

An interesting observation is the total elimination of radioactivity from the body after administration of toluene and xylene in 24 hours and 48 hours, respectively, and the complete extractability of toluene and xylene metabolites from the tissues, notably the liver and kidney, at all survival times. Toluene has generally failed to cause adverse hepato-renal effects in animals (52, 103, 343). Phenolic metabolites, which are probably further biotransformation products of initially formed epoxides, have been found at administration of high doses of toluene only (147) and may possibly be involved in causing the hepato-renal injuries reported from heavy, chronic intentional or occupational exposure to toluene (142, 159, 355, 437). Toluene induced kidney and liver injuries therefore seem to be possibly occurring only at massive exposure. The dose of toluene administered to mice for autoradiography is only about 0.25 ml/kg, as calculated from the elimination studies below, which is considerably less than the dose reported to increase urinary organic sulfate in rats (147). The distribution picture of toluene metabolites also closely resembles that of benzoic acid. Similarly, xylene has not been linked to hepato-renal injuries in animals (65, 198) or man (51). Only very high doses of xylene to guinea pigs caused elevations of hepatocellular enzymes in serum (103). Benzene is generally not considered a serious hepato-renal toxic agent. However, several animal studies reporting histochemical liver and kidney changes (203, 206, 207, 213) coupled to ultra-structural changes of mitochondria and smooth and rough endoplasmic reticulum (204, 205, 210, 358) and elevation of serum levels of hepatocellular enzymes (473) support a role of

benzene in causing liver and kidney injuries. Centrilobular liver necrosis has been found in cases of chronic human benzene poisoning (18, 261). Autoradiography of dried, evaporated sections as compared to dried, evaporated and extracted sections revealed a small but clearly visible amount of firmly bound radioactivity in both the kidney and liver at all survival times starting at 30 minutes after inhalation. Moreover, the radioactivity was not evenly distributed within the liver, suggesting a localized, presumably centrilobular, distribution of firmly bound metabolites. The actual irreversible binding of radioactivity from ^3H -benzene to the liver and kidney in mice (406) and of a benzene metabolite to rat liver DNA (259) has been reported. Benzene metabolites, although not firmly bound, were also interstitially localized in the testicle up to 2 hours after inhalation. There are reports of increases in testicular weight and histochemical changes, including lipid accumulation in Leydig cells, in the testes of rats exposed to benzene (189, 475). Metabolites of benzene and xylene were also found in the lung, localized to the bronchi at 2 hours after inhalation. Only benzene has so far possibly been connected with pulmonary injury causing histochemical changes in rabbit lung (418). The metabolites may have been formed in the liver and transported to the lung but benzene biotransformation has been shown to take place in lung microsomes from hamsters, rats and rabbits (173) and rat and rabbit lung microsomal preparations have been demonstrated to biotransform p-xylene (61, 172).

As shown by low-temperature autoradiography as compared to autoradiography of dried, evaporated sections, only benzene

itself was present in the bone marrow after inhalation. This is in sharp contrast to the study by Andrews *et al.* (20) reporting high levels of both benzene and its metabolites in the bone marrow of Swiss albino mice after subcutaneous injection of ^3H -benzene. The amount of benzene metabolites in the bone marrow was nine times that found in the blood and six times that found in the liver over an 8 hour period after injection of 880 mg/kg ^3H -benzene. Benzene metabolites have even been shown to be irreversibly bound, though in minute amounts, to mouse bone marrow (406). The effects of benzene on red blood cell precursors seem to be dose dependent as shown by Lee *et al.* (245) using the ^{59}Fe -incorporation technique. A dose of 88 mg/kg benzene had no effect whereas 440 mg/kg or 2200 mg/kg benzene significantly decreased 24 hour ^{59}Fe -utilization in mice. The elimination studies of benzene reported below indicate that roughly 90% of the 5 μl benzene added to the inhalation apparatus is retained by the mouse, which corresponds to a dose of about 160 mg/kg. However, intraperitoneal injections of 1 ml/kg (880 mg/kg) or 3 ml/kg (2640 mg/kg) ^{14}C -benzene did not give any significant amounts of benzene metabolites in the bone marrow as evidenced by autoradiography of dried, evaporated sections. The possibility that benzene metabolites may accumulate in the bone marrow during repeated administration of benzene was investigated in mice given up to 18 daily injections of 1 ml/kg ^{14}C -benzene intraperitoneally and subject to autoradiography of dried, evaporated sections and dried, evaporated and extracted sections. The autoradiograms did not reveal any such accumulation. In addition, intraperitoneal administration of benzene might be expected to make

