[Chem. Pharm. Bull. 19(8)1681—1690(1971)]

UDC 547.562.31.09 : 547.292.09 : 615.31.033.076.9

Uptake by Brain and Distribution of Radioactivity after Intravenous Administration of ^{14}C-Labelled Meclofenoxate in Mice

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(Received February 17, 1971)

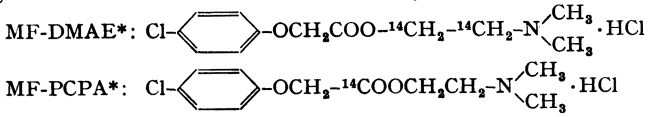
The distribution of radioactivity after intravenous administration of MF-PCPA*,[2] MF-DMAE*, PCPA*, and DMAE* was investigated by autoradiography and radioactivity measurement. The uptake of the label by brain was emphasized. Radioactivity was high in brain immediately after the injection of either MF-PCPA* or MF-DMAE*, but was negligible after the administration of the constituents. Radioactivity in brain remained relatively high even at 24 hr after administration of MF-DMAE* while it was gradually disappeared after the injection of MF-PCPA*. The distribution pattern of the label in whole body was very similar each other when animals were given MF-PCPA* and PCPA*, or when given MF-DMAE* and DMAE* except for the rapid condensation of the labelled MF into the brain.

A number of pharmacological investigations have been reported concenring on MF[2] in relation to central nervous system,[3–6] endocrines[7–10] and autonomic nervous system.[11–12] Since MF is a brain stimulator, the uptake by brain of the drug would be of particular importance and interest. In addition, DMAE, a constituent of MF, is also a brain stimulant[13] and is known to be converted to choline derivatives[14] as well as being present in brain.[15]

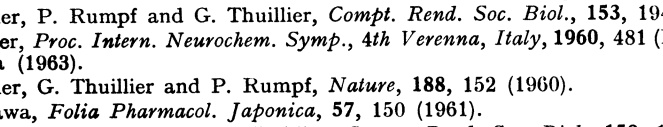
A preliminary study was reported from our laboratory concerning the distribution of radioactivity after oral and intravenous administration of MF-PCPA*.[16] In this paper, systematic autoradiographic studies with the use of labelled MF and its constituents, are reported with the emphasis on the uptake of the drug by brain.

1) Location: *Enokicho, Suita, Osaka.*

2) Abbreviations in this paper are as follows; MF: meclofenoxate hydrochloride (β-dimethylaminoethyl *p*-chlorophenoxyacetate hydrochloride), DMAE: β-dimethylaminoethanol hydrochloride, PCPA: *p*-chlorophenoxyacetic acid, DMAE*: DMAE-1,2-^{14}C, PCPA*: PCPA-carboxyl-^{14}C, MF-DMAE*: MF labelled by DMAE* and MF-PCPA*: MF labelled by PCPA*.



MF-DMAE*: Cl-C$_6$H$_4$-OCH$_2$COO-^{14}CH$_2$-^{14}CH$_2$-N(CH$_3$)$_2$·HCl



MF-PCPA*: Cl-C$_6$H$_4$-OCH$_2$-^{14}COOCH$_2$CH$_2$-N(CH$_3$)$_2$·HCl

3) J. Thuillier, P. Rumpf and G. Thuillier, *Compt. Rend. Soc. Biol.*, **153**, 1941 (1959).
4) J. Thuillier, *Proc. Intern. Neurochem. Symp., 4th Verenna, Italy*, **1960**, 481 (Pub. in 1960); *Chem. Abstr.*, **59**, 1527a (1963).
5) J. Thuillier, G. Thuillier and P. Rumpf, *Nature*, **188**, 152 (1960).
6) N. Ishikawa, *Folia Pharmacol. Japonica*, **57**, 150 (1961).
7) R. Roudier, L. Baginski and J. Thuillier, *Compt. Rend. Soc. Biol.*, **158**, 1019 (1964).
8) V. Petkov, *Minerva Medica*, **58**, 516 (1967).
9) I. Ishihara and Y. Yokoo, *Ann. Report Res. Inst. Environ. Med.* (*Kankyo-igaku Kenkyusho Nenpo*), **21**, 132 (1970).
10) I. Kobayashi, T. Yamada and K. Shichijo, *Metabolism*, **18**, 800 (1969).
11) J. Nickel, U. Breyer, B. Claver and G. Quadbeck, *Arzneimittel-Forsch.*, **13**, 881 (1963).
12) G. Quadbeck, B. Claver and G. Minot, *Arzneimittel-Forsch.*, **14**, 563 (1964).
13) C.C. Pfeiffer, E.H. Jenny, W. Gallagher, R.P. Smith, W. Jr. Bevan, K.F. Killam, E.K. Killam and W. Blackmore, *Science*, **126**, 610 (1957).
14) C. Arton and M. Crowder, *Federation Proc.*, **8**, 180 (1949).
15) C. Honegger and R. Honegger, *Nature*, **185**, 530 (1960).
16) Y. Mitta, M. Kishi and Y. Minaki, *Radioisotopes*, **16**, 29 (1967).

