showing the presence of sex chromatin varies according to the type of tissue examined. Ranges vary from 52 to 85 percent, in skin biopsies, to as low as 20 to 64 percent for exfoliated cells. Embryonic tissue (amniotic fluid cells) has shown a range of 34 to 66 percent (*4, 7*). In view of these observations, we feel that the results obtained in the present investigation allow us to suggest that the placental septa are composed for the most part of cells which are of maternal origin (*8*).

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19 July 1957

Stimulant Effect of 2-Dimethylaminoethanol—Possible Precursor of Brain Acetylcholine

Pfeiffer and Jenney (*1*) have found that the tertiary amines arecoline, pilocarpine, and eserine will inhibit the conditioned avoidance response in rats protected peripherally with atropine methyl nitrate. Arecoline, when used in atropine methyl nitrate-protected schizophrenic patients, will produce a "lucid interval" similar to that of amobarbital or carbon dioxide (*1, 2*). These findings initiated a new research program (*3*) designed to find longer acting parasympathomimetic agents or precursors of acetylcholine which might affect the central nervous system. Two quaternary nitrogen compounds, acetylcholine and methacholine, probably owing to their slow transit across the blood-brain-barrier, are without effect on the conditioned avoidance response. Furthermore, Mayer and Bain (*4*) have used the tertiary and quaternary analogs of a convulsant, fluorescent acridone, to delineate the blood-brain-barrier, and Koelle and Steiner (*5*) find that the quaternary nitrogen analog of a phosphate ester of thio-choline inhibits the peripheral but not the cerebral cholinesterase, while the tertiary amine congenor inhibits both. These observations are not compatible with the concept that choline acts as an effective precursor of cerebral acetylcholine, because choline may be limited in its transport across the membranal barriers.

The lack of pharmacological potency of choline chloride in man is evidenced in the treatment of patients with liver disease, where as much as 10 g per day may be given without any discernible acetylcholinelike pharmacodynamic effect (*6*). Since choline is a methyl donor, one might expect the tertiary analog to be readily formed *in vivo*. However, choline as a methyl donor has been reported to go to dimethyl glycine by way of the intermediate, betaine (*7*), and, therefore, it may not provide a tertiary amine precursor for the synthesis of cerebral acetylcholine. For this reason, possible tertiary amine precursors of acetylcholine, including 2-dimethylaminoethanol (DMAE), have been studied extensively in mammals, including man, in these laboratories.

The pertinent publications on DMAE can be summarized briefly as follows: Krayer *et al.* (*8*) find that DMAE in a dose of 200 mg/800 ml volume counteracts the cardiac failure produced by pentobarbital sodium. Biochemical studies [Korey *et al.* (*9*)] indicate that DMAE is acetylated at the same rate as is choline by choline acetylase, and that acetyl-DMAE has less than 0.01 of the activity of acetylcholine on the frog rectus preparation. In chronic feeding experiments on growing chicks on a choline-deficient diet, Jukes and Oleson (*10*) report that DMAE will partially substitute for choline. In a *Neurospora* cholineless mutant, however, Jukes and Dornbush (*11*) show that DMAE substitutes completely for choline as a growth factor. Reid (*12*), working with choline-deficient guinea pigs, finds that DMAE will substitute completely for choline, whereas aminoethanol, N-methylaminoethanol, betaine, dimethyl glycine, and methionine are ineffective.

The following pharmacological observations have been made (*13*). The oral

Table 1. Effect of choline and DMAE on convulsant thresholds when 1.0 g per day is given orally for 2 weeks. Twelve mice were used in each group, and 0.5 percent pentylenetetrazol was infused intravenously at the rate of 0.05 ml/10 sec. $t = 2.392$ for difference between means of choline and DMAE. $p = < 0.05$.

Treatment	Lethal convulsion (ml/mouse)
Controls	0.457 ± 0.11
Choline	0.433 ± 0.09
DMAE	0.342 ± 0.11

LD_{50} of DMAE tartrate is 3.1 ± 0.16 g/kg, mouse, and 2.6 ± 0.1 g/kg, rat. With these large doses, animals show respiratory depression and frequently die of pulmonary edema. In pharmacodynamic studies on anesthetized dogs, DMAE tartrate has approximately 0.002 of the vasodepressor effect of acteylcholine, 0.1 of that of pilocarpine, and 0.5 of that of choline chloride. The muscarinic effect on the guinea pig uterus is approximately 0.1 and on the rabbit ileum, 0.025 of that of choline chloride (*13*). Rats and other species tolerate and grow normally on daily doses of 50 or 100 mg/kg for 6 months or longer. Higher doses (500 mg/kg a day) produce a small but statistically significant lowering of the hematocrit, hemoglobin, and eosinophil count. The animals grow normally, but occasional deaths occur from maximal seizures which appear in both rats and mice after 3 to 4 weeks of dosage. Mice treated chronically at 500 mg/kg a day are also more susceptible to audiogenic seizures (*13*). Daily oral dosage of DMAE significantly lowers the threshold for seizures from intravenously infused pentylenetetrazol (Tables 1 and 2). These data correlate with those obtained in curarized nonanesthetized cats, in which DMAE demonstrated some antagonism of the effects of pentobarbital on the electroencephalogram. The incidence of low-voltage fast activity in the electroencephalogram, similar to that obtained during arousal, was increased

Table 2. Effect of choline and DMAE on convulsant thresholds when they are fed to mice as a 0.03*M* solution in drinking water for 31 days. Ten mice were used in each group, and 0.5 percent pentylenetetrazol was infused intravenously at the rate of 0.05 ml/10 sec. $t = 5.3295$ for difference between means of choline and DMAE. $p = < 0.001$.