a larger proportion of the administered amount of benzene available for biotransformation in the liver as compared to the inhalational route and it has also been shown that benzene increases the rate of its own biotransformation in mouse liver microsomes (156). Attempts to increase benzene biotransformation with phenobarbital, which has been shown to occur both *in vitro* (408) and *in vivo* (192), or depletion of glutathione, involved in trapping the presumably formed reactive benzene oxide, with diethyl maleate, similarly did not increase the amount of benzene metabolites in the bone marrow. Whole-body autoradiography therefore seems to rule out the affinity of benzene metabolites for bone marrow as a cause of benzene hematotoxicity. However, the possibility remains that benzene metabolites may be present in a very small amount in the bone marrow; enough to cause bone marrow toxicity but not to be registered by whole-body autoradiography. Benzene may also be an agent toxic for bone marrow in itself, as suggested by Ikeda and Ohtsuji (192). Similarly, phenol, the main benzene metabolite, did not show any affinity for bone marrow, which was also observed by Andrews *et al.* (20). Administration of phenol to animals has also failed to induce signs of bone marrow toxicity (277, 293). The reasons for the large discrepancy between the results of whole-body autoradiography and the investigation by Andrews *et al.* (20), as regards the amount of benzene metabolites in the bone marrow, are obscure, although it might be argued that the collection of bone marrow from such a small animal as the mouse and the following extraction procedures used to separate between benzene and its metabolites may involve several uncertain factors. Some kind of intra-

species or sex variation seems unlikely in that both studies were performed with male albino mice.

Elimination studies

Uptake. The doses of ^{14}C -labeled benzene, toluene and xylene administered to mice by inhalation were calculated from the differences between the amounts of radioactivity added to the inhalation apparatus and the amounts recovered by repeated washings of the apparatus with scintillation fluid. These values were used to calculate the uptake of the three solvents. The uptake of benzene was about 91% whereas the uptake of toluene and xylene was about 60% and 31%, respectively (Table II:1).

Elimination in exhaled air. About 44% of the administered dose of benzene was eliminated unchanged in the exhaled air in 8 hours whereas toluene and xylene were exhaled to a considerably less extent; about 10% and 3.4% of the administered doses, respectively (Table II:1). The time course of the cumulative elimination of the three solvents in the exhaled air is shown in Fig. II:9. About 98% of the exhaled amount of benzene was recovered during the first 3 hours whereas the recovery of the same proportion of the exhaled amount of toluene required 5 hours. The expiration of m-xylene was yet somewhat slower, 98% being recovered in 7 hours. A very small part of the administered dose of benzene was exhaled as $^{14}\text{CO}_2$ (Table II:1) beginning immediately after the inhalation. The cumulative elimination curve of CO_2 from benzene showed no plateau towards the end of the experiment in contrast to the cumulative

Table II:1. The uptake of ^{14}C -labeled benzene, toluene and m-xylene by mice and the elimination of radioactivity in exhaled air and urine during 8 hours after inhalation. Mean values $\pm$ S.E.M.

solvent	inhaled amount	uptake %	dose μCi (mg/kg)	eliminated in exhaled air		eliminated in urine $\text{dpm} \cdot 10^3$ (% of dose)
	μCi (μl)			unchanged $\text{dpm} \cdot 10^3$ (% of dose)	as CO_2 dpm (% of dose)	
benzene (n=4)	1.30 (5)	91.0 ± 1.1	1.18 ± 0.01 (159.9 ± 2.0)	1136.5 ± 63.3 (43.7 ± 2.4)	3145 ± 230 (0.12 ± 0.01)	769.1 ± 27.8 (29.6 ± 1.1)
toluene (n=4)	1.45 (10)	60.5 ± 9.6	0.87 ± 0.14 (209.4 ± 33.1)	189.5 ± 30.3 (9.9 ± 1.6)	-	1305.9 ± 81.8 (67.9 ± 4.3)
m-xylene (n=3)	1.15 (10)	31.4 ± 1.3	0.36 ± 0.02 (108.5 ± 4.5)	26.8 ± 1.8 (3.4 ± 0.2)	-	528.7 ± 5.3 (66.2 ± 0.6)

