Absorption, Disposition, and Excretion of ^{3}H-Mineral Oil in Rats

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The fate in rats of ^{3}H-mineral oil was studied after both oral and i.p. administrations. Five hours following a single oral dose of 0.66 ml. of ^{3}H-mineral oil/Kg. about 1.5 per cent of the dose had been absorbed unchanged, and an additional 1.5 per cent of the dose was found in the carcasses as ^{3}H-nonmineral oil substances. The ^{3}H-mineral oil concentration in the carcasses decreased at first rapidly to 0.3 per cent within 2 days post-treatment, and much more slowly, thereafter, to 0.1 per cent of the amount administered by day 21. The absorption and excretion of mineral oil after chronic administration orally was similar to the absorption and excretion after a single dose. The physiologic disposition of the drug, 5 hr. after an oral dose, indicated that the liver, fat, kidney, brain, spleen, and carcass contained ^{3}H-mineral oil. After the i.p. administration of labeled mineral oil, it was excreted very slowly; 11 per cent was found in the feces during the first 8 days post-treatment; only trace quantities were found in the urine. The identity of the mineral oil following oral administration that had been isolated from tissues and excreta was made on the basis of thin-layer chromatography of tissue extracts. The similarity of the physical properties of the extracts of animals that had received mineral oil, i.p. or p.o., supported the view that it was mineral oil that had passed through the walls of the gut. The nonmineral oil nature of the substances containing ^{3}H after the administration of ^{3}H-water indicated that other substances were not mimicking ^{3}H-mineral oil in the assay. ^{3}H-mineral oil was shown to have exchanged ^{3}H with other substances. Metabolism of ^{3}H-mineral oil to more polar substances may also have occurred. The incorporation in the oil of dioctyl sodium sulfosuccinate, an emulsifying agent, tended to increase the amount of ^{3}H-mineral oil absorbed.

MINERAL OIL, liquid petrolatum U.S.P., is a mixture of thousands of compounds (1); it has been employed for over 50 years as a lubricant laxative. Despite its widespread and often chronic use, little is known of the physiologic disposition of this compound. Several reports have been published which have dealt with this problem, but the lack of a suitable assay and the indefinite composition of the mineral oil employed in the various studies made such studies difficult to evaluate, especially quantitatively (2–7). A comprehensive bibliography on this subject has been recently prepared (8). From the work of Stetten (5) and Bernhard and Scheitlin (6) it appeared that 5 to 25% of the administered doses to rats had been absorbed. This report contains data on the absorption, distribution, and excretion of mineral oil by rats that had received an oral or intraperitoneal dose of tritiated mineral oil, randomly labeled.

METHODS

Tritiated mineral oil (^{3}H-MO) which met the standards for liquid petrolatum U.S.P., except for the presence of radioactivity, specific activity 1.52 mc./ml., was administered to Sprague-Dawley and Holtzman rats of either sex at a dose of 0.66 ml./Kg., an amount equivalent to the recommended human dose. In studies of the fate of mineral oil after chronic administration, nonlabeled mineral oil was administered for 31 consecutive days at a dose of 0.66 ml./Kg. Tritiated mineral oil was given as the final dose on the 32nd day, and the animals were sacrificed at intervals thereafter. The effect on absorption of the oil of adding a wetting agent, dioctyl sodium sulfosuccinate, was studied. Radioactivity was measured in the alimentary tracts, carcasses, feces, and urines 24 hr. after the oral administration of tritiated mineral oil containing 1.7 or 8.3 mg./ml. of dioctyl sodium sulfosuccinate.

To study the excretion of the oil rats were placed in metabolism cages where food and water were provided *ad libitum*. Urine and feces were collected daily and stored at −15° until assayed.

In the distribution studies rats were anesthetized with ether and killed by exsanguination at suitable intervals following drug administration. After the hair had been removed with an electric clipper, the alimentary tract was exposed by a midline incision, carefully isolated, and ligated a few millimeters from the oral and anal ends. The tract and the remainder of the rat, defined as carcass, were separately weighed and homogenized with 4 vol. of toluene. In several studies visceral organs were removed for individual assays.

For the assays of radioactivity in the treated animals, tissues, as well as feces, were extracted with toluene 3 times. The residue of each sample, after toluene extraction, was extracted once with *p*-dioxane. Radioactivity that had been extracted from the tissues or excreta was measured in a liquid scintillation spectrometer after portions of the ex-

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tracts had been added to toluene or dioxane scintillation solutions.[1] Radioactivity that could not be extracted from the feces or tissues was measured by combusting portions of the extracted residue by the oxygen flask technique of Kelly *et al.* (10). Radioactivity in urine was measured by counting an aliquot of the urine in the dioxane scintillation solution directly.

