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Percutaneous absorption, disposition, metabolism, and excretion of ^{14}C-labelled Cyoctol in humans after a single dermal application

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Summary

^{14}C-labelled cyoctol was applied in an ethanolic solution to the forearm of four young healthy volunteers for 8 h under non-occluding conditions, to investigate its absorption, disposition, and excretion after a single dermal application. Urine and feces were collected to determine the absorbed amounts of radioactivity, while ipsi- and contralateral blood samples were taken in order to obtain an insight into the kinetics of the penetration and elimination processes. The application area was stripped with cellophane adhesive tape at three different time points to study the retention of cyoctol in the stratum corneum. Cyoctol was found to be absorbed by human skin at levels of approx. 10% of the applied dose and appeared to penetrate at a relatively fast rate. Radioactivity accumulated neither in the skin nor in any other site in the body as total recovery values were close to 100% at 120 h after removal of the dose. Radioactivity was eliminated primarily through the kidneys (> 99%) in the form of polar metabolites. Another interesting phenomenon was that cyoctol was completely metabolized when passing through the skin as no unchanged Cyoctol could be detected in ipsilateral plasma samples. This may eliminate the occurrence of adverse systemic effects after dermal application.

Introduction

Cyoctol, or 6-(5-methoxyhept-1-yl)bicyclo [3.3.0]octan-3-one (Chantal Pharmaceutical Corp., Los Angeles, CA, U.S.A.), is a new androgen receptor blocking agent (Ford et al., 1987) currently undergoing extensive clinical investigation for the treatment of acne vulgaris, alopecia and keloid scar tissue (Johnson and Orenberg, 1987; Strick et al., 1989).

Pharmacological studies suggest that this anti-androgen effect occurs with topical administration only as systemically absorbed cyoctol is devoid of anti-androgen activity. This might be caused by an extensive first-pass metabolism after oral intake, so that insufficient amounts of unchanged drug can penetrate from the systemic circulation into the skin, or the parent drug does not reach the target tissue at all. Therefore, topical administra-

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tion of cyoctol seems to be the most logical route of administration in clinical practice.

In order to assess its body burden after topical application, a clinical study was performed in which cyoctol was dosed to the forearm of healthy volunteers for 8 h under non-occlusive conditions. As the metabolism of cyoctol was unknown, ^{14}C-labelled cyoctol had to be used. The absorption was determined by measuring the amounts of radioactivity in the excreta, while ipsi- and contralateral blood sampling and tape stripping of the stratum corneum at different time points provided information on the rate of the absorption process.

Materials and Methods

Chemicals

^{14}C-labelled cyoctol was obtained from Chantal Pharmaceutical Corp. The chemical structure including the position of the carbon-14 label is indicated in Fig. 1. The radiochemical purity of the test compound was determined by RP-HPLC to be at least 97.0%.

The stock solution of the labelled compound consisted of ethanol (96%)/distilled water (75 : 25 v/v), and contained approx. 1.5% cyoctol. The specific activity of [^{14}C]cyoctol was 12.1 mCi/mmol. All other chemicals were of analytical grade and were obtained from Merck, Darmstadt, F.R.G.

Preparation of the dosage

The stock solution was used as such. The amount of radioactivity per mg in the solution was assessed in triplicate by mixing approx. 10 mg, accurately weighed, with approx. 10 ml of methanol and counting the total amount of radioactivity.

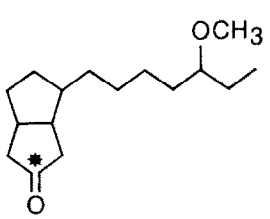


Fig. 1. Structural formula of cyoctol or 6-(5-methoxyhept-1-yl)bicyclo[3.3.0]octan-3-one. The asterisk indicates the position of the carbon-14 label.

Study design

Four young male healthy volunteers participated in this study. Prior to commencing the study, each volunteer was subjected to a standard physical examination program and a complete medical history was taken. All volunteers gave their written informed consent. The studies were performed in accordance with FDA's Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) regulations and guidelines.

Blank urine, feces (if possible), and blood samples were collected prior to the start of the study.

