Table II. Induction of enzymes by cortisone in livers of carbon tetrachloride treated mice

	Time with CCl_4 prior to hormone			
	0 h		16 h	
	TT	TO	TT	TO
Control	5.17 ± 0.04	1.44 ± 0.02	5.17 ± 0.04	1.44 ± 0.02
Cortisone	9.53 ± 0.30	2.29 ± 0.02	9.53 ± 0.30	2.29 ± 0.02
CCl_4	7.67 ± 0.20	0.97 ± 0.10	4.40 ± 0.30	0.19 ± 0.07
CCl_4 + Cortisone	11.63 ± 0.02	2.06 ± 0.03	5.10 ± 0.70	0.17 ± 0.05

Details of the experimental design are given in Table I, except that all assays were carried out 4 h after hormone administration. The procedures for determination of liver tyrosine transaminase (TT) and tryptophan oxygenase (TO) activities are well established and have been described in detail previously[4,11]. The enzyme activities are expressed as μg *p*-hydroxyphenyl-pyruvic acid/mg liver/10 min and μg kynurenine/mg liver/h for TT and TO, respectively. All values represent average of 5–6 separate determinations ± the standard error of the mean.

enhanced transcriptional activity is a prerequisite for enzyme induction and this may be selectively amplified by the steroid even when RNA synthesis in general is otherwise suppressed. As the latter remains lowered for protracted periods of time, and as the available messenger RNA is presumably exhausted, both the endogenous homeostasis of enzymes, and their induction by the hormone, are gradually impaired. It has previously been established that TO is not inducible by the endogenously released corticoids at any time after CCl_4 administration[4]. So induction of TT in such animals under selected conditions (Table II; 4) could reflect differences in the relative stabilities of mRNAs for these 2 enzymes. This thesis explains the observation that synthesis of uracil-rich, DNA-like RNA (presumably mRNA) can be seen within 10–20 min after cortisone administration in rats and therefore precedes both the enzyme induction and the general ribosomal RNA synthesis[8], leading eventually to liver hypertrophy and increased RNA content. A similar relationship has previously been observed in hepatoma cell cultures[9,10]. In any event, these studies form the first evidence that, in mammalian liver, induction of selected enzymes by a corticosteroid can occur at a time when the stimulatory effect of the hormone on the synthesis of total RNA is completely eliminated.

It should be remembered that CCl_4 rapidly and markedly alters Kupffer cell function in the liver[4]. Since the cortisone inducible enzymes are found in liver parenchymal cells, it had previously been suggested that initial information processing may occur in the reticuloendothelial system of the animal (for a brief review see[11]). The manner in which this is accomplished remains to be determined, but it is clear that the information thus gathered would be of unusual singificance in delineating the control, organisation and expression of genetic information in mammalian liver.

Résumé. Chez des souris traitées par le tétrachlorure de carbone l'induction de quelques enzymes sélectives par la cortisone s'est produite à un moment où la synthèse de l'ARN total était inférieure au niveau observé chez les témoins.

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Biochemical Changes of Brain and Liver in Neonatal Offspring of Rats Fed Monosodium-L-Glutamate

Much attention has recently been focused on some of the possible hazards of high levels of monosodium-L-glutamate (MSG) given by injection or fed to several species of experimental animals. These effects range from obesity and neuroendocrine disturbances to sterility and brain lesions[1–8]. In a previous study[9] we attempted to establish a biochemical basis for the action of MSG by feeding it to rats at levels up to 20% of the diet and then measuring the concentrations of a number of brain and liver constituents. Analysis of liver indicated that dietary MSG had no effect on protein, RNA, DNA, glutamate, lactate, malate or α-glycerophosphate. Concentrations of glutamate, glutamine, aspartate, DNA and protein and activity of glutamic decarboxylase (GAD) in brain remained constant while γ-aminobutyric acid (GABA) concentrations were significantly decreased in animals fed MSG. The rats ingesting MSG exhibited increased irritability, which may be related to decreased levels of brain GABA[9,10].

The purpose of the present investigation was to study the effects of dietary MSG on some selected brain and liver metabolites of second generation neonatal rats born to parents fed a diet supplemented with 10% MSG.

