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DISTRIBUTION KINETICS OF TRITIATED THYMIDINE IN MARMOSETS*

by

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Tritiated thymidine (H^3-TDR) has been used extensively as a label for DNA in autoradiographic studies of cell population kinetics. In mammalian tissues the autoradiographs are usually analyzed under the assumption that the introduction of H^3-TDR into the organism results in "pulse labeling", i.e. the time interval in which the label is available is negligible compared to the duration of the DNA synthesis phase (S-phase). The mechanism involved in the uptake of label into nuclear DNA is also assumed to satisfy the conventional tracer conditions, viz.: 1) labeled precursor mixes with unlabeled endogenous precursors in such a way that the cells are unable to distinguish labeled from unlabeled precursors; 2) the presence of exogenous precursors, labeled or unlabeled, does not affect the time at which cells enter DNA synthesis, the rate of synthesis or the duration of synthesis. The number of cells labeled equals the number of cells in S-phase at the time of injection only if these conditions are satisfied. It is, therefore, clearly important to know for how long H^3-TDR remains available in the bloodstream after its administration.

Several workers have investigated the fate of H^3-TDR in mammalian organisms. *Messier & Leblond* (1960) found that the con-

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centration of H^3-TDR in the bloodstream of rats reached a peak 20 minutes after subcutaneous injection. This was followed by a rapid fall, and a total plasma clearance was obtained after 60 minutes. *Quastler & Sherman* (1959) reported, on the basis of grain counts, that thymidine uptake into intestinal crypt cells of mice was almost completed 16 minutes after intraperitoneal injection. *Rubini et al.* (1960) followed the fate of H^3-TDR in the bloodstream of humans after intravenous injection and found that more than 90 % of the injected thymidine was either incorporated into DNA or catabolized 1—2 minutes after the injection. A grain count analysis of autoradiographs from bone marrow cells showed that the maximal grain count was not reached until 60 minutes after injection, but that only a small increase was found between 10 and 60 minutes. *Quastler & Kember* (1963) studied the clearance of H^3-TDR from the peritoneal cavity in mice by rinsing the peritoneal cavity with Ringer's solution at various times after intraperitoneal injection and determining the H^3-activity in the peritoneal washings. According to this investigation, the half-life of the tritiated thymidine in the peritoneal cavity was less than 1 minute. Recently *Staroscik et al.* (1964) investigated the availability time of H^3-TDR after intravenous administration in mammary tumor cells of mice. From the derivative of the grain count development, these workers suggest an availability time of approximately 60 minutes.

It is not surprising that differences are found in the results of various investigators. Clearance from the blood plasma and from the peritoneal cavity cannot be expected to be the same; the differences in methods of administration, viz. subcutaneous, intraperitoneal and intravenous, are also likely to yield variations in the rate of uptake of the tracer. If, however, one intends to use grain counts to evaluate quantitative changes in the labeled DNA content of cells, information on the rate of uptake and of possible variations in this rate is of considerable importance.

The four-factor model of the ultimate metabolic steps leading to DNA synthesis (*Quastler,* 1963) and the elaboration and experimental test of this concept (*Quastler et al.,* 1964) show that the amount of label incorporated into DNA is a function not only of the total amount of H^3-TDR injected, but also of its specific activity and its rate of uptake. For the reproducibility of

grain counts it is consequently of importance to use a method of administration of tracer that not only satisfies the conditions of pulse labeling, but also leads to the most consistent rate of uptake.

Intraperitoneal injection of H^3-TDR has so far been used in most animal experiments carried out for autoradiographic purposes. The intraperitoneal injection is easy to carry out, even in the "hot-box", and the results of *Quastler & Kember* (1963) and of *Quastler & Sherman* (1959) suggest a fast rate of uptake by this injection method. There are, however, certain disadvantages related to this method of injection that could endanger the quantitative reproducibility of grain counts in autoradiographs, e.g. the sensitivity to irritation of the mesothelium of the peritoneal cavity. *Quastler & Kember* (1963) showed that an injection of physiological saline at body temperature prior to the H^3-TDR injection slows down the disappearance rate of the thymidine from the peritoneal cavity. These workers also found that the rate of uptake is influenced by the volume of the injection. Another factor not to be overlooked, especially when dealing with small animals, is the possibility of giving the injection not into the peritoneal cavity, but into fat tissue, or directly into the gut. Intravenous injection seems a more attractive route of administration but it is difficult to perform in the "hot-box" as a standard method. An alternative way of injecting the H^3-TDR would be intramuscularly.

The purpose of the present investigation is to compare the intravenous, the intraperitoneal and the intramuscular administrations of H^3-TDR by following the fate of the H^3-TDR in the blood plasma and by analyzing the grain count distributions on autoradiographs. The grain count analysis is based on the assumption that the grain counts of labeled cells are proportional to the content of labeled DNA in these cells and that the amount of labeled DNA will continue to increase as long as the precursor is available in the bloodstream.

MATERIAL AND METHODS

The experiment was carried out on 18 marmosets. The marmoset has been used previously in autoradiographic studies by *Skougaard & Beagrie* (1962). This monkey has proved useful for

Fig. 1. Cotton topped marmoset, oedipomidas oedipus. Male, 340 g.

tracer work, combining a small size (200– 400 g body weight) with the characteristics of a primate.

The marmoset is of the family Hapaloidae. According to *Hill* (1957) the definition of the marmoset is: "Small, aboreal, quadropedal platyrrhius of squirrel-like habit with a soft pellage, long

nonprehensile tail; with ovoid cranium, dental formula $\frac{2}{2}\frac{1}{1}\frac{3}{3}\frac{2}{2}$ = 32, with large ears (often adorned with tufts), with fore-limbs shorter than hind-limbs, with the manus and pes elongated and terminating in digits provided (except for the hallux) with pointed falcate nails, with the pollex not opposable but provided with a flat nail." *Hill* (1957) described 40 varieties of the species. In the present study the cotton topped marmoset, oedipomidas oedipus, was used (Fig. 1).

The H^3-TDR had a specific activity of 3.6 curie per mmole, with the tritium located in the methyl group of the pyrimidine ring, the solution containing 1 mc H^3-TDR per ml. The C^{14}-thymidine (C^{14}-TDR) had a specific activity of 30.0 mc per mmole and was labeled in the 2 position of the pyrimidine ring. The solution contained 0.1 mc C^{14}-TDR per ml.

The following procedure was used to obtain the blood samples. The left femoral artery was carefully dissected free in the femoral triangle and closed by placing a clamp on the vessel at the proximal end of the incision. The wall of the artery was pierced with a Keith abdominal needle (1¾), and a polyethylene catheter (P.E. 10) was directed into the artery. After release of the arterial clamp, the catheter was pushed without difficulty into the aorta for some 10 cm (Fig. 2). The other end of the tube was connected to a ½ ml tuberculin syringe via a 27 gauge hypodermic needle. Catheter, needle and syringe had previously been heparinized. This system allowed normal blood circulation even in the leg distal to the insertion, and it was possible to obtain ½ ml blood samples in 15 seconds whenever necessary. The operation was carried out under ether anaesthesia combined with a prior injection of tranquilizer (Sernyl, Park Davies and Co.). After the insertion of the catheter, the ether anaesthesia was discontinued. The effect of the tranquilizer was now sufficient to keep these highly excitable animals calm and apparently without discomfort for as long as 48 hours.

The marmosets were given 5 ml saline intraperitoneally after 6 hours and again after 12, 24 and 36 hours. A glucose concentrate was given perorally by pipette every 3 hours. The bleeding caused by the operation was negligible and a few seconds after the catheter was inserted no bleeding at all took place.

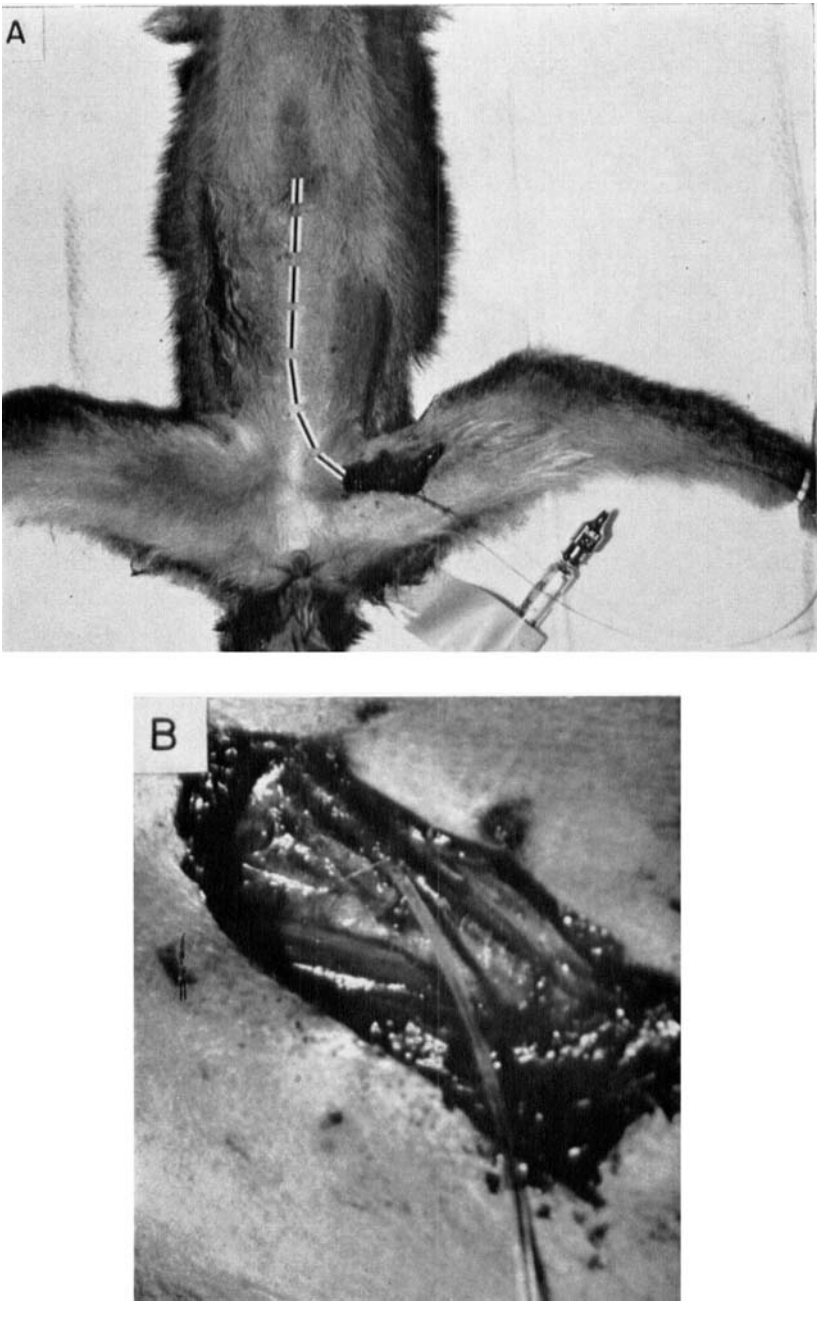


Fig. 2. A. The polyethylene catheter in position in aorta as indicated by the dotted line. B. The incision and the insertion of the tubing into the femoral artery.

Table 1

Marmosets and tracers used in the experiment listed with the time intervals between injection and bloodsamples and between injection and biopsies.