The study consisted of a single application of approx. 50 μl of the stock solution, containing approx. 0.7 mg of drug and approx. 35 μCi [^{14}C]cyoctol. The volar aspect of the right forearm was shaven 4 days prior to the start of the study. The application area was outlined by an adhesive template (Ensure-it, Deseret Medical, Sandy, UT, U.S.A.; 10×14 cm) from which an inner rectangle of 4×6 cm^2 had been removed. Dosing was performed using a glass microsyringe. The exact amount applied was calculated from the weight of the syringe before and after dosing and the radioactivity per unit weight of stock solution. After administration, the dosage was spread over the entire application area, using a metal spatula. Thereafter, the area was covered with a dome under non-occlusive conditions. The dosage and dome were left in place for 8 h.

After this time period, during which the volunteers were seated with their arms resting on a bench, the dome and template were removed. Subsequently, the remainder of the dosage was removed by wiping the skin with dry gauze pads. The boundaries of the treated area were marked and the skin was thoroughly rinsed with ethanol sponging six times. The area was left uncovered for about 1 h until the first tape stripping procedure (see below), after which it was covered by a dry gauze pad till the next stripping procedure. All items expected to contain radioactivity, including the dome and template, were saved for radioactivity analysis.

During the study, blood pressure, heart rate,

respiration rate, and body temperature were measured twice a day.

After completing the study, all volunteers again passed a full physical examination.

Sample collection

Urine, blood, and feces were collected at regular intervals during the 8 h application period and for 120 h following removal of the dosage.

From each arm, two consecutive blood samples (5 and 10 ml in volume for whole blood and plasma, respectively) were collected in heparin-containing polypropylene tubes. They were drawn from both arms simultaneously; ipsilaterally (right arm) and contralaterally (left arm).

All urine, blood, plasma, and feces samples were stored at $-20\,^{\circ}C$ until analysis.

Tape stripping, which removes a layer of stratum corneum, was performed on days 1, 2, and 3, at 1, 23, and 45 h after removal of the dosage, according to the procedure described previously (Wiechers et al., 1990). This was carried out using commercial cellophane translucent adhesive tape of 9 mm width (3M Co., Leiden, The Netherlands). A total of 28 strips, approx. 6 cm in length, was sequentially adhered to and removed from the same transverse portion of the treatment site. Four sequential strips were placed in one scintillation counting vial and saved for radioactivity analysis (series 1). A second set of strips was obtained from another site, right next to the previously stripped site. This time, however, all 28 strips were combined in one brown-glassed gallipot (series 2). This procedure was repeated on days 2 and 3 on adjacent sites, so that at the end of the third day over 90% of the application area had been stripped of its stratum corneum. Bathing and showering was not allowed until after the last stripping on day 3, to avoid any loss of radioactivity from the skin surface.

Sample treatment

Radioactive material in the swabs, gauze pads to remove non-absorbed material, template, dome, and spatula was extracted using methanol and analyzed for radioactivity. Determinations were performed 10-fold. The external recovery was defined as the sum of the amounts recovered in these samples, but excluded the material adhering to the spatula which was directly subtracted from the dose.

To the tapes combined in groups of four intended for total radioactivity assessment (series 1), 18 ml of scintillation cocktail was added. The vials were shaken for 16 h to extract all radioactive material and counted. The second group of tapes, all combined per volunteer, was treated as previously described in detail and analyzed for total radioactivity and metabolic profile (Wiechers et al., 1989).

Whole blood (0.25 ml) was solubilized by adding 1 ml of a mixture of Soluene-350 (Packard Technologies, Groningen, The Netherlands) and isopropanol (1 : 2 v/v), then decolorized by means of 0.5 ml of hydrogen peroxide, and counted after 2 days.

Plasma was analysed after adding 4 ml scintillation cocktail to a 1 ml aliquot.

After assessing the volume and pH of each urine fraction, 1 ml per fraction was separated and counted after the addition of 3 ml scintillation cocktail.

Feces samples were weighed and freeze-dried using a Hetosicc CD-52 lyophilizer (Heto, Birkerup, Denmark), and the residues were powdered and homogenized. A 500 mg aliquot was combusted in a Tri-Carb sample oxidizer (Packard Technologies) and the resulting carbon dioxide was absorbed in 10 ml Carbosorb II. The latter was mixed with 11 ml Permafluor (both Packard Technologies) as the scintillation cocktail and the mixture counted.

Analytical procedures

Determination of radioactivity was performed using a Packard B4450 Liquid Scintillation Counter (Packard Technologies) and RiaLuma (Lumac, Landgraaf, The Netherlands) as the scintillation cocktail unless stated otherwise. Samples were counted for either 5 or 10 min, depending on the amount of radioactivity anticipated in the sample in question, or to a statistical accuracy of 0.5%. Data were converted to disintegrations per s (Bq) after assessing the extent of quenching in each individual sample by using an external standard.

