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EFFECT OF TEA BREW ON THE URINARY EXCRETION AND TISSUE – DISTRIBUTION OF ^{14}C–CAFFEINE IN THE RAT

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Abstract : The effect of tea brew on the excretion and tissue distribution of (1–methyl–^{14}C) caffeine was investigated. Following oral administration of (1–methyl–^{14}C) caffeine to male and female rats, the radioactivity was excreted (approx. 62% and 70% respectively) mainly in the urine, the greater part of the excretion occuring during 12 – 24h period; small amounts (approx. 7% and 9% respectively) were found in the urine. In contrast, the oral administration of (1–methyl–^{14}C) caffeine with tea brew resulted in an enhanced (approx. 70% and 78%) urinary excretion of radioactivity. The major urinary excretion again occuring during 12 – 24h period. Major differences were apparent in the tissue-distribution studies. The radioactivity in the stomach declined faster when caffeine was administered with tea than when pure caffeine was administered. In contrast, the radioactivity profile for blood showed a higher specific activity over a longer period when caffeine was administered with tea. The overall studies indicate that tea brew, probably reduce tissue uptake of caffeine and enhances urinary excretion.

1. Introduction

Caffeine is a widely consumed pharmacologically active food constituent. It occurs naturally in tea, coffee and is also used as a food additive as in chocolates and cola type soft drinks. Caffeine is a central nervous system stimulant which increases cardiac output, induces diuresis and results in a variety of psycho-pharmacological effects.[11, 15]

Until recent times, caffeine was considered as a safe food additive. However, due to recently developed evidence concerning the capacity of caffeine to cause birth defects, the concern of the Food and Drug Administration of the United States (USFDA) has shifted from caffeine's potential behavioural effects to its potential teratogenicity.[12] For example, intraperitoneal administration of caffeine to pregnant mice at dose levels of 200–250 mg/kg was embryotoxic and affected palate and limb development of the fetuses.[13, 14, 18, 20] A single subcutaneous injection of mice with 200 mg/kg of caffeine produced hematoma, clubfoot and digital defects, while higher dosages (300–400 mg/kg) produced offsprings that exhibited digital and facial deformities and muscular development disorders.[25]

Similar but variable teratogenic effects have been observed in rats[21] and rabbits.[3] The Food and Drug Administration while reviewing these data[7] indicated the possibility that at sufficiently high level of exposure, well above what humans are exposed to in the diet, caffeine can cause birth defects in animals. Subsequent studies sponsored by the FDA have further confirmed the potential teratogenic properties of caffeine.[8]

The results of these studies received considerable publicity resulting in a citizens petition to the USFDA requesting that all caffeine containing beverages be labelled as harmful to health.[5] So far the investigations by USFDA have been confined to the usage of caffeine as a food additive and regulations are under consideration by USFDA according to which all caffeine containing manufactured beverages are required to be labelled as harmful to health.

In contrast, caffeine occurs naturally in both coffee and tea. In coffee, caffeine is chemically bound with chloregenic acid to form K-caffeine-chloregenic-acid complex[16] whereas in tea, caffeine is thought to be complexed with polyphenolic substances which bind very strongly with caffeine.[6, 22] This complex is insoluble in cold water at neutral or acid pH and may influence the rate of assimilation of caffeine.[24] Thus it has been claimed[1, 9, 10] that caffeine is assimilated more slowly from tea than from coffee or aqueous solutions and that this may modify the pharmacological action of caffeine in tea in such a way as to reduce or eliminate any known harmful effects. The claim has, however, been disputed[17] as a result of observations on the levels of plasma caffeine following the administration of tea or coffee to human subjects.

Thus it seems, that the scientific data presently available on the role of tea and its components on the pharmacological effects of caffeine is rather diffuse and conflicting. The need for the availability of such data is ever increasing in view of the teratogenic effects of caffeine as previously described. Furthermore these investigations may provide evidence against any possible extension to tea, of regulations applicable to synthetic beverages containing caffeine.

2. Materials and Methods

2.1 General

All chemicals used were of an analytical grade. (1–Methyl–^{14}C)– caffeine was obtained from New England Nuclear (Boston, Mass, USA). Samples of Broken Orange Peckoe (B.O.P.) tea were gifts from the Tea Research Institute, Talawakelle, Sri Lanka.

2.2 Preparation of (1–Methyl–^{14}C)–caffeine

(1–Methyl–^{14}C) caffeine (specific radioactivity 0.10 m ci/ml) was diluted with cold caffeine and recrystallised from water to constant activity. The radio-purity was further confirmed by radiochromatography. The product had a specific activity of 0.21 u ci/mg.

2.3 Preparation of tea brew

Tea (10 g) was placed in a flat bottom Quickfit flask (1 l). Boiling water (400 ml) was added to it and the contents were refluxed for 10 min. At the end of this period the mixture was filtered through muslin cloth into a Volumetric flask (500 ml). The tea residue was washed with warm distilled water (approx. 4 x 25 ml) and these washings were used to make upto the mark (500 ml) of tea brew. The concentration of caffeine in this prepared tea brew was 0.1062 mg/ml.

2.4 Experimental animals

In all experiments Sprague-Dawley rats (200 ± 15 g body wt.) maintained on a standard laboratory diet were used.

