ribosomes produced by these hepatotoxic agents are 80 S ribosomes, which upon high-potassium treatment are dissociated into 40 S and 60 S subunits.

The present study on the dissociability of the ribosomes suggests that the fragmentation of messenger RNA is an unlikely mechanism of rapid polysome disaggregation by dimethylnitrosamine and lasiocarpine. It also excludes the possible activation of tissue ribonuclease as the mechanism of polysome disaggregation by these agents, which is consistent with the finding of Mizrahi and Emmelot[11] that liver ribonuclease activity is not elevated after dimethylnitrosamine treatment.

The small amount of ribosomes observed in the normal liver was not entirely dissociated in 0·3 M KCl. This suggests that the experimental procedure artificially fragmented the polysomes present in the normal liver, producing monosomes. The particular sample of lasiocarpine treatment had some polysomes remaining (Fig. 2e) and this may have similarly led to the formation of monosomes through artificial fragmentation, resulting in the presence of residual monosomes in the high-potassium treatment while the majority of ribosomes were converted into ribosome subunits (Fig. 2f).

As shown in the present study, the evaluation of dissociability of ribosomes in mammalian tissue may be helpful in exploring the mechanism of polysome disaggregation as to whether or not it is due to fragmentation of polysomes.

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Effect of GABA and 3-aminopropane sulphonic acid on the labelling of brain amino acids from [U-^{14}C] glucose in the conscious mouse

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IN A recent publication[1] the effects of intraventricularly injected excitatory and inhibitory amino acids on the cerebral metabolism of [U-^{14}C]glucose in mice anaesthetized with pentobarbitone sodium (Nembutal) were reported. In these experiments the metabolic effects of the amino acids were superimposed upon a cerebral metabolism which was already profoundly modified by the anaesthetic. In addition, the depression of nervous activity produced by the anaesthetic limited the dose of the depressant amino acids which could be injected without severe hypoxic consequences. Nevertheless, the conclusion was reached that the inhibitory amino acids had metabolic effects which were consistent with a depression of oxidative processes, and that the effects were similar in this respect to those produced by the barbiturate itself.

To substantiate these conclusions, a limited series of similar experiments has now been conducted on conscious mice. The labelled glucose and the inhibitory amino acids were injected intraventricularly, and all methods were the same as previously described[1] except that the anaesthetic was omitted and

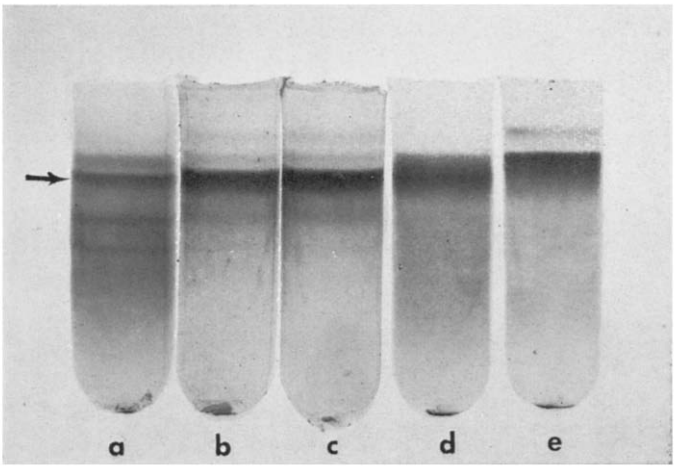

FIG. 1. Ribosome–polysome profiles of mouse livers in photopolymerized linear sucrose gradients The arrow indicates the position of the 80 S band. (a) Normal liver sample, 25 mM KCl treatment; (b) RNase-treated sample from normal liver, 25 mM KCl treatment; (c) RNase-treated sample, 0·3 M KCl treatment; (d) 3 hr after injection of puromycin, 25 mM KCl treatment; (e) 3 hr after injection of puromycin, 0·3 M KCl treatment.