Experimental

Labelled Compounds——Preparation of MF-PCPA* (0.55 mCi/mM) and PCPA* (0.55 mCi/mM) was described previously.[16] DMAE* was the product of New England Nuclear Corp. (Boston, Mass.) with the specific activity of 1.0 mCi/mM. MF-DMAE* was synthesized from DMAE* and *p*-chlorophenoxyacetylchloride. In brief, 49.8 mg of DMAE* and 101.3 mg of the latter compound were refluxed in benzene for 2 hr and chilled. The formed precipitate was recrystalized from ethanol–ether mixture to obtain 92.5 mg of MF-DMAE* (yield 79.3%, specific activity 1.0 mCi/mM). The radiochemical purity was checked by the dilution analysis and paper chromatography (upper phase of *n*-BuOH: AcOH: water=8:1:10, Rf=0.62) and was found to be 99.5 and 100%, respectively.

Procedures——Male dd mice weighing about 20 g were used throughout the experiments. Administration was done by the intravenous injection of the solution in 0.9% sodium chloride of the labelled compound and suitable additives as indicated, with the dosage of 0.4 mM/kg. The dosage of radioactivity of MF-PCPA* and PCPA* was 220 μCi/kg, and that of MF-DMAE* and DMAE*, 200 μCi/kg (*i.e.* these two were diluted with non-labelled respective compound). After administration, mice were given food and water *ad libitum*. For quantitative uptake studies, mice were killed by decapitation. 0.1 ml portion of bleeding blood was dissolved in Hyamine hydroxide[17] and radioactivity was determined. Whole brain was rapidly dissected out, frozen on dry ice and weighed. Brains from animals given PCPA* or MF-PCPA* were treated with the previously described method[18] with slight modifications and their total radioactivity was determined. Brains from other animals were homogenized and fractionated into acid soluble fraction with ice cold 5% perchloric acid, lipid fraction with ethanol–ether mixture and ether, and insoluble residue which mainly consists of protein, according to the standard procedure. The radioactivity of each soluble fraction was determined on 1—5 ml portion. Insoluble protein residues were treated with the described method[18] modified slightly.

Radioactivity was determined with a Tri-Carb Model 3380 liquid scintillation spectrophotometer. Bray's solution[19] and toluene scintillator solution[20] were used as phosphor.

For autoradiographic studies, mice were killed by immersion into dry ice–acetone mixture under light ether anaesthesia. The following procedure was Ullberg's technique[21] modified by Matsuoka and Kashima.[22] Frozen mice were cut into sections 40 μ thick. The sections mounted on adhesive tape were frozen-dried and exposed to X-ray film (Fuji No. 200), which was processed after 8—9 days unless otherwise stated.

Result

Quantitative Studies on Uptake of Radioactivity by Brain

Table I shows the concentration of radioactivity in whole brain and blood at 5 min and 24 hr after intravenous injection of the labeled compounds.

It is evident that the concentration of the total radioactivity in the brain at 5 min after MF-DMAE* administration was very close to that after MF-PCPA* injection. The concentration of the drug in the brain was calculated as high as 0.3 mg of MF/g tissue and the radioactivity in the whole brain was amounted several per cent of the administered dosage. Since MF is the ester of equimolar amount of DMAE and PCPA, the results suggest that intact MF passed from blood into brain, or that similar amount of DMAE and PCPA after the degredation of MF in blood was incorporated into the tissue as MF is relatively labile in aqueous solution. It is also possible that both events were paraleled.