Materials and methods. Holtzman weanling rats were fed Purina laboratory chow alone or supplemented with 10% MSG for 100 days. The rats were mated on a one-to-

Table I. Body, brain and liver weights[a] of rats during postnatal development

Days	Control			Treated		
	Body weight	Brain weight	Liver weight	Body weight	Brain weight	Liver weight
1	7.21 ± 0.26	0.2871 ± 0.0058	0.280 ± 0.011	7.39 ± 0.20	0.2939 ± 0.0088	0.288 ± 0.009
2	8.40 ± 0.26	0.3400 ± 0.0063	0.336 ± 0.010	8.64 ± 0.27	0.3488 ± 0.0089	0.332 ± 0.009
3	10.32 ± 0.17	0.4148 ± 0.0068	0.410 ± 0.011	9.47 ± 0.23	0.4003 ± 0.0085	0.358 ± 0.018
5	13.13 ± 0.52	0.5442 ± 0.0082	0.485 ± 0.016	13.59 ± 0.57	0.5882 ± 0.0154	0.453 ± 0.019
10	25.55 ± 0.39	1.0132 ± 0.0117	0.796 ± 0.017	24.43 ± 0.38	1.0000 ± 0.0127	0.726 ± 0.020
21	51 ± 3	1.4242 ± 0.0243	1.934 ± 0.137	52 ± 1	1.4356 ± 0.0187	1.963 ± 0.061

[a] Each value is the average of 10 rats and is expressed as g ± S.E.M. The control rats were born of parents fed Purina chow. Treated rats were born of parents fed Purina chow supplemented with 10% MSG. All 20 control rats conceived and 17 of the 20 treated rats conceived; the average litter sizes were 9.7 ± 0.4 and 10.2 ± 0.5 pups/litter, respectively.

one basis for a 7-day period and the resultant offspring (first generation; F_1) after weaning were continued on the same diet as their respective parents for 100 days. At this time the F_1 generation rats were mated as described above and 10 neonatal offspring (second generation; F_2) from each group were sacrified at days 1, 2, 3, 5, 10 and 21. Brains were removed for determinations of GAD, GABA, glutamate, aspartate, protein and DNA by previously described methods[11]. Livers were assayed for RNA, DNA, protein and glutamate. The stomach contents of the 5-day-old rats were also assayed for glutamate as an indicator of transfer of MSG by way of mothers' milk.

Mean values, standard errors and their significance were calculated according to the Student's *t*-test.

Results. No significant differences were noted in conception rate, pups per litter or body, brain and liver weights between offspring of control and MSG-fed rats (Table I) during the first 21 days of postnatal development. As seen in Figure 1, the brain-to-body weight ratio was significantly higher ($P < 0.05$) for treated rats at day 3, whereas the liver-to-body weight ratio was lower than that of controls at days 3 and 5 ($P < 0.05$). These differences disappeared before the neonatal rats were weaned.

Figure 2 shows that the protein content of the brains of treated rats parallels that of the controls. The DNA concentrations in brains of control and treated rats were remarkably similar (Figure 3) and, when expressed as

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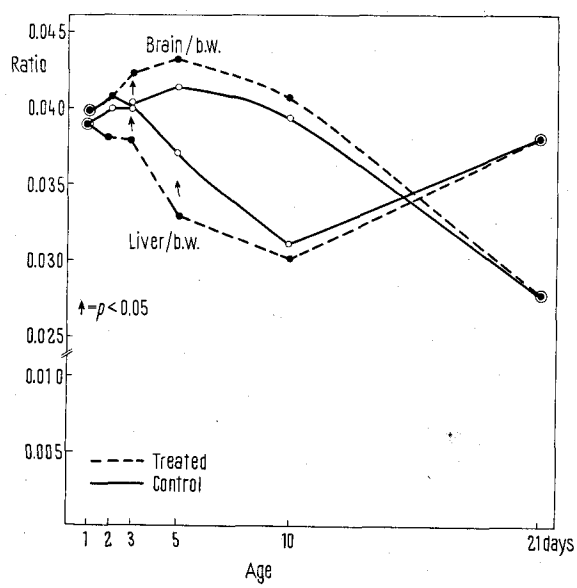

Fig. 1. Effect of 10% dietary MSG on brain-to-body weight and liver-to-body weight ratios of F_2 generation neonatal rats.

