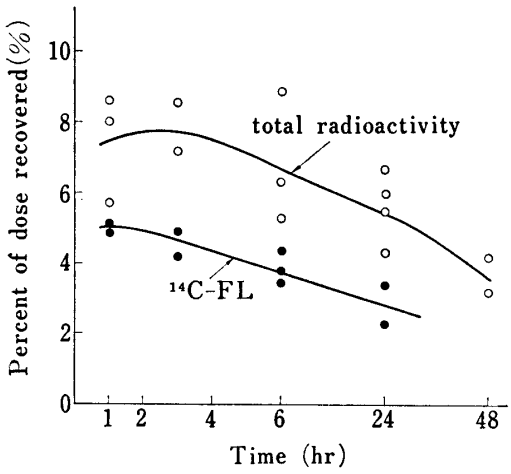


Fig. 4. Incorporation of Radioactivity into Skin of Mice after Application on the Skin of 0.2% ^{14}C-Fluocinolone Acetonide Cream

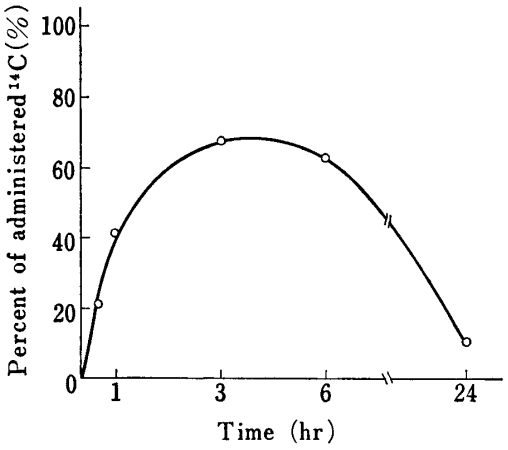


Fig. 5. Changes of Radioactivity in Gastrointestinal Contents after Subcutaneous Injection of ^{14}C-Fluocinolone Acetonide into Mice

(Each point represents the mean of 3—4 animals.)

the total amount of radioactivity found into the skin was about 7.5% of the dose after 60 min and about 6% at 24 hr after application.

Excretion

Fig. 5 and 6 show the changes of radioactivity in gastrointestinal contents, and the cumulative excretion of radioactivity in urine and faeces after subcutaneous injection of ^{14}C-FL into mice. The radioactivity in gastrointestinal contents was detectable soon after injection and 21% of the administered radioactivity was found there after 30 min. The percent recovery of administered ^{14}C reached 67% at the peak at 3 hr and decreased down to about 10% at 24 hr. Consequently, a large amount of radioactivity was excreted into the faeces and the cumulative amount was as high as 82% after 24 hr. The urinary excretion of radioactivity was as low as 6.2% after 24 hr and increased to 7.5% after 48 hr.

The determination of $^{14}CO_2$ in the expired air revealed that the cumulative amount of radioactivity at 24 hr was no less than 1.2% of the given amount. The biliary excretion

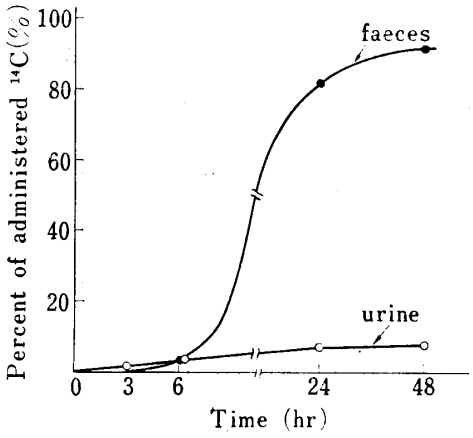


Fig. 6. Cumulative Excretion of Radioactivity in Urine and Faeces after Subcutaneous Injection of ^{14}C-Fluocinolone Acetonide into Mice

—○—: urine —●—: faeces
(Each point represents the mean of 5 animals.)