Animal no.	Type of inject.	Tracer	Time of blood samples minutes after inject.	Time of biopsies minutes after inject.
1	i. v.	H^3-TDR	1, 3, 4, 10, 20, 60, 120, 240	1, 7, 30, 60, 120
2	i. v.	H^3-TDR	1, 3, 5, 10, 30, 60, 120, 180, 360, 18h, 24h, 48h	2, 10, 30, 45, 60, 120
3	i. v.	H^3-TDR	1.5, 6, 15, 30, 60, 120	2, 5, 10, 25, 45, 90
4	i. p.	H^3-TDR	2, 5, 10, 19, 30, 45, 60, 90, 120	1, 6, 22, 40, 60, 120
5	i. p.	H^3-TDR	1, 5, 10, 15, 30, 60, 120, 240, 360, 480	1, 10, 20, 45, 60, 120
6	i p.	H^3-TDR	1, 5, 15, 30, 60, 120, 240, 420	1, 5, 15, 30, 120
7	i. m.	H^3-TDR	1.5, 3, 5, 10, 15, 25, 45, 60, 90, 120	7, 15, 30, 60, 90
8	i. m.	H^3-TDR	2, 10, 30, 60, 120, 240, 360	2, 10, 60, 120
9	i. m.	H^3-TDR	1, 5, 10, 20, 40, 60, 120, 240	5, 15, 30, 60, 90, 120
10	i. p.	C^{14}-TDR	1, 5, 11, 25, 45, 75, 150, 245	
11	i. p.	THO	5, 20, 120, 24h, 48h, 120h	
12	i. p.	THO	10, 60, 120, 24h, 72h, 144h	
13	i. v.	H^3TDR	3, 10, 25, 45, 90	
14	i. v.	H^3TDR	5, 15, 35, 60	
15	i. p.	H^3TDR	3, 10, 21, 45, 120	
16	i. p.	H^3TDR	5, 15, 30, 60	
17	i. m.	H^3TDR	3, 12, 43, 75	
18	i. m.	H^3TDR	6, 18, 35, 65	

After the catheter was established, the H^3-TDR was injected either intravenously, intramuscularly or intraperitoneally (Table 1, animal nos. 1—9). In the 3 marmosets injected intravenously, the injections were given in the right femoral vein. The intramuscular injections were given in the right thigh muscle. The dose of the injected H^3-TDR was 2 μc per g of body weight and

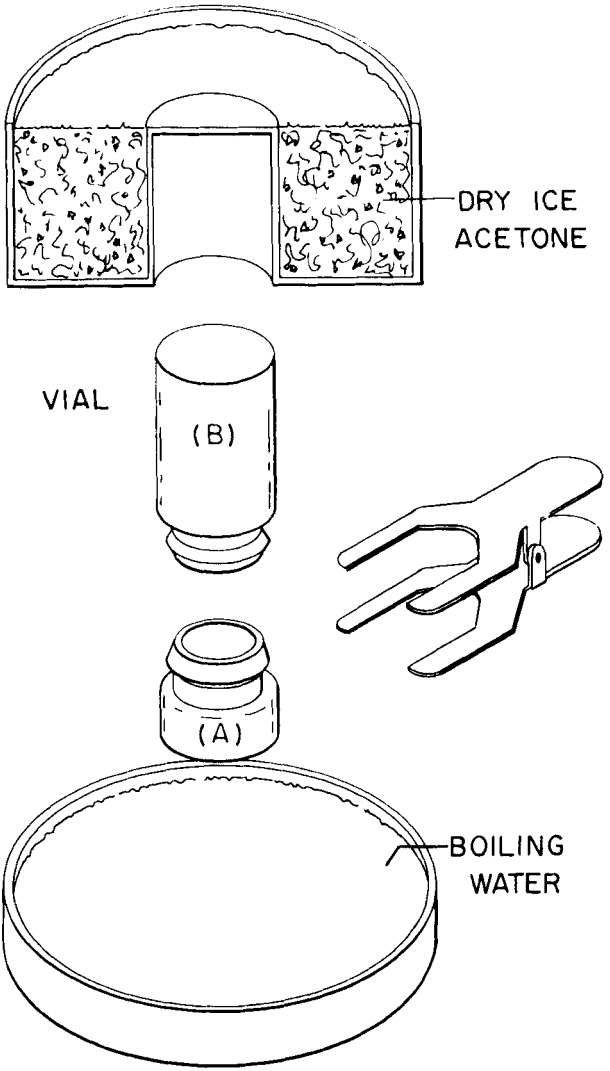


Fig. 3. Device used to collect volatile plasma components in the liquid scintillation counting vial (B). When sealed by the clamp the two vials, A and B, fit closely.

of the C^{14}-TDR, 0.2 μc per g. Prior to the injections, the thymidine solution was preheated to body temperature.

Blood samples were taken at intervals from 1 minute to 48 hours after the thymidine injections (see Table 1). After removal

of needle and plunger, the syringe containing the blood sample was sealed at both ends by rubber stoppers. The samples were then centrifuged in the syringes for 30 minutes at 3000 rev/min; 100 λ of the plasma were pipetted into a counting vial, and 3.9 ml absolute alcohol and 16 ml 0.3 % phenylbiphenyloxadiazole (PBD) xylene were added to the plasma samples. Exposure of the samples to air was kept at a minimum to avoid exchange of volatile tritium components. From the remaining plasma, 100 λ were pipetted to a small vial (Fig. 3 A) 20 mm high and specifically made for this purpose. The opening of this vial was immediately sealed to a standard vial (B) by a clamp (see Fig. 3). The fitting surfaces of both vials were of ground glass to give an exact fit. The vials were now placed A in boiling water and B in a copper cap containing a dry ice-acetone mixture. After 5 minutes, the vials were taken apart and vial B, now containing the volatile plasma components, was quickly sealed. Absolute alcohol and PBD xylene were added as previously described.

The tritium activity of both vials, viz. those containing the plasma samples as well as those containing the volatile components, was determined in a liquid scintillation spectrometer (Packard Tri-Carb Control Model 314). In order to determine the efficiency of the spectrometer, a standard was made by diluting 25 λ H^3-TDR solution with distilled water to a volume of 100 ml. The activity of 100 λ of the dilution was determined as described. All samples, with the exception of a few with a very low activity, were counted for a period of time sufficient to reach a minimum of 10^4 counts. Background and standard were determined for each day of counting and corrections to the results made accordingly. Prior to this experiment a known amount of tritiated water was transferred from vial A to vial B by the above method and was shown to condense in the B vial without any loss of activity.

In 3 of the animals the urine was collected through a catheter at the end of the experiment. The abdomen of the animal was opened to collect all urine present and the volume of the urine was measured. None of the 3 marmosets had urinated during the course of the experiment. In 1 marmoset an injection of 0.2 μc C^{14}-TDR per g body weight was given intraperitoneally. The activity of the plasma was determined after acid (HCl) addition to

approximately pH 4, in order to remove possible contents of C^{14}-labeled carbonates or bicarbonates.

Paper chromatography

For the purpose of analyzing the nonvolatile H^3-labeled plasma components, 6 marmosets were injected with 2 μc H^3-TDR per g body weight (Table 1, animal nos. 13--18). Two of these animals were injected intravenously, 2 intraperitoneally, and 2 intramuscularly. Two ml blood samples were taken 3, 10, 22, 45, and 90 minutes after the injection. The blood samples were immediately cooled to 4°C and after centrifuging, the plasma proteins were precipitated with trichloracetic acid. After centrifuging, the supernatant was carefully neutralized to pH 7.0 with 1 N sodium hydroxide, lyophilized and reconstituted to 200 μl. This solution was then analyzed by ascending paper chromatography using ethyl acetate, water and formic acid solvent. Nonradioactive thymidine and thymine carriers were added to the sample. The spots containing these components were identified under ultraviolet light, cut out and the radioactivity determined in the liquid scintillation spectrometer using Bray's solution. [Naphthalene, 2,5-diphenyloxazole, para-bis 2,5-phenyl-oxazolyl benzene, methanol, ethylene glycol and p-dioxane (*Bray* 1960)]. In addition to the thymidine and thymine spots, the columns were cut in 2 cm pieces and the activity of each piece determined.

Tritiated water injection

For the determination of the total body water phase in the marmosets, 2 animals were each injected intraperitoneally with tritiated water (THO) (Table 1, animal nos. 11 and 12). By counting a diluted sample in the scintillation spectrometer, the activity of the THO was determined to be 24×10^5 cpm/100 λ or 0.14 mc/ml. Blood samples were taken at time intervals as shown in Table 1 by the technique already described. After the first samples were obtained, the needle was removed, the tube sealed by a knot, the incision sutured, and the whole area carefully bandaged. No bleeding took place and the animals showed no sign of discomfort. After about 2 hours they recovered from the anaesthesia and moved freely in the cage. They ate and drank ap-

parently normally and did not lose weight. The H^3-activity of the blood plasma was determined as described.

In 2 of the marmosets, gas phase counting was carried out on lyophilized samples of liver, kidney, muscle and skin. For each of these organs the total weight of the organ and the weight of the sample before and after lyophilizing was determined. The H^3-activity of the samples was assayed in a glass proportional counting tube according to the method described by *Christman* (1957). The liver, kidney and muscle samples were not rinsed in order to preserve water-soluble nonvolatile H^3-labeled components in the tissue. The samples from the skin, taken from 4 different areas of the body and a sample from the gut, were weighed and then fixed in 10 % neutral buffered formalin for 48 hours, rinsed in running water 24 hours and then lyophilized. This procedure was followed in order to remove all catabolic H^3-labeled products and assay only the H^3-labeled DNA of the skin.

Autoradiography of biopsies

At different time intervals after the H^3-TDR injection, biopsies were taken with a "punch biopsy needle" (Table 1). The biopsies were taken from the vestibular attached gingiva immediately apical to the cemento-enamel junction. As no pathological downgrowth of the cuff epithelium was found in these animals, the biopsies did not hold any cells from the epithelial attachment. The tissues were fixed in Carnoy's fixative for 24 hours, transferred to 70 % alcohol at 4°C for 48 hours and then stained *in toto ad modum* Feulgen. Under the dissecting microscope the connective tissue was carefully peeled off and squash preparations made according to the method described by *Wimber & Lamerton* (1963). The squashes were dipped in Kodak NTB liquid emulsion and exposed in moisture-free atmosphere for 11 days following the method of *Elias* (1964). After the photographic processing, grains were counted on a minimum of 1000 cells on each slide. All cells were registered with the number of grains over the nuclei. For the correction of background, autoradiographs of unlabeled squashes were made together with the labeled material. A statistical analysis of the data was performed according to a computer program written by Dr. K. Thompson for the IBM 7094. The

correction for background was made by the computer in accordance with the probability distribution of the number of grains per nucleus following the method described by *Bresciani & Thompson* (1964).

In this study, squash preparations of tissue were preferred to sections because grain counts from a squash give a more accurate expression of the content of the labeled DNA in the nuclei. The reason for this is that all cells in a squash preparation are in direct contact with the emulsion (*Wimber et al.* 1960; *Quastler* 1963).