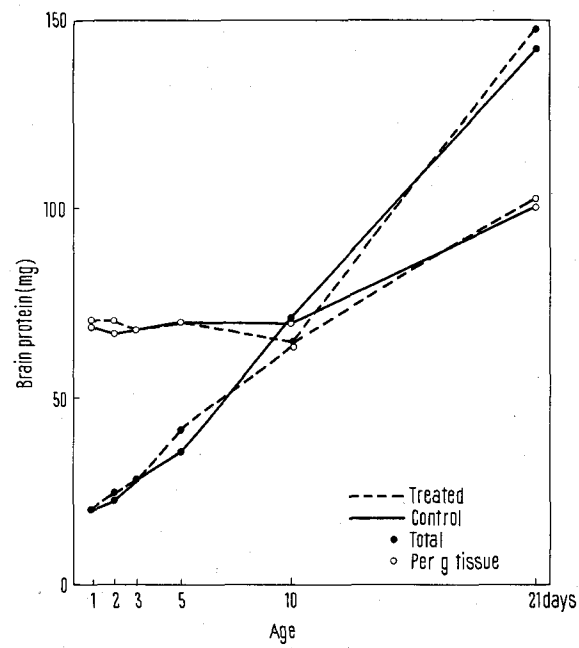

Fig. 2. Effect of 10% dietary MSG on brain protein of F_2 generation neonatal rats.

mg/g tissue, were comparable to adult values at day 5 of postnatal development. Figures 4 and 5 show the concentrations of aspartate, glutamate and GABA in brain. Aspartate and glutamate did not reflect the increased dietary intake of MSG; however, GABA was significantly elevated ($P<0.001$) on day 1. This elevation did not persist and by day 2 the values were down to normal.

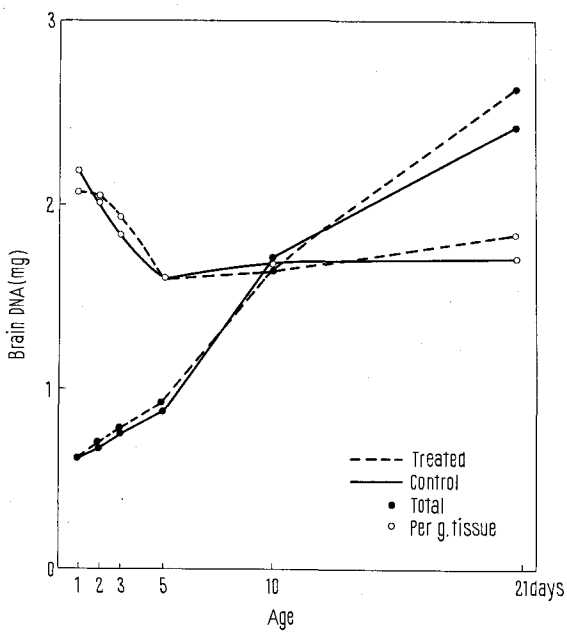

Fig. 3. Effect of 10% dietary MSG on brain DNA of F_2 generation neonatal rats.