This technique made it possible to extract in excess of 98% of the radioactive material from control tissues and from feces to which ^{3}H-mineral oil had been added or from tissues and feces of treated rats.

The presence of ^{3}H-mineral oil in the extracts of the tissues and feces of the treated rats was demonstrated by the use of thin-layer chromatography. The extracts were evaporated at temperatures <45° under reduced pressure to small volumes, usually less than 5 ml. Measured portions were applied to thin-layer chromatography plates of Silica Gel G 250 μ thick. The 30 × 20 cm. plates were activated before use by heating at 120° for 30 min. After activation, the plates were fully developed with Baker's reagent grade benzene to remove interfering impurities (11). The plates were dried at 100° for 5 min. and then spotted. They were developed in hexane–diethyl ether–glacial acetic acid (90:10:1 by volume) (12). ^{3}H-mineral oil was assayed after the plates had been developed by scraping small areas of the chromatogram into vials, adding a toluene or dioxane scintillation mixture, and measuring the radioactivity in a liquid scintillation spectrometer. Under these conditions ^{3}H-mineral oil could be detected on the chromatographic plate at a level of 5×10^{-3} mcg. at R_f 0.90 ± 0.02. The developed plates were visualized when necessary by spraying with 50% aqueous sulfuric acid followed by heating at 100° for 15 min. More polar substances remained at the origin or moved more slowly than mineral oil; they did not, however, separate into discrete areas, but remained as a long smear well separated from the ^{3}H-mineral oil. The quantitative nature of the recoveries was determined by applying appropriate standards to each of the plates before it was developed. Included among the standards were ^{3}H-mineral oil and extracts of control tissues to which ^{3}H-mineral oil had been added at the time the extract was applied to the chromatographic plate.

The lability of the ^{3}H label of the mineral oil was demonstrated by shaking 0.1 ml. of ^{3}H-mineral oil with 1 ml. of aqueous 0.01 *N* HCl. The water layer was carefully separated from the oil and washed 3 times with equal volumes of hexane. The water was vaporized at 95° and atmospheric pressure and condensed on a cold finger chilled with solid CO_2 and acetone. The condensate contained 0.1% of the radioactivity that had been present in the ^{3}H-mineral oil.

In another experiment 80 mg. of ^{3}H-mineral oil was dissolved in 1.0 or 100 ml. of propionic acid. The solutions were allowed to stand at room temperature for 18 hr.; they were then made alkaline by the addition of sodium hydroxide, 1.7 ml. to the first solution, and 17.0 ml. to a 10-ml. aliquot of the second. The alkalinized mixtures were extracted repetitively with heptane until the heptane washes were free of radioactivity. The tritium in the aqueous layers was measured by the use of Bray's solution (9). The aqueous layer of the 1-ml. solution contained 0.89% of the radioactivity, whereas the aqueous layer of the 100-ml. solution contained 3.76%.

Whether other substances accepted ^{3}H and mimicked the chromatographic characteristics of ^{3}H-mineral oil was investigated by administering orally to rats 0.5 ml. of ^{3}H-water with a specific activity of 340 μc./ml., the same dose of radioactivity that had been administered to the rats that had received ^{3}H-mineral oil. The tissues of these animals were subjected to the extraction and chromatographic separation described above for mineral oil.

RESULTS

After the administration of ^{3}H-mineral oil to rats, 85% of the radioactivity extracted from the tissues by toluene proved to have been ^{3}H-mineral oil. Most of the remaining 15% was present in unidentified, more polar substances that remained near the origin of the chromatogram. Of the material extracted with dioxane about 8% proved to have been ^{3}H-mineral oil on the basis of its chromatographic characteristics, the remaining 92% comprised unidentified, more polar substances.

Toluene and dioxane extracts of the carcasses of rats that had been dosed 24 hr. earlier with ^{3}H-water contained 0.3 and 40.9% of the radioactivity, respectively. After the extracts had been concentrated and chromatographed, however, no evidence of substances with the characteristics of ^{3}H-mineral oil was found in any of the tissues; in the carcass, *e.g.*, this was $<5 \times 10^{-4}$ mcg./Gm. based on the specific activity of ^{3}H-mineral oil.