All values have been corrected for the counts retrieved in their respective blank samples.

Metabolic profiling of urine and ipsilateral plasma was performed on a Nucleosil C18 5 μm RP column (150 × 4.6 mm, i.d.), using linear gradient elution by means of two Waters M510 pumps (Millipore, Etten-Leur, The Netherlands), controlled by an Adalab data acquisition/control system (Interactive Microware, State College, PA, U.S.A.). The gradient went from 100% phosphate buffer 0.01 M, pH 6.8, to 100% methanol in 20 min and was followed by a 10 min methanol flush, the flow rate being 1.0 ml/min.

Results and Discussion

The stock solution of [^{14}C]cyoctol in ethanol (96%)-bidistilled water (75 : 25 v/v) that was used as the dosing solution contained 0.6283 μCi/mg. Using this value and the weight of the dosing solution applied to each volunteer, the total amount of radioactivity applied could be calculated. The values determined, after correction for the amounts of radioactivity retrieved from the spatula used for spreading the dose, are given in Table 1.

The external recovery is also listed in Table 1. It was interesting to note that the gauze pads used to cover the application area between the tape stripping procedures (covering pads) on days 1–2 and days 2–3 contained substantial amounts of radioactivity, as indicated in Table 2. Since the removal of non-absorbed radioactivity after the 8 h exposure had been essentially complete with the final ethanol spongings containing no radioactivity, the amounts now retrieved in these covering pads conceivably have been absorbed previously. Consequently, the radioactivity found in these pads can be considered to represent part of the percutaneous absorption. Outward transcutaneous migration of compounds, i.e. from the inside of the body to the outside, has been described (Peck et al., 1988), and this may well explain the relatively high levels of radioactivity retrieved in these covering pads. As the amounts of radioactivity in the skin decline with time, the outward migration decreases similarly. Unfortunately, no differentiation could be made between migration from the stripped and unstripped part of the application area.

The individual contributions of the various samples to the total recovery are listed in Table 3. The mean recovery of [^{14}C]cyoctol-derived radioactivity during the entire study period was 105.15 ± 3.39%. This indicates complete recovery of all radioactivity within acceptable limits for accuracy and precision. It should be noted that a study like

TABLE 2

Amounts of radioactivity (μCi) retrieved in gauze pads covering the application area (covering pads) between the tape stripping procedures on days 1 and 2 (days 1 – 2) and between days 2 and 3 (days 2 – 3)

Volunteer no.	Radioactivity		
	Days 1–2	Days 2–3	Total
1	0.4007	0.0393	0.4400
2	0.8286	0.0886	0.9172
3	1.1811	0.0615	1.2426
4	1.3529	0.2326	1.5855

TABLE 1

Dose and amounts of ^{14} C-labelled cyoctol-derived radioactivity retrieved in the various samples (μCi)

Volunteer no.	Dose	Radioactivity retrieved in				
		External Recovery	Tapes	Urine	Feces	Covering pads
1	27.053	25.269	0.178	1.290	0.009	0.440
2	36.616	33.282	0.250	3.969	0.006	0.917
3	34.382	33.297	0.209	1.978	0.013	1.243
4	35.876	35.095	0.411	1.747	0.010	1.586

this comprises a good many samples of different nature and with vastly different amounts of radioactivity that may easily result in cumulative errors. The urine and feces data also indicate that all absorbed radioactivity has been excreted and that accumulation of [^{14}C]cyoctol-derived radioactivity in the body does not occur.

The percutaneous absorption of cyoctol was calculated from the sum of the amounts retrieved in the urine, feces, tape strippings and the covering pads, and amounted to 10.4 ± 2.8% of the dose applied. This is much lower than the values determined previously in rats and rabbits in which percutaneous absorption of 90–100% was observed (Selim et al., 1985).

The amounts of radioactivity retrieved in both tape stripping series have been combined and are listed in Tables 1 and 3. Fig. 2 shows the disposition of radioactivity in the stratum corneum, as obtained in the first part of the tape stripping procedure. In this three-dimensional plot the *X*-axis shows the tape number which is indicative of the depth in the stratum corneum. The *Y*-axis refers to the amount of radioactive material retrieved in the samples and the different days are shown along the *Z*-axis. The rapid decline as observed when going from day 1 to 2 to 3 indicates that radioactivity does not accumulate in the stratum corneum. Unfortunately, the decline in levels of radioactivity with increasing depth in the skin does not provide information on the rate of percutaneous absorption, however, such data can be obtained from the whole blood and plasma values.

