2-^{14}C-1-Allyl-3,5-diethyl-6-chlorouracil I: Synthesis, Absorption in Human Skin, Excretion, Distribution, and Metabolism in Rats and Rabbits

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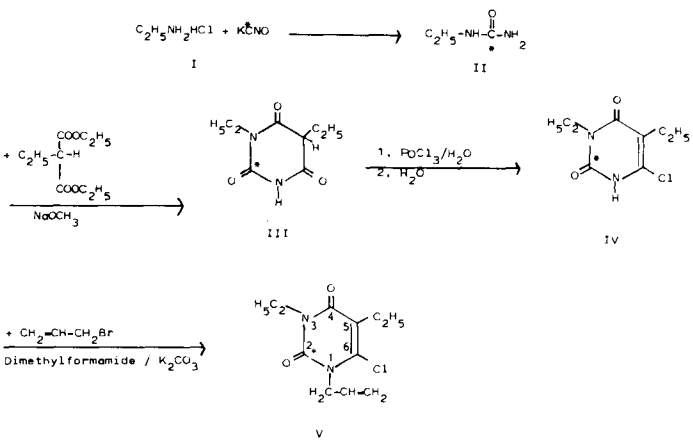
Abstract □ With ^{14}C-potassium cyanate as the starting material, 2-^{14}C-1-allyl-3,5-diethyl-6-chlorouracil was synthesized for *in vitro* and *in vivo* absorption studies in human skin and for metabolic studies in rats and rabbits. The radioactivity in the horny layer, epidermis, and dermis of the human skin was determined after different intervals of time, and the radioactivity excreted in the urine was measured by collecting samples for 5 days from a patient and also under occlusion conditions. Almost 90% of the radioactivity remained on the surface and ~6.28% penetrated and was systemically absorbed. Over a 5-day period, a total of 3.25% was excreted. Almost 3% was systemically absorbed and cumulated in the system. After intraperitoneal application in male and female rats, most of the radioactivity was excreted in the feces and urine, with female rats excreting more in the urine than male rats. The radioactivity rose in the organs in the first 3 hr and then decreased. At the end of 144 hr, no appreciable radioactivity could be found in the organs and tissues, except in the carcass where the cumulation was maximum (1%). After intravenous injection in rabbits, most of the radioactivity (80%) was excreted in the urine and only 4% in the feces. At the end of 96 hr, ~3% was cumulated in the body. The drug was quantitatively metabolized in both rats and rabbits: Metabolite 1 (70–85%), Metabolite 2 (10–15%), Metabolite 3 (5–10%), and Metabolite 4 (0.3%).

Keyphrases □ 2-^{14}C - 1 - Allyl-3,5-diethyl-6-chlorouracil—synthesis, absorption in human skin, excretion, distribution and metabolism, rats, rabbits □ Excretion—2-^{14}C-1-allyl-3,5-diethyl-6-chlorouracil, synthesis, absorption in human skin, distribution and metabolism, rats, rabbits □ Metabolism—2-^{14}C-1-allyl-3,5-diethyl-6-chlorouracil, synthesis, absorption in human skin, excretion, distribution, rats, rabbits □ Absorption—2-^{14}C-1-allyl-3,5-diethyl-6-chlorouracil, synthesis, human skin, excretion, distribution, metabolism, rats, rabbits

1-Allyl-3,5-diethyl-6-chlorouracil[1] (V) has shown promising therapeutic activity against herpes simplex and vaccinia virus (1, 2). It is used for the external treatment of herpes and other viral infections of the skin and mucous membranes. Its absorption in human skin and excretion, distribution, and metabolism in rats and rabbits were studied using ^{14}C-V.



Scheme I

[1] Acluracil, Robugen GmbH, 7300 Esslingen/Neckar, West Germany.

EXPERIMENTAL[2]

Synthesis—For absorption studies in human skin and for metabolism studies, ^{14}C-V, specific activity 0.073 mCi/mmole was synthesized (Scheme I). The starting material was ^{14}C-potassium cyanate[3], specific activity 0.082 mCi/mmole.

^{14}C-N-Ethylurea (II)—A solution of ^{14}C-potassium cyanate (1.0 g, 12.33 mmoles) in 2 ml of water was added to ethylaminehydrochloride (I) (0.95 g, 11.77 mmoles) in water (1 ml) at 80° with constant stirring. The water was completely removed and the residue dried to give 1.72 g of ^{14}C-II (90.0% yield), mp 91–92° [lit. (3) mp 90–93°].

