between the end of the methotrexate injections and the commencement of asparaginase, probably enabling the dividing fraction of the tumour to be reconstituted from the resting cells.

The R_1 lymphoma, in its response to anti-tumour agents, appears to be a suitable model for studying drug combinations likely to be effective in the treatment of acute lymphoblastic leukaemia. In view of the use of both methotrexate and asparaginase in the treatment of this disease, it would seem important that these two agents should always be administered consecutively and not simultaneously.

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Inhibition of monoamine oxidation in brain by monoamine oxidase inhibitors

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MANY data have accumulated in recent years on the inhibition of monoamine oxidase (MAO) by hydrazide and non-hydrazide inhibitors resulting in an elevated level of biogenic amines in brain.[1–7] Contrary to these, observations were also made indicating that trans-phenylcyclopropylamine (trans-PCP) and iproniazid failed to elevate brain level of tryptamine.[8,9] The earlier work on enzymatic reduction of tetrazolium salts or other redox indicators in the presence of amines[10–12] has been extended by others,[13–15] although it is not known whether the amine dehydrogenating system (MADH) is identical with MAO or not. However, histochemical studies using tetrazolium salt reduction in the presence of monoamines have always been described as MAO activity in the literature.[16–21] A preliminary account of certain differences observed in the reaction mechanism of monoamine oxidation catalysed by rat brain MAO and MADH systems and their response to inhibitors like iproniazid, trans-PCP and pargyline is described in this communication.

Whole brain homogenates of adult albino rats were prepared as a 10 per cent suspension in 0·25 M sucrose. The reaction mixture for MAO assay contained 0·02 M phosphate buffer pH 6·5, 0·0125 M semicarbazide pH 6·5, 0·01 M tyramine and 50 mg of tissue homogenate in a final volume of 2 ml. Aldehyde formed was measured at 420 mμ by the method of Green and Haughton[22] as described previously.[23] The reaction mixture for MADH assay consisted of 0·025 M phosphate buffer pH 7·0, 0·5 mg neo-tetrazolium chloride (NTC), 0·01 M tyramine and 100 mg of tissue homogenate in a final volume of 2 ml. NTC reduction was measured at 520 mμ according to the method of Lagnado and Sourkes.[14] Air was used as the gas phase in aerobic experiments and anaerobic experiments were carried out *in vacuo* in Thunberg tubes.[24] Incubations were carried out at 38° for 30 min with a preincubation period of 5 min unless stated otherwise. When the inhibitors were added, they were allowed to incubate with the enzyme for 20 min prior to addition of the substrate. Ammonia formed in these systems was determined by the manometric method of Braganca *et al.*[25] followed by nessler-isation. All values are corrected for appropriate blanks without any added substrate. *In vivo* experiments were performed by administering daily to groups of six rats intraperitoneal injections of MAO

inhibitors (50 mg/kg of iproniazid or 2 mg/kg of trans-PCP or pargyline) for 3 days, while the six control rats received injections of normal saline for 3 days. On the fourth day the animals were sacrificed and the enzyme activities were determined.

Anaerobiosis increased MADH activity whereas MAO activity was completely abolished (Table 1). The formation of ammonia and aldehyde during oxidation of amines by MAO is dependent on a high partial pressure of oxygen for saturation[10,26] and these substances are not formed anaerobically. Oxygen can not be replaced by electron acceptors like NTC. However even aerobically, MAO system with added NTC produced ammonia and aldehyde but there was no formazan production possibly due to the inhibition of formazan production by semicarbazide.[14] On the other hand MADH system produced no ammonia anaerobically, suggesting that ammonia is not a product of this system. The formation of ammonia in MADH system aerobically, though less than that formed in MAO system may be due to the activity of MAO system operating here.

The degree of *in vitro* inhibition of MAO and MADH systems by different concentrations of iproniazid, trans-PCP and pargyline which are highly inhibitory to MAO is shown in Table 2. Excepting iproniazid at concentrations of 1×10^{-4}M and above (cf. ref. 14) these drugs failed to inhibit NTC reduction *in vitro*. Results of *in vivo* administration of these inhibitors as shown in

TABLE 1. MAO AND MADH ACTIVITIES OF RAT BRAIN HOMOGENATE

Systems	Aerobic incubation			Anaerobic incubation		
	*E_{420}	E_{520}	Ammonia (μg)	E_{420}	†E_{520}	Ammona (μg)
1. MAO	0·205 ± 0·008 (123·6 ± 4·83)	—	6·40 ± 0·091	Nil	—	Nil
2. MADH	—	0·190 ± 0·015	6·25 ± 0·089	—	0·215 ± 0·007	Nil
3. System 1 + NTC (0·5 mg)	0·205 ± 0·008 (123·6 ± 4·83)	Nil	6·50 ± 0·091	Nil	Nil	Nil

* Figures in parentheses represent μg *p*-hydroxyphenylacetaldehyde 2:4-dinitrophenylhydrazone formed which is determined from a calibration curve using twice crystallised dinitrophenylhydrazone.[22,28]

† NTC reduction in these experiments was measured after 15-min incubation.
Data are expressed as mean of six experiments ± S.D.