Treatment	Lethal convulsion (ml/mouse)
Controls	0.525 ± 0.09
Choline	0.525 ± 0.10
DMAE	0.250 ± 0.11

by DMAE, and the threshold at which reticular stimulation induced this type of cortical activity was lowered (*13*). In standardized tests of emotionality, mice on DMAE 0.05 percent in drinking water (*ad libitum*) showed an increase in emotionality which was significantly different from the control mice. Maze learning of rats and mice on DMAE is not facilitated (*13*).

In rats and dogs, DMAE in doses up to 7.5 mg/kg does not produce an antidiuretic effect or a change in renal hemodynamics (*13*). Single doses of 500 mg/kg of DMAE do not protect mice from lethal doses of *d*-tubocurarine, decamethonium, atropine, or hexobarbital.

Since DMAE may occur in natural foods and may regulate the synthesis of acetylcholine, the cautious clinical trial of various salts of DMAE was initiated. Total oral doses as low as 10 to 20 mg of DMAE base per day produce, in 7 to 10 days, a mild and pleasant degree of central nervous system stimulation which is characterized by lessened daytime fatigue and sounder sleep, but fewer hours of sleep are needed. Doses above 20 mg/day may result in increased muscle tone (most evident in the neck, masseter, and quadriceps muscles) and insomnia. (This observation contrasts sharply with that for choline, where 10 g per day is devoid of stimulant action.) Our initial trial of DMAE in schizophrenic patients was at a dose level of 250 mg per day. These patients showed increased motor and verbal activity and insomnia. After 4 weeks the daily dose was reduced to 50 mg per day because of weight loss in the 12 male patients (average 5-lb loss), although urine and blood studies disclosed no abnormality. Since then, 40 to 50 patients have been on therapy for 1 year, during which time the laboratory findings have been within normal limits (*13*). The possible beneficial effect of DMAE therapy in the chronic schizophrenic patient is a gradual one and requires 6 months or more of therapy. We postulate that DMAE therapy can be made more effective in the chronic schizophrenic when biochemical adjuvants are found which will increase acetylcholine synthesis.

In patients other than schizophrenic, DMAE produces relief of periodic headache, functional bowel distress, and chronic fatigue syndromes. DMAE therapy has not made asthmatic patients worse and has not precipitated peptic ulcer symptoms but has slightly increased the incidence of grand mal seizures in two epileptic patients. The incidence of petit mal seizures is, in contrast, diminished by DMAE therapy. The central nervous system stimulation which occurs in man is not accompanied by a rise in blood pressure, a rise in body temperature, or a change in the plasma level of protein-bound iodine. Pharmacological and biochemical studies designed to elucidate the exact mechanisms of action of DMAE are now in progress.

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23 July 1957

Evidence that Glutamine Is a Precursor of Asparagine in a Human Cell in Tissue Culture

Previous work from this laboratory (*1–3*) has shown that glutamine is required for the growth of a wide variety of mammalian cells in tissue culture and that, in the case of the HeLa strain human carcinoma cell, it is utilized for protein synthesis without prior degradation. This conclusion was based in part on the observation that, when the cells were grown on a medium containing L-glutamine labeled with C^{14} in the carbon chain and N^{15} in the amide N, the glutaminyl residues of the protein had the same ratio of C^{14} to N^{15} as did the glutamine in the medium. Aside from the amide N of glutamine, the only constituent of the cell protein which contained appreciable N^{15} under these conditions was the amide N of asparagine (*2*) (Table 1). Unlike glutamine, asparagine was not an essential nutrient for these cells, and the close parallel between their N^{15} contents suggested the possibility that glutamine amide N might be a precursor of the asparagine of the protein. However, since the glutamine in the medium was degraded to glutamic acid and ammonia during the culture of the cells (*2*), these data offered no way of deciding whether the pathway between glutamine and asparagine proceeded by way of free ammonia.

Table 1. N^{15} content of the amide groups of the glutamine and asparagine of HeLa cell protein. The N^{15} content of the isolated amide N is referred to that of the precursor as 1. The values are corrected for the amount of protein present at the start of the experiment. The starting glutamine in various experiments contained from 2 to 8 atoms percent excess N^{15}; the NH_4Cl contained 20 atoms percent excess N^{15} in each instance. The conditions of cell growth, the determination of N^{15}, and the isolation of the glutamine and asparagine amide N from enzymatic hydrolyzates of protein by means of glutaminase and asparaginase have been described (*3*).

Precursor in medium	Expt. No.	Relative atoms percent excess in the isolated amide N	
		Glutamine	Asparagine
L-glutamine amide-N^{15}	1	1.2	1.2
	2	1.1	0.82
	3	1.1	0.86
	4	1.1	0.86
$N^{15}H_4Cl$	5	0.012	0.008
	6	0.012	0.008
	7	0.008	
	8	0.008	

If the incorporation of N^{15} into the amide group asparagine does involve ammonia as an intermediate, then culture of the cells in a medium containing $N^{15}H_4Cl$ should lead to labeled asparagine, provided that the ammonia in the medium is available to the cell for biosynthetic processes. Salzman in this laboratory has, in fact, shown (*4*) that when HeLa cells are grown with $N^{15}H_4Cl$, the intracellular soluble pool of ammonia is heavily labeled with N^{15}. Consequently, the data in Table 1, indicating that $N^{15}H_4Cl$ in the medium is not incorporated into the asparagine of the protein to any significant extent, indicate that free ammonia is not an intermediate between glutamine and asparagine. At least, if the transfer of the amide nitrogen of glutamine to asparagine does involve ammonia, then this ammonia is