2-^{14}C-3,5-Diethylbarbituric Acid (III)—Diethyl ethylmalonate (2.17 g, 11.72 mmoles) was added dropwise to 5 ml of a 30% methanolic sodium methoxide solution. The reaction mixture was refluxed for 30 min with constant stirring. The methanolic solution (3 ml) of ^{14}C-II (1.72 g, 19.52 mmoles) was then added. The reaction mixture was refluxed for 5 hr at 100° with constant stirring under anhydrous conditions. After cooling, the solvent was evaporated to dryness and the residue was dissolved in 10 ml of hot water. The solution was acidified to pH 1–2 with 2.2 ml of concentrated hydrochloric acid and extracted with chloroform (5 × 15 ml). The solvent was removed and the residue crystallized from ether to give 2.16 g of 2-^{14}C-III (47.6% yield), mp 92–93° [lit. (4) mp 93–94°].

2-^{14}C-3,5-Diethyl-6-chlorouracil (IV)—Phosphorus oxychloride (2 ml) was added dropwise to a mixture of III (2.16 g, 11.73 mmoles) and water (0.1 ml). The reaction temperature was slowly raised to 100° with constant stirring, and after heating for 90 min it was cooled. The remaining phosphoryl chloride was decomposed carefully with 6 ml of water. The cooled mixture was stirred for 1 hr and allowed to stand in the refrigerator overnight. The white crystalline precipitate was filtered off, washed with ice cold water, and dried to give 1.23 g of 2-^{14}C-IV (52.1% yield), mp 168° [lit. (5) mp 167–169°].

2-^{14}C-1-Allyl-3,5-diethyl-6-chlorouracil (V)—Potassium carbonate (2.235 g), allyl bromide (0.68 ml), and dimethylformamide (12.5 ml) were added to 2-^{14}C-IV (1.23 g, 6.07 mmoles) under anhydrous conditions. The mixture was refluxed with stirring for 2 hr at 90–100° and filtered. The residue was washed with chloroform and the filtrate concentrated. The oily liquid was distilled *in vacuo* (bp 119–120°/0.1 mm) to give 1.17 g of 2-^{14}C-V (79.5% yield), specific activity 0.073 mCi/mmole [lit. (5) bp 118–120°/0.1 mm]. ^{1}H-NMR and mass spectra obtained were consistent with structure V. Radiochemical purity was checked by radioscanning of the TLC plates.

Radioactivity Measurements—The radioactive zones on TLC plates were located with a thin-layer scanner[4]. The radioassay in the solution was carried out in a two-canal liquid scintillation counter[5], using a scintillation solution of 1000 ml of dioxane, 180 g of naphthalene, 8 g of 2,5-diphenyloxazole, and 0.1 g of 1,4-bis[2-(4-methyl-5-phenyl-oxazolyl)] benzene. The external standardization technique was employed.

Application—*Human Skin*—A 0.4% solution of ^{14}C-V was made in water using 3% of a solubilizing agent[6]. For *in vitro* studies, 20 μl = 4.9 mg was applied on an area of 7 cm^2. For *in vivo* studies, 50 μl = 4.9 mg of a 0.1% solution of ^{14}C-V was applied on an area of 28 cm^2.

Rats and Rabbits—A 0.15% solution of ^{14}C-V was made in sterile water with 3% of a solubilizing agent[6]. Male and female Wistar rats (200–220 g) were given 7.5 mg/kg body weight of ^{14}C-V solution by intraperitoneal injection. The animals were kept in metabolic cages which enabled the collection of feces and urine separately. They were given free access to

[2] Melting points were taken on a Tottoli (Büchi, Switzerland) apparatus and are uncorrected.

[3] New England Nuclear Corp., Boston, Mass.

[4] Dr. Rudolf Berthold Co., Wildbad, West Germany.

[5] Tri-Carb model 3950, Packard Instrument Co., La Grange, Ill.

[6] Cremophor RH 40, BASF, Ludwigshafen, West Germany.