RESULTS

H^3-plasma activity

The H^3-activities in the plasma from the marmosets, measured by the scintillation spectrometer are shown in Tables 2—10 and some of the data are plotted on semilogarithmic scales in Figs. 4—6. Figure 4 demonstrates the results from one of the intra-

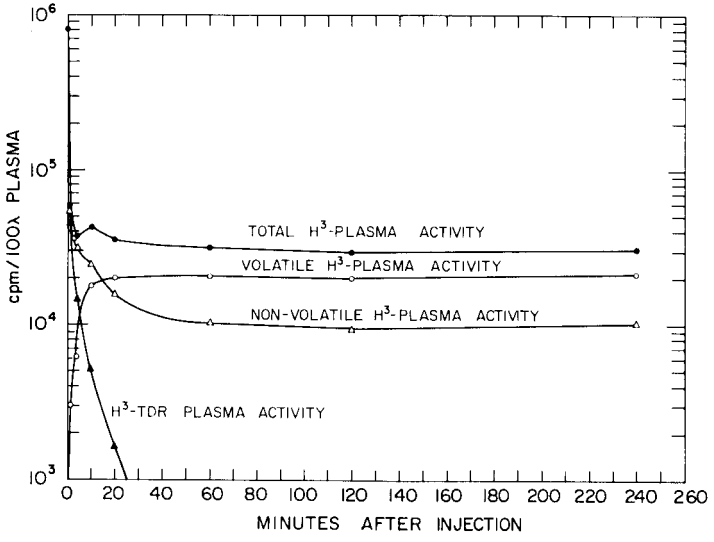


Fig. 4. Semilogarithmic graph showing the H^3-activities in the blood plasma vs. time after intravenous injection of H^3-TDR. The total and the volatile H^3-activities are measured in a liquid scintillation spectrometer. The non-volatile H^3-activity is determined as the difference between the total and volatile activities. The H^3-TDR activity is determined from the nonvolatile activity as described on page 24.

venously injected marmosets. The total H^3-activity per 100 λ plasma shows a decrease from nearly 60,000 counts per minute (cpm) 1 minute after the injection, to 35,000 cpm after 3 minutes. This fall in activity becomes even more striking when the initial activity is calculated. This is readily done when the plasma volume and the efficiency of the tritium counting in the scintillation counter are known. The plasma volume in monkeys, according to *Overman & Feldman* (1957) and *Bender* (1955), is between 36 and 42 ml per kg body weight. The counting efficiency was determined to be 8 % through counting a standard of known activity. These data indicate an initial H^3-activity of over 800,000 cpm if all the injected thymidine were equally distributed in the blood plasma. In other words, more than 90 % of the injected thymidine has disappeared from the circulating blood within the first minute after intravenous injection. The curve shows a slight increase in activity from 3 to 10 minutes followed by a fall until a "plateau" of 30,000 cpm is reached after 30 minutes. In one of the marmosets the experiment was continued for 48 hours, and in this case the plateau was found to be constant from 60 minutes

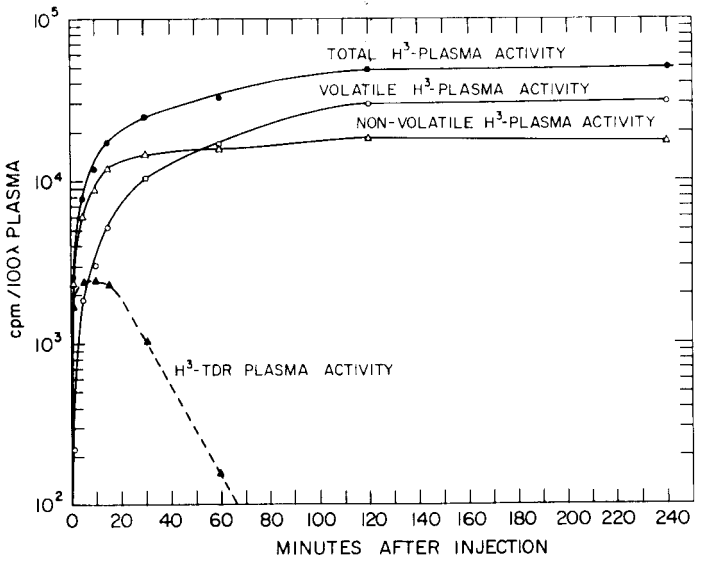


Fig. 5. Semilogarithmic graph showing the H^3-activities in the blood plasma vs. time after intraperitoneal injection of H^3-TDR.

to 8 hours after which it decreased slowly. The total fall in activity in this last period was approximately 20 %. The volatile activity rose from 3000 cpm at 1 minute to a plateau of 20,000 cpm 20 minutes after the injection. This plateau shows a slight but steady increase over the period observed.

The nonvolatile plasma H^3-activity, found as the difference between the total and the volatile activities, shows a rapid fall during the first minutes, a less steep slope in the interval between 4 and 30 minutes and then reaches a level of approximately 10,000 cpm.

Figure 5 shows the curves from an intraperitoneally injected marmoset. In this animal the total, as well as the volatile H^3-activity, increased for more than 2 hours after the injection. As in the case of the intravenously injected animals, the data here

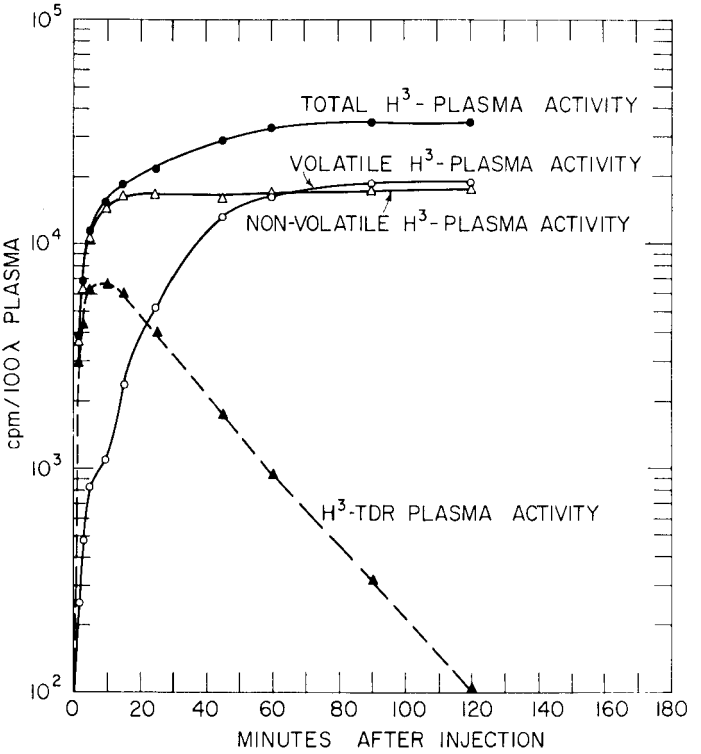


Fig. 6. Semilogarithmic graph showing the H^3-activities in the blood plasma vs. time after intramuscular injection of H^3-TDR.

Table 2

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1	58.286	3.059	55.227
3	36.011		
4	37.229	6.235	30.994
10	42.643	17.740	24.903
20	35.417	19.943	15.474
60	31.194	20.867	10.327
120	29.109	20.016	9.093
240	31.360	21.091	10.269

Table 3

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1	57.498	2.324	55.174
3	35.916	5.991	29.926
5	20.981	8.788	12.193
10	25.091	11.332	13.759
30	25.682	14.337	11.345
60	24.628	17.885	6.743
120	25.032	18.446	6.586
180	24.283	18.433	5.850

Table 4

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1.5	56.508	6.152	50.356
6	52.834	16.095	36.739
15	46.968	23.088	23.880
30	44.896	27.389	17.507
60	46.839	30.418	16.421
120	46.160	31.157	15.003

Tables 2—4 showing the total, volatile and nonvolatile H^3-plasma activities from the blood samples of the intravenously injected marmosets No. 1—3. All activities are given as cpm/100 λ plasma.

Table 5

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
2	3.019	228	2.791
5	16.640	1.291	15.349
10	21.216	3.493	17.723
19	32.948	8.162	24.786
30	38.851	13.168	25.683
45	42.428	21.312	21.116
60	48.502	27.482	21.020
90	51.348	30.710	20.638
120	51.624	31.281	20.343

Table 6

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1	2.572	221	2.351
5	7.921	1.862	6.059
10	11.954	3.017	8.937
15	17.240	5.180	12.060
30	24.996	10.510	14.486
60	32.643	16.696	15.947
120	48.071	29.550	18.521
240	48.526	30.912	17.614
360	48.922	31.023	17.899

Table 7

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1	1.480	333	1.147
5	14.328	2.393	11.935
15	41.561	12.462	29.099
30	52.666	20.417	32.249
60	57.193	31.138	26.055
120	57.585	37.388	20.197
240	56.535	38.511	18.024

Tables 5—7 showing the total, volatile and nonvolatile H^3-plasma activities from the blood samples of the intraperitoneally injected marmosets No. 4—6.

Table 8

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1.5	3.910	252	3.658
3	6.862	486	6.376
5	11.600	827	10.773
10	15.726	1.086	14.640
15	18.372	2.369	16.003
25	21.612	5.211	16.401
45	29.007	13.270	15.737
60	33.055	16.179	16.876
90	34.930	17.425	17.505
120	34.861	17.393	17.468

Table 9

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
2	4.170	126	4.044
10	12.356	650	11.706
30	23.388	2.817	20.571
60	32.348	7.380	24.968
120	41.349	19.686	21.663
240	51.080	30.589	20.491
360	52.514	31.874	20.640

Table 10

Minutes after injection	Total H^3-activity	Volatile H^3-activity	Nonvolatile H^3-activity
1	799	196	603
5	7.023	1.362	5.661
10	16.211	3.320	12.891
20	25.095	6.758	18.337
40	35.450	17.467	17.983
60	42.842	23.926	18.916
120	45.690	25.619	20.071
240	46.161	26.492	19.669

Tables 8—10 showing the total, volatile and nonvolatile H^3-plasma activities from the blood samples of the intramuscularly injected marmosets No. 7—9.

show a constant plateau for the total and a slight increase of the volatile activity over several hours. The nonvolatile activity reached its peak of 18,000 cpm after 2 hours and decreased slowly after this time.

In Fig. 6 the corresponding data from one of the intramuscularly injected marmosets are demonstrated. This graph is of the same general form as the one shown in Fig. 5. However, the nonvolatile activity is seen to constitute a higher percentage of the total activity than was the case for the intravenously and the intraperitoneally injected animals. This was consistent for all 3 intramuscularly injected marmosets.

Total body water

The data from the 2 marmosets injected intraperitoneally with THO are seen in Table 11 and plotted on a semilogarithmic scale in Fig. 7. The THO appears to be in equilibrium and presumably evenly distributed over the total body water phase of the animal shortly after the injection. The plasma activity measured in the first samples taken 5 minutes to 2 hours after the injection shows for animal no. 11 that 100 λ plasma had an activity of 37×10^2

Table 11

Marmosets No. 11 and 12 tritiated water injection.

Sample no.	Time after THO inject.	cpm./100 λ plasma-B	Time after THO inject.	cpm./100 λ plasma-B
1	5 m	3670	5 m	3577
2	20 m	4060	25 m	3492
3	120 m	3602	3 h	3594
4	48 h	2833	24 h	2872
5	5 d	1404	48 h	2608
6	10 d	773	4 d	1572
7			6 d	1360
8			8 d	936
9			10 d	679

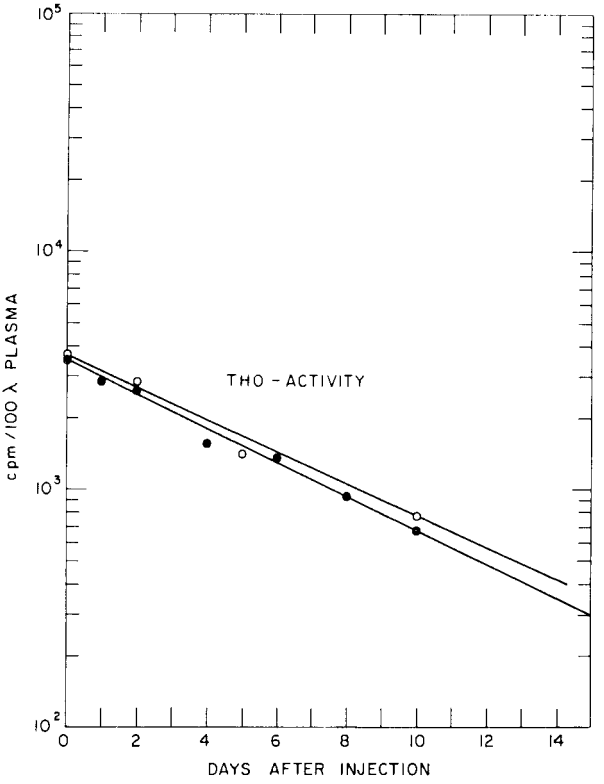


Fig. 7. Semilogarithmic graph of the H^3-activity measured in 2 marmosets injected intraperitoneally with THO. In both animals the activity corresponding to 50 % of the initial activity is found approximately 4.5 days after injection.

cpm. The injected activity being 24×10^3 cpm per g of body weight, 1 g of body weight will contain $\frac{24 \times 10^3}{37 \times 10^2}$ times 100 λ or 66 % water. The same calculation for animal no. 12 yields 69 % total body water. This result is in agreement with the total body water estimation by *Cooper et al.* (1958). These workers find an average of 61 % total body water in man.

The semilogarithmic graph (Fig. 7) indicates an exponential fall in the H^3-activity, with a biological half-life for THO in marmosets of 4.5 days.