However, as shown in the same table, the concentration of the label was very low in brain at 5 min after the administration of the equimolar mixture of DMAE* and PCPA or DMAE and PCPA*. As blood level was sufficiently high, the blood–brain barrier conceivably prevented the mere mixture of the constituents to enter the brain. These results support the first of the three possibilities mentioned above. That is, it could be concluded that only intract MF could easily penetrate into brain through the barrier. Here, the discrepancy of the concentration

17) R.J. Herberg, *Anal. Chem.*, **32**, 42 (1960).
18) H. Miyazaki, Y. Minaki and Y. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **14**, 1298 (1966).
19) G.A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
20) Technical Mannual of Packard Instr.
21) S. Ullberg, *Proc. 2nd UN Intern. Conf. Peaceful Uses Atomic Energy*, **24**, 248 (1958).
22) O. Matsuoka and M. Kashima, *Radioisotopes*, **15**, 195 (1966).

ratio, brain/blood, between the MF-DMAE* and MF-PCPA* given animal may be explained by the different clearance rate of DMAE*- and PCPA*-compounds from blood: as shown in the following autoradiographic studies, DMAE*-compounds disappeared from blood much faster than PCPA*-compounds.

At 24 hr after administration, the concentration of the label in brain and blood were nil in the animals given PCPA* and MF-PCPA*, coinciding with the results of the preliminary report[16] which showed almost complete excretion of the radioactivity within 24 hr after administration of PCPA*-compounds. When MF-DMAE* was given, the radioactivity in brain remained high and considerable amount was incorporated into lipid fraction at 24 hr. The results suggest the active anabolism of DMAE moiety of MF after the cleavage of the ester bond. Higher amount of DMAE* was taken up by brain at 24 hr than at 5 min and lipid fraction contained more radioactivity than acid soluble fraction. The results concerning on the uptake of DMAE* by brain are essentially in accord with the results of Groth, *et al.*[23] who studied on the role of DMAE as a precursor of chloine derivatives. Low radioactivity in brain at 5 min and delayed uptake of significant label implies the presence of some transport system of DMAE or its metabolite to the tissue. Radioactivity was much higher when it was administered as MF-DMAE* than when given as DMAE* at 24 hr. This would be resulted from the higher uptake of the label shortly after administration of MF-DMAE*.

Table I. Concentration of Radioactivity in Brain and Blood 5 min and 24 hr after Administration of Labelled Compounds

Labelled compound injected	Time after injection	Concentration of radioactivity in Brain			Blood total	Brain/Blood
		Acid sol.	Lipid	Total		
		$\times 10^4$ dpm/g tissue				
MF-DMAE*	5 min	111	2	113	33	3.4
	24 hr	21	24	45	6	7.5
MF-PCPA*	5 min			133	96	1.4
	24 hr[a]			0	0	—
DMAE*+PCPA[b]	5 min	8	0	8	17	0.5
	24 hr	7	11	18	6	3.0
DMAE+PCPA*[b]	5 min			6	93	0.1
	24 hr[a]			0	0	—

a) Value from one mouse while others are mean of two animals.
b) equimolar mixture containing sodium hydroxide to dissolve PCPA
All values are corrected to the case of 200 μCi/kg administration. Almost no radioactivity was detected in the insoluble fraction of brain from animals given DMAE*-compounds. See Experimental section in the text for details.

Distribution of Radioactivity after MF-PCPA* Administraition

White areas in the autoradiograms of Fig. 1—17 were radioactive. The results obtained by the autoradiographic studies are qualitatively summarized in Table II. Readers should refer Table II as well as autoradiograms for the resolution of the distribution of radioactivity. The distribution of radioactivity at 1 and 5 min, and 1 and 4 hr after the administration of MF-PCPA* are shown in the autoradiograms of Fig. 1—4. Evidently, the radioactivity was mainly concentrated in whole brain and spinal cord 1 min after administration of MF-PCPA*, indicating that MF was specifically taken up by central nervous system. Radioactivity was also high at 5 min in the nerve tissues and the autoradiogram (Fig. 2) corresponds with the quantitative data cited in Table I. In accordance with each other, radioactiviry level in bood

23) D.P. Groth, J.A. Bain and C.C. Pfeiffer, *J. Pharmacol. Exptl. Therap.*, **124**, 290 (1958).