The dispositions of ^{3}H-mineral oil and of tritium-containing substances other than mineral oil in rats that had received 0.66 ml. of tritiated drug/Kg. orally, either acutely or after chronic administration, are illustrated in Fig. 1. It is apparent that more than 80% of the total dose had not been absorbed before it was excreted in the feces. The highest concentration of mineral oil that had been absorbed by the rats at any time was 13.8 mcg./Gm. (1.6% of the dose); this was found 5 hr. after administration. Twenty-four hours after the dosing, the small amount of ^{3}H-mineral oil remaining in the rats was still largely in the alimentary tract, about 4.5% of the quantity administered; the carcass at that time contained about 0.6%, or 5.2 mcg. of mineral oil/Gm. of tissue. These levels continued to fall steadily, 48 hr. after the administration the levels of ^{3}H-mineral oil in the gastrointestinal tract and in the carcass had dropped to 0.7 and 0.3% of the dose, respectively.

Figure 1 shows that rats treated daily for 31 days with unlabeled mineral oil prior to the terminal dose of ^{3}H-mineral oil gave no evidence of facilitated absorption. The concentrations of ^{3}H-mineral oil found in the alimentary tracts and in the remainders of the animals were similar whether the rats had received the drug acutely or chronically.

[1] The toluene scintillation solution contained 4 Gm. of PPO, 0.05 Gm. of POPOP in 1 L. of toluene. The dioxane scintillation solution of Bray (9) contained 4 Gm. of PPO, 0.2 Gm. of POPOP, 60 Gm. of naphthalene, 20 ml. of ethylene glycol, 100 ml. of absolute methanol diluted to 1 L. with *p*-dioxane.

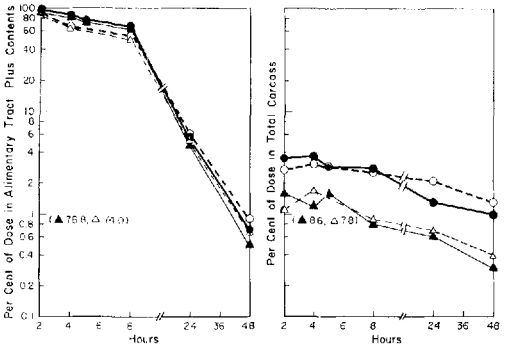

Fig. 1.—Disposition of radioactivity in alimentary tracts plus contents or in the carcasses of acutely or chronically dosed rats following an oral dose of tritiated mineral oil 0.66 ml./Kg. Each point represents the data from two animals in all the acute experiments except 24 hr. at which time five animals were assayed; three animals were assayed for each point in the chronic experiments. The difference between total ^3H and the ^3H-MO represents the radioactivity contained in substances other than ^3H-mineral oil. The figures in parentheses represent 1% of the dose expressed as mcg. of mineral oil/Gm. of tissue. Key: ●, total ^3H, acute; ○, chronic; ▲, ^3H-MO, acute; △, chronic.

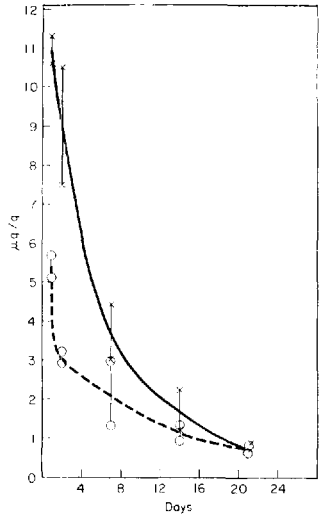

Fig. 2.—Long-term retention of ^3H-mineral oil in the carcasses of rats following a single oral dose of 0.66 ml./Kg. The curves have been drawn through the means obtained at each interval. Each mean was based on two animals as shown. Key: ———, total ^3H-substances; – – – –, ^3H-MO.

That the ^3H in the mineral oil exchanged with other substances in the rat accounted for the difference between the values of radioactivity in the ^3H-mineral oil and total radioactivity. The possiblity could not be ruled out that metabolism of ^3H-mineral oil may have contributed to the non-mineral oil substances that contained ^3H. In the alimentary tract the ratio of radioactivity of ^3H-mineral oil to the radioactivity of other substances was about 10:1 during the period when most of the drug was still in the animal; it had dropped to about 8:1 at 24 hr. and to 3:1 at 48 hr. post-treatment.