2.5 Dosage and Administration of Drugs

In experiments with pure caffeine, (1–methyl–^{14}C)–caffeine (specific activity 0.21 u ci/mg) was dissolved in distilled water (10 mg/ml). When caffeine was administered with tea, (1–methyl–^{14}C)–caffeine (specific activity 0.21 u ci/mg) was dissolved in the tea brew (10 mg/ml) prepared as described previously. The tea brew containing dissolved caffeine was then incubated for 30 min at 37°C before administration. The endogenous caffeine content (0.1062 mg/ml) in tea was assumed to be negligible. Different preparations were separately administered via a stomach tube while the animal was under light diethyl ether anaesthesia.

2.6 Radiochemical techniques

Radioactivity counting was carried out on a Beckmann LS100C liquid scintillation counter. For aqueous samples, a dioxan system was used, comprising naphthalene (60 g), 2,5–diphenyloxazole (4 g), 1,4–bis–(5–phenyl–oxazol–2–yl)–benzene (0.2 g), methanol (100 ml) and ethylene glycol (20 ml) made upto 1 litre with dioxan. Heterogenous samples (e.g. tissue homogenates) were counted for radioactivity in the dioxan system after gelling (5% w/v) with Cab–O–Sil (Packard Instrument Co., Wembly, Middx., U.K.).

2.7 Excretion Studies

After administration of the appropriate preparation of caffeine, the animals (6 males and 6 females for each experiment) were placed in individual metabolism cages, where urine and faeces were collected separately after 6h, 12h, 24h and 48h and were assayed for radioactivity as previously described.

2.8 Tissue-distribution Studies

Rats were separately administered with either pure caffeine or caffeine with tea. Animals were killed at 1h, 3h, 6h, 12h, 24h and 48h after the drug treatment. Tissues were rapidly excised, dried on filter paper and weighed. Samples of tissues (0.1 – 1.0 g) were homogenised in sucrose (0.32M, 5 – 40 ml) and aliquots of homogenates were assayed for radioactivity as described above.

3. Results and Discussion

3.1 Excretion Studies

The urinary excretion of radioactivity following the oral administration of (100 mg/kg body wt.) (1–methyl–^{14}C) caffeine and (1–methyl–^{14}C) caffeine with tea was investigated in both male (n=6) and female (n=6) rats. Urine and faeces were collected after 6h, 12h, 25h and 48 hours. The results are given in Table 1.

Following oral administration of (1–methyl–^{14}C) caffeine to male rats approx. 62 percent of the radioactivity appeared in the urine over the 48h experimental period, the major proportion (33 percent) appearing during 12 – 24h period. The excretion pattern in the female, though not markedly different from male animals, showed an enhanced urinary recovery (approx. 70 percent during 48h). In both sexes appreciable amounts (7 – 9 percent) were present in faeces. Although this may be interpreted as evidence for either biliary or intestinal excretion of administered radioactivity, the possibility of some contamination of faeces by the urine cannot be ruled out. The overall urinary and faecal excretion of administered radioactivity in the male was approx. 69 percent, while in the female, it was approximately 79 percent. However, the analysis of this data by student t-test showed no statistically significant sex difference in the excretion of administered radioactivity.

In contrast, when (1–methyl–^{14}C) caffeine was administered with tea, the total urinary recovery of radioactivity during 48h in the male and female was approx. 67 percent and 78 percent respectively, once again

Table 1 Recovery of radioactivity after the administration of ^{14}C –labelled caffeine

Rats (n=6) were administered either (1–methyl–^{14}C) caffeine or (1–methyl–^{14}C) caffeine (100 mg/kg body wt.) with tea orally. Urine and faeces were collected over 48h and samples were assayed for radioactivity. The results are given as Mean ± SEM.

Collection period		Percentage Administered Radioactivity			
		Caffeine		Caffeine with tea	
		Male (n=6)	Female (n=6)	Male (n=6)	Female (n=6)
Urine	0 – 6h	8.28 ± 3.77	12.35 ± 3.78	12.18 ± 6.81	10.04 ± 0.98
	6 – 12h	12.18 ± 1.78	12.18 ± 2.18	13.46 ± 2.24	10.93 ± 0.52
	12 – 24h	33.64 ± 4.13	32.10 ± 4.95	28.94 ± 4.16	34.44 ± 1.97
	24 – 48h	8.01 ± 1.05	12.85 ± 2.43	10.73 ± 3.12	23.07 ± 4.85
	sub total	62.01 ± 4.63	69.46 ± 7.93	67.3 ± 2.83	78.43 ± 1.56
Faeces	0 – 24h	3.265 ± 0.35	3.78 ± 0.52	3.41 ± 1.01	3.42 ± 1.10
	24 – 48h	3.48 ± 0.54	5.79 ± 0.67	3.83 ± 0.28	3.54 ± 1.39
	sub total	6.74 ± 0.46	9.34 ± 0.73	7.25 ± 1.02	6.97 ± 0.30
	Grand total	68.74 ± 4.67	79.04 ± 8.66	73.44 ± 3.08	85.45 ± 1.56

the major proportion appearing during 12 – 24h period. Faeces contained approx. 7 percent. The overall recovery of administered radioactivity in the male was approx. 73 percent while in the female it was approx. 85 percent. It is also apparent that the incorporation of ^{14}C–labelled caffeine into tea infusion, resulted in an enhancement of urinary recovery of administered radioactivity by approx. 5 and 9 percent in the male and female respectively. This latter observation may suggest that caffeine in tea is absorbed to a lesser extent by the tissues than pure aqueous solution of caffeine and this effect appears to be more pronounced in the female than in the male. This possibility is further strengthened by the fact that the urinary excretion of administered radioactivity by the female during 24 – 48h period increased from approx. 10 percent to approx. 23 percent as a result of incorporation of labelled caffeine into tea infusion.