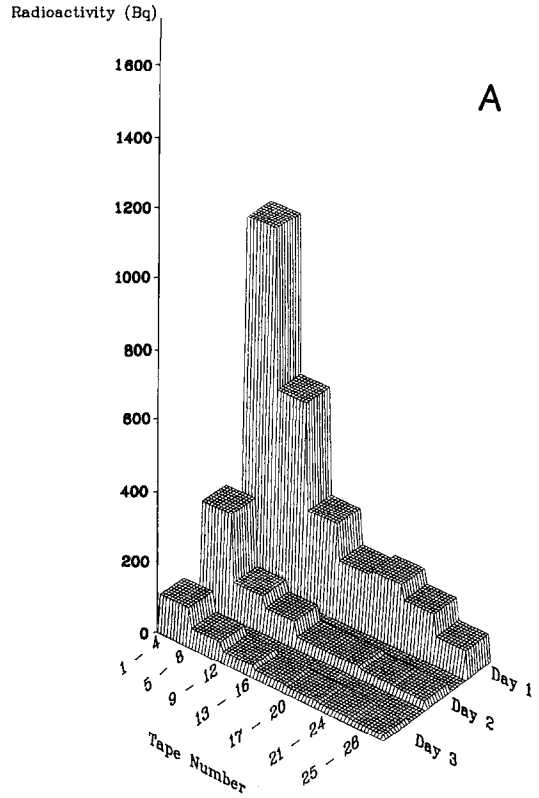
The levels of radioactivity in whole blood were negligible, usually not exceeding twice the standard deviation of the background, and therefore were too low to allow accurate determination. It should be noted that it is a known phenomenon that analytical procedures for whole blood are less sensitive than those for plasma.

The levels of [^{14}C]cyoctol-derived radioactivity in ipsi- and contralateral plasma samples are shown in Fig. 3. Ipsilateral plasma levels are expected to be high as they are measured in the efferent blood vessels of the application area, immediately following percutaneous absorption and prior to systemic dilution. Corresponding contralateral levels may be lower due to systemic dilution and uptake into various organs. Therefore, the difference in ipsi- and contralateral plasma samples reflects the rate of percutaneous absorption. In Fig. 3 this difference is clearly visible and indicates that cyoctol is relatively rapidly absorbed through human skin. The systemically circulating amounts of radioactivity (contralaterally) are so low that the large majority of the samples do not exceed twice the standard deviation of the background. Maximal ipsilateral plasma levels are obtained at 6, 8, 6, and 10 h after application of the dose for volunteer 1, 2, 3, and 4, respectively, again indicating that accumulation in the stratum corneum does not occur and that penetration is relatively fast. Contralateral

TABLE 3

Recovery of ^{14}C-radioactivity, expressed as percentage of the applied dose (%)

Sample type	Volunteer no.				Mean ± S.D.
	1	2	3	4	
External recovery	93.40	90.89	96.84	97.82	94.7 ± 3.2
Percutaneous absorption					
Urine	4.77	10.84	5.75	4.87	6.6 ± 2.9
Feces	0.03	0.02	0.04	0.03	0.03 ± 0.01
Tapes	0.66	0.68	0.61	1.15	0.78 ± 0.25
Covering pads	1.63	2.50	3.61	4.42	3.0 ± 1.2
Total percutaneous absorption	7.09	14.04	10.01	10.47	10.4 ± 2.8


Radioactivity (Bq)
A
1600
1400
1200
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800
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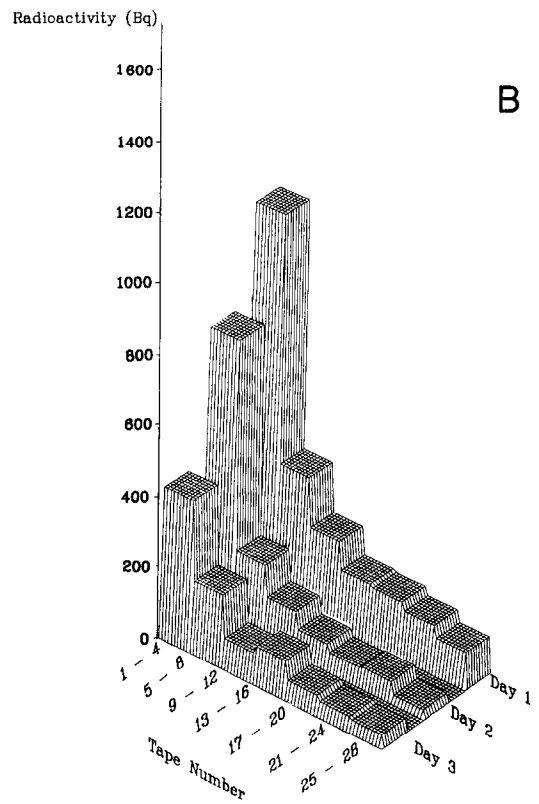
1 – 4
5 – 8
9 – 12
13 – 16
17 – 20
21 – 24
25 – 28
Tape Number
Day 1
Day 2
Day 3