Paper chromatography

The results from the paper chromatography are seen in Tables 12—17 and the percentage that the H^3-TDR constitutes of the nonvolatile activity as a function of time is seen in Fig. 8. It is evident from all 6 animals that the degradation of thymidine is rapid; after 3—5 minutes only half of the nonvolatile H^3-activity in the plasma is H^3-TDR, and after 30—40 minutes, less than 10 %. The curves from the intravenously and intraperitoneally injected animals are almost identical, whereas the data from the intramuscularly injected marmosets show a higher percentage of H^3-TDR in the nonvolatile plasma activity at all points than was found in any of the other groups. The largest difference is found between 20 and 60 minutes after injection. In this time interval the H^3-TDR percentage is about 3 times higher following intramuscular injection than after the other 2 methods of administration.

The activities on the chromatographic paper outside the thymidine and thymine spots, were identified according to the R_f values tabled by *Fink et al.* (1956). No attempt was made to separate the activities due to dihydrothymine and β-ureidoisobu-

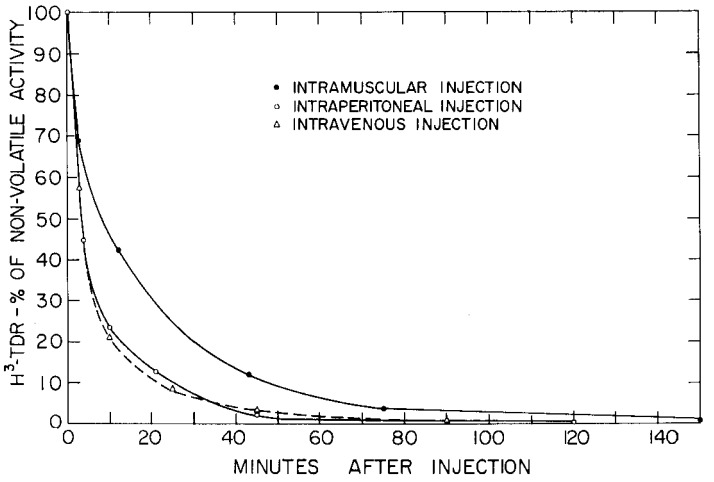


Fig. 8. Graph showing the percentage of H^3-TDR found in the nonvolatile H^3-activity by paper chromatography of blood samples as a function of time after injection of H^3-TDR.

Table 12

Paper chromatography analysis: Marmoset No. 13 intravenous injection.

Min. after injection	H^3-activity							
	H^3-TDR		H^3-T		H^3-DHT + H^3-BUIB		H^3-BAIB	
	cpm	% of total	cpm	% of total	cpm	% of total	cpm	% of total
3	10229	57.2	4667	26.1	2450	13.7	536	3.0
10	2620	21.1	2583	20.8	5825	46.9	1391	11.2
25	802	8.2	812	8.3	6102	62.4	2061	21.1
45	360	3.1	430	3.7	7661	66.0	3163	27.3
90	45	0.7	32	0.5	3058	47.2	3343	51.6

Table 13

Paper chromatography analysis:
Marmoset No. 14 intravenous injection.

Min. after injection	H^3-activity		
	H^3-TDR		Total
	cpm	% of total	
3	15567	63.2	24614
15	1961	16.8	11613
30	563	6.4	8765
60	63	0.9	7118

Table 14

Paper chromatography analysis: Marmoset No. 15 intraperitoneal injection.

Min. after injection	H^3-activity							
	H^3-TDR		H^3-T		H^3-DHT+H^3-BUIB		H^3-BAIB	
	cpm	% of total	cpm	% of total	cpm	% of total	cpm	% of total
4	738	41.8	438	26.6	399	24.2	73	4.4
10	1884	23.2	2037	25.1	3008	37.0	1190	14.6
21	1455	12.8	468	4.1	6909	60.8	2536	22.3
45	332	2.5	106	0.8	8816	66.4	4027	30.3
120	33	0.3	66	0.6	3977	36.4	6851	62.7

Table 15

Paper chromatography analysis:
Marmoset No. 16 intraperitoneal injection.

Min. after injection	H^3-activity		
	H^3-TDR		Total
	cpm	% of total	
5	1039	32.0	3247
20	339	7.9	4299
60	42	0.8	5104
120	—	—	4769

Table 16

Paper chromatography analysis: Marmoset No. 17 intramuscular injection.

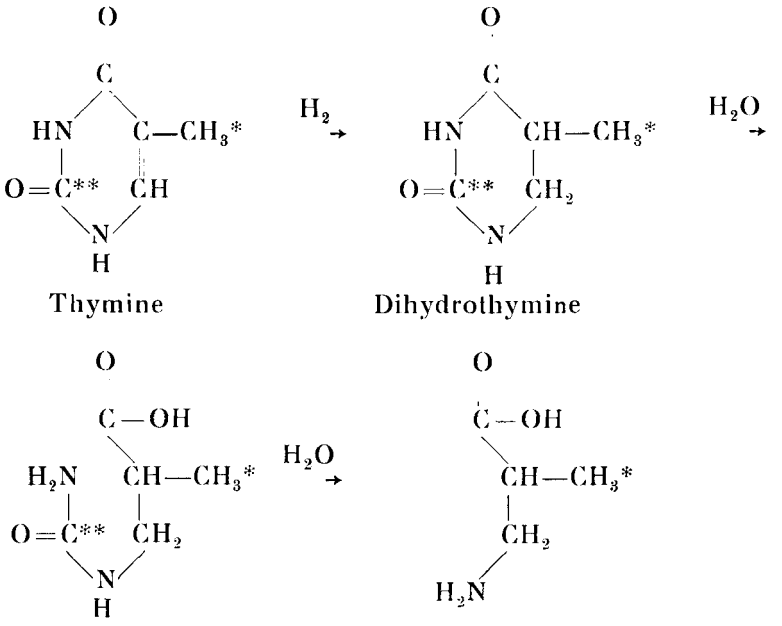
Min. after injection	H^3-activity							
	H^3-TDR		H^3-T		H^3-DHT + H^3-BUIB		H^3-BAIB	
	cpm	% of total	cpm	% of total	cpm	% of total	cpm	% of total
3	1104	68.9	122	7.6	266	16.6	111	6.9
12	1427	42.6	267	8.0	1374	41.0	282	8.4
43	680	12.1	232	4.1	3152	56.2	1543	27.5
75	177	3.7	166	3.4	2928	60.7	1550	32.2
150	56	0.9	37	0.6	2282	36.9	3809	61.6

Table 17

Paper chromatography analysis:
Marmoset No. 18 intramuscular injection.

Min. after injection	H^3-activity		
	H^3-TDR		Total
	cpm	% of total	
5	649	73.3	885
20	514	30.6	1681
60	123	6.1	2008
120	43	1.4	3086

tyric acid, the R_f values of these components being 48 and 47, respectively, for the solvent used. The identity of the nonvolatile H^3-labeled plasma components found in this experiment is in accordance with the catabolism of thymidine as demonstrated by *Friedkin & Roberts* (1954) and by *Fink et al.* (1956). *Friedkin & Roberts* show enzymatic degradation of thymidine to thymine by thymidine phosphorolase, and *Fink et al.* demonstrate the catabolism of thymine to β-aminoisobutyric acid via the following pathway:



It is evident that all these components will contribute to the nonvolatile H^3-activity after injection of H^3-methyl-TDR. According to *Potter* (1959) tritium labeled thymine (H^3-T) does not label DNA and therefore the same is true for H^3-dihydrothymine (H^3-DHT), H^3-β-ureidoisobutyric acid (H^3-BUIB), and H^3-β-aminoisobutyric acid (H^3-BAIB). The data listed in Tables 12,

*position of H^3-label in H^3-methyl-TDR
**position of C^{14}-label in C^{14}-2-TDR

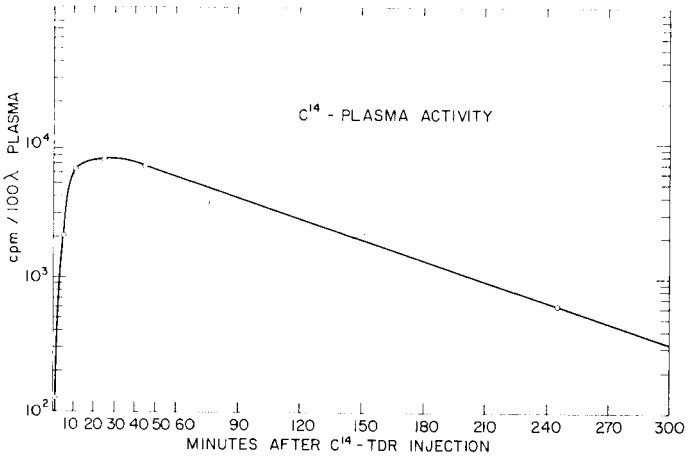


Fig. 9. C^{14}-activity per 100 λ plasma plotted on a semilogarithmic scale as a function of time following intraperitoneal injection of C^{14}-TDR.

14 and 16 show that only a small fraction of the nonvolatile activity is due to H^3-T. The predominating H^3-labeled components appeared to be H^3-DHT and H^3-BUIB in the first 60—90 minutes after injection. The concentration of H^3-BAIB, however, seemed to be increasing and accounted for more than 50 % of the nonvolatile activity after this period.

This is confirmed by the results from the marmoset injected with C^{14}-2-TDR. Following an intraperitoneal injection of H^3-methyl-TDR the nonvolatile activity increases to a maximum and thereafter remains constant for a period of several hours (Fig. 5). The fall in the activity seen in the data from the C^{14}-2-TDR injected animal (Fig. 9) therefore demonstrates the increasing concentration of BAIB, because this component is labeled following the H^3-methyl-TDR injection but unlabeled after the C^{14}-2-TDR injection.

H^3-TDR plasma concentration in marmosets

By applying the H^3-TDR percentages shown in Fig. 8 to the nonvolatile plasma activities listed in Tables 2—10, it is possible to calculate the tritium activity in the plasma due to H^3-TDR as a function of time after injection. The data obtained in this way

Table 18

H^3-TDR activities in marmosets No. 1—9.

Minutes after H^3-TDR injection	H^3-TDR activity cpm/100 λ plasma								
	Intravenous			Intraperitoneal			Intramuscular		
	1	2	3	4	5	6	7	8	9
1	45838	45794			1693	826			513
1,5			38271				2963		
2				1675				3033	
3		17118					4399		
4	14567								
5		4877		5986	2363	4655	6356		3340
6			12859						
10	5255	2903		4785	2413		6588	5268	5801
15			3510		2291	5529	5921		
19				3470					
20	1625								5501
25							3936		
30	884	907	1190	1798	1014	2257		3908	
40									2338
45	342			529			1731		
60	155	101	246	210	159	261	940	1398	1059
90	73			103			315		
120		18	45	41	37	40	104	65	120

are seen in Table 18 and plotted in the semilogarithmic graphs in Figs. 4—6 and 10—12.

The plasma clearance of H^3-TDR following intravenous injection appears to be extremely rapid. This fall in activity is not only due to incorporation of H^3-TDR into DNA and catabolism. A considerable dilution takes place as the thymidine mixes with the extracapillary tissue fluid. Information on whether the thymidine distributes over the total body water or the extracellular fluid is not available. It seems reasonable to assume, however, that the thymidine will be rapidly distributed in the extracellular fluid.