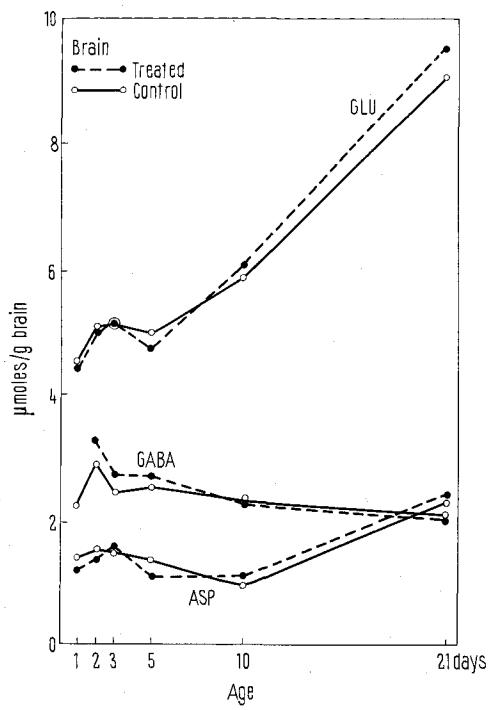

Fig. 4. Effect of 10% dietary MSG on concentrations of glutamate (GLU), aspartate (ASP) and γ-aminobutyric acid (GABA) in brains of F_2 generation neonatal rats.

GAD, the enzyme responsible for the formation of GABA, did not change significantly in treated rats; activity varied from 62 to 100 µmoles GABA formed/g protein/h during the 21-day postnatal period.

The results in Table II show that 10% MSG in the diet had no effect on liver protein, RNA-P, DNA-P or glutamate.

Examination of the stomach contents of 5-day-old rats showed that offspring of parents fed the MSG diet had 20% more free glutamate than controls (0.574 µmole as compared to 0.476 for controls; $P<0.05$). Treated offspring had rough, shaggy-hair coats which became normal during the third week.

Discussion. Feeding MSG to rats through several generations had no effect on reproductive function as measured by conception rate and pups born per litter. These data are in agreement with earlier reports from this agency[12] and other laboratories who studied the rat[13]. Furthermore, no differences were noted in the development of the rat during the postnatal period as measured by brain, liver and body weights. The increased brain-to-body weight ratio at day 3 and decreased liver-to-body weight ratios at days 3 and 5 for rats fed MSG were transient effects from which the animals recovered by day 10.

DNA concentrations in brain reflect cell number and size, and a decrease is associated with neuron deficiency[14]; the values found in this study were not different for control and MSG-fed rats. Protein concentrations in brain, as well as those of DNA, are indicative of growth and development of this organ and are markedly decreased in mal-

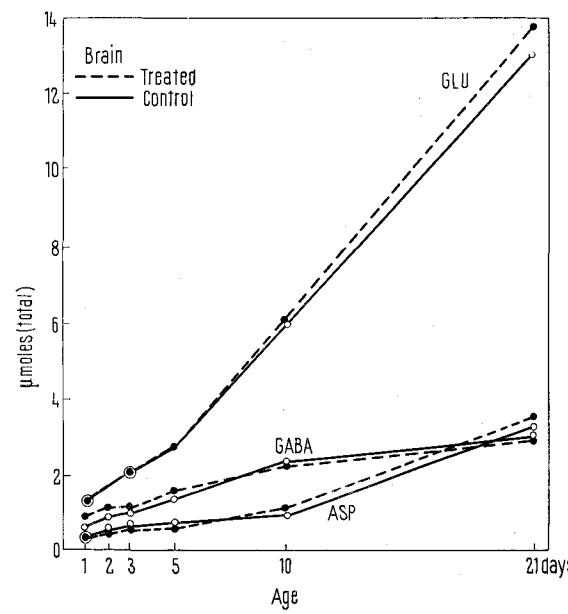

Fig. 5. Effect of 10% dietary MSG on total brain content of glutamate (GLU), aspartate (ASP) and γ-aminobutyric acid (GABA) in F_2 generation neonatal rats.

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Table II. Liver protein, RNA-P, DNA-P and glutamate[a] in rats during postnatal development

Days	Control				Treated			
	Protein	RNA-P	DNA-P	Glutamate	Protein	RNA-P	DNA-P	Glutamate
1	138 ± 8	0.913 ± 0.037	0.294 ± 0.029	4.27 ± 0.24	127 ± 2	0.956 ± 0.031	0.295 ± 0.017	3.46 ± 0.11
2	112 ± 2	0.965 ± 0.031	0.288 ± 0.017	4.41 ± 0.15	122 ± 7	0.899 ± 0.022	0.314 ± 0.025	4.31 ± 0.17
3	146 ± 12	1.072 ± 0.065	0.380 ± 0.014	4.21 ± 0.17	130 ± 9	1.107 ± 0.041	0.400 ± 0.010	4.05 ± 0.22
5	134 ± 12	1.035 ± 0.022	0.344 ± 0.009	3.57 ± 0.25	128 ± 3	1.067 ± 0.016	0.314 ± 0.009	4.28 ± 0.29
10	149 ± 5	0.942 ± 0.014	0.280 ± 0.011	3.22 ± 0.09	166 ± 12	0.962 ± 0.032	0.284 ± 0.008	3.43 ± 0.07
21	171 ± 8	0.930 ± 0.164	0.251 ± 0.036	4.09 ± 0.21	166 ± 5	0.954 ± 0.133	0.266 ± 0.029	4.51 ± 0.25