In the carcass, however, the evidence for metabolism of the oil or for exchange of ^3H from the ^3H-mineral oil to other substances was more apparent. At 24 and 48 hr. post-treatment the ratios of the radioactivity in ^3H-mineral oil to the radioactivity of other substances were 1:2 and 1:3, respectively.

The long-term retention of mineral oil in the carcasses of rats is summarized in Fig. 2. The initial

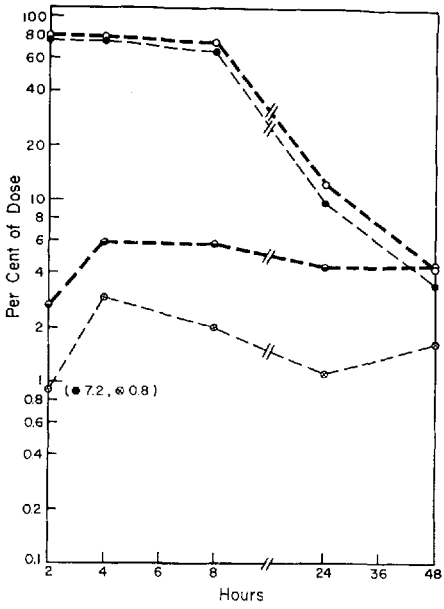

Fig. 3.—Disposition of radioactivity in the alimentary tracts plus contents or in the carcasses of rats chronically treated orally with mineral oil at various periods after the administration of a final dose of 0.066 ml. of ^3H-mineral oil. (See text for details.) Each point represents an assay of a single animal. The figures in parentheses represent 1% of the dose expressed as mcg. of mineral oil per Gm. of tissue. Key: ○, total ^3H, alimentary tracts plus contents; ◒, total ^3H, carcass; ●, ^3H-MO, alimentary tract plus content; ×, ^3H-MO, carcass.

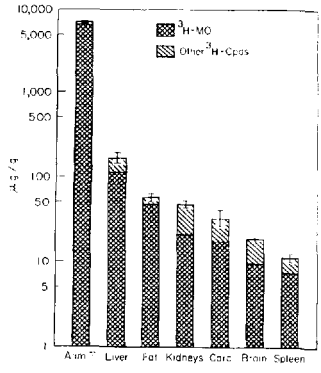

Fig. 4.—Disposition of radioactivity 5 hr. after oral administration to rats of tritiated mineral oil 0.66 ml./Kg. Height of each bar indicates the mean concentration per Gm. of tissue. Bars indicate the range of the actual values (n = 2).

sharp drop in the concentration of mineral oil may have been due to the elimination of absorbed mineral oil in the bile and also to the effects of exchange of ^3H. The slope of the curve of ^3H-mineral oil changed sharply by day 2, and, thereafter, it reflected a much slower elimination of the mineral oil. The convergence of the curve denoting the total radioactivity with the curve for ^3H-mineral oil attested to the slower rate of exchange or other change in the ^3H-mineral oil components after day 2. This was reasonably anticipated since all the ^3H atoms in the mineral oil were not equally active; those that were most active would exchange with H more rapidly than the rest. Significant concentrations of the mineral oil, 0.7 mcg./Gm. (0.1% of the amount administered), were detected in the carcasses 21 days after the single oral dose.

Evidence that the amount of mineral oil absorbed was dependent on the dose given was obtained in a chronic experiment in which rats received orally 0.066 ml. of ^3H-mineral oil/Kg., one-tenth of the usual dose. This experiment was carried out by administering orally 0.66 ml. daily of unlabeled mineral oil/Kg. for 31 consecutive days. On the 32nd day the animals received orally one-tenth the usual dose, 0.066 ml. of ^3H-mineral oil/Kg.; they were sacrificed at intervals after the final dose. The results of this experiment (Fig. 3) indicated that the carcasses of the rats that had received the smaller dose contained about 1% (0.8 mcg./Gm.) of the ^3H-mineral oil that had been administered 24 hr. earlier, whereas the carcasses of the rats that had received the normal dose contained after a comparable interval 0.7% (5.5 mcg./Gm.) of the amount administered. These data seemed to offer preliminary evidence that the passage of mineral oil through the gut wall resulted from diffusion. Clearly, additional animals would need to be tested before a firm conclusion could be reached.