No data are available at the present time on the order of magnitude of the extracellular tissue fluid volume in monkeys. According to *Steele et al.* (1956) the glucose space in dogs is 27 % of the

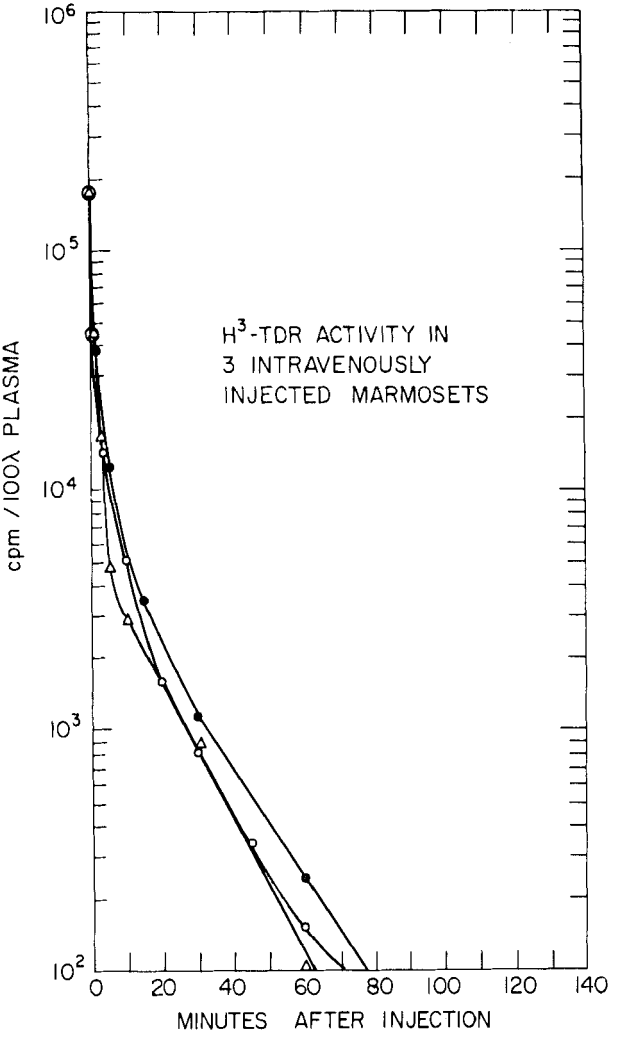


Fig. 10. The calculated H^3-TDR activity per 100 λ plasma vs. time after intravenous injection.

body weight and *Nichols et al.* (1953) have determined the inulin space in humans to be 17.5 % of the body weight. These workers define the inulin space as the plasma water + the rapidly equilibrating water: "the volume of water within a tissue or body which is extracellular, extravascular, not associated with collagen or

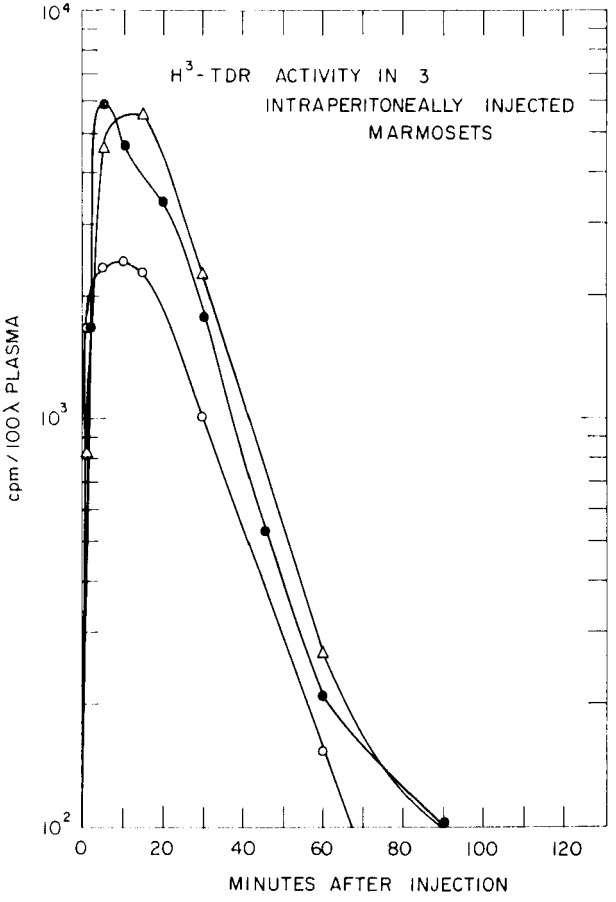


Fig. 11. The calculated H^3-TDR activity per 100 λ plasma vs. time after intraperitoneal injection.

elastin, and is assumed to equilibrate rapidly with the plasma concentration of substances like inulin."

Thus it would seem reasonable to assume that the space where the thymidine will be found in the marmoset is of the order of 20 % of the body weight. On this assumption, provided all the injected thymidine were evenly distributed, the initial H^3-activity would be 177,000 cpm/100 λ. The fall in the H^3-TDR activity within the first minute after intravenous injection due to DNA incorporation plus catabolism is consequently about 75 %. After 10 min-

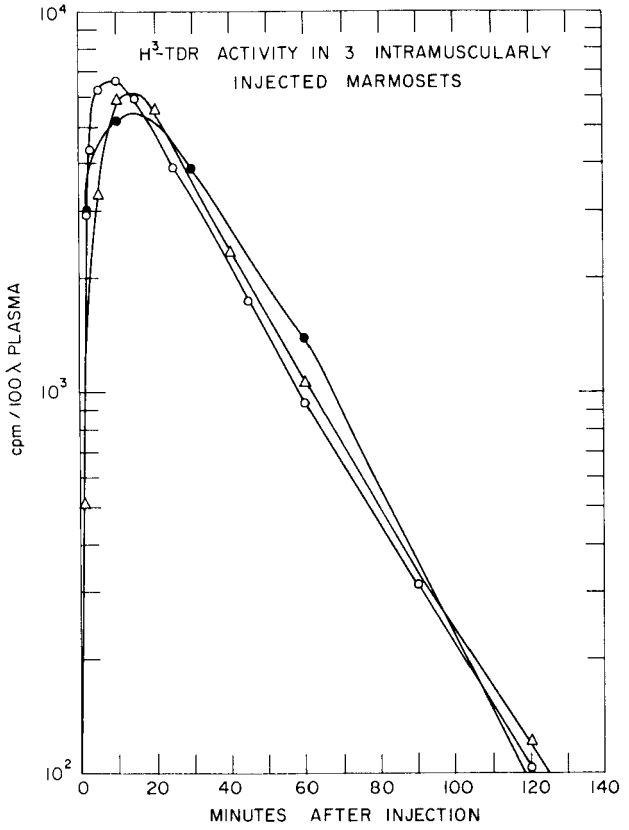


Fig. 12. The calculated H^3-TDR activity per 100 λ plasma vs. time after intramuscular injection.

utes only 3 % of the H^3-TDR is left and after 60 minutes, less than 0.1 %.

The H^3-TDR curves demonstrated in Figs. 5 and 11 for the intraperitoneally injected marmosets show an increase in the activity during the first few minutes until a peak is reached after 10 minutes. The slope in the following part of the curve is similar to the slope found during the same time interval in the curves from the intravenously injected animals. The H^3-TDR activity 60 minutes after injection averages 167 cpm/100 λ plasma in the intravenously injected marmosets and 210 cpm/100 λ in those intraperitoneally injected.

The curves from the intramuscularly injected marmoset in Fig. 12 also indicate a peak in the thymidine concentration 10 minutes after injection, but the maximum is higher than that found in the curve in Fig. 11. Further, the fall in the H^3-TDR activity is slower following intramuscular injection. After 60 minutes the concentration is still approximately 20 % of maximum and the absolute value averages 1132 cpm/100 λ, some 5 times higher than that found in the intraperitoneally or intravenously injected animals. This finding is in accordance with the observation that the nonvolatile plasma activity is relatively higher after intramuscular injection and that the H^3-TDR constitutes a higher percentage of the nonvolatile H^3-labeled components than was the case following intraperitoneal or intramuscular injections.

From Table 18 it will appear that the maximal thymidine concentration in some cases can be higher in an intraperitoneally than in an intramuscularly injected marmoset. This is the case when a rapid uptake occurs from the peritoneal cavity as, for example, in marmoset no. 4 (Table 5) where the nonvolatile plasma activity reaches a high level a few minutes after injection.

An increased rate of uptake into the plasma will result not only in a higher nonvolatile H^3-activity in the first minutes after the injection, but also in an increase in the H^3-TDR fraction of this activity (Fig. 8). From Tables 2—10 and 18 and Figs. 10—12, it is evident that the rate of uptake varies more among the intraperitoneally injected marmosets than within the other groups.

Quantitative evaluation of the fate of H^3-TDR

A. Catabolic H^3-labeled plasma components

The catabolic activities found in the blood plasma (i) the volatile activity, which is assumed to be tritiated water (THO) and (ii) the nonvolatile activity, which after a period of 2 hours will be due to catabolic products only.

1. *Tritiated water.* — The THO will be evenly distributed over the total body water phase of the animals. The fraction of the injected H^3-TDR that is degraded to THO can, therefore, be found through the following calculation:

Let the THO activity be A cpm per 100 λ plasma. A cpm/100 λ $= A \times 10^2 \times \frac{1}{8 \times 60}$ disintegrations per second (dps)/100 λ (counting efficiency 8 %) 1 g of tissue contains 680 mg or 6.8×100 λ water. Therefore, the THO activity per g of tissue will be:

$$A \times 10^2 \times \frac{1}{8 \times 60} \times 6.8 \text{ dps} = A \times 1.42 \text{ dps.}$$

The injected activity, 2 μc per g $= 2 \times 3.7 \times 10^4$ dps/g. The percentage of the injected activity degraded to THO will therefore be:

$$\frac{A \times 1.42 \times 10^2}{2 \times 3.7 \times 10^4} \% = A \times 10^{-3} \times 1.92 \%.$$

The THO percentages calculated in this way are listed in Table 19. These figures vary in the 9 marmosets from 33.3 % to 71.6 %, the average for the intravenously injected animals being 44 %, for the intraperitoneally injected, 63 % and for the intramuscularly injected, 48 % of the injected activity. The average for all nine animals was 52 %.

Table 19

Catabolic H^3-activities as % of injected activity 120 minutes after injection.

	Intravenous			Intraperitoneal			Intramuscular		
	1	2	3	4	5	6	7	8	9
THO	38.3	35.3	59.6	59.7	56.6	71.6	33.3	58.6	50.7
Nonvolatile H^3-activity	5.8	3.3	8.4	11.4	10.0	10.1	9.8	11.6	11.0
Urine			27.5		24.3	15.2	26.3	14.2	
Liver			4.0		4.3			3.8	

2. *Nonvolatile activity.* — A similar evaluation of the nonvolatile components is possible if it is known how these are distributed in the animals. It seems reasonable to assume the same distribution as for thymidine, viz. in the extracellular fluid. This assumption is supported by the data from the tritium assay of the muscle tissue. Practically no labeled DNA will be found in this tissue. Therefore, the nonvolatile activity measured 2 hours after H^3-TDR injection will be due to H^3-labeled catabolic components in the tissue fluid.

The activity in 1 mg lyophilized muscle tissue was 1230 dpm. One g tissue contains 320 mg dry components and an activity of 320×1230 dpm $= 3.9 \times 10^5$ dpm. The nonvolatile activity found in the blood plasma in the same animal was:

$$15003 \text{ cpm}/100\ \lambda = 15 \times 10^3 \times 10^2 \times \frac{1}{8} \text{ dpm}/100\ \lambda = 1.9 \times 10^5$$

dpm/100 λ. In other words, in 1 g tissue an activity corresponding to that of 210 λ plasma is found, which is in accordance with the assumption of the nonvolatile H^3-activity being distributed in the extracellular water space of approximately 20 % of the body weight.

The percentage of the injected H^3-TDR activity being catabolized to nonvolatile catabolic components will, for a nonvolatile activity of B cpm/100 λ plasma, be:

$$\frac{2 \times B \times 10^2 \times 10^2}{2 \times 3.7 \times 10^4 \times 60 \times 8} \% = B \times 5.6 \times 10^{-4} \%.$$

The percentages calculated in this way are listed in Table 19 showing an average of 9.0 % for the 9 marmosets, the intravenously injected averaging 5.8 %, the intraperitoneally, 10.5 % and the intramuscularly injected, 10.8 % of the injected activity.