3.2 Tissue-distribution studies

In an attempt to quantify, on a time-related basis, the relative distribution of radioactivity in major tissues and also to ascertain whether there was any specific accumulation of radioactivity in any given tissue, a tissue-distribution study was undertaken. The relative patterns of distribution of radioactivity following separate oral administration of (1–methyl–^{14}C) caffeine and (1–methyl–^{14}C) caffeine with tea, in blood and in the whole homogenates of stomach, liver, heart, kidney, spleen are illustrated in Figures 1, 2 and 3.

The results are expressed as percentage of administered radioactivity/ g wt. of tissue. The radioactivity in the stomach declined rapidly, the rate of this decline being faster when caffeine was administered with tea. Following the administration of (1–methyl–^{14}C) caffeine, the radioactivity in the blood reached a peak at 3h, and then declined sharply. In contrast, administration of labelled caffeine with tea, although resulted in a peak blood level at 3h, there was no sharp decline after 3h, but the level was maintained at a higher specific radioactivity for approx. 20 hours than when caffeine alone was administered. This observation may suggest that although caffeine in tea is rapidly absorbed from gastro-intestinal tract, its removal by the tissues from the blood, is slower than when caffeine only is administered.

It is apparent from Figures 2 and 3 that small but detectable amounts of radioactivity were associated with all tissues investigated, under both conditions of caffeine administration. However, it is important to dissociate any contribution made by labelled blood perfusing the organ from that of the actual tissue content of radioactivity. Thus a prerequisite for evidence of a greater tissue accumulation must be the occurrence of a greater specific radioactivity in the organ-tissue than in the blood. Examination of Figure 2 indicate that during 6 hours after the administration of (1–methyl–^{14}C)