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Table I—Relative and Absolute Concentrations in Various Tissues After Application *In Vitro* of 2-^{14}C-V [a]

Penetration time, min	Tissue	Quantity Applied, %	Tissue under 1 cm^2 of Surface, μg/cm^2	Respective Tissue, mg/ml	mmole[b]	Rejection, %
100	Horny layer	10.06	111.5	55.72	229.6	
	Epidermis	0.36	3.98	0.249	1.026	88.54
	Dermis	0.90	10.00	0.0965	0.381	
300	Horny layer	7.35	84.4	40.70	167.7	
	Epidermis	0.97	10.8	0.672	2.77	87.52
	Dermis	3.10	34.4	0.318	1.31	
1000	Horny layer	5.40	59.8	29.92	123.3	
	Epidermis	1.31	14.5	0.904	3.73	84.64
	Dermis	7.97	88.2	0.817	3.36	

[a] Application of 7.75 mg of 2-^{14}C-V/7 cm^2 of human skin. [b] Millimole per liter tissue.

Table II—Relative and Absolute Concentrations in Various Tissues or Urine After *In Vivo* Application of 2-^{14}C-V [a]

Penetration time, min	Tissue or Urine	Quantity Applied, %	Tissue under 1 cm^2 of Surface, μg/cm^2	Respective Tissue, mg/ml	mmole[b]	Rejection, %
100	Horny layer	12.97	22.69	11.35	46.74	
	Epidermis	0.93	1.63	0.102	0.42	83.11
	Dermis	1.94	3.39	0.031	0.13	
1000	Horny layer	13.54	23.70	11.85	48.83	
	Epidermis	0.78	1.36	0.085	0.35	75.10
	Dermis	1.69	2.95	0.027	0.11	
	Urine 5 days	3.24 total				
1000 under occlusion	Urine 5 days	9.375 total				78.23

[a] Application of 4.9 mg of 2-^{14}C-V/28 cm^2 of human skin. [b] Millimole per liter tissue.

Table III—Elimination of Radioactivity in Feces and Urine of Male and Female Rats After Intraperitoneal Administration of 2-^{14}C-V [a]

Sample	Hours	Average Percent of Applied Dose ±*SD* Male	Female
Feces	8	14.4 ± 4.3	10.2 ± 3.8
	16	30.7 ± 5.6	24.7 ± 5.2
	24	4.9 ± 2.2	2.9 ± 1.8
	48	5.1 ± 1.4	5.8 ± 1.6
	72	9.7 ± 1.9	8.6 ± 1.7
	96	1.2 ± 0.9	0.3 ± 0.1
	120	0.1 ± 0.03	0.3 ± 0.08
	144	0.04 ± 0.01	0.1 ± 0.08
	Total	66.1 ± 0.58	52.9 ± 1.1
Urine	8	3.6 ± 1.5	7.3 ± 1.8
	16	16.4 ± 2.4	21.3 ± 2.8
	24	2.0 ± 0.9	6.8 ± 1.7
	48	2.1 ± 0.8	2.3 ± 0.6
	72	2.4 ± 1.2	3.6 ± 1.4
	96	1.9 ± 0.8	1.8 ± 0.8
	120	0.1 ± 0.05	0.6 ± 0.1
	144	0.01 ± 0.005	0.1 ± 0.03
	Total	28.5 ± 0.09	43.8 ± 0.1

[a] Average of six animals; administration of 7.5 mg of 2-^{14}C-V.

Table IV—Elimination of Radioactivity in Feces and Urine of Male Rabbits After Intravenous Administration of 2-^{14}C-V [a]

Sample	Hours	Average Percent of Applied Dose ±*SD*
Feces	24	3.6 ± 1.4
	48	0.5 ± 0.1
	72	0.2 ± 0.1
	96	0.1 ± 0.07
	Total	4.4 ± 0.1
Urine	24	80.1 ± 6.8
	48	6.1 ± 2.6
	72	1.5 ± 0.8
	96	0.6 ± 0.2
	Total	88.3 ± 2.4

[a] Average of 3 animals; administration of 15.0 mg of 2-^{14}C-V.

food[7] and water and were kept at 22° and 55% humidity. The feces and urine were collected daily over a period of 144 hr. For studying the distribution, the rats were killed at different time intervals and all organs and tissues were removed separately. All samples were frozen at −20° and taken out before use. New Zealand male rabbits (2 kg each) were given a dose of 15 mg/kg body weight by intravenous injection into the ear vein. Feces and urine were collected daily over a 96-hr period. At the end of this period they were killed and organs and tissues removed and frozen.