3. *Nonvolatile activity in liver and kidney.* — It is generally accepted that the catabolism of thymidine takes place mainly in the liver (*Potter* 1961), and it can therefore be expected that a high activity will be found in this organ. The H^3-activity per mg lyophilized liver tissue was found to be 8800 dpm, about 7 times higher than the activity found in the muscle tissue. The total activity in the liver represented 4.5 % of the injected activity.

The activity in the kidney was also considerably higher than in muscle tissue; 4700 dpm per mg lyophilized tissue. The total activity in the kidney approximated ½ % of the injected H^3-activity.

4. *Activity in the urine.* — The activity in the urine 2 hours after the injection was high, varying from 800,000 cpm/100 λ to 1,400,000 cpm/100 λ. The total activity in the urine varied from 14—27.5 % of the injected activity.

The data in Table 19 show that the sum of the H^3-labeled catabolic products determined in plasma, urine, liver and kidney accounts for 88—99 % of the injected activitv

B. Estimation of H^3-labeled DNA

Several workers have shown that under physiological conditions mitotic activity takes place predominantly in the blood-forming tissues and in the epithelia covering the outer and inner surfaces of the body (*Hughes et al.* 1958; *Messier & Leblond* 1960; *Smellie* 1955).

In a classification of the cell populations in the rat *Messier & Leblond* show 3.6 % labeled cells in the skin and 15.1 % in the duodenal epithelium. *Quastler & Sherman* (1959) report 40—48 % labeled cells in the progenitor intestinal crypt epithelium of mice. It seems reasonable to assume that the skin and the gut together represent considerably more than 50 % of the mitotic activity in the body, and that, therefore, the H^3-DNA content in these tissues will give an estimate of the amount of labeled DNA in the whole body.

The lyophilized samples from the skin of the marmoset showed an activity of 50—70 dpm/mg and from these figures it was calculated that the total H^3-DNA content of the skin accounts for less than 0.1 % of the injected activity.

The activity found in the gut was considerably higher: 1600 dpm/mg lyophilized tissue, the total activity approximately 0.5 % of the injected activity. A sample from the oral mucosa showed an activity of 250 dpm/mg. These figures confirm the impression that only a small fraction of the injected H^3-TDR is being utilized in DNA metabolism. In the marmoset it is less than 1 %.

Grain counts

A. Distribution

The grain count distribution on the autoradiographs of the biopsies is exemplified in the histograms seen in Fig. 13 from one of the intramuscularly injected marmosets. It is evident that the number of cells with high grain counts increases with the time interval between injection and biopsy. After 7 minutes, no labeled cells are found with more than 9 grains, after 15 minutes the maximum is 18 grains, after 30 minutes, 45 grains, after 60 minutes, 57 and after 90 minutes, 68 grains over the most intensely

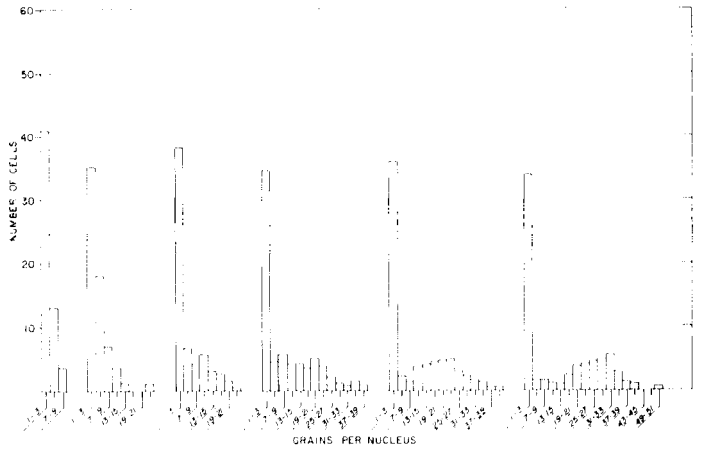


Fig. 13. Histograms showing the grain count distributions in autoradiographs from 6 biopsies taken from the same marmoset at different times after intramuscular injection of H^3-TDR.

labeled nuclei. The same pattern of the labeling was found in the animals injected intravenously or intraperitoneally although the increase in the labeling intensity seemed more rapid in these categories. Figure 14 shows 3 samples of labeling from squash autoradiographs.

1. *"Low-labeled" cells.* — It will appear from the histograms that a high percentage of the labeled cells even in the late biopsies were carrying 1—3 grains only. This category of labeled cells was found on all autoradiographs examined, but the percentage was somewhat lower in the late biopsies from the intravenously and intraperitoneally injected animals.

These "low labeled" cells can be due to the following factors: a) background, b) self-absorption caused by the low range of H^3-β-rays, c) short contact between the cells in DNA synthesis and the labeled precursor, d) slow rate of DNA synthesis 1) during periods of the S-phase or 2) outside the proper S-phase (G-phase labeling), e) low concentration of the labeled precursor or f) a slow turnover of DNA not related to mitotic activity.

a. The data in the present study were corrected for background. It is, however, a possibility that background over a preparation of labeled material might be higher than that found on unlabeled

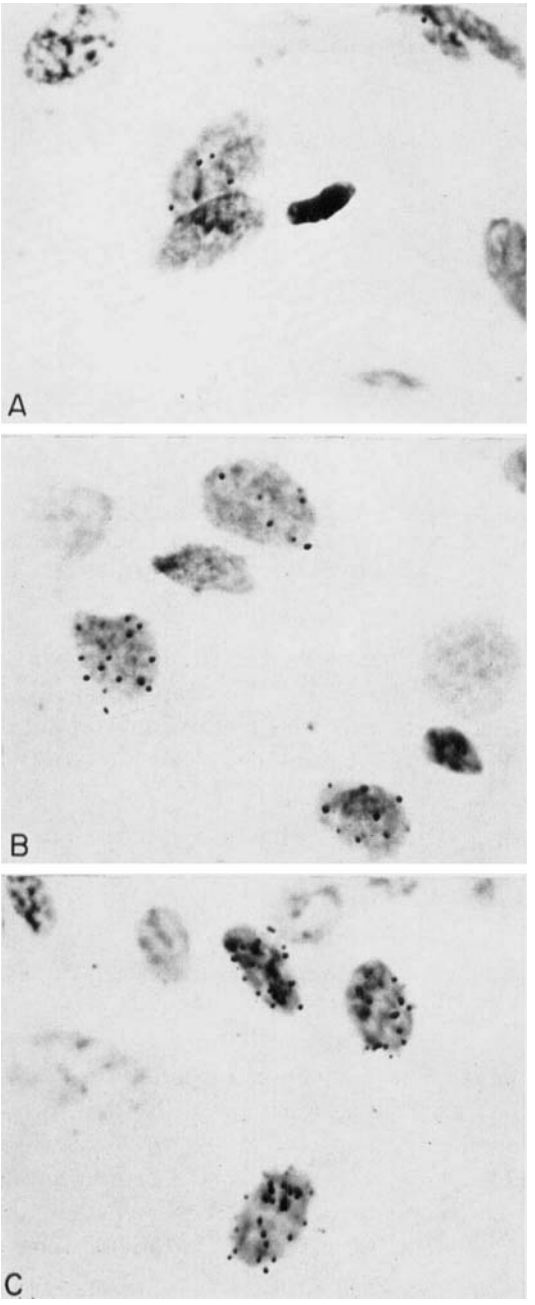


Fig. 14. Labeled cells from squash autoradiographs of biopsies from gingival epithelium taken 7, 30 and 120 minutes after intramuscular injection of H^3-TDR.

material. In order to determine the correct background, an analysis was made of the distribution of grains over and outside the nuclei on the unlabeled control slides. It was assumed that the same distribution of background grains would be found on the labeled autoradiographs and further assumed that on these, all grains outside the nuclei were due to background. It was thus possible from the number of grains outside the nuclei to determine the background. This analysis showed that in only a few cases were backgrounds slightly higher on the labeled than on the control autoradiographs; corrections were made accordingly. It can be assumed, therefore, that the low labeled cells in the histograms are not due to background.

b. In the squash preparations the cells are in equal and close contact with the emulsion. Therefore, the grain count on the labeled cells on the autoradiographs can be expected to be proportional to the contents of labeled DNA in the nuclei (*Quastler* 1963, *Wimber et al.* 1960). This is in contrast to autoradiographs of sections in which the distance from the labeled parts of the nuclei to the emulsion can vary considerably compared to the average range of the β-rays, so that the self-absorption of the β-rays will vary (*Maurer & Primbsch* 1964).

c. The low labeling of the early biopsies is due to the fact that the cells in S-phase have only brief exposure to the H^3-TDR. In the later biopsies the same will be true for cells leaving S-phase shortly after injection and for cells entering S-phase a short time before biopsy. Considering that the duration of the S-phase in the oral epithelia of marmosets is about 7 hours (*Skougaard & Beagrie* 1962), this would only account for a small fraction of the total number of labeled cells and therefore not explain that more than 50 % of the labeled cells belong to this category.

d. 1) Recent results by *Bresciani* (1964) suggest that the rate of DNA-synthesis during the S-phase is not constant. However, the part of the S-phase where the synthesis rate is slow is short compared to the length of the total S-phase, and therefore this feature will only account for an equally small fraction of the total number of labeled cells.

2) *Quastler & Wimber* (1963) have shown that a low rate of DNA-synthesis may take place outside S-phase in intestinal crypt

cells of mice. This phenomenon would provide an explanation for the many low-labeled cells if the period of G-phase, where labeling occurs, is sufficiently long. Contrary to the possibilities mentioned under b., c. and d. 1, the G-phase labeling would explain the lack of continuity in the labeling distribution.

e. The data from the present investigation show a higher frequency of low-labeled cells in the biopsies from the intramuscularly injected marmosets than in those from the other groups. Also, it is a fact that the intramuscularly injected animals show a longer period of time with a relative low plasma concentration of H^3-TDR. It would thus seem reasonable to assume a connection between these observations, i.e. that some of the low-labeled cells in the late biopsies from the intramuscularly injected animals are caused by a period of low H^3-TDR concentration.

f. The type of low-labeled cells described by *Pelc* (1963) as indicating a slow DNA turnover not related to mitotic division is mainly described in nondividing tissues and the grain count is found by Pelc to be only 2 % of the grain count found in S-phase labeling. This is $\frac{1}{5}$ to $\frac{1}{10}$ of the low-labeling intensity found in the marmoset biopsies. It will be assumed therefore that the type of low-labeling described by *Pelc* is not found in the present material from the oral epithelium of the marmoset.

None of these categories of low-labeled cells represent cells synthesizing DNA at a normal rate during the period of time the labeled precursor was available. It was therefore decided to score as labeled, only cells carrying 4 or more grains over the nuclei. An exception to this rule was made for the early biopsies taken less than 5 minutes after the injection of thymidine. On these autoradiographs, all cells with one or more grains after correction for background were considered labeled.

B. Mean grain count

The labeling intensity as a function of the time interval between injection and biopsy can be followed by determining the mean grain count from the autoradiographs. Figure 15 shows the mean grain counts from the biopsies of marmosets nos. 1, 5, and 7, exemplifying the 3 routes of administration. As expected, the

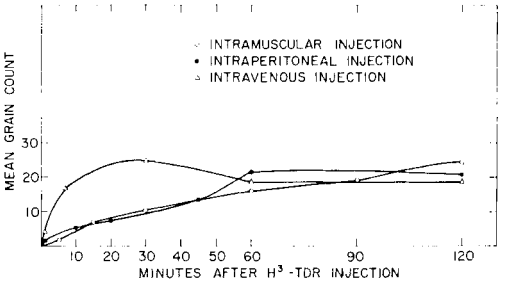


Fig. 15. The mean grain count of gingival epithelium biopsies as a function of time following intravenous, intraperitoneal and intramuscular injection of H^3-TDR.

mean grain count in all 3 animals increased with time. This increase is most rapid in the biopsies from the intravenously injected marmoset. Ten minutes after injection, the mean grain count in this animal had reached the same level as at 60 and 120 minutes after injection. The highest value of the mean grain count was found 30 minutes after injection. This apparent dilution of the labeling intensity between 30 and 60 minutes is clearly not due to cell division, but is an expression of a variability in the yield of autoradiographic grain count — in this case within the same animal following the very same injection. This feature will be discussed later.