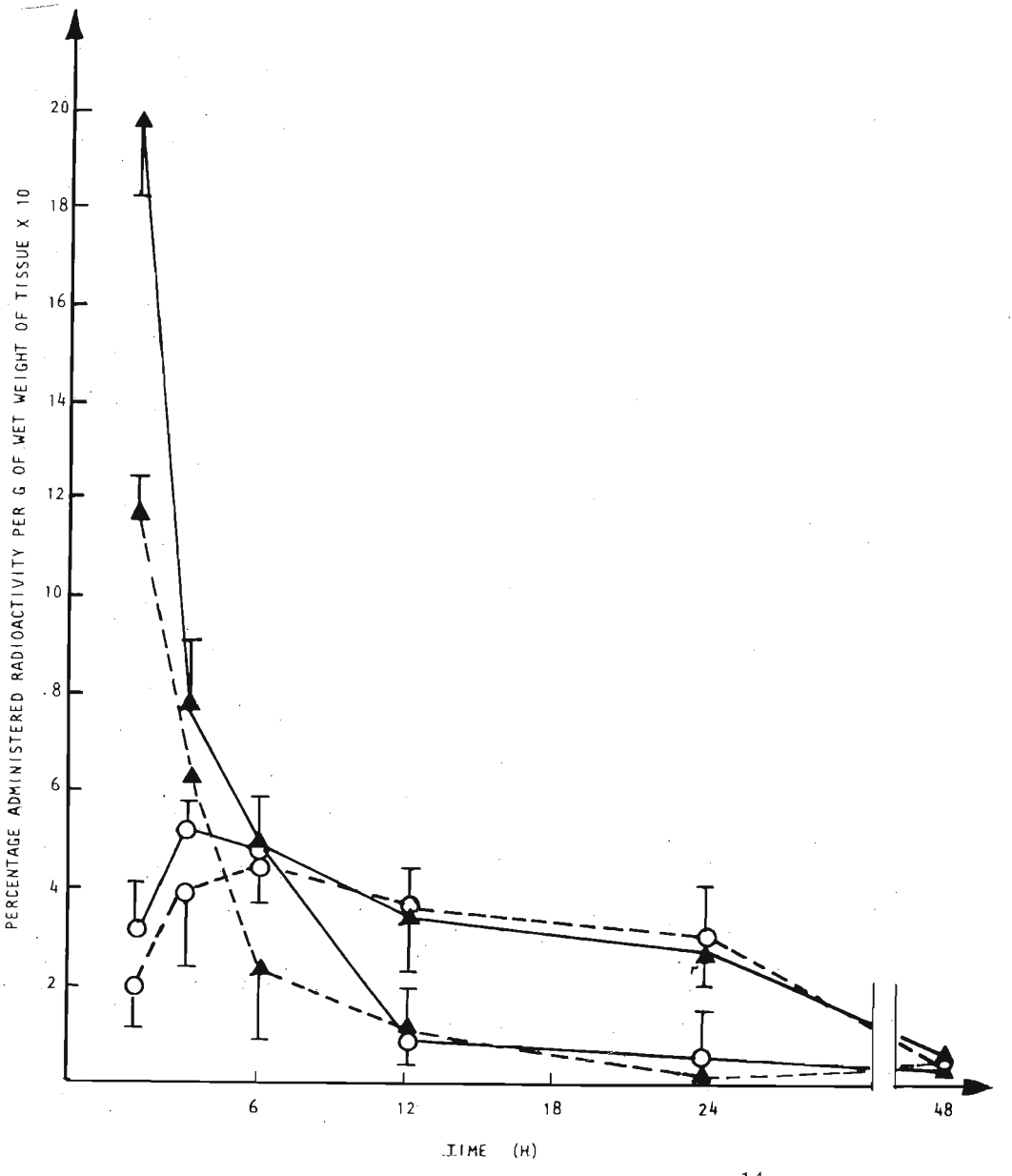

Figure 1 Tissue radioactivity after the administration of ^{14}C–labelled caffeine

Rats (n=6) were given (1–methyl–^{14}C) caffeine (100 mg/kg body wt.) orally. Tissues were removed after various time intervals and assayed for radioactivity (% of administered radioactivity/g wet weight of tissue, Mean ± SEM).

○ Blood; ▲ Stomach; ——— (1–methyl–^{14}C) caffeine; ....., (1–methyl–^{14}C) caffeine with tea.

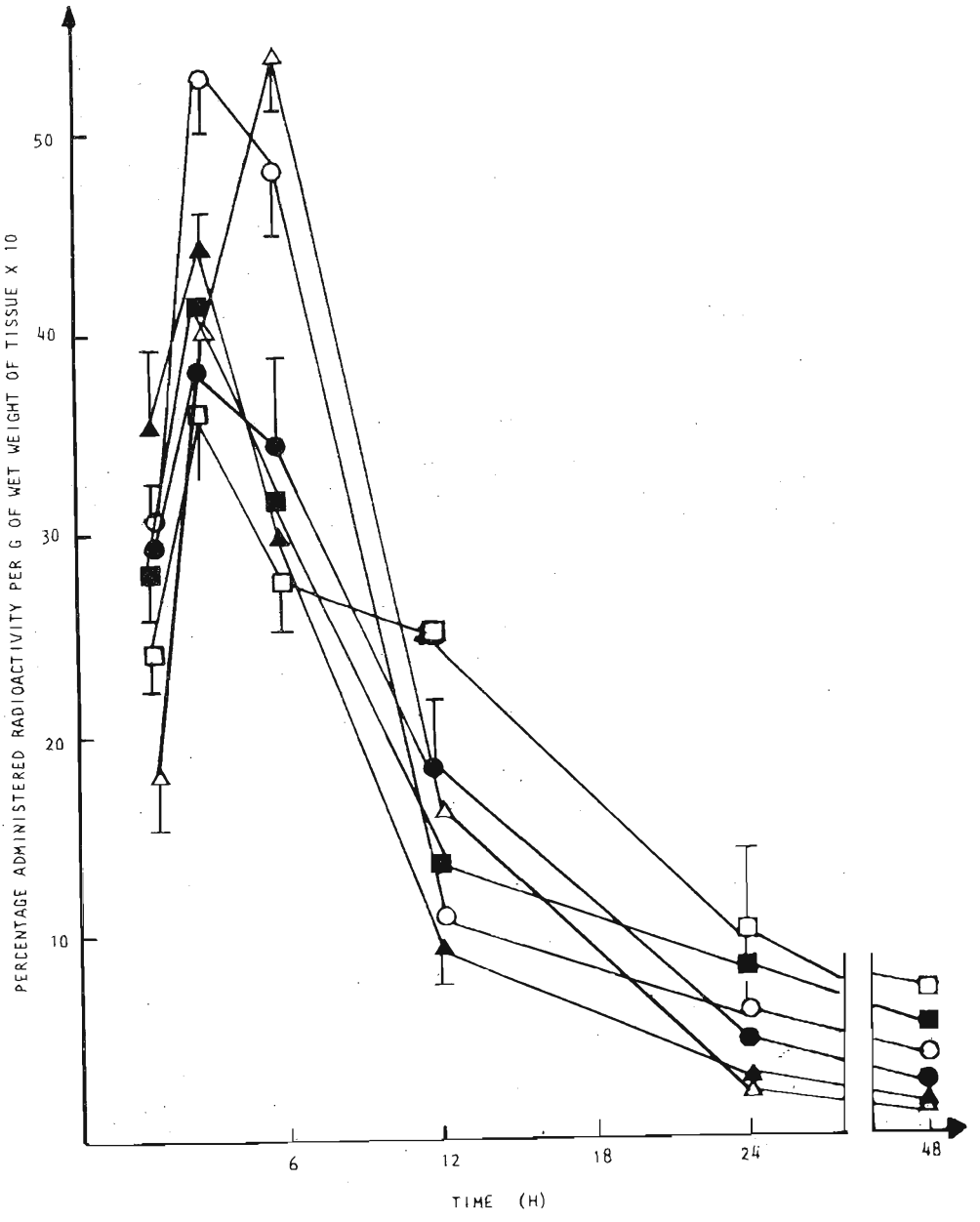

Figure 2 Tissue radioactivity after the administration of ^{14}C–labelled caffeine

Rats (n=6) were given (1–methyl–^{14}C) caffeine (100 mg/kg body wt.) orally. Tissues were removed after various time intervals and assayed for radioactivity (% of administered radioactivity/g wet wt. of tissue, Mean ± SEM).