Preparation of the Skin—In Vitro *Investigation*—The subcutaneous fat was carefully removed from skin mammary ablations shortly after surgery. Three different specimens of appropriate size were obtained. For each penetration period one specimen was used. The skin was gently cleaned and subcutaneous fat was removed. An area of 7 cm^2 was marked out. The solution of ^{14}C-V was applied for 90 sec by gently rubbing with a glass spatula. Subsequently, the specimen was stretched in a draft-free penetration chamber according to published procedures (6, 7). The subcutaneous side was in contact with gently agitated saline. The glass chamber was kept at a constant temperature of 32° for the indicated time period (penetration time).

In Vivo *Investigations*—Two patients were included in this study. A solution of ^{14}C-V was applied gently to a 28-cm^2 area of healthy skin, and the treated area was protected against unintentional abrasion by a wire cage under free access of air.

Processing of the Skin and Urine—In Vitro—After 100-, 300-, and 1000-min penetration time, the surplus of ^{14}C-V on the surface was removed gently with dry cotton. The specimens were then fastened on a rubber stopper and the horny layer removed by repeated stripping with adhesive tape[8] until a polished shining layer was visible, indicating that all horny skin was removed. Each of the ~20 strips was placed in a separate vial. After removal of the horny layer, a 28-mm^2 sample was punched out and cut horizontally on a freeze microtome[9]. The 16 slices of 10-μm thickness first-sectioned represented the epidermis. The remaining part representing the dermis was cut into 40-μm slices. Thus, ~70 samples were obtained in each experiment with one penetration time and were counted separately. Each sample was placed in a scintillation glass and 0.3 ml of 0.5 *N* NaOH was added. The closed glasses were kept

[7] Altromin, Altromin, Lage/Lippe, West Germany.

[8] Tesafilm, Beiersdorf, Hamburg, West Germany.

[9] Jung, Heidelberg, West Germany.

Table V—Tissue Distribution of Radioactivity in Male and Female Rats [a] at Different Time Intervals After Intraperitoneal Administration of 2-^{14}C-V

Specimen	Sex	Average Percent of Applied Dose, hr										
		1	3	5	8	16	24	48	72	96	120	144
Blood	Male	1.82	1.13	1.23	1.05	0.40	0.60	0.56	0.40	0.65	0.28	0.17
	Female	3.06	3.22	0.42	1.01	0.65	0.98	0.90	1.48	0.27	0.19	0.10
Liver	Male	1.94	1.66	1.47	1.32	0.38	0.31	0.02	0.28	0.08	0.06	0.08
	Female	2.53	3.61	1.43	0.86	0.27	0.71	0.40	0.33	0.18	0.04	0.21
Testes/ovary	Male	0.32	1.26	0.47	0.15	0.04	0.10	0.40	0.96	0.04	0.04	0.10
	Female	0.58	0.25	0.23	0.21	0.01	0.03	0.35	0.33	0.28	0.08	0.08
Kidneys	Male	1.04	0.63	0.65	1.05	0.18	0.21	0.22	0.41	0.08	0.12	0.06
	Female	0.79	0.94	0.53	0.38	0.01	0.24	0.45	0.25	0.07	0.18	0.05
Gut	Male	13.93	13.28	7.79	5.27	0.39	0.73	0.05	0.32	0.05	0.04	0.06
	Female	11.27	6.30	5.75	10.15	1.09	2.26	0.61	0.51	0.18	0.26	0.07
Spleen	Male	0.23	0.24	0.38	0.07	0.02	0.07	0.38	0.44	0.19	0.01	0.02
	Female	0.05	0.24	0.56	0.06	0.12	0.07	0.24	0.23	0.10	0.03	0.10
Stomach plus duodenum	Male	1.54	1.16	0.40	0.74	0.08	0.04	0.12	0.12	0.11	0.20	0.01
	Female	0.27	0.17	0.,34	0.21	0.12	0.12	0.25	0.54	0.11	0.16	0.01
Heart	Male	0.94	0.31	0.45	0.04	0.08	0.03	0.76	0.20	0.09	0.08	0.08
	Female	0.10	0.30	0.10	0.03	0.02	0.07	0.23	0.53	0.14	0.14	0.11
Lungs	Male	0.71	0.37	0.18	0.37	0.36	0.04	0.36	0.36	0.21	0.10	0.08
	Female	0.25	0.34	0.28	0.20	0.07	0.08	0.23	0.42	0.08	0.15	0.17
Brain	Male	0.71	1.08	0.68	0.14	0.08	0.11	0.07	0.18	0.38	0.04	0.04
	Female	1.20	0.15	0.09	0.03	0.04	0.05	0.29	0.15	0.11	0.06	0.12
Abdominal fat	Male	1.03	0.41	0.90	0.71	0.09	0.10	0.44	0.22	0.04	0.40	0.04
	Female	7.15	8.80	0.54	1.34	0.24	0.52	0.37	0.18	0.07	0.60	0.06
Muscles	Male	3.30	2.71	3.40	1.76	0.51	2.01	1.10	0.99	0.37	0.18	0.18
	Female	3.10	1.86	2.30	2.31	0.68	3.24	1.40	1.29	0.16	0.17	0.21
Subcutaneous fat	Male	1.39	0.32	0.85	0.89	0.03	0.29	0.43	0.07	0.04	0.10	0.05
	Female	1.02	6.31	0.93	0.22	0.11	0.34	0.34	0.36	0.04	0.10	0.06
Contents of gut	Male	45.97	60.81	65.48	61.14	13.49	3.82	0.70	0.56	0.45	1.12	0.10
	Female	45.26	47.96	59.32	47.43	5.84	2.57	1.27	1.32	0.41	1.14	0.05
Carcass	Male	20.25	9.84	11.55	14.37	6.27	5.71	2.60	3.42	1.67	1.08	1.02
	Female	19.52	14.96	20.86	13.38	17.99	9.16	5.15	5.48	2.64	0.92	1.21

