located within the cell in a form able to participate in an exchange process. The data do not permit an explanation, at the molecular level, of the difference between the transport mechanisms in the two types of cells. It is possible that a carrier protein, localized in the cell membrane, is altered in the m^t cells.

The folic acid reductase of m^t cells had an unaltered "affinity" for methotrexate, was of equal activity in s and m^t cells, and behaved similarly during fractionation. At 10^{-5} M methotrexate, s cells were capable of taking up 10 times the amount of methotrexate required to titrate the total folic acid reductase activity within the cell. Fractionation of such cells revealed that the methotrexate was only in small part associated with this enzyme; the major fraction was dialyzable, whereas a minor fraction was tightly bound to material without folic acid reductase activity. In addition, the intracellular methotrexate from s cells incubated for a period of 18 hr at a completely inhibitory, but much lower, concentration of the drug (3×10^{-8} M) was recovered as extracellular drug by incubation of the cells for 6 hr in growth medium free of methotrexate. The quantity of methotrexate recovered suggested that the inhibitor can be freed from the folic acid reductase of these cells.

The specificity of the mutagenic events affecting transport in m^t cells was indicated by the observation that the nutritional requirements for very high levels of folic acid (and of thymidine, hypoxanthine, and serine in the presence of inhibitory levels of methotrexate), were unchanged in the m^t cells. In addition, the sensitivity of the m^t cells to selected antimetabolites during reproduction in culture (cytosine arabinoside, 6-azauridine, and 5-fluorodeoxyuridine) was unchanged; furthermore, methotrexate was not significantly inactivated by either line of cells.

The levels of folic acid reductase activity of s and m^t cells was not altered by growth of the cells in the presence of partial or completely inhibitory levels of methotrexate, a finding which suggested that enzyme induction did not occur in either line in response to the drug. Similar findings concerning enzyme induction had been reported for those mutants that become resistant by virtue of an increased level of folic acid reductase activity.[5] However, m^t cells had an increased nutritional requirement for DL-leucovorin. It has also been demonstrated that 10^{-5} M DL-leucovorin inhibits the uptake of 3×10^{-8} M methotrexate by sensitive and resistant cells. Thus, it is possible that the transport mechanism for methotrexate may also be concerned with the entry of other derivatives of folic acid into the cell.

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Department of Pharmacology,
Yale University School of Medicine,
New Haven, Conn., U.S.A.

GLENN A. FISCHER

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The subcellular distribution of substance of P and 5-HT in brain

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SEPARATION of the crude mitochondrial fraction (P2) from brain homogenates in a density gradient of 0·32, 0·8 and 1·2 M sucrose solutions produces three subcellular fractions: a light myelin fraction, a nerve ending fraction of intermediate density and a denser mitochondrial fraction.[1] Pharmacological analyses of these fractions show that acetylcholine (ACH)[2, 3] and substance P [2] are mainly located in the nerve ending fraction. However in guinea-pig brain homogenates which contain 1 mM iproniazid, 5-HT is mainly recovered from the mitochondrial and also from the microsomal fractions.[2] Preliminary experiments on rat brain homogenates, which did not contain iproniazid, indicated that 5-HT

was recovered from the same subfractions of P2 which contained the ACH. Therefore further experiments were carried out to investigate this difference, and to compare the distributions of ACH, substance P and 5-HT in the fractions obtained by separation in a more complex density gradient consisting of 0·32, 0·8, 1·0, 1·2 and 1·4 M sucrose solutions. It has been reported that the use of such a gradient allows separate cholinergic and non-cholinergic nerve ending fractions to be obtained.[4]

Table 1 shows the results obtained in experiments on a rat and a guinea-pig brain homogenate, which contained eserine ($2·7 \times 10^{-5}$ M) and were prepared in a piston press homogeniser. Iproniazid

TABLE 1. SUBCELLULAR DISTRIBUTION OF ACH, SUBSTANCE P AND 5-HT IN BRAIN

Fraction	Approx. density sucrose (M)	Rat						Guinea-pig			
		ACH		5-HT		Sub. P		ACH		5-HT	
		(%R)	RSA	(%R)	RSA	(%R)	RSA	(%R)	RSA	(%R)	RSA
P1		(20)	0·71	(17)	0·61	(25)	0·9	(12)	0·86	(5)	0·36
P2		(62)	1·2	(42)	0·78	(55)	1·0	(57)	1·0	(35)	0·64
P3		(18)	1·0	(41)	2·3	(20)	1·1	(31)	1·0	(60)	2·0
A	0·32–0·8	(10)	0·28	(16)	0·45	(18)	0·5	(27)	0·54	(52)	1·0
B	0·8–1·0	(34)	3·1	(20)	1·8	(14)	1·3	(27)	3·9	(27)	3·9
C	1·0–1·2	(52)	1·9	(42)	1·4	(68)	2·4	(36)	1·8	(21)	1·1
D	1·2–1·4	(4)	0·27	(21)	1·4	(0·0)	0·0	(9)	0·7	(0·0)	0·0
E	>1·4	(0·0)	0·0	(0·0)	0·0	(0·0)	0·0	(1)	0·1	(0·0)	0·0
%P2		54		101		75		68		125	
P2 content/g brain		1·5 μg		84 ng		27 units		1·3 μg		47 ng	