The physiologic disposition in various organs and in fat 5 hr. after a single oral dose of 0.66 ml. of ^3H-mineral oil/Kg. is summarized in Fig. 4. The livers contained relatively high concentrations of the mineral oil at that time, 110 mcg./Gm., 0.5% of the dose. The concentrations in the fat, kidneys, brains, and spleens were relatively low; they ranged from 50 to 8 mcg./Gm. It was calculated that these organs contained a total of less than 0.2% of the dose. The remainder of the carcasses[2] contained 17.5 mcg./Gm., or 1.6% of the dose.

As was indicated earlier, the major portion of the dose, 75%, still remained in the alimentary tracts 5 hr. after dosing. Very little alteration in the ^3H-mineral oil occurred during that period. Of the radioactivity in the alimentary tracts, only 5% was found in substances other than mineral oil. In the carcass of the rat, however, the concentration of ^3H-nonmineral oil substances was somewhat higher. This may have been due to metabolism of the oil, to the preferential absorption of substances other than mineral oil that had been labeled with ^3H, by exchange in the gut, or to the increased opportunity for exchange after absorption of the mineral oil.

[2] The carcass in Fig. 4 represented the remainder of the animal after the other organs and tissues discussed above had been removed.

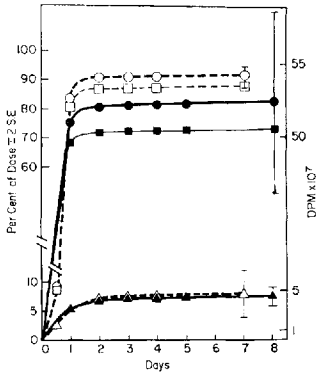

Fig. 5.—Cumulative excretion of radioactivity by rats orally dosed with tritiated mineral oil 0.66 ml./Kg. Each point in the chronic experiment represents the mean for two animals. Each point in the acute experiment represents the mean for four animals, except days 1 and 2, at which times 11 and six animals, respectively, were used. The difference between total ^3H and ^3H-mineral oil represents DPM of substances other than mineral oil, which contained ^3H, expressed as per cent of the administered dose. Only trace quantities of mineral oil were found in the urine, 0.3–2.5 × 10^{-3}% of the dose in the first 24 hr. after administration. Key: ▲, total ^3H in urine, acute; △, in urine, chronic; ●, in feces, acute; ○, in feces, chronic; ■, ^3H-MO in feces, acute; □, in feces, chronic.

The cumulative excretion of radioactivity following oral administration of the drug is illustrated in Fig. 5. The major portion of the drug was excreted during the 24-hr. period after the dose had been administered. About 80% of the dose was recovered in the feces of acutely dosed rats during the first 2 days after treatment. Levels of radioactivity recovered in the feces of chronically treated animals were slightly, but not significantly higher, than the values for acutely treated animals. Two standard errors about each point were calculated. Typical values are given about the points at days 7 and 8. As with the radioactivity in the alimentary tract over 90% of the ^3H in feces was in the form of mineral oil.

Seven to 8% of the radioactivity administered was excreted in urine during the week following drug administration. Samples collected for an additional week contained an additional 1 to 2% of the dose. Several urine samples were exhaustively extracted with toluene; the toluene extracts were carefully concentrated and portions of the concentrate were chromatographed. In five separate experiments less than 2% of the radioactivity in the urine excreted during the first day after dosing was extractable by toluene; the amount of ^3H-mineral oil in the urine varied between 0.3 and 2.5 mcg./day (0.3–2.5 × 10^{-3}% of the dose).

The fate of mineral oil administered i.p. was studied, not only to provide information about the disposition of the oil after this route of administration, but also to learn if any difference could be detected between the original ^3H-mineral oil and the ^3H-mineral oil isolated from the tissues of the body after the drug had passed through the walls of the gut.

The results of this experiment (Table I and Fig. 6)

TABLE I.—CONCENTRATION OF ^{3}H-MINERAL OIL AND NONMINERAL OIL SUBSTANCES CONTAINING ^{3}H IN THE TISSUES OF RATS AFTER THE ADMINISTRATION OF ^{3}H-MINERAL OIL[a]

	mcg./Gm.			
	Oral		I.p.	
Tissue	^{3}H-Mineral Oil	^{3}H-Nonmineral Oil Substances	^{3}H-Mineral Oil	^{3}H-Nonmineral Oil Substances
Liver	21.7	19.3	432.5	31.2
Kidney	3.3	6.2	174.9	70.8
Brain	3.4	2.7	6.5	2.9
Fat	21.4	4.0	20,235.2	<1.0

[a] Rats received 0.66 ml. of ^{3}H-mineral oil/Kg. orally (n = 2) or i.p. (n = 3). Twenty-four hours later they were sacrificed and the tissues assayed as described in the text. The specific activity of the nonmineral oil substances was assumed to have been the same as that of ^{3}H mineral oil.

revealed that the mineral oil isolated after an i.p. injection exhibited the same characteristics in the extraction and assay as that isolated after oral administration; this provided additional evidence that mineral oil had been absorbed from the gut.