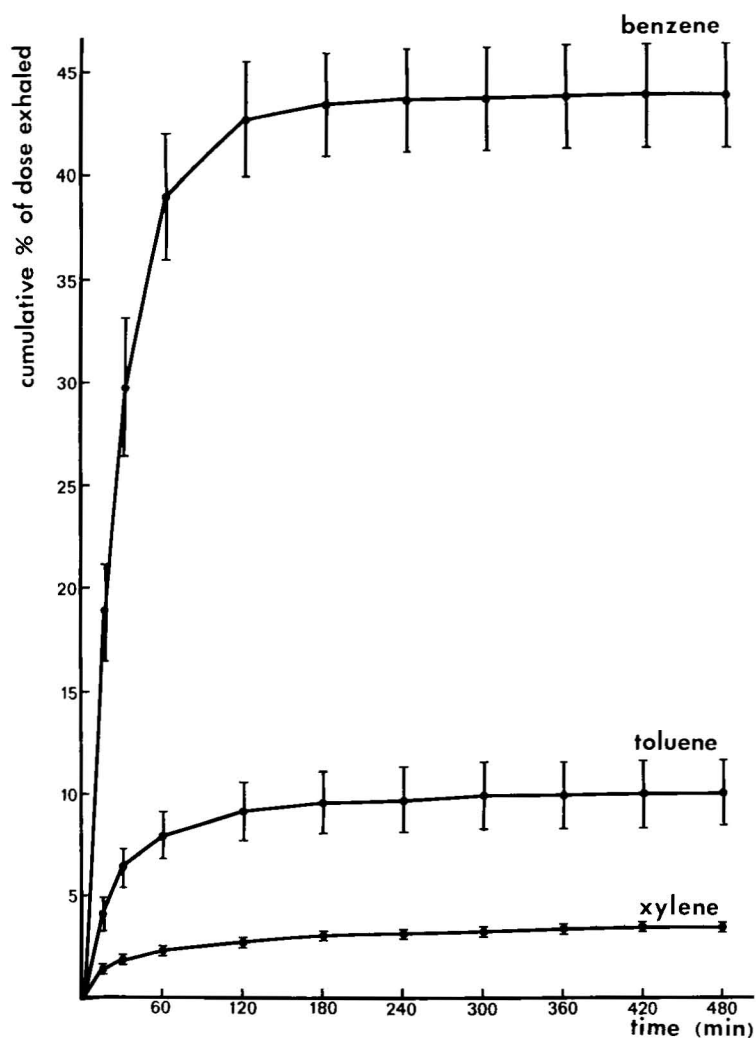


Fig. II:9. The cumulative elimination of benzene (n=4), toluene (n=4) and m-xylene (n=3) in the exhaled air. Mean values $\pm$ S.E.M.

elimination curve for exhaled unchanged benzene. No radioactivity was recovered as ^{14}CO . The elimination rates of benzene, toluene and xylene were calculated as mean % of dose exhaled per minute and plotted against time in a semi-logarithmic diagram (Fig. II:10). Computerized non-linear regression analysis according to the method of least squares of the experimental data yielded three-exponential decay curves for all solvents, characterized by an initial rapid