Fig. 1

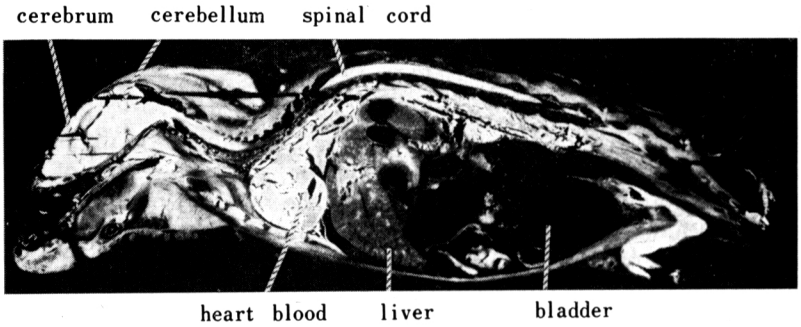


Fig. 2

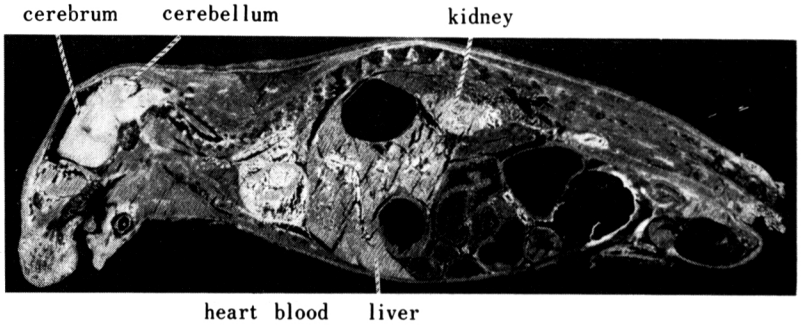


Fig. 3

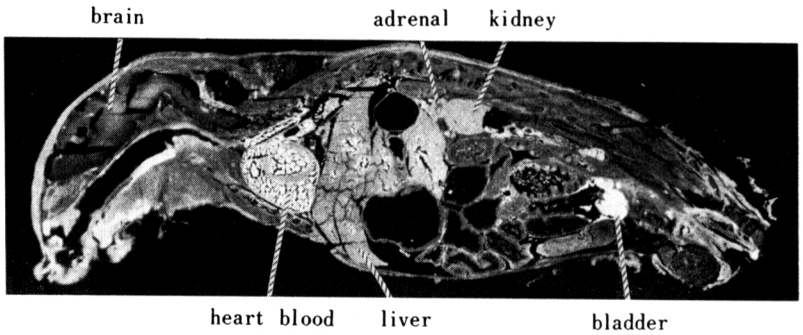


Fig. 4

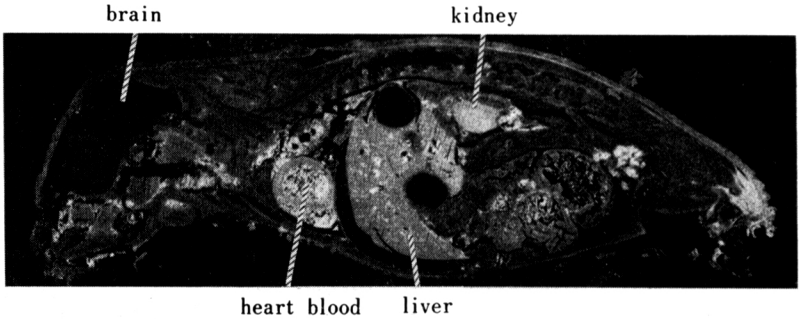


Fig. 1—4. Autoradiograms Showing the Distribution of Radioactivity at 1 min (1), 5 min (2), 1 hr (3) and 4 hr (4) after Administration of MF-PCPA*, Respectively

was close to that in whole brain. However, radioactivity in the brain gradually disappeared later and became almost negligible at 4 hr while that in blood remained still high at that time. Fig. 5 is the enlarged autoradiogram showing the distribution of radioactivity in the brain of the animal at 1 min after MF-PCPA* injection. The concentration of the label were high in cerebral cortex, striatum, thalamus, hippocampus and cerebellar cortex. White matter contained lower concentration of radioactivity.

As compared with the high and rapid uptake of the lablel by central nervous system, uptake by other tissues, although relatively high, were considerably nonspecific. The result is consistent with that of the preliminary report[16] that tissue affinity of radioactivity after MF-PCPA* administration were high and relatively nonspecific. Aderenal, kindney and urinary bladder concentrated slightly higher radioactivity. The latter two indicate that the label was excreted into urine *via* renal tissue. The uptake by adrenal gland will be briefly discussed in the next section.