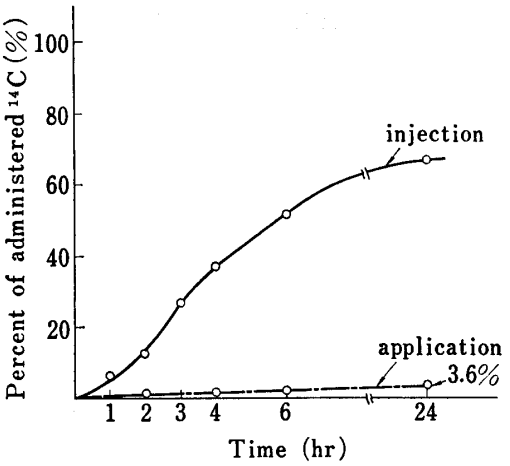


Fig. 7. Cumulative Excretion of Radioactivity in Bile after Subcutaneous Injection of ^{14}C-Fluocinolone Acetonide Solution into Rat and after Application on the Skin of Rat of 0.2% ^{14}C-Fluocinolone Acetonide Cream

(Each point represents the mean of 3 animals.)

in rats after administration of ^{14}C-FL was shown in Fig. 7. The cumulative excretion of radioactivity into bile after subcutaneous injection was 67% of the administered radioactivity in 24 hr, whereas the amount of ^{14}C excreted into bile after dermal application was only 3.6% in the same period.

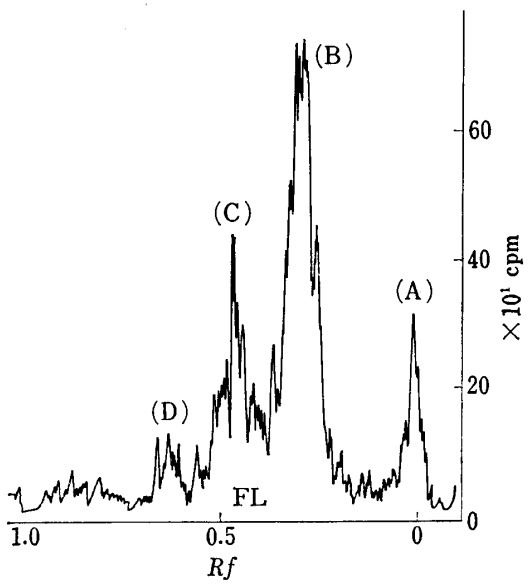


Fig. 8. Thin-Layer Chromatogram of Radioactivity in Liver extracted with Chloroform 2 Hours after Subcutaneous Injection of ^{14}C-Fluocinolone Acetonide into Mouse

solvent system; *n*-butyl acetate–chloroform–acetone (4:4:2)
plate: Kieselgel GF_{254}

Metabolism

The liver homogenate after subcutaneous injection of ^{14}C-FL into mice was extracted with chloroform, and ^{14}C-FL and its metabolite were separated by means of TLC with a solvent system of *n*-butyl acetate–chloroform–acetone. Fig. 8 shows the radioactinogram at 2 hr after injection. Four spots on the TLC radioactinogram were observed near at the origin (peak A), at *Rf*: 0.28—0.32 (peak B), at *Rf*: 0.43—0.48 (peak C ^{14}C-FL) and at *Rf*: 0.55—0.6 (peak D). The peak B was the largest of all.

The metabolites remained in water phase after chloroform extraction were separated by TLC (*n*-butyl acetate–benzene–methanol; 4:4:3 system). The radioactive peaks of the liver sample obtained 60 min or 2 hr administration were five of following *Rf*; 0.0, 0.10, 0.35, 0.65 and 0.72. On those of liver samples examined 6 and 24 hr after administration, the peak at the origin became higher and other peaks except that of *Rf* 0.65 were markedly lowered.

When the radioactive substance seen around the original area at 2 hr was extracted with water and treated with β-glucuronidase, 34% of the radioactivity was extracted into the chloro-

Table II. Percentage of ^{14}C-Fluocinolone Acetonide (^{14}C-FL) in the Radioactivity in the Liver, Gastrointestinal Contents and Urine after *s.c.* Injection of ^{14}C-FL into Mice

	Percentage of ^{14}C-FL (%)					
	30 min	60 min	2 hr	6 hr	24 hr	0—24 hr
Liver	3.8 (0.36)	9.5 (0.97)	6.7 (0.23)	6.0 (0.16)	4.8 (0.03)	
Gastrointestinal contents				2.1 (0.73)	2.6 (0.56)	
Urine						12.2 (0.27)

( ): Percent of dose recovered.
Each value indicates the mean of 3 animals.

form phase. When the concentrated solution of the chloroform phase was developed on thin-layer plate with a solvent system of *n*-butyl acetate–chloroform–acetone, more than 80% of the radioactivity was recovered in the area of ^{14}C-FL (*Rf*: 0.43—0.48) and about 10% in the area with *Rf* 0.32. These results indicated that the radioactive substances near at the origin of chromatogram consisted mainly of glucuronates of FL and the metabolite.

