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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hepatic glutathione reductase activity by the method of WENDELL[7]. Protein was determined by the method of LOWRY et al.[8] Assays were also performed with FAD added to the methemoglobin reductase assay in a final concentration of 1 μM and to the glutathione reductase assay in a final concentration of 4 μM.

Results and discussion. At the time of sacrifice, riboflavin deficiency was readily apparent in rats of Group 1, who showed rough coats and a failure to gain weight[9,10]. Weight-gains of rats receiving the deficient diet lagged significantly behind those of animals whose diets were sufficient in riboflavin ($p < 0.001$)[11]. Furthermore, riboflavin deficiency of Group 1 animals was corroborated biochemically by measuring the activity of hepatic glutathione reductase. The Table shows that activity of this enzyme of animals in the deficient group was significantly below that of animals receiving adequate riboflavin intake ($p < 0.001$). However, erythrocyte methemoglobin reductase activity was not affected by riboflavin deficiency (Table). Slight augmentation of enzyme activity was observed in all groups with addition of FAD in vitro, but the increases were insignificant. The above findings show that assay of erythrocyte methemoglobin reductase activity is not a valid method for detection of riboflavin deficiency.

Enzyme assayed	Groups[a]	Mean specific activity[b]	Standard error	t[c]	p[d]
Erythrocyte methemoglobin reductase	1	4.7	0.7	No significant difference	
	2	4.0	0.7		
	3	4.9	0.6		
	4	5.1	0.7		
Hepatic glutathione reductase	1	31.5	4.8	3.82	< 0.001
	2	50.2	4.5		
	3	57.7	5.9		
	4	50.5	2.8		

[a] Six rats in each group: 1. Riboflavin-deficient diet; 2. Deficient diet with riboflavin supplementation; 3. Regular diet with riboflavin supplementation; 4. Regular diet. [b] Activity of methemoglobin reductase is expressed as nmoles methemoglobin reduced/min/mg Hb, and activity of glutathione reductase is expressed as µmoles glutathione reduced/min/g protein. [c] t = Student's t-test for comparison of difference between means of enzyme activities of deficient and combined non-deficient groups. [d] p = p-value as determined by two-tailed test.

Zusammenfassung. Bei Ratten mit Riboflavin-Mangel wurde eine normale Aktivität der NADH-abhängigen Methämoglobinreduktase festgestellt.

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Side-Chain Hydroxylation in the Biosynthesis of β-Ecdysone (20-Hydroxyecdysone) in the Blowfly *Calliphora stygia*

From a study[1] of the metabolism of 25-deoxyecdysone in the blowfly *C. stygia* it was concluded that side-chain hydroxylation probably precedes modification of the steroid nucleus in the biosynthesis of β-edysone (20-hydroxyecdysone) from cholesterol. To test this conclusion we have now studied the metabolism in *C. stygia* of [26-^{14}C]-25-hydroxycholesterol and [22-^{3}H-]-(22R)-22-hydroxycholesterol.

A colloidal suspension of [26-^{14}C]-25-hydroxycholesterol (37.0×10^6 dpm, 60 mCi/mmole) in water containing 0.5% sodium oleate was injected into 3rd instar larvae of *C. stygia* (100 insects) 6–9 h prior to puparium formation together with [1-^{3}H]-cholesterol (145×10^6 dpm, 10 Ci/mmole) to serve as a reference standard (^{3}H:^{14}C = 3.9:1). The prepupae were collected 6–9 h after puparium formation (about 18 h incubation), homogenized in ethanol and the β-ecdysone isolated as described previously[2]. As observed earlier[3], [1-^{3}H]-cholesterol was found to be incorporated into β-ecdysone (0.016%). However, the ^{3}H:^{14}C ratio of the purified β-ecdysone isolated was 20:1, indicating that 25-hydroxycholesterol, if incorporated, was incorporated to a much lower extent than cholesterol. Recently it was reported[4] that 25-hydroxycholesterol is not metabolised to β-ecdysone in seedlings of *Podocarpus elatus*.

[22-^{3}H]-(22R)-22-hydroxycholesterol (1×10^9 dpm, 2.46 Ci/mmole, radiochemical purity 98%), prepared by reduction of 22-ketocholesterol with sodium borotritide[5] was incubated in *C. stygia* and the β-ecdysone isolated as before. After extensive purification the β-ecdysone fraction was chromatographed with non-radioactive β-ecdysone. The curve of the UV-absorption plotted against elution volume then coincided with a weak peak of radioactivity which corresponded to an incorporation of not more than 0.0001%, that is about 1/100 of that obtained with cholesterol.

The cholesterol pool in *C. stygia* is very large[3] and probably very much larger than any of its more polar

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