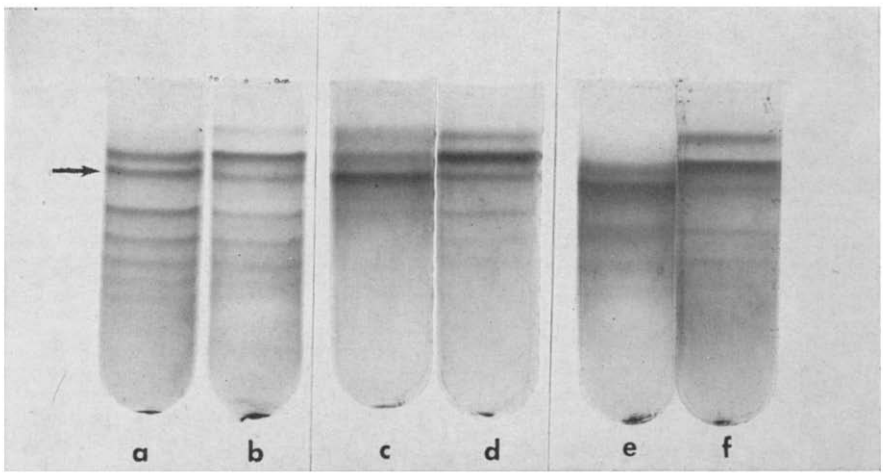

FIG. 2. Ribosome–polysome profiles of mouse livers in photopolymerized linear sucrose gradients (composite photograph). The arrow indicates the position of the 80 S band. (a) Normal liver sample, 25 mM KCl treatment; (b) normal liver sample, 0·3 M KCl treatment; (c) 1 hr after injection of dimethylnitrosamine, 25 mM KCl treatment; (d) 1 hr after injection of dimethylnitrosamine, 0·3 M KCl treatment; (e) 1 hr after injection of lasiocarpine, 25 mM KCl treatment; (f) 1 hr after injection of lasiocarpine, 0·3 M KCl treatment.

TABLE 1. EFFECTS OF GABA AND 3-APS ON THE LABELLING OF ENDOGENOUS BRAIN AMINO ACIDS FROM PRECURSOR [U-^{14}C]GLUCOSE IN CONSCIOUS MICE

	Control (n = 3) total acid-soluble radioactivity (dis/min/g × 10^{-3})	% Change induced by	
		GABA (n = 3)	3-APS (n = 2)
Total amino acids	33·8 ± 4·12	−27·2 ± 4·9*	−14·9 ± 8·8
Glutamate	18·5 ± 2·3	−27·0 ± 7·5	−17·4 ± 10·5
Aspartate	3·59 ± 0·58	−31·2 ± 3·8*	−17·1 ± 11·5
Glutamine	4·64 ± 0·84	−51·9 ± 2·87†	−25·9 ± 8·3
GABA	2·20 ± 0·51	−18·2 ± 9·1	−26·5 ± 1·3*
Alanine	3·28 ± 0·38	+30·8 ± 13·6	+52·3 ± 9·6

Intraventricular injections were made with the aid of a restraining frame described by Clarke *et al.*[9] The control solution contained [U-^{14}C]glucose (18 μCi/ml, 335 mCi/mmole) dissolved in isotonic saline. Other solutions contained the same amount of [U-^{14}C]glucose, and either GABA (50 mM dissolved in 125 mM NaCl) or 3-APS (5 mM dissolved in 148 mM NaCl). The pH of the solutions was adjusted to 7·4 with NaOH. Injection volume, 10 μl in each case. Mice killed 5 min after injection by immersion in Arcton (ICI, Macclesfield, England) at −150°. All extraction methods as previously described.[1] Significance of effects evaluated by Student's t-test; *P < 0·05; † P < 0·01.

higher doses of γ-aminobutyric acid (GABA) and 3-aminopropane sulphonic acid (3-APS) were used in order to ensure a satisfactory depression of CNS activity. This was manifested as general muscular flaccidity and immobility. The results are expressed in terms of the proportion of the total acid-soluble radioactivity which was incorporated into each of the labelled amino acids (Table 1) and the ratios of the incorporation into each amino acid relative to that into glutamate (Table 2). The results are not expressed in terms of specific radioactivities in view of the unknown changes occurring in amino acid pool sizes in tissue localized close to the injection site and because, as previously, no changes in whole brain levels of the endogenous amino acids were detected. The changes induced by the two inhibitory amino acids were consistent in each of the separate experiments conducted, but statistical significance was sometimes low because of the small n-value. In view of the trauma associated with the injections it was not considered justified to use a larger number of animals.