○ , Blood; △ , Heart; ● , Testes;
▲ , Kidney; ■ , Liver; □ , Spleen

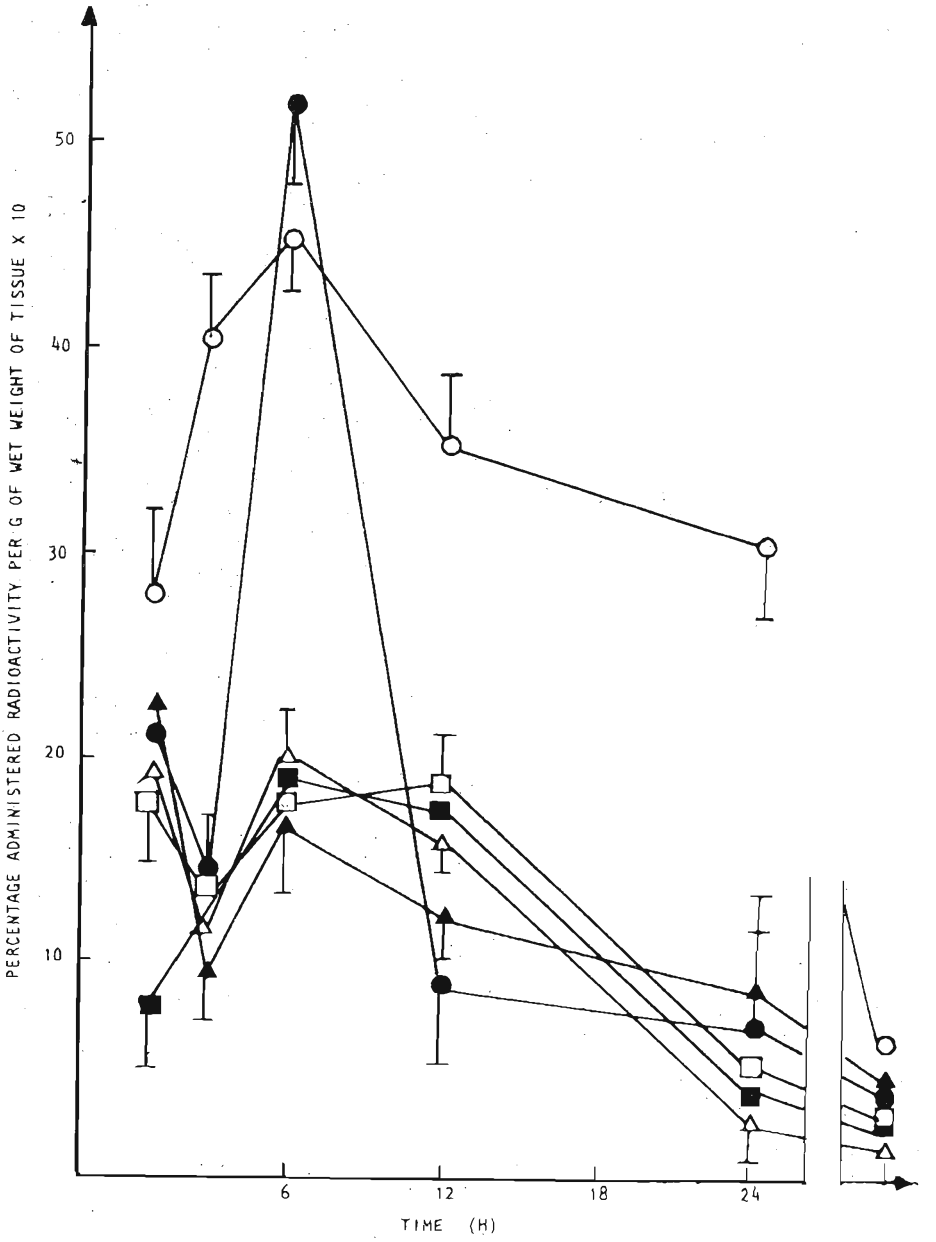

Figure 3 Tissue radioactivity after the administration of ^{14}C–labelled caffeine with tea

Rats (n=6) were given (1–methyl–^{14}C) caffeine (100 mg/kg body wt) with tea. Tissues were removed after various time intervals and assayed for radioactivity (% of administered radioactivity/g wet wt of tissue, Mean ± SEM)

○ , Blood; △ , Heart; ● , Testes;
▲ , Kidney; ■ , Liver; □ , Spleen

caffeine, no tissue did attain a level of radioactivity that is significantly greater than that in the blood. At 12h the tissue content of radioactivity in the spleen, testes and heart was higher than that in the blood. However, in view of the small percentage of administered radioactivity associated with each of these tissue at this time, it may not reflect any specific accumulation. These data indicate that, as reported for the mouse,[19] caffeine is not specifically accumulated by any of the rat tissues investigated.

In contrast, the administration of (1–methyl–^{14}C) caffeine along with the tea infusion resulted in a further significant decrease in the uptake of radioactivity by all tissues except the testes. Examination of Figure 3 clearly indicate that in no tissue except the testes, did the specific radioactivity exceed that in the blood at any time during the 48 hour experimental period. In the testicular tissue the level of radioactivity at 6h was higher than that in the blood but transient.