[a] Each value is the average of 5 rats and is expressed as mg/g liver ± S.E.M. for protein, RNA-P and DNA-P and as μmole/g liver for glutamate. Control rats were born of parents fed Purina chow. Treated rats were born of parents fed Purina chow supplemented with 10% MSG.

nutrition[15]; these concentrations were also unaffected by dietary MSG.

In a previous study[16], in which rats were fed MSG at levels up to 20% of the diet for 15 weeks, we found that GABA concentrations were reduced but GAD activity was not affected. In the present study, we found that mothers' milk from MSG-fed rats contained 20% more free glutamate than did controls, resulting in increased concentrations of GABA in brains of offspring at day 1. ADKINS et al.[17] reported a similar increase in free glutamate of milk from MSG-fed rats. The transient rise in GABA probably resulted in 'activation' of enzymes responsible for the metabolism of GABA and therefore the GABA concentrations were decreased to control values by day 2. Brain aspartate and glutamate were unresponsive to MSG in the diet.

Liver protein, RNA-P, DNA-P and glutamate levels were also independent of dietary MSG. The high activities of transaminases and oxidases which metabolize glutamate in liver are sufficient to maintain glutamate concentrations at 4 μmole/g liver. The activities of the corresponding enzymes in the brain will be reported in a future paper.

These generation studies are in general agreement with our earlier reports on effects of dietary level of MSG in that we were unable to find changes in biochemical components of brain and liver. We have now reached the F_4 generation with no further effects noted except for the rough, shaggy-hair coat which persists for approximately 30 days.

Zusammenfassung. Nachweis, dass die Ernährung von Ratten mit Monosodium L-Glutamat (MSG) im Laufe der ersten 21 Tage nach der Geburt keinen Einfluss auf die Entwicklung sowie auf das Körper-, Gehirn- und Lebergewicht hatte. Diätetisches MSG hatte ausserdem keinen Effekt auf Protein, Aspartat und Glutamat im Gehirn.

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Erythrocyte NADH-Methemoglobin Reductase Activity in Experimental Riboflavin Deficiency

Measurement of activity of enzymes requiring vitamin cofactors has been shown to be a sensitive and specific means of detecting vitamin deficiencies[1-3]. A flavin-dependent enzyme, NADH-dependent methemoglobin reductase, has been proposed as a possible indicator of the status of riboflavin nutrition[4]. Activity of the enzyme in erythrocytes from normal subjects is enhanced approximately two-fold by addition of flavin-adenine-dinucleotide (FAD) to the assay system[4], but activity of the enzyme from riboflavin-deficient subjects has not been studied. The purpose of this paper was to study erythrocyte NADH-methemoglobin reductase activity in riboflavin deficient rats, and to determine the effect of addition of FAD upon activity of the enzyme.

Materials and methods. 24 Sprague-Dawley weanling rats were divided into 4 groups. Groups 1 and 2 were fed a riboflavin-deficient diet (Nutritional Biochemicals). Groups 3 and 4 were given a regular balanced diet containing 794 mg of the vitamin per kg. Supplements of 80 μg of riboflavin were given by s.c. injection every other day to rats in Groups 2 and 3 in accordance with established riboflavin requirements[5]. 2 rats from each group were sacrificed on days 21, 23 and 26 following initiation of the diets. Blood was collected by cardiac puncture, and the liver was removed. Erythrocyte methemoglobin reductase activity was assayed by the method of HEGESH et al.[6] and

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