It may be seen from Table I that the concentrations of labeled material in the brain were relatively unaffected by the route of administration. It is also apparent that after oral administration the nonmineral oil fractions of the total radioactivity, found in the respective tissues, were greater than after i.p. administration.

The excretion of mineral oil after an i.p. injection was found to have been relatively slow. Only 11% of the dose of radioactivity was excreted in the feces during 8 days post-treatment (Fig. 6). About 95% of the radioactivity in the feces was ^{3}H-mineral oil. The chronic i.p. administration of mineral oil did not alter the pattern of excretion exhibited after a single acute i.p. dose. Because of the relatively wide variation from animal to animal there was no significant difference in the quantities excreted in the feces following a single acute injection or following chronic i.p. administration of the drug.

The urine excreted during the first 8 days post-treatment contained 8% of the total radioactivity injected. During the first 24 hr. after i.p. dosing 0.4 mcg. of ^{3}H-mineral oil was excreted in the urine (0.3×10^{-3}% of the dose).

The influence of an emulsifying agent on the absorption and disposition of mineral oil was tested by treating rats with ^{3}H-mineral oil containing 1.7 or 8.3 mg. of dioctyl sodium sulfosuccinate/ml. The results of this experiment are summarized in Fig. 7. Total radioactivity excreted in the urine and feces, which accounted for the major portion of the dose, was not significantly altered from control values by the addition of dioctyl sodium sulfosuccinate to the mineral oil. When one considered only the relatively small fraction of the dose that had been absorbed, however, the levels of ^{3}H-mineral oil in the carcasses were found to have been higher in both groups that had received mineral oil containing the emulsifying agent.

In terms of total radioactivity in the carcasses both preparations containing the additive seemed better absorbed than the oil alone. The increased quantities of ^{3}H-mineral oil and of ^{3}H-nonmineral oil substances found in the carcasses amounted to a total of 0.7% of the dose, a barely significant increase. In terms of ^{3}H-mineral oil in the carcasses, the presence of the additive produced concentra-

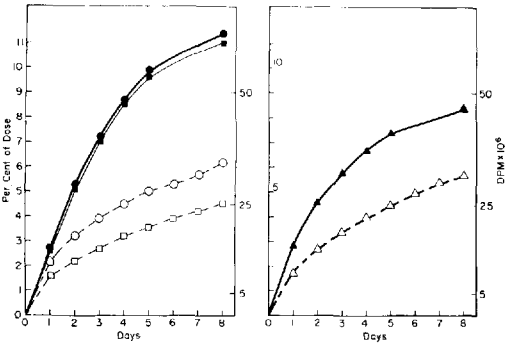

Fig. 6.—Cumulative excretion of radioactivity by rats intraperitoneally dosed with tritiated mineral oil, 0.66 ml./Kg. Each point represents the mean of three to five animals. A trace of mineral oil was found in the urine, 3.0×10^{-4}% of the amount administered in the first 24 hr. after dosing. Key: ▲, total ^{3}H in urine, acute; △, in urine, chronic; ●, in feces, acute; ○, in feces, chronic; ■, ^{3}H-MO in feces, acute; □, in feces, chronic.

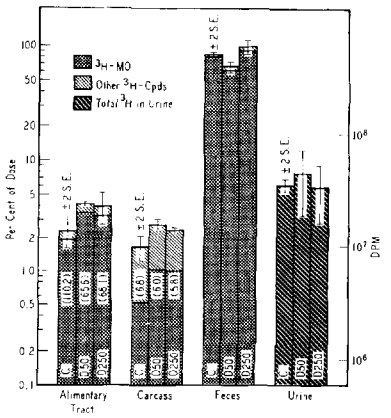

Fig. 7.—Disposition of radioactivity in rats 24 hr. after oral administration of ^{3}H-MO (C) or tritiated mineral oil containing dioctyl sodium sulfosuccinate, 1.7 mg./ml. (D50) or 8.3 mg./ml. (D250). Height of each bar indicates the mean percentage of the dose recovered. In control animals the means ±2 S.E. are listed (n = 3). In experimental animals, the mean, and the value for each animal are given (n = 2). The figures in parentheses represent 1% of the dose expressed as mcg. of mineral oil per Gm. of tissue.

tions that were almost 2 times higher than those found in the control group—6.1 and 5.8 mcg./Gm. in the groups that had received the low and high dose of the emulsifying agent, respectively, and 3.4 mcg./Gm. in the control group.