The observation of a general reduction in the tissue uptake of radioactivity following the administration of ^{14}C–labelled caffeine with tea, is in agreement with the claim[1, 9, 10] that caffeine is assimilated more slowly from tea than from aqueous solutions of caffeine. This possibility is further supported by the examination of area (Figures 4, 5 and 6) under each radioactive profile for different conditions of caffeine administration. Higher area for the radioactivity of stomach (Figure 4) throughout the experimental period when caffeine was administered with tea demonstrates slow gastro-intestinal absorption when compared with pure caffeine. Examination of Figure 5 confirms lack of specific accumulation of caffeine or its radioactive metabolites by any of the tissues under investigation. Several important observations emerge from Figure 6. Firstly, it provides further evidence for decrease in tissue uptake of radioactive when ^{14}C–caffeine was administered with tea. Secondly, perhaps most important, the total area for testicular radioactivity is less than that for blood confirming the high level of radioactivity associated with the testes (Figure 3) was definitely transient and not a reflection of specific accumulation of caffeine or its radioactive metabolites by the testes.

The overall results of excretion and tissue-distribution of caffeine may suggest that, as reported for the mouse,[4] caffeine is rapidly excreted by the rat possibly as unchanged[2] and metabolic products. The marked lowering in the tissue-uptake of radioactivity and the maintenance of a higher specific radioactivity in the blood over a longer period following the administration of labelled caffeine with tea, while providing further support for the claim[1, 9, 10] that caffeine is assimilated more slowly from tea than from aqueous solution, it may also throw some light on the ability of tea to reduce the possible harmful effects of caffeine. It was also apparent that following the administration of labelled caffeine with tea, the clearance of radioactivity from the gastrointestinal tract was faster than when pure caffeine was administered. In contrast, the clearance of radioactivity from

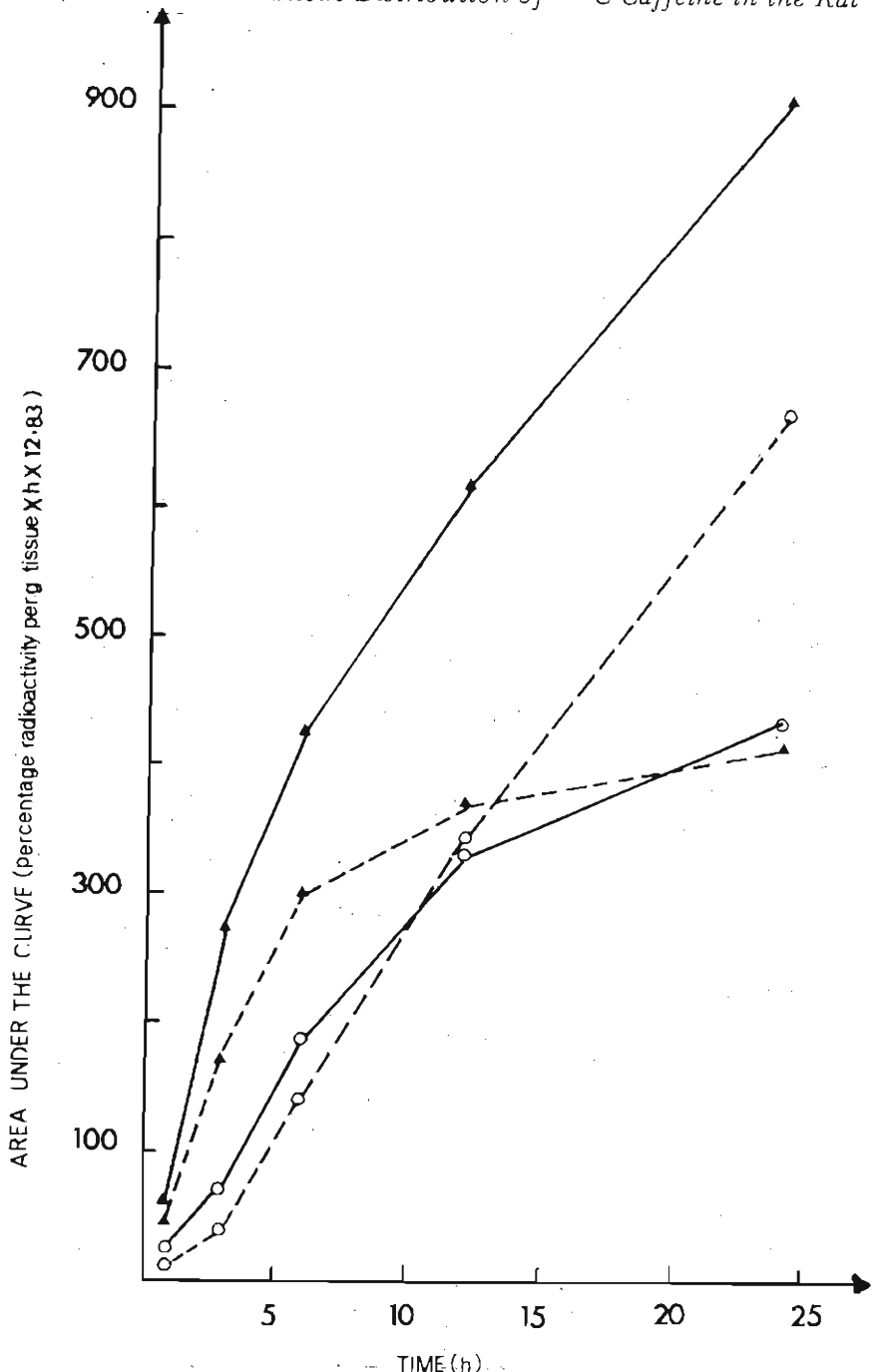

Figure 4 Area under the radioactive profiles following the administration of caffeine