[a] Average of six animals; administration of 7.5 mg of 2-^{14}C-V.

Table VI—Tissue Distribution of the Radioactivity in Male Rabbits [a] 96 hr After Intravenous Administration of 2-^{14}C-V

Specimen	Average Percent Applied Dose
Bones	1.88
Carcass	1.18
Muscles	0.09
Liver	0.07
Intestinal content	0.40
Heart	0.01
Kidney	0.03
Lungs	0.03
Brain	0.01
Blood	0.04
Total	3.73

[a] Average of three animals; administration of 15.0 mg of 2-^{14}C-V.

for 6 hr in an incubator at 75°. Scintillation solution then was added to each sample and the radioactivity measured.

In Vivo—Two penetration times (100 and 1000 min) were selected. The surplus ^{14}C-V was removed and the horny layer sampled as indicated above. A specimen of 28 mm^2 was punched out from the excised area and sliced in the same manner as described for *in vitro* experiments. By day urine was sampled over a 4-hr period and during the night over an 8-hr period; each volume was measured. Samples of 0.3 ml were taken and after addition of hyamine, added directly to the scintillation cocktail.

Penetration Under Occlusion—In a third patient, the pathological skin condition was simulated by occlusion technique, which causes hydration of the horny layer and thus facilitates the penetration. The horny skin was completely removed and 50 μl/28 cm^2 of 0.10% ^{14}C-V was applied and covered with occlusive film[10] for 1000 min, which helped the maximum penetration.

Calculation for Absorption in Human Skin—Subsequent calculations of disintegrations per minute of the percentage of the applied quantity and absolute quantities per layer of skin were performed using a computer program. Absolute quantities are given in micrograms within the respective layer calculated for a 1-cm^2 surface, in micrograms per milliliter of volume of the layer, and in millimoles (calculated on the basis of 1 liter of skin volume). The following tissue thicknesses were taken in calculation: horny layer, 20 μm; epidermis, 160 μm; and dermis, 1.5 mm.

Animal Sample Preparation—*Feces, Organs, and Tissues*—For qualitative and quantitative determination of the radioactivity, the feces samples were dried at 60°, powdered, homogenized[11], and extracted with methanol in a soxhlet apparatus for 48 hr. The organs and tissues were cut into fine pieces and extracted with methanol for 72 hr according to procedures described previously (8, 9). An aliquot (0.1 ml) of the samples was taken up for required measurements.

Urine—An aliquot (0.1–0.2 ml) was mixed directly into the scintillation solution and measured for radioactivity (10).

