The results described above indicate that, if acetylcholine were the sole material with acetylcholine-like activity in the brain extract, then it would have been possible to isolate and identify it, as was done for the equivalent amount of added crystalline acetylcholine chloride. This was not observed. It is therefore unlikely that the original material could have been acetylcholine. The results obtained, however, strongly support the previous findings of Hosein and Koh[17] that the material with acetylcholine-like activity in narcotized rat brain extracts is probably acetyl-l-carnityl Co*A*.

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Effect of Adenosylethionine on 5-Methyltetrahydrofolate-homocysteine Transmethylase

THE 5-methyltetrahydrofolate-homocysteine transmethylase of *Escherichia coli* requires catalytic quantities of *S*-adenosyl-L-methionine[1] and a vitamin B_{12} derivative for activity[2]. It was of interest to determine the effect of *S*-adenosyl-L-ethionine on this reaction since this compound can substitute for adenosylmethionine in certain enzyme-catalysed reactions such as the formation of the ethyl derivatives of phospholipid choline, creatine, histidine and carnosine[3,4]. Adenosylethionine bromide was isolated from rat liver after ethionine feeding[5]. It behaved as a single spot on a paper chromatogram with 1-butanol–acetic acid–water (60:15:25, v/v/v) as the solvent. After boiling in 0·1 N sodium hydroxide for 1 h a chromatogram of the digest yielded spots for ethionine, homoserine and adenine[5]. Adenosylmethionine iodide was purchased from Calbiochem, Los Angeles, Calif. It was found (Table 1) that adenosylethionine could not replace adenosylmethionine in the reaction under investigation. When added simultaneously at equal concentration little or no effect was observed. Significant inhibition was observed when adenosylethionine was pre-incubated with the enzyme or when higher concentrations of adenosylethionine were employed.

Table 1. EFFECT OF ADENOSYLETHIONINE ON 5-METHYLTETRAHYDROFOLATE-HOMOCYSTEINE TRANSMETHYLASE

Reaction mixture: *d.l*,L-5-methyltetrahydrofolate, 3·5 μmoles; D,L-homocysteine, 20 μmoles; DPHN, 1 μmole; FAD, 0·4 μmole; K_2PO_4 buffer, *p*H 7·4, 200 μmoles; 5-methyltetrahydrofolate-homocysteine transmethylase (partially purified from *E. coli* (ref. 2)), 2·7 mg; protein containing 150 mμg vitamin B_{12}. The total volume was 2 ml. Incubation was carried out at 37° C under hydrogen in Thunberg tubes for 1·5 h. Methionine was determined microbiologically with *Leuconostoc mesenteroides* (ATCC 8042). The assay was not affected by adenosylethionine.

Adenosyl-ethionine (μmoles)	Adenosyl-methionine (μmoles)	L-methionine (μmoles)	Inhibition (per cent)
	Experiment I		
—	—	0	—
—	0·03	0·50	—
—	0·06	0·77	—
—	0·12	0·81	—
0·03	—	0	—
0·06	—	0	—
0·12	—	0	—
	Experiment II		
—	0·06	0·62	—
0·06	0·06	0·62	—
0·06*	0·06	0·46	26
0·06	0·06*	0·57	8
0·12	0·06	0·36	42
0·24	0·06	0·32	48

* Pre-incubated with enzyme for 15 min. In all the other experiments both adenosylethionine and adenosylmethionine are added before enzyme.
A zero time value of 0·12 μmole L-methionine has been subtracted from each of the above values.

At present no compound is known which will replace adenosylmethionine in this system. Adenosylhomocysteine, thiomethyladenosine and *S*-methylmethionine were inactive[6]. The precise role of adenosylmethionine in this reaction remains uncertain.

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Trans to *Cis* Isomerization of Urocanic Acid

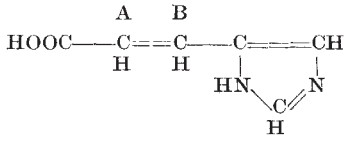
IRRADIATION of natural and synthetic urocanic acid with ultra-violet light results in the formation of a new product which has been identified by melting-point determination, ultra-violet and infra-red spectroscopy, and chromatography[1–3]. By analogy with cinnamic acid this has been thought to be a *trans* to *cis* isomerization. A similar change occurs in the urocanic acid of mammalian epidermis *in vivo* when the animals are exposed to ultra-violet light[4]. Using nuclear magnetic resonance it has been possible to demonstrate more conclusively that this ultra-violet-induced transformation is a *trans-cis* isomerization.

Commercial urocanic acid was shown by paper chromatography to contain trace amounts of a contaminant with the same R_F as the product formed by irradiation with ultra-violet light. A 0·1 per cent solution of urocanic acid at *p*H 6·0 was irradiated with stirring at 0° C with a General Electric sunlamp (1×10^6 ergs cm^{-2} sec^{-1}). Paper chromatography was performed on the concentrated solution in propanol and ammonia (3 : 1), and showed two spots with R_F values of 0·51 and 0·70—the former being identical to the major spot in the original material and the latter to the trace component. These compounds were separated by differential water solubility; their physical properties are shown in Table 1. Nuclear magnetic resonance spectra were obtained on a 10 per cent solution in deuterium oxide with a Varian *A*60 instrument. The chemical shifts of the doublets corresponding to the various olefinic protons and the spin–spin coupling constants are indicated. The structural assignments are based on the known effect of structure on the physical properties enumerated. Cinnamic acid, for example, shows a change in the coupling constant from 15·8 c.p.s.