Distribution of Radioactivity after MF-DMAE* Administration

Fig. 6—11 are the autoradiograms showing the distribution of radioactivity at 1 min—24 hr after MF-DMAE* administration. Coinciding with the results obtained above, brain took up significant amount of radioactivity immediately after injection. Unlike the case of MF-PCPA* administration, the detailed distribution of the label in the brain was obscure and the radioactivity in the tissue remained significantly high even at 24 hr after injection. The results are

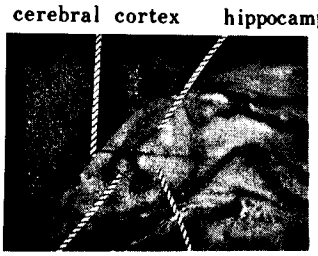


Fig. 5. Enlarged Autoradiogram Showing the Distribution of Radioactivity in Brain at 1 min after Administration of MF-PCPA*

exposed for 4 days

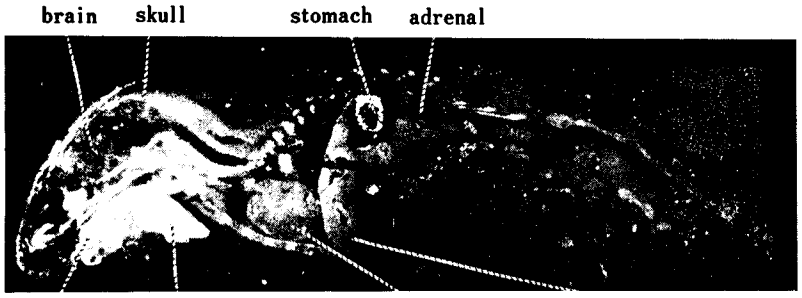


Fig. 6

Fig. 7

Fig. 8

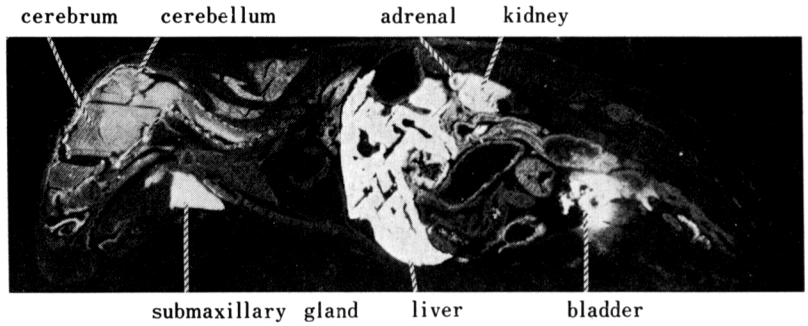


Fig. 9

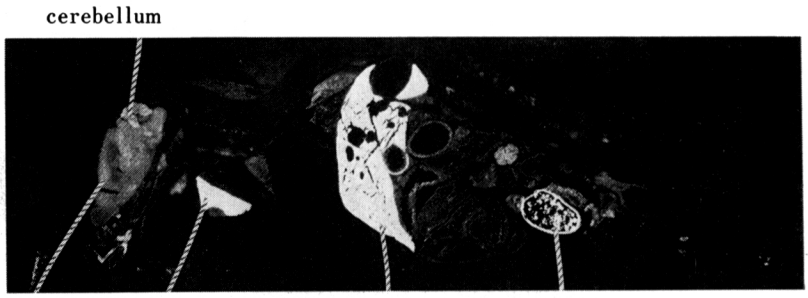


Fig. 10

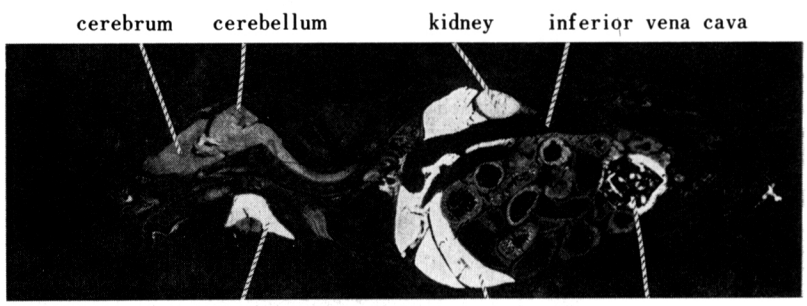


Fig. 11

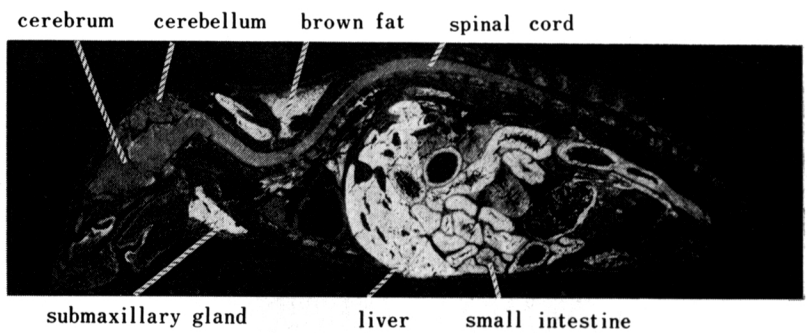


Fig. 6—11. Autoradiograms Showing the Distribution of Radioactivity at 1 min (6), 5 min (7), 1 hr (8), 2 hr (9), 4 hr (10) and 24 hr (11) after Administration of MF-DMAE*, Respectively