The mean grain counts from the intraperitoneally and intramuscularly injected marmosets consistently increase to reach a maximum 60 minutes after the injection for the intraperitoneally injected and 120 minutes for the intramuscularly injected group.

C. Mean grain count compared to plasma H^3-TDR content

Provided all other variables are constant and tracer conditions are met, the content of labeled DNA in the cell nuclei, expressed as mean grain count, should be proportional to the amount of labeled thymidine which has passed through the bloodstream by the time of biopsy. This quantity is equal to the time integral of the plasma H^3-TDR concentration. The curves of plasma H^3-TDR

Table 20

Marmoset no.	Type of injection	Exponential function	Integral 0—120 counts per 100 λ	$\pm \delta$	Mean grain count	$\pm \delta$
1	i.v.	$5.6 \times 10^4 \times e^{-t/17} + 12.4 \times 10^4 \times e^{-t/1.3}$	316,000		18.9	
2	i.v.		361,000		26.1	
3	i.v.	$5.6 \times 10^4 \times e^{-t/15.6} + 12.4 \times 10^4 \times e^{-t/1.09}$	263,000		15.2	
Average	i.v.	$5.6 \times 10^4 \times e^{-t/16} + 12.4 \times 10^4 \times e^{-t/1.2}$	313,000	49,000	20.0	5.5
4	i.p.	$1.5 \times 10^4 \times (e^{-t/13.9} - e^{-t/4.5})$	132,000		16.1	
5	i.p.	$6.8 \times 10^3 \times (e^{-t/16.1} - e^{-t/5.9})$	75,000		10.6	
6	i.p.	$1.6 \times 10^4 \times (e^{-t/13.9} - e^{-t/4.3})$	156,000		20.6	
Average	i.p.	$1.3 \times 10^4 \times (e^{-t/14.5} - e^{-t/4.5})$	121,000	41,000	15.7	5.0
7	i.m.	$1.0 \times 10^4 \times (e^{-t/26.3} - e^{-t/2.8})$	225,000		27.8	
8	i.m.	$1.15 \times 10^4 \times (e^{-t/28.3} - e^{-t/7.4})$	224,000		16.4	
9	i.m.	$1.2 \times 10^4 \times (e^{-t/26} - e^{-t/4.3})$	236,000		24.6	
Average	i.m.	$1.1 \times 10^4 \times (e^{-t/26} - e^{-t/4.2})$	228,000	6,800	22.9	5.8

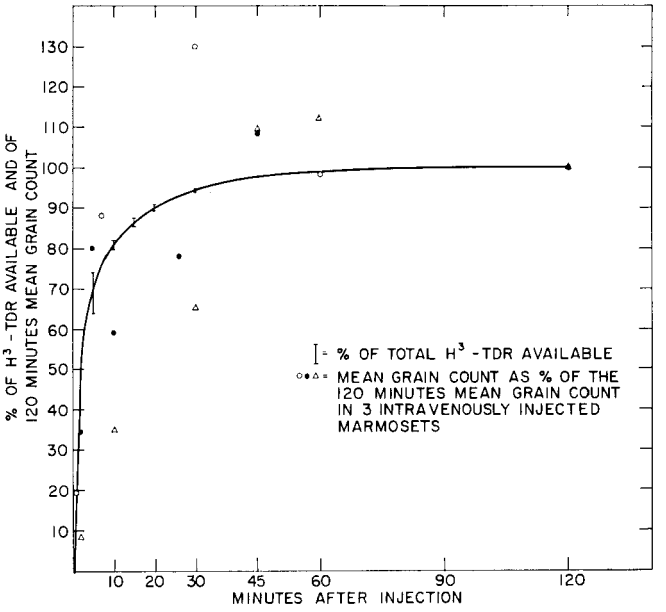


Fig. 16.

Figs. 16—18. The percentage increase in the cumulative H3-TDR activity and the corresponding mean grain counts in percentage of the 120 minute mean grain count as a function of time after injection.

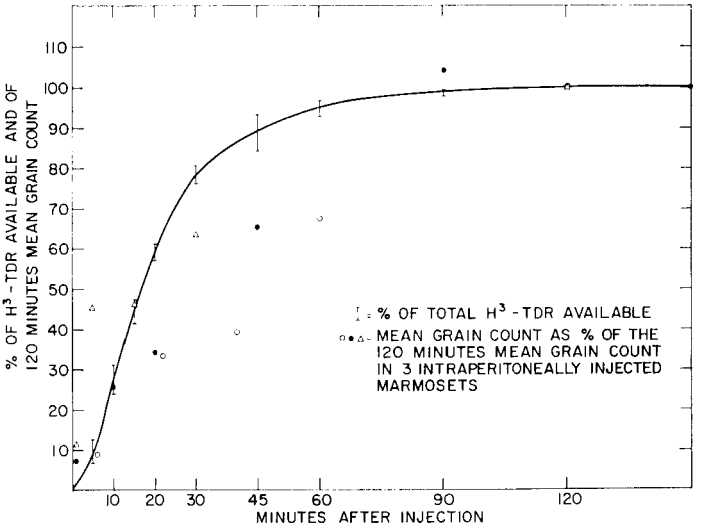


Fig. 17.

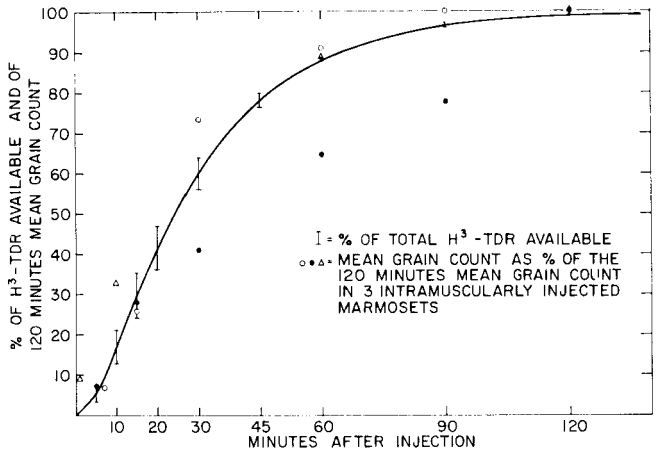


Fig. 18.

activity (Figs. 10—12) closely approximate double exponential functions of the form:

$$y = y_1 \times e^{-t/\tau_1} + y_2 \times e^{-t/\tau_2}$$

for intravenous injection and

$$y = y_0 \times (e^{-t/\tau_1} - e^{-t\tau_2})$$

for intraperitoneal and intramuscular injection. The specific functions for the individual curves have been established by conventional graphical procedures, and are listed in Table 20.

The amounts of H^3-TDR that have been available in the bloodstream at any time t will be proportional to the integrals from 0 to t of these functions. In Table 20 the functions, their integrals from 0 to 120 minutes and the mean grain count from the 120-minute biopsies are listed for the 9 marmosets.

In Figs. 16—18 the percentage increase in the mean grain counts and in the available H^3-TDR is plotted as a function of time for each of the 3 categories. The variability in the yield of grain count from one animal to another is considerably larger

than the variability found in the cumulative plasma activities. The agreement between the two types of data is closest in the intramuscularly injected marmosets.

Evaluation and comparison of the 3 routes of administration

A. Intravenous injection

The H^3-TDR curves for the intravenously injected marmosets agree with the results of *Rubini et al.* (1960) in humans with respect to the rate of plasma clearance. In both cases, an extremely rapid fall in activity with a half-time of less than one minute is followed by a slower exponential decline to effective plasma clearance by 60 minutes. The total tritium activity curves for plasma are also similar in the two experiments. Marmosets and humans both show a brief increase in plasma tritium activity 3 to 10 minutes after injection. This significant feature may be the result of overflow consequent to flooding of the precursor pool mentioned by several workers (*Rubini et al.* 1962, *Feinendegen & Bond* 1962, *Miller et al.* 1964). Another possible explanation is a time factor in thymidine catabolism in the liver. The observed retention of tritium activity in the liver (Table 19) provides some support for this explanation.

There is, however, a major difference between the human and marmoset data. In man, the volatile H^3-activity reaches the same level as the total H^3-activity after 1—2 hours, bringing the nonvolatile activity after this time to zero. In marmosets, an almost constant nonvolatile activity persists for several hours. Furthermore, the fraction of this nonvolatile activity which is H^3-TDR, rapidly falls to low values. Five minutes after injection, less than 50 % is H^3-TDR and after 25 minutes less than 10 % (Fig. 8). The paper chromatography analysis indicates that this long-lasting nonvolatile H^3-activity is caused by H^3-BAIB and other thymidine catabolites (Tables 12—17). In man and in mice, H^3-BAIB is only found in the urine after intravenous injection of H^3-TDR (*Rubini et al.* 1960, *Cronkite* 1964). We presume, therefore, that the persistence of nonvolatile activity in the blood plasma reflects an ability of the marmoset kidney to retain thymidine catabolites such as BAIB.

In the intravenously injected group of marmosets, both the total

amount of H^3-TDR available for uptake and the time course of availability are different from those in the two other groups. The total amount available is 2.6 times larger after intravenous injection than after intraperitoneal injection, and most of this difference occurs during the first few minutes. Ten minutes post injection, the fraction of the eventual total H^3-TDR available which has already been available is 80 % (250,000 counts per 100 λ plasma) for the intravenous group, but only 27 % (33,000 counts per 100 λ plasma) for the intraperitoneal group. After 10 minutes the H^3-TDR curves are almost identical in the two groups.

The mean grain counts from the intravenously injected animals are also higher than those from the intraperitoneal group, but only 25 % higher; the average mean grain count in the last biopsy in the intravenous group is 20 grains per nucleus compared to 16 grains per nucleus in the intraperitoneal group. These figures indicate that the cells in the intravenously injected animals have not been able to utilize efficiently the higher concentrations of H^3-TDR available during the first minutes after injection. This is consistent with the results of *Quastler et al.* (1964) who have shown that in mice the mean grains per nucleus does not increase proportionally with the intraperitoneally injected dose if this exceeds 8 μc per g of body weight for the specific activity of thymidine used in the present experiment. An intraperitoneal injection of 8 μc H^3-TDR per g body weight in mice would result in a maximal H^3-TDR plasma activity of approximately 120,000 cpm/100 λ plasma (*Skougaard & Stewart* 1965) which thus defines the upper limits of the tracer region for H^3-TDR in these units. No data are available defining the tracer region for H^3-TDR in marmosets, but we assume that the initial H^3-TDR concentration of 177,000 cpm/100 λ exceeds this limit and that this explains the relatively low grain count following this type of injection. The extremely rapid change in the H^3-TDR concentration during the first few minutes after intravenous injection is also likely to reduce the reproducibility of the autoradiographs since even slight changes in the local blood supply will cause considerable variations in the total amount of labeled precursor available to the cells during this time. This explains why some of the earlier biopsies from the intravenously injected animals showed a higher grain count than the later biopsies from the same animal.

B. Intraperitoneal and intramuscular injection

In the intramuscularly injected group the total amount of H^3-TDR available for uptake is greater than in the intraperitoneal group (228,000 counts per 100 λ compared to 121,000 counts per 100 λ) and the plasma H^3-TDR activity decreases more slowly. This difference in the cumulative plasma H^3-TDR between the two groups is caused by the more rapid catabolism of H^3-TDR following intraperitoneal injection as demonstrated in Fig. 8. A possible explanation of this could be the fact that a major part of the thymidine after intraperitoneal injection enters the blood circulation via the vena porta system and the liver, resulting in a high rate of catabolism following this route of administration. The differences in the amount of H^3-TDR available and in the availability time are reflected in the grain counts. The mean grain count is higher in the intramuscular group and, in contrast to the intraperitoneal group, continues to increase for more than 60 minutes after injection. Also, as a result of the long availability time, the number of lightly labeled cells is higher following the intramuscular injection. The variability in plasma H^3-TDR is seen to be smaller in the intramuscular group, the standard deviation being 4000 counts per 100 λ compared to 21,000 counts per 100 λ in the intraperitoneally injected group, and as seen in Figs. 17 and 18, the scatter in grain count reflects this.