The distributions are expressed as the percentage of the total recovered activity in each fraction (%R) and as the relative specific activity (RSA), which is the ratio of %R to the percentage of nitrogen recovered in that fraction. %P2 is the total recovered activity in A, B, C, D and E expressed as a percentage of the activity of P2. The amounts of 5-HT and ACH in P2 are expressed in terms of the complex with creatinine sulphate and as the bromide respectively.

was omitted from the sucrose solutions since in its presence (1 mM) there was obvious aggregation of the particles and the layers obtained in the density gradient differed markedly from those obtained in its absence. As in the previous experiments,[2] a large proportion of the 5-HT was found in the microsomal fraction (P3) and the relative specific activity, which refers the 5-HT content of a fraction to its nitrogen content, was higher than that for P2 or the nuclear fraction (P1). In contrast to the previous findings, on subfractionation of P2 the 5-HT was recovered from fractions lighter than mitochondria. This agrees with recently reported observations,[5] in which the 5-HT was assayed by a fluorimetric method and the suspension media did not contain iproniazid. Thus it is concluded that the different results previously obtained with guinea-pig brain were due to the presence of iproniazid, which in some way interfered with the sedimentation characteristics of the 5-HT containing particles. Since iproniazid does not affect the distribution of ACH[2] and a large proportion of the 5-HT is found in the microsomal fraction, these results suggest that the particles which contain the 5-HT may be different from those which contain the ACH.

The distribution of substance P in P1, P2, P3 and the subfractions of P2 was similar to that of ACH. Thus fraction D, the non-cholinergic nerve ending fraction of de Robertis *et al.*,[4] contains little or no 5-HT or substance P.

Department of Physiology,
Australian National University,
Canberra

R. W. RYALL

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Lack of effect on Myleran derivatives on the synthesis of uridine nucleotides and glycogen

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THE pyrimidine moieties of nucleic acids are derived, directly or indirectly, from uridine 5′-diphosphate or -triphosphate (UDP, UTP), which arise from uridine 5′-monophosphate (UMP). In liver UMP is normally formed, under the action of orotidylate pyrophosphorylase (Code No. EC 2.4.2.10) and orotidylate decarboxylase (EC 4.1.1.23), from orotic acid, which arises from carbamylaspartic acid by two enzymic steps (dihydroorotase, EC 3.5.2.3, and dihydroorotate dehydrogenase, EC 1.3.3.1). An alternative pathway,[1] perhaps particularly important in liver tumours,[2] is from uridine under the action of uridine kinase (EC 2.7.1.21). Derivatives of UMP are important not only for nucleic acid synthesis but also for other processes such as synthesis of glycogen, the latter arising from UDPglucose by the action of UDPglucose-α-glucan glucosyltransferase (EC 2.4.1.11). The possibility that certain of these reactions in liver may be influenced by dimethylmyleran (2:5-dimethanesulphonoxyhexane, DM) or mannitolymyleran (1:6-dimethanesulphonylmannitol, MM) has now been examined, with negative results.

TABLE 1. TESTS OF MYLERAN DERIVATIVES FOR EFFECTS ON CERTAIN ENZYME PROCESSES

The values represent the mean percentage difference from controls, and are followed, where more than three experiments were done, by the standard error of the mean (number of experiments in parentheses). Values shown thus in square brackets represent mean activity in controls, as μmole/g/min.

	Derivative	Effect when injected	Effect *in vitro*
Orotate formation from carbamylaspartate, "free' activity [0·002]	DM		−17% (1)
Uridine nucleotide formation: from orotate* [0·008]	DM	+24 (3) ⎫ −1	+18 (6) ⎫ +6
	MM	−26 (3) ⎭ ±22%	−19 (3) ⎭ ±12%
Uridine nucleotide formation: from uridine [0·03]	DM		−15 (2) ⎫ 0
	MM	−18% (3)	+8 (4) ⎭ ±10%
Glycogen formation from UDPglucose [0·7]	MM	−18% (2)	−7% (2)

* Data for the individual products (UMP, UDP, UTP and UDPglucose) have been combined; there were no selective effects on the yield of any one product, or on the amount of uridine formed.

Young adult male rats of the Institute albino strain were given two intraperitoneal injections, each 6 mg/kg body wt., of DM in arachis oil or of MM in saline solution; the first injection was given 4 days and the second 1 day before killing. Alternatively, DM as a fine suspension in 0·25 M sucrose solution or MM dissolved in sucrose solution was added to liver samples from untreated rats prior to homogenizing; the amount was usually such as to give 0·5 mg/ml in the medium. Enzyme activities were assayed as in work described elsewhere.[2] The formation of uridine nucleotides from orotate was assayed essentially according to Stone and Potter,[3] with liver homogenates freed from nuclei and mitochondria. For uridine kinase, the procedure was similar to that used in Reichard's laboratory.[4] Glycogen synthesis was assayed essentially as in Leloir's laboratory,[5] with unfractionated homogenates to which UDPglucose labelled in the glucose moiety was added.