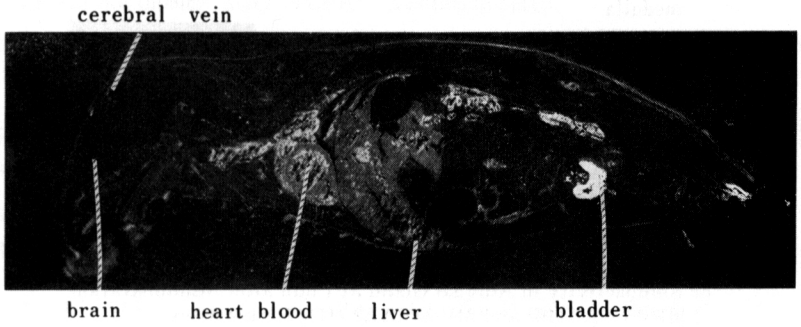


Fig. 12. Autoradiogram Showing the Distribution of Radioactivity at 5 min after administration of PCPA*

Fig. 13

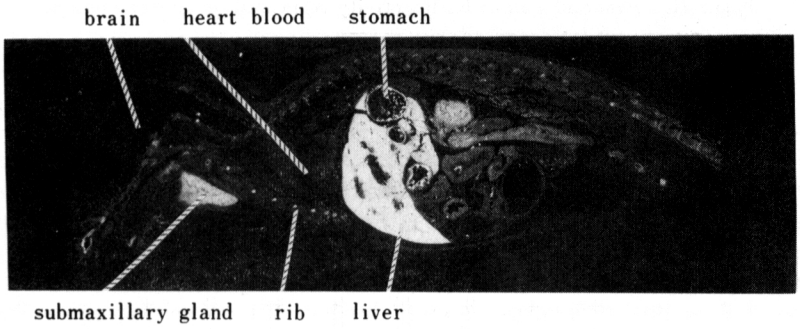


Fig. 14

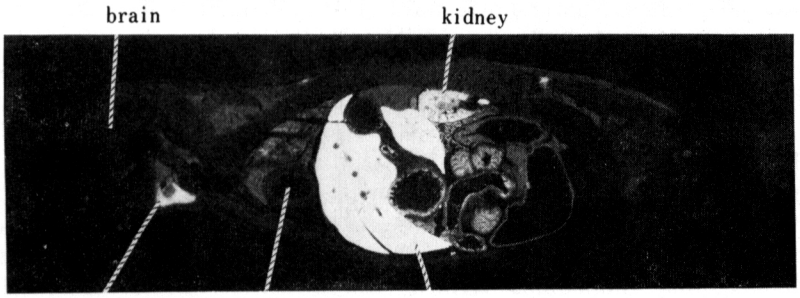


Fig. 15

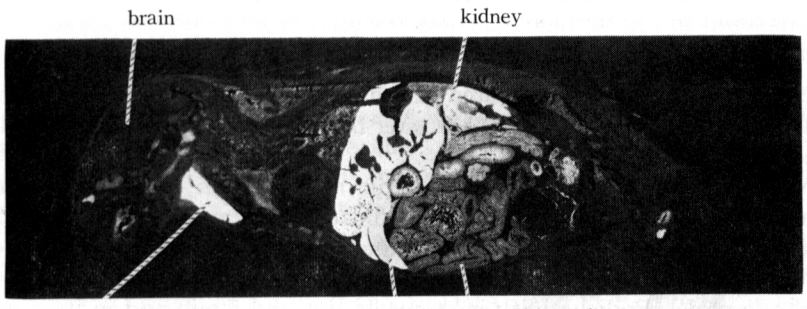


Fig. 13—15. Autoradiograms Showing the Distribution of Radioactivity at 5 min (13), 1 hr (14) and 4 hr (15) after Administration of DMAE*, Respectively

Fig. 16

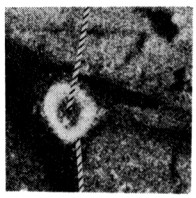


Fig. 17

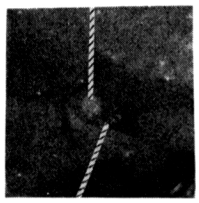


Fig. 16—17. Enlarged Autoradiograms Showing the Distribution of Radioactivity in Adrenal Gland at 1 min after Administration of MF-PCPA* (16) and MF-DMAE* (17), Respectively

Fig. 16 is one exposed for 4 days.