Radioactivity (Bq)
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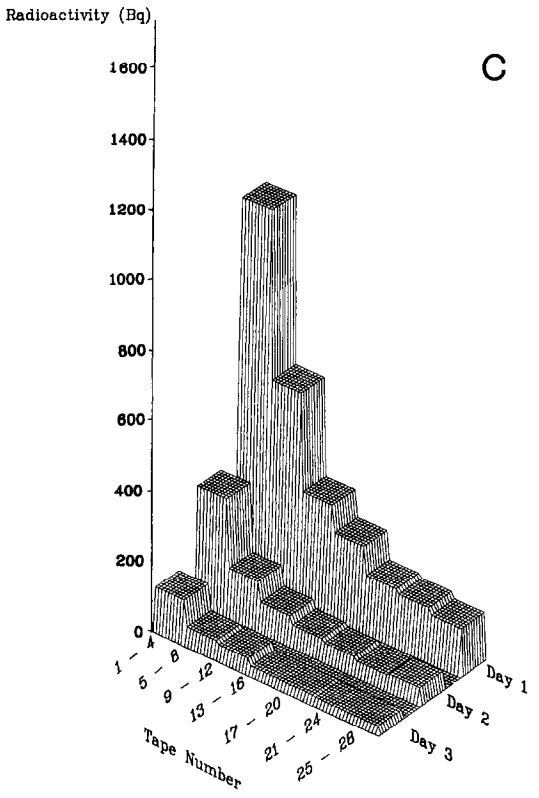
Tape Number
Day 1
Day 2
Day 3


Radioactivity (Bq)
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Tape Number
Day 1
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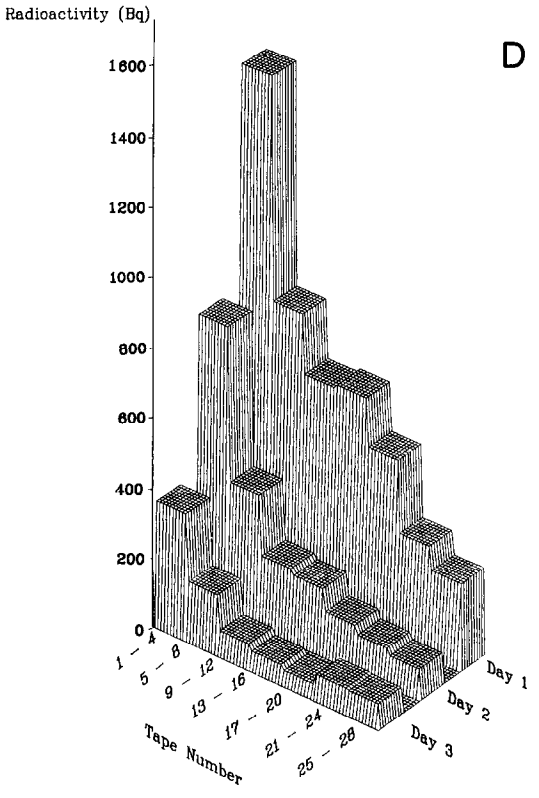
Radioactivity (Bq)
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Tape Number
Day 1
Day 2
Day 3

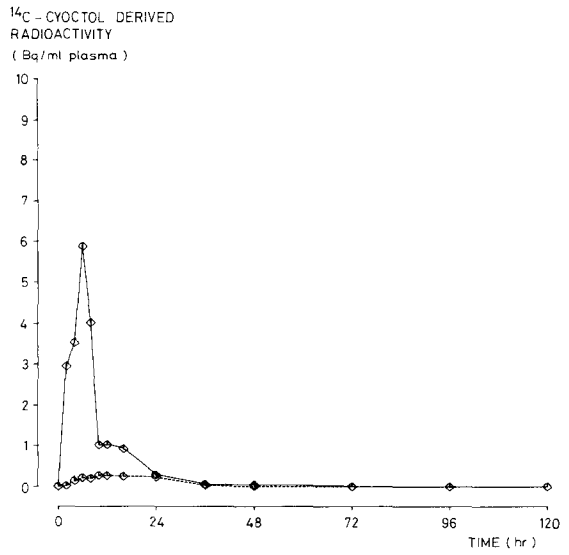

Fig. 3. Ipsi- and contralateral plasma levels of [^{14}C]cyoctol-derived radioactivity (continuous and broken line, respectively) of volunteer 3.