, Blood; , Stomach

——— , (1–methyl–^{14}C) caffeine

. . . . . , (1–methyl–^{14}C) caffeine with tea

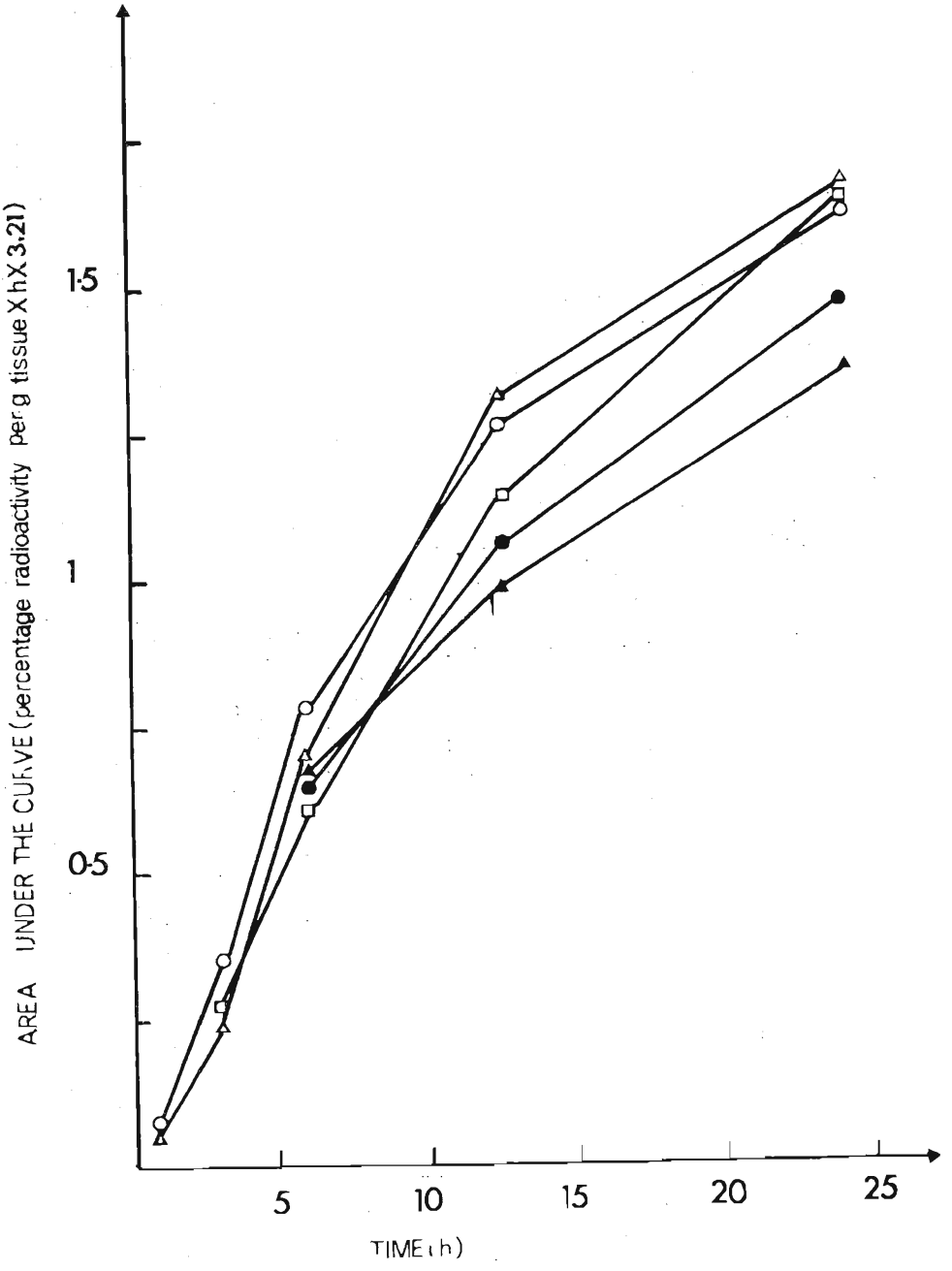

Figure 5 Area under the radioactive profiles following the administration of (1—methyl—^{14}C) caffeine