TABLE II. Rise and Fall of Radioactivity Appeared on Autoradiograms

Organs	MF-PCPA*				MF-DMAE*						PCPA*		DMAE*	
	1 min	5 min	1 hr	4 hr	1 min	5 min	1 hr	2 hr	4 hr	24 hr	5 min	5 min	1 hr	4 hr
Brain	###	###	+	±	##	##	##	##	##	##	−	−	±	±
Spinal Cord	###	###	+	±	##	##	##	##	##	+	−	−	±	±
Blood	###	###	###	##	##	##	−	−	−	±	###	+	−	±
Lung	###	##	##	+	##	##	+	##	##	##	##	+	+	##
Spleen	±	+	+	+	+	##	+	+	+	##	+	+	+	##
Bone Marrow	±	+	+	+	##	##	+	##	+	##	+	##	##	##
Stomach	±	+	+	+	###	###	##	##	##	+	+	###	##	+
(Content)	−	−	−	−	###	###	##	##	+	−	−	###	##	±
Small Intestines	±	+	+	+	±	##	##	##	+	##	+	+	##	##
(Content)	−	±	±	±	−	##	+	+	±	±	−	±	±	±
Large Intestines	±	+	+	+	±	##	+	##	+	##	+	+	+	##
(Content)	−	−	−	−	−	±	±	−	±	±	−	±	−	−
Liver	+	+	+	+	+	###	###	###	###	###	##	###	###	###
Adrenal Cortex	###	##	##	+	±	±	+	+	##	##	##	−	+	##
Medulla	±	±	+	±	##	##	±	+	+	+	−	+	+	+
Submaxillary Gland	##	+	+	+	###	###	###	###	###	###	+	###	###	###
Kidney	##	##	##	##	+	###	###	###	##	###	###	##	##	###
Urinary Bladder	−	+	###	###	−	###	###	###	###	##	##	###	###	##
Bone	+	+	+	+	###	###	###	+	±	+	+	##	±	+
Muscle	##	##	+	+	###	##	+	+	+	+	+	+	+	+

###: highest radioactivity, ##: higher activity, +: high activity, ±: low activity and −: no activity

compatible with the results in Table I and also indicating that PCPA* portion of MF disappeared from brain after the cleavage of the ester bond while DMAE* portion was anabolically metabolized in the brain.

However, bone, submaxillary gland, tongue and stomach condensed higher radioactivity than nerve tissue at 1 min. Bone lost radioactivity almost completely at 2 hr (Fig. 9). The radioactivity content in submaxillary gland was continuously high in the experimental period. Significantly high radioactivity in tongue at 1 min may suggest that the secretion of the label occurred. Radioactivity in stomach at 1 and 5 min and in its content suggests that the label was derived from submaxillary gland *via* mouth or that excretion of the label into stomach cavity from gastric mucosa took place. It is noteworthy that brown fat and gastrointestinal tracts took up high radioactivity at 24 hr after administration.

Distribution of Radioactivity after PCPA* Administration

Fig. 12 shows the distribution of the label at 5 min after PCPA* injection and corresponds with the data in Table I. Although blood in cerebral vein contained significant radioactivity, brain took up undetectable amount of it. The pattern of the distribution in other tissues was very similar to that after MF-PCPA* administration. This also supports that esterified PCPA does enter brain and free PCPA does not.

Distribution of Radioactivity after DMAE* Administration

Fig. 13—15 are the autoradiograms showing the distribution of the label after DMAE* injection. The radioactivity in brain was negligible at 5 min after administration with slight activity in blood. This is also compatible with the results in Table I. However, though being very low, the label in brain was gradually accumulated as shown in Fig. 14 and 15. Except for the uptake by brain the distribution was very similar to that after MF-DMAE* administration: high uptake by submaxillary gland, mouth, stomach and its constant at early period, kidney and intestinal tracts. Bone also took up relatively high radioactivity at 5 min but lost it at 1 hr.