Chromatography—Analytical TLC was performed on 20 × 5-cm (0.25 mm) silica gel plates[12]. Solvent systems were (v/v): (*a*) petroleum ether–ethyl acetate (8:2); (*b*) petroleum ether–ethyl acetate (5:5); (*c*) cyclohexane–acetone (7:3); (*d*) chloroform–ether (6:4).

Gas Chromatography—Analytical GLC determinations were performed on a gas chromatograph[13] equipped with a flame-ionization detector. The chromatographic column was glass tubing (1.5 m × 0.4-mm i.d.) packed with 3% SE-30 on 100–120 mesh Varaport 30. The operating conditions were: injection port temperature, 230°; oven temperature, 200°; detector temperature, 250°; nitrogen (carrier gas) flow rate, 20 ml/min.

Enzymatic Hydrolysis of the Urine—The rabbit urine was adjusted to pH 7 with acetic acid and then extracted with ether for 96 hr in a liquid–liquid extractor, removing ~90% of the radioactivity (Extract A). The rest of the radioactivity could not be removed with organic solvents and was probably due to conjugates. The urine after ether extraction was concentrated to half of its volume and adjusted to pH 5.5 with concentrated acetic acid. It was diluted to half of its volume with acetate buffer. The enzymatic hydrolysis was done with 0.01 ml of β-glucuronidase and arylsulfatase[14]/ml of urine at 37° for 72 hr and extracted with ether for 96 hr (Extract B).

RESULTS AND DISCUSSION

Absorption in Human Skin—*In Vitro*—Table I shows that most of the applied ^{14}C-V (85–90%) remained on the skin surface or was washed

[10] Leukoflex, Beiersdorf, Hamburg, West Germany.

[11] Waring Blender, Bosch, Stuttgart, West Germany.
[12] Silica gel HF_{254}, E. Merck AG, Darmstadt, West Germany.
[13] Aerograph 1740, Varian.
[14] Glusulase, Boehringer, Mannheim, West Germany.

Table VII—TLC R_f Values of the Metabolites of V

	Solvent	R_f Metabolite 1	Metabolite 2	Metabolite 3	V
(*a*)	Petroleum ether–ethyl acetate (8:2)	*S*[a]	*S*	*S*	0.45
(*b*)	Petroleum ether–ethyl acetate (5:5)	0.38	0.63	0.71	0.85
(*c*)	Cyclohexane–acetone (7:3)	0.16	0.43	0.59	0.75
(*d*)	Chloroform–ether (6:4)	0.30	0.70	0.78	*F*[b]

[a] Start. [b] Front.

Table VIII—Ratio in Percent of Various Metabolites of V in Feces [a] and Urine [b] of Male and Female Rats

Sample	Sex	Percent Metabolite 1	Metabolite 2	Metabolite 3
Feces	Male	68	20	12
	Female	65	22	13
Urine	Male	85	10	5
	Female	88	8	4

[a] The feces of various time intervals were mixed together. [b] The urine of various time intervals were pooled together.

in the penetration chamber. Over a 1000-min period, however, ~8% penetrated into the dermis, which acted as a further transport system in the body. The absolute quantity within the respective layers calculated for a 1-cm^2 surface area showed the accumulation of the radioactivity in the dermis, the thickest tissue. The absolute values calculated with an equal tissue volume, however, showed a surprisingly higher molar concentration after a 1000-min period in all tissues. The rejection rate included the percentage of the radioactivity in the saline of the penetration chamber and on the surface of the skin which was wiped off with cotton.

In Vivo—The results are shown in Table II. Here also it is evident that most of the ^{14}C-V remained on the skin surface. The concentration in the horny layers was somewhat higher than *in vitro* studies; in the epidermis it was almost the same. However, the concentration in the dermis *in vivo* was much less than *in vitro* after a penetration time of 1000 min. This is because the circulation system does not play a role *in vitro*, and the drug accumulated in the excised skin. The difference (6.28%) between these *in vivo* and *in vitro* dermal concentrations after a penetration time of 1000 min could be considered as resorption of the drug through the circulatory system. It was more clear when the absolute concentration *in vitro* and *in vivo* was compared. The fact that ^{14}C-V was systemically absorbed in the system was also confirmed by the excretion of the radioactivity in the urine. Over a 5-day period, 3.24% of the activity was excreted after which it was negligible or had completely stopped showing that at least 3% of ^{14}C-V accumulated in the body. The difference between 100- and 1000-min studies showed that a steady state of penetration (*i.e.*, equilibrium between the passage of the substance from the horny skin and its further transport in the circulatory system) relatively soon had reached a standstill; probably it required <100 min. Because of the importance of absorption and excretion of ^{14}C-V, occlusion conditions were employed in a third patient to allow maximum penetration for a period of 1000 min. Similarly, 9.4% of the radioactivity (Table II) could be detected in the urine.