For the purpose of grain count analysis on squash autoradiographs, intramuscular administration seems to offer advantages with regard to reproducibility, especially when the category or low labeled cells is discarded. If, however, the purpose is to analyze autoradiographs of sections for determining the labeling index, this method of injection is less appropriate in the marmoset. The fact that the H^3-TDR concentration one hour after intramuscular injection is still sufficient to cause labeling adds inaccuracy to calculations based on the labeling index. The conventional formula for determination of the duration of the mitotic cycle time is:

$$T_c = \frac{T_s \times N_c}{N_s} \text{ hours,}$$

where T_s = duration of the S-phase, N_s = number of labeled cells,

N_c = total number of cells involved in the cycle and T_c = cycle time. The N_s one hour following intramuscular injection of H^3-TDR is not equal to the number of cells in S-phase at the moment of injection, however, but includes the cells that have moved into the S-phase during the availability time. A corrected formula with respect to this factor would then be:

$$T_c = \frac{(T_s+1)\times N_c}{N_s} \text{ hours.}$$

Following all types of injection, the variability in the mean grains per nucleus exceeds the variability in the H^3-TDR plasma activity data (Figs. 16—18). This indicates that the variations in availability are only partly responsible for the poor reproducibility in H^3-TDR autoradiography. The small fraction of H^3-TDR utilized in DNA metabolism means that minor variations in the route from the injection site to the S-phase cells will cause a relatively large variation in the specific activity of the DNA precursors in the surroundings of the cells. Since this specific activity is the final deciding factor for the labeling intensity, a high variability of labeling is to be expected for this reason.

SUMMARY

The H^3-activity in the blood plasma of 9 marmosets was analyzed by liquid scintillation counting following intravenous, intraperitoneal or intramuscular injection of tritiated thymidine (H^3-TDR). The volatile plasma components were collected by distillation and counted separately. The total volatile and nonvolatile plasma activities reached plateau levels after 1 to 2 hours in all 9 marmosets. These levels were maintained for several hours. The nonvolatile plasma activity was further analyzed by paper chromatography into several components: H^3-TDR, H^3-T, H^3-DHT, H^3-BUIB and H^3-BAIB. The H^3-TDR component decreased rapidly; 20 minutes after injection only 10 % of the nonvolatile plasma H^3-activity was H^3-TDR.

The H^3-TDR contents of the blood plasma were determined as a function of time after injection. After intravenous injection the plasma H^3-TDR level decreased from an initial maximum concentration to negligible values after about 60 minutes. In the intra-

peritoneally injected animals, the H^3-TDR concentration increased to a maximum 10 minutes after injection, and then fell to zero by 60 minutes post injection. In the intramuscularly injected marmosets, peak levels were also found 10 minutes after injection, but the fall in activity thereafter was slower than in the other groups. Total plasma clearance was not attained until 120 minutes after injection.

By measuring plasma tritium activity in the blood plasma following THO injection, the total body water phase of the marmosets was found to be 67.5 % of the body weight. The biological half-life of THO in the marmoset was found to be 4.5 days.

The volatile activity in the plasma after H^3-TDR injection consisted entirely of THO, and was found to average 51.5 % of the injected H^3-TDR activity. The nonvolatile plasma tritium activity averaged 9 % of the injected activity. The activity found in the urines averaged 21.5 % and that in the liver and kidney 4 % of the injected activity. It was possible to account for up to 99 % of the injected H^3-TDR in these terms. In agreement with these results the total labeled DNA in skin and gut accounted for less than 1 % of the H^3-TDR injected.

Autoradiographs were prepared from squash preparations of gingival biopsies taken at various time intervals after the H^3-TDR injection. The highest mean grain counts were obtained following intramuscular injection, although the amount of H^3-TDR available in the bloodstream was highest following intravenous injection and lowest after intraperitoneal injection. It is suggested that the extremely high H^3-TDR concentration immediately after intravenous injection exceeds the limits of the tracer region so that H^3-TDR is incorporated less efficiently.

For all 3 routes of injection, the grain count data showed more scatter than the values for the H^3-TDR plasma concentration, but the scatter was least for the intramuscular injection.

The variability in the grain counts may be due to the small percentage of H^3-TDR actually incorporated into DNA, since minor variations in catabolism will cause relatively large variations in the small fraction incorporated.

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RÉSUMÉ

CINÉTIQUE DE DISTRIBUTION DE LA THYMIDINE TRITIÉE CHEZ LES OUISTITIS

L'activité H^3 dans le plasma sanguin de 9 ouistitis a été analysée par le compteur à scintillation liquide après injection intraveineuse, intrapéritonéale ou intramusculaire de thymidine tritiée (H^3-TDR). Les composants volatils du plasma furent recueillis par distillation et comptés séparément. Les activités plasmatiques totale, volatile et non volatile atteignent des plateaux après 1 à 2 heures chez les 9 ouistitis. Ces valeurs se maintiennent plusieurs heures. L'activité non volatile fut en outre analysée par chromatographie sur papier en plusieurs composants: H^3-TDR, H^3-T, H^3-DHT, H^3-BUIB, et H^3-BAIB. L'activité H^3-TDR diminue rapidement; 20 minutes après l'injection l'activité H^3-TDR ne représente plus que 10 % de l'activité H^3 non volatile du plasma.

Les taux de H^3-TDR du plasma furent déterminés en fonction

du temps après l'injection. Après injection intraveineuse le taux plasmatique de H^3-TDR diminue d'une concentration initiale maximale à des valeurs négligeables après environ 60 minutes. Chez les animaux injectés par voie intrapéritonéale, la concentration de H^3-TDR s'élève pour atteindre un maximum 10 minutes après l'injection et ensuite tombe à zéro 60 minutes après cette injection. Chez les ouistitis injectés par voie intramusculaire, des valeurs maximales furent aussi trouvées 10 minutes après l'injection, mais ensuite la diminution de l'activité est plus lente que dans les autres groupes. La disparition de l'activité ne fut obtenue que 120 minutes après l'injection.

Par la mesure de l'activité du tritium plasmatique après injection de THO l'eau totale du corps chez les ouistitis représente 67,5 % du poids du corps. La demi-vie biologique de THO chez les ouistitis est de 4,5 jours.

L'activité volatile du plasma après injection de H^3-TDR est constituée entièrement de THO et on a pu montrer qu'elle atteint en moyenne 51,5 % de l'activité de la thymidine tritiée injectée. L'activité tritiée non volatile du plasma atteint en moyenne 9 % de l'activité injectée. L'activité trouvée dans les urines atteint en moyenne 21,5 % et dans le foie et les reins 4 % de la dose injectée. Il est possible dans ces conditions de pouvoir retrouver jusqu'à 99 % de la thymidine tritiée injectée. En accord avec ces resultats tout l'ADN marqué dans la peau et le tube digestif representait moins que 1 % de la thymidine injectée.

Des autoradiographies furent faites de squash de biopsies gingivales prélevées à differents intervals de temps après l'injection de H^3-TDR. Les "mean grain counts" les plus élevés furent obtenus après injection intramusculaire quoique le taux de H^3-TDR disponible dans le courant sanguin fut le plus élevé après injection intraveineuse et le plus bas après injection intrapéritonéale. Ceci suggère que les concentrations extrèmement élevées de H^3-TDR immédiatement après l'injection intraveineuse dépasse les limites de la zone de marquage de sorte que H^3-TDR est incorporée moins efficacement.

Pour les 3 voies d'injection le comptage des "grains" a montré plus de dispersion que les valeurs des concentrations plasmatiques de H^3-TDR mais la dispersion fut la moindre pour la voi intramusculaire.

La variabilité des comptages de grains peut être due au faible pourcentage de H^3-TDR incorporée dans l'ADN, puisque des variations minimes du catabolisme causeront des variations relativement importantes de la petite fraction incorporée.

ZUSAMMENFASSUNG

VERTEILUNGSKINETIK VON TRITIUM-THYMIDIN IN MARMOSETS

Bei 9 Marmosets wurde nach intravenöser, intraperitonealer und intramuskulärer Injektion von Tritium-Thymidin (H^3-TDR) die H^3 Aktivität des Blutplasmas im Flüssigkeits-Scintillationszähler gemessen. Die flüchtigen Plasmakomponenten wurden abdestilliert und getrennt gemessen. Die gesamte flüchtige und nichtflüchtige Plasmaaktivität erreichte ein Plateau nach 1 bis 2 Stunden bei allen 9 Marmosets. Dieser Spiegel wurde für mehrere Stunden aufrechterhalten. Die nichtflüchtige Plasmaaktivität wurde mittels Papierchromatographie in mehrere Komponenten aufgetrennt: H^3-TDR, H^3-T, H^3-DHT, H^3-BUIB und H^3-BAIB. Die H^3-TDR-Komponente nahm schnell ab; 20 Minuten nach Injektion bestanden nur noch 10 % der nicht flüchtigen Plasma H^3-Aktivität aus H^3-TDR.

Der H^3-TDR Gehalt des Blutplasmas wurde als Funktion der Zeit nach der Injektion bestimmt. Nach intravenöser Injektion fällt der H^3-TDR-Plasmaspiegel von einer anfänglich maximalen Konzentration innerhalb 60 Minuten auf vernachlässigbare Werte ab. Bei intraperitoneal injizierten Tieren stieg die H^3-TDR Konzentration 10 Minuten p. i. auf ein Maximum an und fiel innerhalb 60 Minuten p. i. auf Null ab. Nach intramuskulärer Injektion wurden Spitzenwerte ebenfalls in 10 Minuten erreicht, die Aktivität fiel jedoch langsamer ab als bei den anderen Gruppen. Erst 120 Minuten nach Injektion war das Plasma völlig frei von H^3-TDR Aktivität.

Durch Injektion von THO und anschliessende Messung der Tritiumaktivität im Blutplasma wurde das Gesamt-Körperwasser bei Marmosets mit 67,5 % des Körpergewichts bestimmt. Als biologische Halbwertszeit von THO im Marmoset wurde 4,5 Tage gefunden.

Die flüchtige Aktivität im Plasma nach H^3-TDR Injektion bestand gänzlich aus THO. Sie machte im Mittel 51,5 % der inji-

zierten Aktivität aus. Die nichtflüchtige Plasmaaktivität betrug durchschnittlich 9 % der injizierten Aktivität. Im Harn wurden durchschnittlich 21,5 % in Leber und Niere 4 % der injizierten Aktivität gefunden. Danach konnten bis zu 99 % der injizierten H^3-TDR Aktivität wiedergefunden werden. In Übereinstimmung mit diesen Ergebnissen betrug die DNS Aktivität in Haut und Darm weniger als 1 % der injizierten H^3-TDR Aktivität.

Zu verschiedenen Zeiten nach H^3-TDR Injektion wurde Zahnfleischgewebe entnommen und zu Quetschpräparaten verarbeitet zur Herstellung von Autoradiographien. Die höchsten durchschnittlichen Silberkornzahlen wurden nach intramuskulärer Injektion gefunden, obwohl die grösste Menge H^3-TDR im Blutstrom nach intravenöser Injektion verfügbar war und die geringste nach intraperitonealer Injektion. Es wird angenommen, dass die extrem hohe H^3-TDR Konzentration unmittelbar nach der intravenösen Injektion die Grenzen der Tracer Region überschreitet, so dass im Verhältnis weniger H^3-TDR eingebaut wird.

Die Ergebnisse der Silberkornzählung streuten bei allen drei Injektionsarten mehr als die Werte für die H^3-TDR Plasmakonzentration. Die Abweichungen waren jedoch bei intramuskulärer Injektion am geringsten.

Die Streuung der Silberkornzahlen könnte bedingt sein durch den geringen Prozentsatz von H^3-TDR, der tatsächlich in die DNS eingebaut wird, denn geringe Abweichungen im Abbaustoffwechsel werden grosse Schwankungen des kleinen eingebauten Anteils hervorrufen.

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