Discussion

The results obtained in this study showed a notable chracteristic of blood–brain barrier that MF, the ester, rapidly penetrates into brain while the two constituents, both DMAE and PCPA, do not. Since many drugs acting on the central nervous system are known to be taken up by brain (see review[24] for instance), it could be said that the primary site of action of MF is most likely the central nervous system. If so, it would be reasonable that MF posesses many pharmacological properties on the central nervous system[3–6] and is a brain stimulator. In connection to this, Groth, *et al.*[23] suggested that the delayed uptake of DMAE or its metabolite by brain may be related with its brain stimulating properties because it could be stimulatory only when given chronically.[13] In this respect, the rapid penetration into brain of DMAE molecules after MF injection may partially play part to its pharmacological properties. Also, our findings might make it meaningful to test the action mechanism of intact MF *in vitro* using brain preparation.

As for the whole body distribution of radioactivity, DMAE* and MF-DMAE* or PCPA* and MF-PCPA* showed a relatively similar pattern of distribution except for the rapid condensation by brain of the ester. Therefore, except for the label in the brain, the distribution of radioactivity conceivably shows that of each constituent derived from administered MF in the labelled MF-injected animals. In general, DMAE*-compounds showed somewhat specific distribution and their clearace from blood was very rapid. In contrast, PCPA*-compounds remained high in blood and their distributions were more or less nonspecific. Here, the observation on the uptake of the radioactivity by adrenal gland is also of interest. MF-PCPA* (Fig. 16) was highly concentrated in adrenal cortex after administration while MF-DMAE* (Fig. 17) was significantly taken up by medulla. Interestingly, with the passage of time radioactivity derived from MF-DMAE* became higher in cortex (Fig. 8). Though there are pharmacological evidences concerning the effect of MF on adrenal functions,[8–9] they are reportedly regulated by the central nervous system influenced by the drug. MF in such organ as thalamus(Fig. 5) may affect on the function.

In conclution, based on the findings in the present study, we consider that the pharmacological properties of MF maybe explained by the action of three components. First is the action on the central nervous system of MF itself and, of DMAE and PCPA present in brain after the cleavage of the ester. Other two are the effects of DMAE and PCPA released by the breakdown

24) Y. Sato, *Pharmacometrics* (*Oyo Yakuri*), **4**, 535 (1970).

of MF in, perhaps, blood. Kobayashi, *et al.*[10] recently reported that PCPA derived from MF made free thyroxine available to tissue cells by the interference with the hormone-plasma protein interaction. The event is quite likely to occur *in vivo* since PCPA remained high in blood after administration of MF. Unfortunately, no such clear information is available concerning on the mechanism of MF effects in *in vitro* system though Thuillier[4] presented the evidence thst MF, neither DMAE nor PCPA, acted on the central nervous system. Although some part of brain stimulatory properties of MF could be explained by the rapid uptake of DMAE molecule by the tissue, the exact mechanism through which DMAE can be a brain stimulant is aloso still obscure. The results in the present study would be usefull for the detailed biochemical and molecular-pharmacological examiniation of action mechanism of MF and derived constituents.

Acknowledgement We wish to thank Drs. S. Ose, T. Mizuma and H. Takamatsu for their support to this work, Messrs. Y. Mitta, M. Kishi, T. Fujii and H. Suzuki for their discussion and collaboration.

Additional Note (Received June 4, 1971) We re-examined the concentration of label in brain and bone at 5 min after the intravenous administration of MF-DMAE* as described in the text. As for the bone sample, skull was subjected for the determination of radioactivity because it contained more radioactivity than brain in the corresponding autoradiogram (Fig. 7). The result on the brain was same as that given in Table I, but contrary to Fig. 7, the concentration of label in the skull was only 30% of that in the brain. This is the same order as blood level. So the autoradiogram Fig. 7 does not exactly correspond to the quantitative data in Table I as for the 5 min distribution of MF-DMAE*.

To explain the discrepancy, it is most likely that considerable amount of DMAE* formed in the brain, being volatile, disappeared during the freeze-drying period from whole body section. After the administration of MF, brain contained intact MF, DMAE and PCPA at 1 min, and mainly latter two at 5 min (unpublished data), which supports the explanation. It can be also accounted that the detailed distribution of label in the brain given MF-DMAE* was more obscure than that given MF-PCPA*. Sato[25] pointed out that whole body autoradiography, in a certain case, may make it possible to estimate the volatile metabolite by the disappearance of label from the autoradiogram.

The detailed metabolism of MF will be reported elsewhere.

25) Y. Sato, *Pharmacometrics* (*Oyo Yakuri*), 3, 77 (1969).