plasma levels were far too low to obtain an impression of the plasma half-life of elimination. Assuming only unchanged cyoctol to be present in the ipsilateral plasma samples, the maximal levels are between 1.8 and 4.6 ng/ml, which is sufficiently low that this radioactivity can be neglected in the total recovery of radioactivity listed in Table 1.

Absorbed radioactivity is predominantly excreted in the urine (99.5 ± 0.2%). Fig. 4 shows the excretion of radioactivity in the individual urine fractions of volunteer 2. Most of the radioactivity is excreted within the first 12 h of the experiment, indicating a rapid elimination of absorbed radioactivity that appears to be complete for all volunteers at the end of the study, i.e. 120 h after removal of the dose. The semi-logarithmic plot of the excretion rate of [^{14}C]cyoctol-derived radioactivity vs midpoint time of urine collection showed a biphasic pattern. The α-phase elimination half-life was 10.0 ± 3.3 h (mean ± S.D.) and that of the β-phase was 21.2 ± 3.4 h. It should be noted that these values were calculated on the basis of total

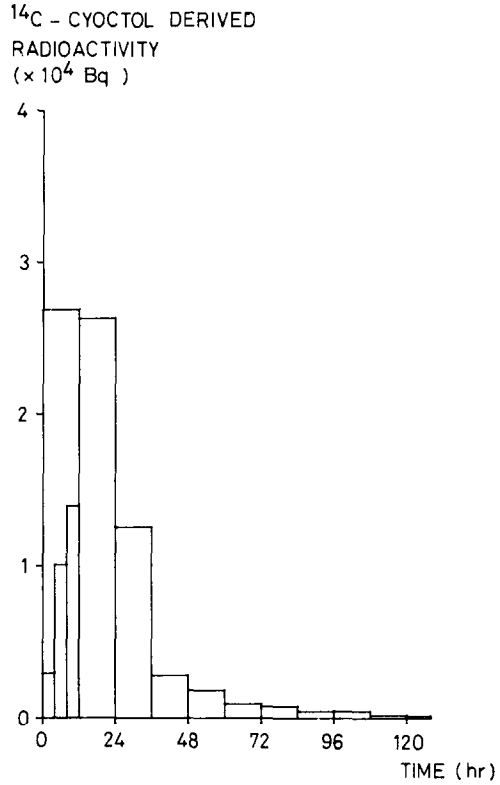

Fig. 4. Urinary excretion of [^{14}C]cyoctol-derived radioactivity of volunteer 3. See text for details.

amounts of radioactivity excreted during a certain time interval rather than on the quantities of individual metabolites. Nevertheless, they indicate that cyoctol and/or cyoctol-derived compounds containing the labelled carbonyl group are fairly rapidly eliminated from the systemic circulation.

The amounts of radioactivity retrieved in the feces samples are given in Table 1. The sublimated water, removed from the feces samples by lyophilization, contained no radioactivity. The efficiency of the combustion process was 95%, and the memory effect was less than 0.01%. The amounts in the individual samples indicated that fecal excretion was complete as well, but due to the low number of data points, no further pharmacokinetic analysis could be performed.

Fig. 2. Disposition of [^{14}C]cyoctol-derived radioactivity in the stratum corneum. See text for details. A–D correspond to volunteers 1–4, respectively.

Radiochromatograms of urine and ipsilateral plasma samples demonstrate that radioactivity is not present as unchanged cyoctol, but as more polar metabolites in the urine and a less polar product in ipsilateral plasma (chromatograms not shown). This indicates complete metabolic conversion of cyoctol during skin passage. Although the identity of the metabolite is as yet unknown, it may well be devoid of anti-androgen activity (De Zeeuw et al., 1990). If the latter can be confirmed, the dermal application of cyoctol would have an added advantage in that the complete metabolic conversion of the drug prior to reaching the general circulation would exclude adverse systemic effects.

Acknowledgments

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