Animal Excretion—*Rats*—Table III shows the excretion of the radioactivity in feces and urine of male and female rats up to 144 hr. A total of 66.1% of the activity was excreted by the male rats in the feces and 28.5% in the urine. On the other hand, the female rats excreted ~52.9% in the feces and 43.8% in the urine. These different excretion rates in the feces and urine could be due to different physiological conditions in both sexes and the female rats excreted a greater quantity of urine than male rats. However, both male and female rats excreted >95% of the radioactivity (male 94.6%, female 96.7%) in the feces and the urine.

Rabbits—Table IV shows the excretion of the radioactivity in the feces and urine of male rabbits up to 96 hr. Most of the radioactivity (88.1% in urine and 3.6% in feces) was excreted after 24 hr. Excretion was slow and at the end of 96 hr, a total of 93% of the activity was excreted in the feces and the urine. This showed that in both rats and rabbits, the kidneys are one of the main elimination organs in the excretion of V and its metabolites.

Animal Distributions—*Rats*—Table V gives the values for male and female rats as percentages of applied dose. The radioactivity increased in all organs and tissues in the first 3 hr and then decreased. In blood the fall of the activity was slow and remained at a constant level between 16 and 72 hr. This was in agreement with urine where maximum excretion took place between these periods. This shows that metabolism of ^{14}C-V was complete after 16 hr and the maximum blood concentration was reached. However, some differences in values in male and female rats were recorded, showing the different metabolism rates in the two sexes. In the kidneys, the main organ of excretion, the radioactivity rose and decreased during various time intervals which can be explained due to throwback mechanisms. In all organs and tissues, however, the radioactivity decreased smoothly, except for a slight rise between 48 and 72 hr, which was due to redistribution phenomenon of these organs. In the liver, the main organ of biotransformation, no concentration of the radioactivity was observed after 5 hr. The high concentration of the activity in the intestine was due to feces that contained high activity and was subsequently excreted. This was further confirmed by contents of the intestinal tract which contained high activity and was ultimately excreted with feces. This shows that the liver plays an important part in the biotransformation and the activity in the feces originated mainly from the activity *via* the bile. At the end of 144 hr, <0.1% of the activity was found in all the organs and tissues of both male and female rats, except in the carcass, where values between 1.02 and 1.21% were recorded. In the carcass of both male and female rats, high radioactivity was accumulated even after 1 hr of application of ^{14}C-V and then decreased.

Rabbits—Table VI gives the values in the organs and tissues at the end of 96 hr. Except in the bones and carcass, very little activity could be found in the other organs and tissues. A total of 3.7% was found in the body after 96 hr.

Metabolism—*Rats*—The feces and the urine of rats showed on the TLC scanner Metabolites 1, 2, and 3 but no unchanged ^{14}C-V. Table VII gives the R_f values of the metabolites compared with ^{14}C-V.

The ratio of these metabolites in feces and urine of both male and female rats is given in Table VIII. Metabolite 1 is the major metabolite in the feces and urine of both male and female rats. The GLC investigations of the prepurified feces and urine showed the presence of another metabolite, 4, in 0.2% concentration. In the increasing order of their retention time in GLC, the metabolites were classified as follows: Metabolite 4 > Metabolite 1 > Metabolite 2 > Metabolite 3.

Rabbits—The combined rabbit urine after extraction with ether (Extract A) showed the presence of Metabolites 1, 2, and 3 in the ratio of 8.5:1.0:0.5. GLC showed the presence of Metabolite 4 in 0.3% concentration. The urine of the rabbits after enzymatic hydrolysis (Extract B) gave Metabolite 1 in 3% yield which showed that it occurred partially as a conjugate. Because of less radioactivity in the feces and many impurities, it could not be investigated properly.

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