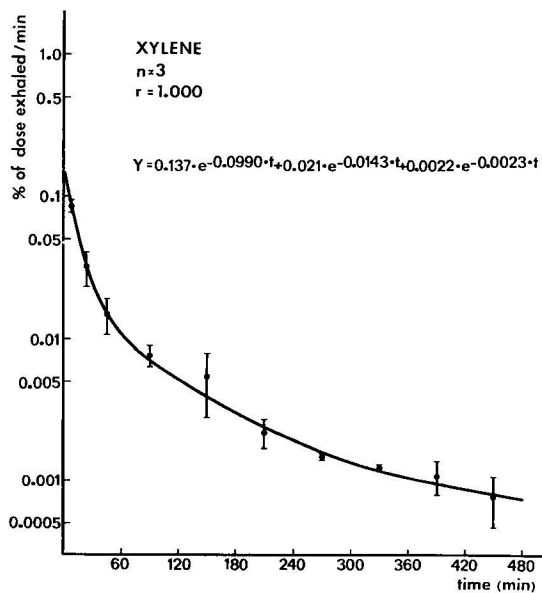
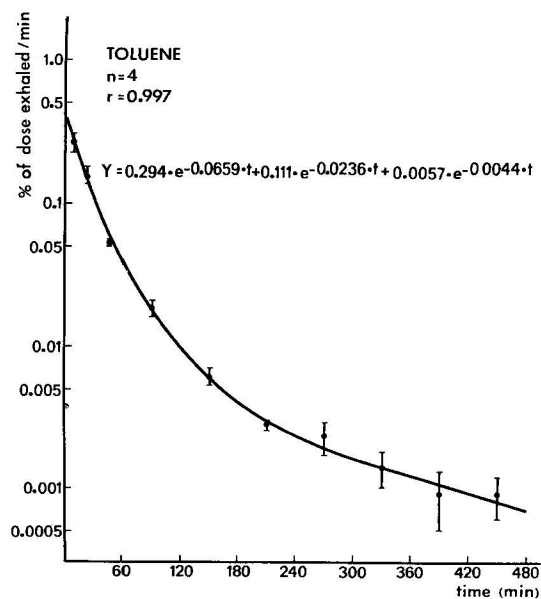
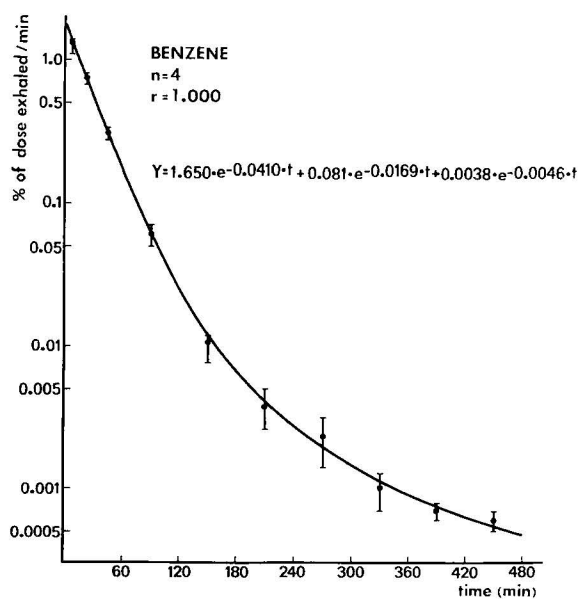


Fig. II:10. Elimination rate curves for benzene, toluene and xylene in the exhaled air.
Mean values $\pm$ S.E.M.

decrease of elimination rate (0-45 min) followed by a slow decrease (45-210 min) and an even slower decrease (210-480 min). Fig. II:10 shows that excellent fits were obtained between experimental and computer calculated data for the excretion rates of benzene, toluene and xylene in the exhaled air. The computer derived equation for the rate of excretion of benzene in the exhaled air was:

$$Y = 1.650 \cdot e^{-0.0410 \cdot t} + 0.081 \cdot e^{-0.0169 \cdot t} + 0.0038 \cdot e^{-0.0046 \cdot t}$$

with apparent half-lives of:

$$t_1 = 16.9 \text{ min}, t_2 = 41.1 \text{ min}, t_3 = 151.6 \text{ min}$$

for toluene:

$$Y = 0.294 \cdot e^{-0.0659 \cdot t} + 0.111 \cdot e^{-0.0236 \cdot t} + 0.0057 \cdot e^{-0.0044 \cdot t}$$

$$t_1 = 10.5 \text{ min}, t_2 = 29.4 \text{ min}, t_3 = 158.7 \text{ min}$$

and for xylene:

$$Y = 0.137 \cdot e^{-0.0990 \cdot t} + 0.021 \cdot e^{-0.0143 \cdot t} + 0.0022 \cdot e^{-0.0023 \cdot t}$$

$$t_1 = 7.0 \text{ min}, t_2 = 48.6 \text{ min}, t_3 = 300.2 \text{ min}$$

Elimination in urine. About 66% of the dose of ^{14}C -m-xylene was recovered in the urine in 8 hours. The radioactivity from inhaled ^{14}C -benzene and ^{14}C -toluene was eliminated in the urine in amounts corresponding to about 30% and 68% of the respective doses (Table II:1).