Table 1

	R_F	M.p. (° C)	λ Max (mμ) at pH 1	Chemical shifts (p.p.m.*) HA	HB	Coupling constant c.p.s.
Trans	0·51	224	267	6·37	7·27	16
Cis	0·70	180–184	270	6·18	7·07	13

* Down field relative to 3-(trimethylsilyl) propylsulphonic acid (Na salt) as internal standard.



to 12·3 c.p.s. for the olefinic protons of the *trans* and *cis* isomers respectively[5]. It is of interest that the extinction coefficient of the *cis* isomer of cinnamic acid is much less than the *trans*[6], while those of urocanic acid are quite similar.

Because of the large amount of material needed for the nuclear magnetic resonance studies it was necessary to use isotope techniques to identify the new ultra-violet light absorbing product formed in epidermis following irradiation. Guinea-pigs were injected with 20 μc. of L-histidine-^{14}C (uniformly labelled) intraperitoneally and 24 h later they were depilated with wax and given a minimal erythema dose of ultra-violet light with a 'Hanovia' hot quartz lamp. The epidermis was separated by heating the skin to 50° C for 1 min and the urocanic acid isolated on a 'Dowex-1 formate' column as previously described[7]. Two radioactive compounds were identified by chromatography and autoradiography. The spots were eluted from the paper and chromatographed with the *cis* and *trans* isomers to which they corresponded exactly.

The data presented confirm that natural urocanic acid consists of the *trans* isomer contaminated with some *cis* and that following irradiation there is a *trans* to *cis* isomerization. This same reaction also occurs in the epidermis where urocanic acid is present at a high concentration.

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Cortisone-induced Lipaemia and Hepatic Steatosis in the Male Rat

It has previously been reported that female rats develop lipaemia and fatty livers during treatment with cortisone[1], and, more recently, the pathogenesis of this has been traced to a markedly enhanced mobilization of fatty acids from adipose tissue[2]. The mechanism thus seems quite different from that accounting for the fat accumulation in the livers of female, but not of male, rats during ethionine administration[3], and it is not reversed or prevented by measures which are effective during ethionine treatment[2]. Nevertheless, it is important to clarify whether the effect could also be observed in male rats.

Male albino 190–200 g rats were treated in a manner identical to that used previously for female rats[1]. All rats received tube feedings three times daily of a liquid diet; control rats gained weight well (average weight 247 g after 3 weeks). Experimental rats received 6·25 mg cortisone intramuscularly daily, while controls received saline. The experimental rats lost weight (average weight 170 g after 3 weeks). Animals were killed after 3 weeks; serum and liver lipids were extracted and total glycerides estimated by the van Handel–Zilversmit procedure[4]. Liver protein content was determined on a saline homogenate by the method of Oyama and Eagle[5].

Results of lipid analysis are shown in Table 1. The magnitude of rise of serum and hepatic lipids is similar to that seen in female rats[1]. Thus, it seems clear that no sex difference can be demonstrated for the fatty acid mobilizing properties of cortisone.

Table 1

	Serum glyceride*	Liver glyceride†
Untreated controls (6)	1·42 ± 0·24	0·208 ± 0·06
Cortisone-treated, pair-fed animals (3)	3·42 ± 0·18	0·850 ± 0·30

* mmoles/l. of serum.
† mmoles/g of liver protein.
All figures are given ± one *S.D.* Figures in parenthesis represent number of animals.

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In vivo Conversion of γ-Hydroxybutyrate into γ-Aminobutyrate

γ-Aminobutyrate (GABA) transaminates in normal brain to succinic semialdehyde[1,2] which in turn oxidates to succinic acid[3].

Moreover, Fishbein and Bessman[4] report that in the soluble fraction of brain homogenate, succinic semialdehyde is reduced to γ-hydroxybutyrate by action of a DPN^+ depending enzyme, indistinguishable from lactic-dehydrogenase; γ-hydroxybutyrate is then lactonized to γ-butyrolactone[5]. γ-Hydroxybutyrate and γ-butyrolactone have been recognized in large amounts in the normal nervous system and they seem the only physiological compounds in the nervous system which have an anaesthetic activity[6].

This communication reports on the variation of γ-aminobutyrate content in rat brain after injection of γ-hydroxybutyrate.

Male albino rats (200 g weight) were injected intraperitoneally with 500 mg/kg of γ-hydroxybutyrate. The rats were killed 2 h after injection and the brain removed and homogenized with 70 per cent alcohol (20 ml./g brain). After centrifugation and extraction with chloroform (10 ml./g brain), the alcohol layer was dried *in vacuo* at 40° C; the residue was dissolved with 1 ml. water/g brain.

γ-Aminobutyrate was separated by paper electrophoresis; 25 or 50 μl. of residue were utilized. Paper: Whatman No. 1; pyridine–acetic acid–water, buffer (250 : 50 : 900); *p*H 6, 12 V/cm, for 1·5 h. Paper strips, dried with hot air, were sprayed with 0·1 per cent ninhydrin in butanol (Fig. 1). A Chromoscan apparatus was used for reading the quantitative measurements. The standard curve