It can be seen from Table 1 that GABA decreased the incorporation of radioactivity from [U-^{14}C] glucose into the total amino acids fraction obtained from the perchloric acid extract of the whole brain. With respect to individual amino acids, the ^{14}C incorporation into glutamine, glutamate, aspartate and GABA was decreased in each case, but that into alanine was increased. The decreased incorporation into glutamate and aspartate, coupled with the increased incorporation into alanine, is consistent with a reduced rate of entry of pyruvate into the Krebs cycle. The fact that glutamine labelling was decreased more than that of glutamate can be explained by isotope dilution effects, if it

TABLE 2. RELATIVE LABELLING OF ENDOGENOUS BRAIN AMINO ACIDS FROM PRECURSOR [U-^{14}C] GLUCOSE IN CONSCIOUS MICE (GLUTAMATE RADIOACTIVITY = 1)

	Aspartate	Glutamine	GABA	Alanine
Control (3)	0·192 ± 0·008	0·249 ± 0·024	0·118 ± 0·015	0·179 ± 0·017
GABA (3)	0·183 ± 0·012	0·164 ± 0·004*	0·129 ± 0·004	0·330 ± 0·064
3-APS (2)	0·198 ± 0·009	0·244 ± 0·014	0·149 ± 0·029	0·305 ± 0·029

Experimental details as described under Table 1. Number of experiments in parentheses Significance of difference from control values calculated by Student's t-test: *P < 0·05.

is assumed that the unlabelled exogenous GABA is taken up mainly into the so-called "small" compartment[2] of tricarboxylic acid cycle intermediates with which the major system of cerebral glutamine synthesis is associated. A similar effect was recently demonstrated with intraventricularly injected L-glutamate and L-aspartate,[1] which are considered to be taken up into and metabolized within this same "small" pool of metabolites.[2] In each case it can be postulated that the uptake and metabolism of the unlabelled amino acids dilutes the radioactivity of the associated tricarboxylic acid cycle intermediates and thus of the glutamate pool associated with glutamine synthesis.

These conclusions regarding the effects of exogenous GABA are consistent with the results obtained by the injection of 3-APS, the synthetic sulphonic acid analogue of GABA which is considerably more potent as a neuronal depressant than the natural inhibitory transmitter.[3,4] A similar degree of CNS depression was obtained with a tenfold lower dose of 3-APS, and it is noteworthy that the metabolic effects are similar in all respects except for the lower decrease observed in the ratio of glutamine to glutamate labelling. This result would be expected if the extra reduction of glutamine labelling caused by GABA is indeed the result of dilution of glutamine-precursor radioactivity as suggested above. It is unlikely that 3-APS would be taken up into and metabolized to Krebs cycle intermediates within the "small" pool rapidly enough to produce a similar effect.

The changes induced by GABA and 3-APS in the labelling of alanine, glutamate and aspartate from [U-^{14}C]glucose are in accord with those previously obtained using anaesthetized mice.[1] The effects are similar to those produced by barbiturates in rat brain[5] and spinal cord[6] and are opposite to those produced by excitant amino acids in mouse brain.[1] Since alanine, glutamate and aspartate are related to keto-acids of the Krebs cycle, the effects can probably be ascribed to some control mechanism of energy metabolism associated with changes in neuronal excitation. It seems likely that the utilization of glucose is closely coupled to the ionic movements underlying electrical activity.[7,8] The detailed nature of such control mechanisms, however, requires further elucidation.

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