○ , Blood; △ , Heart; ● , Testes;
▲ , Kidney; ■ , Liver; □ , Spleen

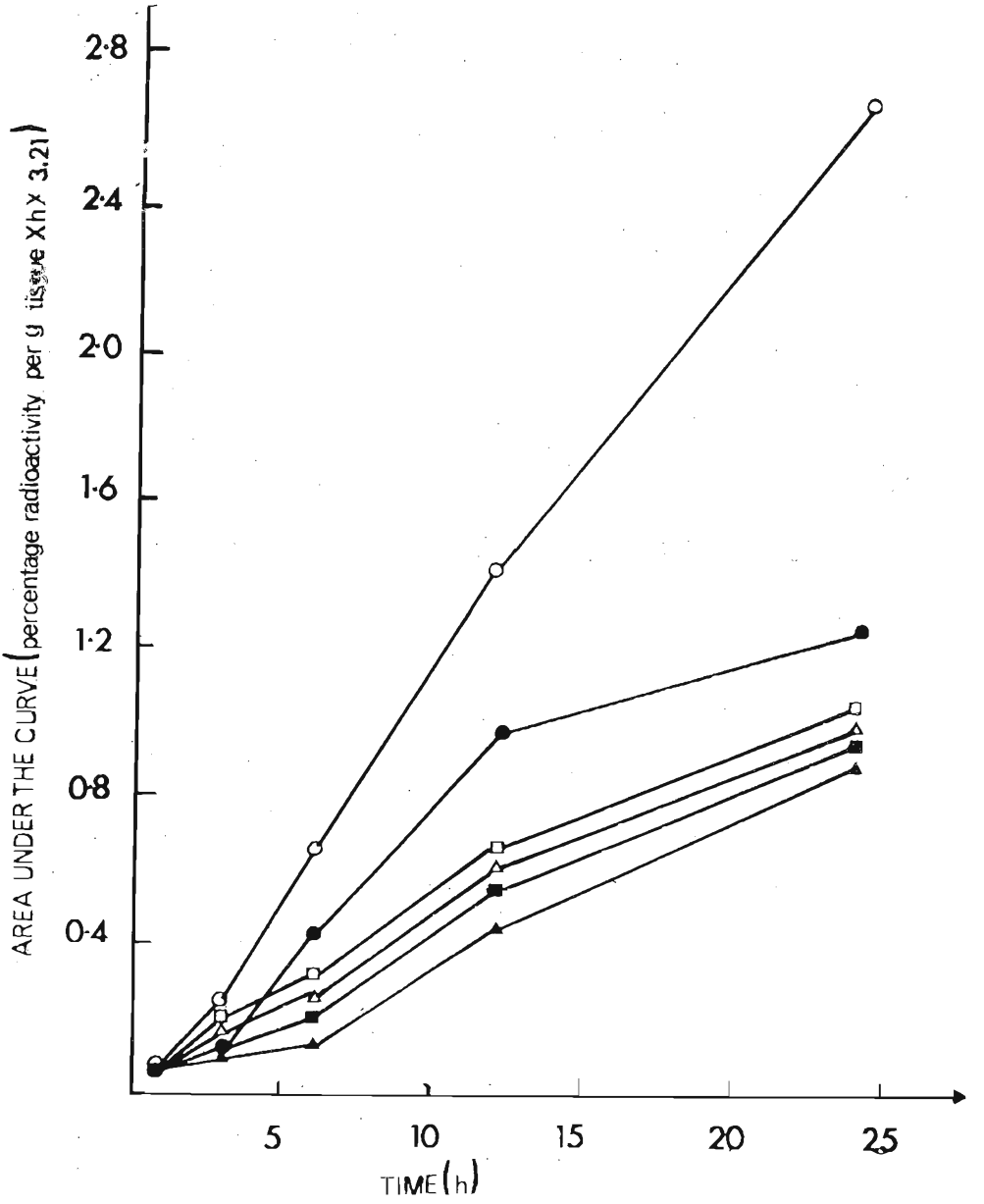

Figure 6 Area under the radioactive profiles following the administration of (1—methyl—^{14}C) caffeine with tea

○ , Blood; △ , Heart; ● , Testes;
▲ , Kidney ■ , Liver; □ , Spleen

the blood was slower when caffeine was administered with tea than when pure caffeine was administered. The observation seems to suggest that, firstly, the mechanism of absorption of pure caffeine from the gastrointestinal tract is different from the mechanism of absorption of caffeine present in tea, and secondly, the mechanism of tissue uptake of caffeine from the bloodstream following its administration with tea is different from that involved in the absorption from the gastrointestinal tract.

Alternatively, it may indicate that some agents present in tea have two opposing effects, one a stimulatory effect on the absorption of caffeine from the gastrointestinal tract, and the second, an inhibitory effect on the uptake of caffeine by the tissue from the bloodstream. It has been reported[23] that caffeine present in tea exists in the form of three complex mixtures, the solubility of which varies considerably from one to another and also dependent on pH. The most soluble complex is thought to be formed by combining caffeine with oxidized theaflavins, the next soluble complex is formed between caffeine and the polyphenolic compounds[26] and the nature of third complex group is unknown.

In order to explain the different rates of clearance of radioactivity as discussed above, it is tempting to suggest that these complex forms of caffeine present in tea while stimulating the absorption of caffeine from the gastrointestinal tract with ease greater than for pure caffeine, the same complex forms may inhibit the tissue uptake of caffeine from the bloodstream.

In conclusion, the results presented in this paper, in addition to the description of distribution and excretion of caffeine in the rat, provide strong evidence of interaction by some components present in tea, on the tissue uptake, accumulation and excretion of caffeine by the rat. It is possible that at least some of these agents are polyphenolic compounds present in tea.

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