DISCUSSION

Although the literature contains reports on the disposition of mineral oil in animals (2, 5, 6), in all of the studies, doses far in excess of those normally employed by patients were given. Administration of high levels of oil was often required in such studies because of the poor specificity and low sensitivity of the methods of assay available. With the availability of a radioactive product it was possible to administer the oil at dosage levels equivalent to those recommended for humans and to assay tissues and excreta with a sensitivity previously unattainable.

Following oral administration approximately half of the radioactivity in the carcasses was shown to have been ^{3}H-mineral oil. The remaining half was ^{3}H-containing substances other than mineral oil which had been formed by the exchange of ^{3}H which had been shown to occur *in vitro*. These nonmineral oil substances might have been absorbed after undergoing an exchange reaction with the mineral oil in the gut, or they might have been formed after absorption. It is also possible that some polar metabolites of ^{3}H-mineral oil may have been included in the radioactive nonmineral oil fraction. That some ^{3}H in the ^{3}H-mineral oil exchanged rapidly was demonstrated *in vitro* by mixing the oil with a dilute aqueous acid solution or by allowing the mineral oil to mix with propionic acid overnight and measuring the ^{3}H content of the aqueous layer after separating the oil. The metabolic alteration of the mixture of saturated hydrocarbons that constituted ^{3}H-mineral oil was not demonstrated by any of the procedures described in this report, yet such a change cannot be ruled out. In any case, the specific activity of the ^{3}H-mineral oil was reduced by the amount of radioactivity found in the nonmineral oil fraction. Since efforts to determine the specific activity of the ^{3}H-mineral oil after it had been absorbed were uniformly unsuccessful because of the low concentrations present, the values of the ^{3}H-mineral oil reported in these experiments have been based on the original specific activity. These data represent, therefore, a minimum concentration of ^{3}H-mineral oil.

If the entire exchange reaction had occurred only after the absorption of ^{3}H-mineral oil, the quantity of oil that had been absorbed would be calculated on the basis of the sum of the radioactivity of the unchanged ^{3}H-mineral oil, the radioactivity of the nonmineral oil substances, the radioactivity found in the urine, and the radioactivity excreted in the bile. It has been calculated that in such a condition the concentration of ^{3}H-mineral oil reported here to have been 17.5 mcg./Gm. in the carcasses (1.6% of the dose) 5 hr. after an oral dose (Fig. 4) would have been increased to not more than 42.5 mcg./Gm. (4% of the dose) by the concentration of nonmineral oil substances containing the tritium in the carcass and by the contribution of radioactivity found in the urine (Fig. 5).

It is likely, however, that the actual value of ^{3}H-mineral oil in the carcasses lies well below the higher figure for a number of the following reasons. Exchange had been demonstrated in the lumen of the gut (Fig. 4). The ready absorption of ^{3}H-nonmineral oil substances had been demonstrated after the administration of ^{3}H-water. The i.p. administration of ^{3}H-mineral oil to rats produced relatively large quantities of ^{3}H-mineral oil in the organs; nevertheless, in comparison with results after oral administration, the concentrations of ^{3}H-nonmineral oil substances were rather low. This suggested that much of the ^{3}H-nonmineral oil substances found in the organs of the rat after the oral dose arose by a reaction that had occurred in the gut prior to absorption.

The failure of the concentration of ^{3}H-mineral oil in the brain to reflect the large amount present in the carcasses after i.p. administration may have been due, to some extent, to an immobility of the oil after it had been injected.

The kidney was able to excrete only trace quantities of mineral oil. This was demonstrated whether the drug had been given orally or i.p. Mineral oil was clearly demonstrated in homogenates of kidneys after either route of administration.

That the excretion of mineral oil after it had been absorbed by the rat was slow is apparent on examination of Fig. 6. The major portion of the dose remained in the animal 8 days following i.p. administration. At that time only 6–10% of the dose had been excreted. It is likely that one route of excretion of mineral oil is in the bile. The relatively slow rate of excretion by this route may have been due in part to the immobility of the oil after it had been deposited in the tissues, a suggestion consistent with that proposed for explaining the low concentrations found in the brains after i.p. administration; or it may have been due in part to the inability of the biliary system to collect it.