Tissue radioactivity levels. The remaining radioactivity in different tissues 8 hours after inhalation is shown in Table II:2. Generally, very low levels of radioactivity were

Table II:2. The retention of radioactivity in selected tissues of mice 8 h after inhalation of ^{14}C -benzene (dose: $4.55 \pm 0.06 \mu\text{l} = 2602.4 \pm 31.8 \cdot 10^3 \text{ dpm}$), ^{14}C -toluene (dose: $6.04 \pm 0.96 \mu\text{l} = 1922.3 \pm 303.8 \cdot 10^3 \text{ dpm}$) and ^{14}C -m-xylene (dose: $3.14 \pm 0.13 \mu\text{l} = 798.8 \pm 33.9 \cdot 10^3 \text{ dpm}$). Mean values $\pm$ S.E.M.

tissue	benzene (n=4)		toluene (n=4)		m-xylene (n=3)	
	dpm/g	total radio- activity(dpm)	dpm/g	total radio- activity(dpm)	dpm/g	total radio- activity(dpm)
brain	245 ± 34	103 ± 18	534 ± 52	197 ± 25	969 ± 238	381 ± 97
liver	4694 ± 392	6101 ± 303	1501 ± 88	1975 ± 207	2906 ± 375	3623 ± 565
lung	2453 ± 216	552 ± 52	1413 ± 204	262 ± 35	1487 ± 159	287 ± 30
spleen	1705 ± 149	169 ± 13	1714 ± 127	144 ± 17	1147 ± 150	97 ± 12
kidney	4235 ± 430	1885 ± 358	1777 ± 215	743 ± 90	2534 ± 173	1372 ± 228
white fat	1409 ± 139	-	1709 ± 289	-	2488 ± 46	-
brown fat	1577 ± 243	-	1724 ± 78	-	2100 ± 243	-
blood	$926 \pm 54^{(1)}$	-	$1738 \pm 253^{(1)}$	-	$1231 \pm 60^{(1)}$	-

(1) dpm/ml

registered in the tissues. Notable is the increasing amount of retained radioactivity in fat in the order benzene, toluene, xylene which is obvious if the fat radioactivities are put in relation to the administered doses. For toluene, no values are higher than that of the blood, indicating that no specific tissues have retained any radioactivity. This is in contrast to benzene, where radioactivity has been retained in the liver and kidney. Slightly higher radioactivity levels in the liver and kidney than that in the blood are also noted for m-xylene, which is probably due to the slower release of m-xylene from fatty tissues and its consequently more prolonged biotransformation as compared to benzene and toluene.

Although varying exposure conditions have been used in determinations of the uptake of benzene (51, 294, 416), toluene (294, 416) and xylene (378, 388) by humans, there is a tendency towards an increasing uptake in the order benzene, toluene, xylene. This is to be expected since one of the main factors governing the respiratory uptake of organic solvents is their blood/gas partition coefficients, which increase in the same order (Appendix 2). In this study quite the reverse order of uptake was noted. Thus, in spite of the higher blood/gas partition coefficients for toluene and xylene compared to that for benzene, as well as the addition of a larger amount of toluene or xylene (10 μ l) to the inhalation apparatus than of benzene (5 μ l), smaller proportions of the added amounts of toluene and xylene were taken up by mice than of benzene. Since the volume of the inhalation apparatus is only about

30 ml, the addition of 5 or 10 μ l of the solvents creates very high atmospheric concentrations. The combination of higher atmospheric concentrations of toluene and xylene with the increasingly irritating properties of toluene and xylene as compared to benzene, remarkably reduced the respiratory frequency of the mice. Thus, another of the main factors involved in respiratory uptake of solvents, the pulmonary ventilation, was very much lower at exposure to toluene and xylene, which probably explains the unexpectedly low uptake of toluene and xylene.