TABLE 2. EFFECT OF CERTAIN MAO INHIBITORS ON RAT BRAIN MAO AND MADH ACTIVITIES *in vitro*

Inhibitors	Final concentrations of inhibitors (M)	Per cent inhibition of		
		MAO	MADH (aerobic)	MADH (anaerobic)
1. Iproniazid	1×10^{-3}	88	70	50
	5×10^{-4}	76	33	23
	1×10^{-4}	65	8	5
	1×10^{-5}	40	nil	nil
2. trans-PCP	5×10^{-4}	100	nil	nil
	1×10^{-4}	90	nil	nil
	1×10^{-5}	76	nil	nil
	1×10^{-6}	67	nil	nil
3. Pargyline	5×10^{-4}	100	nil	nil
	5×10^{-5}	100	nil	nil
	5×10^{-6}	94	nil	nil
	5×10^{-7}	70	nil	nil

Table 3 indicate that only iproniazid produced a slight inhibition of MADH while trans-PCP and pargyline were without any effect on MADH although the corresponding MAO activity was strongly inhibited in all cases.

TABLE 3. EFFECT OF *in vivo* ADMINISTRATION OF CERTAIN MAO INHIBITORS ON RAT BRAIN MAO AND MADH ACTIVITIES

Systems	MAO activity* ($E_{420} \pm$ S.D.)	MADH activity ($E_{520} \pm$ S.D.)	
		Aerobic	†Anaerobic
1. Normal rats	0·205 ± 0·008 (123·6 ± 4·83)	0·190 ± 0·015	0·215 ± 0·007
2. Iproniazid treated rats	0·020 ± 0·006 (12·1 ± 3·43)	0·175 ± 0·008	0·190 ± 0·014
3. trans-PCP treated rats	0·030 ± 0·006 (17·7 ± 3·55)	0·195 ± 0·023	0·210 ± 0·043
4. Pargyline treated rats	0·025 ± 0·005 (14·8 ± 3·11)	0·200 ± 0·009	0·220 ± 0·011

* Figures in parentheses represent μg *p*-hydroxyphenylacetaldehyde 2:4-dinitrophenylhydrazone formed.

† NTC reduction in these experiments was measured after 15 min incubation.

The above results indicate that MADH system is possibly different from MAO and explain the previous observations[8,9] that brain tryptamine level is not elevated by MAO inhibitors, since tryptamine is actively metabolised by brain MADH whereas serotonin is a poor substrate in the tetrazolium reduction system.[27]

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Enzymatic determinants of responsiveness of the LPC-1 plasma cell neoplasm to fluorouracil and fluorodeoxyuridine*

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THE MURINE plasma cell tumor LPC-1 has been examined as a possible model for human plasma cell disease. In a study conducted by Abraham *et al.*[1] 5-fluorouracil (FU) proved to be the most effective of 31 agents tested for anti-neoplastic response. 5-Fluoro-2′-deoxyuridine (FUdR) was much less active. This paper reports results of a study of the biochemical basis for this differential response.

Tumor. The LPC-1 tumor[2] was supplied by Dr. Morris Teller, Sloan–Kettering Institute and was maintained in CDF_1 mice by intraperitoneal or subcutaneous inoculations of 10^5 cells.

Chemicals. Radioactive substrates were purchased from Calbiochem and New England Nuclear Corp. Other chemicals were purchased from Mann Research Laboratories, Sigma Chemical Company and Calbiochem. Unlabeled fluorinated pyrimidines were provided by Hoffman-LaRoche.

Enzyme preparations. Nodules of tumor infiltrating the intestinal mesentery were collected and placed in 2 vol. of 0·05 M potassium phosphate at pH 7·0 containing 5 mM mercaptoethanol, 1 mM EDTA and 3 mM $MgCl_2$. The preparation was homogenized in a Potter–Elvehjem homogenizer and centrifuged at 8000 *g* for 10 min. The supernatant fluid was decanted, then centrifuged at 100,000 *g* for 30 min. The resulting supernatant comprises fraction E_1; the 100,000 *g* pellet forms fraction P. In an alternative procedure, the initial homogenate was subjected to three freeze–thaw cycles followed by brief sonic oscillation, then centrifugation at 30,000 *g* for 30 min. The supernatant fluid resulting from this process represents fraction E_2. All operations described above were carried out at 4° or below.

Enzyme assays. Protein was determined by the method of Lowry *et al.*[3] Uridine phosphorylase (EC 2.4.2.3) was measured at 37° by the procedure of Friedkin and Roberts.[4] Incubation mixtures contained 0·05 M arsenate or phosphate at pH 7·5, 10 mM substrate [2′-deoxyuridine (UdR), uridine (UR), thymidine (TdR), 5-fluorouridine (FUR) or FUdR], 5 mM mercaptoethanol, and 5–6 mg of enzyme protein in a total volume of 0·5 ml. At intervals of 0, 10, 20, 30 and 60 min after addition of enzyme, samples of 100 μl were removed from the mix and diluted with 100 μl of 0·6 M $HClO_4$. The protein was removed by centrifugation and a 100-μl portion of the supernatant fluid was mixed with 100 μl of 1 M KOH. After chilling to precipitate $KClO_4$, the optical density of the solution was read at 290 mμ (substrates: UR and UdR) or 300 mμ (other substrates). Standards were run to determine extinction coefficients of the substrates and products.

Uridine kinase (EC 2.7.1.48) and thymidine kinase (EC 2.7.1.21) were determined by the DEAE disc method of Breitman.[5] Incubations were carried out at 37° using a mixture containing 5 mM

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