The mechanism of the absorption of mineral oil remains unknown. Frazer and Stewart (13) demonstrated that if mineral oil were emulsified with oleic acid and cholesterol to form particles not greater than 0.5 μ in diameter, absorption of large amounts of the oil would occur. These authors commented also that in the absence of emulsification negligible absorption of mineral oil had been noted. The data in this report substantiate the first observation; the presence of an emulsifying agent increased the absorption of mineral oil. With respect to the second observation, these data, contrary to those of Frazer and Stewart (13), indicate that a small but significant amount of mineral oil had been absorbed in the absence of an exogenous emulsifying agent.

The levels of radioactivity in the carcasses of rats chronically treated with ^{3}H-mineral oil were similar to those found after an acute dose. This evidence suggests that chronic administration did not alter the ability of rats to absorb or to excrete mineral oil.

At the dosage level employed in these studies only a mild laxative action was observed. In the chronic studies this effect disappeared 1 or 2 days after initiating the treatment.

The high levels of radioactivity excreted in the feces of the orally dosed rats reflected the poor absorption and the rapid elimination of ^{3}H-mineral oil.

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Enzyme Inhibitors XIV

Syntheses of Some 9-(*m*-Substituted Benzyl)-6-substituted Purines and Their Evaluation as Inhibitors of Adenosine Deaminase

By HOWARD J. SCHAEFFER and R. N. JOHNSON

Recent studies have shown that 9-(*p*-bromoacetamidobenzyl)adenine is an irreversible inhibitor of adenosine deaminase. In order to study the effect of isomers on the inhibition of adenosine deaminase, a variety of reversible inhibitors of adenosine deaminase have been synthesized which are 9-(*m*-substituted benzyl)-6-substituted purines. In addition, it was found that 9-(*m*-bromoacetamidobenzyl)adenine is an irreversible inhibitor of adenosine deaminase, but the rate of irreversible inactivation by the *meta* derivative was lower than that by the corresponding *para* isomer. This decreased rate of irreversible inhibition by 9-(*m*-bromoacetamidobenzyl)-adenine may be rationalized by assuming that in the reversible E...I complex the alkylating group is not positioned as near a nucleophilic group on the enzyme as it is in the case of the corresponding *para* isomer or that the *meta* derivative alkylates a different amino acid on the enzyme than does 9-(*p*-bromoacetamidobenzyl)adenine.

In a recent study, it was found that 9-(*p*-bromoacetamidobenzyl)adenine was an irreversible inhibitor of adenosine deaminase, whereas iodoacetamide was not an irreversible inhibitor of this enzyme (1, 2). Kinetic analysis of the data indicated that the irreversible inhibition of adenosine deaminase by 9-(*p*-bromoacetamidobenzyl)adenine occurred only after the inhibitor had reversibly complexed with the enzyme. In this complex, then, the bromoacetamido moiety of the inhibitor is held near a nucleophilic group on the enzyme, and a reaction related to a neighboring-group reaction occurs with the formation of a covalent bond. Such inhibitors, which Baker has called active-site-directed irreversible inhibitors (3), should be quite specific in their irreversible inactivation of an enzyme. For example, when a comparison is made of isomers of some potential irreversible inhibitors, the environment on the enzyme in which the alkylating or acylating group of the inhibitor is held in the reversible E...I complex (enzyme–inhibitor) could be quite different. Thus, it is possible that the reactive group of one isomer could be held near an appropriate nucleophilic group on the enzyme, whereas the reactive group of an isomeric inhibitor could be held in the reversible E...I complex in such a position that it cannot form a covalent bond with the enzyme. In an attempt to determine the specificity of 9-(*p*-bromoacetamidobenzyl)adenine for adenosine deaminase, it was decided to investigate the possible reversible and irreversible inhibition of adenosine deaminase by some 9-(*m*-substituted benzyl)-6-substituted purines.

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DISCUSSION

Chemistry.—Previous studies have shown that adenosine deaminase (calf intestinal mucosa) has a hydrophobic region to which the 9-alkyl group of some 9-alkyladenines can bind (4). Furthermore, it has been found that some 9-(*p*-substituted benzyl)-6-substituted purines were capable of inhibiting