Andrews *et al.* (20) noted that 71.5% of a subcutaneous dose of 880 mg/kg ^3H -benzene was exhaled unchanged in 4-6 hours with 22.6% of the dose excreted in the urine in 24 hours. A lower dose, 440 mg/kg, was biotransformed to a larger extent (35.8%). Thus, the proportion of a dose of benzene exhaled unchanged by mice seems to decrease with decreasing dose, i.e. there is a limit to the capacity of the mouse to biotransform benzene. In this study 44% of a dose of 160 mg/kg benzene was recovered in the exhaled air. The expiration of benzene in this study was slightly more rapid than that reported by Andrews *et al.* (20), which is explained by the different modes of administration, since subcutaneous injection requires more time for absorption than the inhalational route. Similarly, rabbits receiving increasing oral doses of benzene (0.25-1.0 g/kg) exhaled increasing proportions of the dose (31-60%) (307). Humans exposed to low benzene concentrations (47-106 ppm) for 2-5 hours exhaled 12-42% of the retained amounts (51, 416). Excretion values for unchanged toluene in the exhaled air reported in the literature are lower than those for benzene

both in human (294, 415) and animal (397) experiments. In this study about 10% of a dose of about 210 mg/kg toluene to mice was exhaled unchanged, most of it during the first 4-6 hours, which is less than the amount reported for rabbits receiving 350 mg/kg orally and exhaling 18% of the dose in 12-14 hours (397). No reports of the expiration of xylene by animals have been found in the literature. Humans exposed to 0.4 mg/l m-xylene for 8 hours exhaled 5.8% of the retained amount, most of it during the first 2 hours after exposure (378), which is less than the expiration of benzene (51, 416) and toluene (294, 415) by humans in similar experiments. This is supported by the lower expiration of m-xylene, 3.4% of a dose of about 110 mg/kg in 8 hours, than of benzene and toluene by mice observed in this study. The time course of the expiration of benzene, toluene and xylene shown in Figs II:9 and II:10 and the equations obtained for their elimination rates in the exhaled air give important information about the transfer of the solvents in the body. Similar three-compartment pharmacokinetics have been observed for the expiration of benzene (295, 363) and toluene (363) by humans. The higher fat solubility and probably consequently slower release of m-xylene from the fat depots of the body, as compared to benzene and toluene, reflects itself in the lack of a plateau in the cumulative expiration curve for m-xylene in Fig. II:9 and also in the lower rate constant ($k_3 = 0.0023$) for the third part of the elimination curve for m-xylene, as compared to the corresponding parts of the elimination curves for benzene ($k_3 = 0.0046$) and toluene ($k_3 = 0.0044$), assumed to represent the elimination of the proportion of the amount of

solvents still retained in body fat. This is also in accordance with the results of low-temperature autoradiography where xylene could be registered for the longest period of time in adipose tissues. The first part of the three-exponential elimination rate curves in Fig. II:10 is assumed to be dominated by the elimination of the solvents from blood and well perfused tissues such as the liver, kidney and brain. The elimination rate would be governed by factors such as alveolar ventilation and blood/gas partition coefficients. However, since the well perfused tissue group includes the organs responsible for solvent biotransformation, the rate constants of the first parts of the elimination curves are also influenced by the metabolic clearance of the solvents (363). Or, since the decrease of solvent concentration in respiratory air is parallel to the decrease of solvent concentration in blood, a rapid decrease in respiratory air concentration reflects a rapid decrease in blood concentration for which biotransformation may be responsible (295). The metabolic clearance of toluene by humans is more rapid than that of benzene (107, 363), which may also apply to mice since a higher initial rate constant was obtained for the elimination of toluene ($k_1 = 0.0659$) than of benzene ($k_1 = 0.0410$). The rate constant for the initial elimination of m-xylene is even higher ($k_1 = 0.0990$) which may indicate an even more rapid metabolic clearance of m-xylene. These assumptions also seem to be supported by autoradiographic results since no metabolites of benzene could be registered immediately after inhalation in contrast to toluene and xylene. The initially steeper slopes of the expiratory elimination rate curves for toluene and

xylene may also reflect the more rapid decrease of their blood concentrations as a result of their still continuing transfer to body fat after exposure which is caused by their higher fat solubilities.

The tissue radioactivity levels registered by liquid scintillation counting 8 hours after exposure are in accordance with the autoradiographic distribution pictures where generally very low amounts of radioactivity were seen in the examined organs 8 hours after solvent exposure. The 8 hour autoradiogram were dominated by high concentrations of radioactivity in the intestinal contents for all three solvents. However, the relatively high amounts of radioactivity registered by autoradiography in the liver and kidney after exposure to benzene, some of it shown to be firmly bound, and m-xylene were also registered by liquid scintillation counting. The large urinary excretion of radioactivity, shown to be rapid by autoradiography, was also observed in the elimination studies.

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