Table II shows the percentage of ^{14}C-FL in the total radioactivity in the liver, gastrointestinal contents and 24 hour urine. The parenthesized value are the percentage of unchanged ^{14}C-FL of the total given dose of ^{14}C-FL. The percentage of ^{14}C-FL recovered in the liver is 9.5% of the ^{14}C in liver at 60 min, which corresponds to 0.97% of the given ^{14}C-FL. The percentage of ^{14}C-FL was lower in gastrointestinal contents than in the liver. That in urine was as high as 12.2% which was 0.27% of the given ^{14}C-FL. Most of water soluble metabolite in urine was glucuronides, which were observed at the original area of the TLC.

Fig. 4 shows the amount of radioactivity and ^{14}C-FL in the applied area at different time intervals after the percutaneous administration of ^{14}C-FL. About 7% of the applied radioactivity was absorbed at 60 min and about 65% of it was found in the skin unchanged ^{14}C-FL. The amount absorbed into the skin gradually decreased and the value after 24 hr was about one half of the value after 60 min. The metabolites of ^{14}C-FL were found to consist mainly of glucuronides.

Discussion

Autoradiographic distribution of radioactivity in mice after subcutaneous injection of ^{14}C-FL showed that the highest radioactivity was in the liver and that the concentration was moderate in the myocardium, salivary gland and lung. Radioactivity was scarcely noted in the central nervous system. This result corresponded very well with that of the impulse counting method using liquid scintillation counter.

ARGM at 30 min after injection showed the uptake of radioactivity in the adrenal cortex and medulla but not in the intermediate zone. It was reported in the case of ^{14}C-oestrogen,[10] ^{14}C-testserone,[11] ^{14}C-prognenolone[12] and ^{14}C-progesterone[13] that the highest concentration of radioactivity in adrenal was observed in the cortex and scarcely in the medulla. These results are different from the present results of ^{14}C-FL research.

The cumulative excretion of radioactivity into bile of rats 24 hr after subcutaneous injection was 67% of the administered radioactivity, and 24 hr faeces contained 80% adminis-

10) S. Ullberg and G. Bengtsson, *Acta Endocrinol.*, **43**, 75 (1963).
11) L.-E. Appelgern, *Acta Endocrinol.*, **62**, 505 (1969).
12) L.-E. Appelgern, *Acta Physiol. Scand.*, **71**, *Suppl.*, **301**, 1 (1967).
13) G. Bengtosson, S. Ullberg, N. Wiqvist and E. Diczfalusy, *Acta Endocrinol.*, **46**, 544 (1964).

tered ^{14}C. This fact confirmed that most of the radioactivity in gastrointestinal contents of mice had been excreted through the bile duct.

The amount of $^{14}CO_2$ in the expired air was more than 1.2% of the administered radioactivity during 24 hr. The small amount of $^{14}CO_2$ in expired air indicates that the ^{14}C-acetonide group is scarcely hydrolyzed to ^{14}C-acetone and fluocinolone. Price, *et al.*[14] reported that the amount of $^{14}CO_2$ in expired air for 13 hr was 48% of ^{14}C-acetone administered and Sakumi, *et al.*[15] reported also that 27% of ^{14}C-acetone was recovered in the expired air for 4 hr. It may be considered that cleavage of the acetonide radical from the steroid nucleus occurs scarcely.

Maibac, *et al.*[16] studied percutaneous absorption of ^{14}C-FL in normal human by urine examination and found that ^{14}C-FL in urine was only 0.29% of the given amount. This value was thought to be reasonable from results using mice.

Acknowledgement We wish to express our thanks to Dr. K. Abe, Director of Our Laboratory for his advice throughout this investigation and to Syntex Corporation in Mexico for providing ^{14}C-fluocinolone acetonide.

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15) W. Sakami and Lafaye, *J. Biol. Chem.*, **193**, 194 (1951).
16) H.L. Maibach and R.J. Feldmann, *Clin. Res.*, **14**, (2) 270 (1966).