Effects of early undernutrition and subsequent rehabilitation on acetylcholine levels in rat brain[1]

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Summary. Early undernutrition, induced by feeding mothers a low protein (5%) diet during gestation and/or lactation, resulted in significant deficits in acetylcholine concentration in the rat brain, and these deficits were reversed by subsequent dietary rehabilitation.

The key role of acetylcholine (ACh) as a neurotransmitter has now been well established[3]. The concentration of ACh in the brain varies inversely with the functional activity of the brain. Thus, it is elevated in anaesthesia and sleep[4,5], in deep narcosis[6] and after the administration of CNS depressants[7]. It is decreased in conditions associated with increased brain activity, for example convulsions, electrical stimulation and emotional excitement[4]. The concentration of ACh in the rat brain increases with age up to 100 days or more[7,8]; this increase coincides with the 'growth spurt' of the brain, and this is the time when the brain is more vulnerable to nutritional insults[9,10].

Neonatal undernutrition brought about by litter-size manipulation was not found to alter the concentration of ACh in the rat brain during the weaning period[11]. However, postweaning protein deficiency and also caloric restriction were found to decrease the ACh concentration. Neonatal undernutrition in the rat is also associated with significant deficits in brain DNA, lipids and neurotransmitter amines[9,10,12]. It was therefore logical to investigate the effects of early undernutrition in rats, induced by maternal protein deficiency during gestation and/or lactation, and the subsequent dietary rehabilitation, on brain ACh levels.

Adult female albino rats of proved fertility, weighing 180–200 g (bred at Haffkine Institute), were used for the experiments. They were divided into 3 groups, each of which received a different dietary regimen from the 1st day of conception. The control group (G^+L^+) was fed a 20% protein diet during gestation and lactation. The 2nd group (G^+L^-) was fed a 20% protein diet during gestation and a 5% protein diet during lactation. The 3rd group (G^-L^-) was fed a 5% protein diet during gestation and lactation. Water and diet were provided ad libitum to all the groups. The composition of the diet was essentially similar to that described earlier[13]. For rehabilitation studies, the pups were individually fed on a 20% protein diet for a period of 5 weeks from the age of 22 days.

Pups were killed by decapitation on the 21st day, or after rehabilitation. The whole brain, including the olfactory lobes, was removed within 30 sec. Each brain was homogenized in ice-cold 10% (w/v) trichloroacetic acid (TCA) and processed further for estimation of ACh by bioassay, using frog rectus abdominus muscle, as described earlier[11]. The results are expressed as ACh: μg/g brain ± SEM. The effects of undernutrition were analyzed, using a one way analysis of variance and an F-test to detect differences between the group means. Variables for which the F statistic was significant at the 0.05 level were then subjected to further analysis with a Studentized range test[14].

The results of this study are summarized in the table. The body weights in both the undernourished groups were affected similarly, the deficits being 71 and 73% in the G^+L^- and G^-L^- groups respectively. However, these deficits were greater in the animals of G^-L^- group at 7 and 14 days[15], and thereafter, the pups started eating the mother's diet, thereby diminishing the effects of undernutrition. This observations is in agreement with other reports[16,17]. The brain weights were, however, markedly lower in the G^-L^- group compared with the G^+L^- group, the deficits being 26 and 16%, respectively. The concentration of ACh in the brains of both the undernourished groups were significantly lowered. The deficits in the ACh concentrations were 14 and 24% in G^+L^- and G^-L^- groups, respectively. Our results differ from those reported earlier[11]. In the earlier study, the neonatal undernutrition was induced by litter size manipulation, and therefore not strictly comparable with the present study. Undernutrition in our studies was more severe since it was achieved by feeding the mothers a protein-deficient diet throughout the gestation and/or lactation periods. The difference in the degree of undernutrition may also be due to the fact that the mother nursing a large litter size can compensate to some extent by increasing the food intake (45 g per day), as compared to the control litter size mother (32 g per day). This will not be the case if the mother is fed a low protein diet with an average food intake of 12 g per day. Results of the experiments involving litter size manipulation are likely to be influenced by the factor of maternal stimulation towards the litters, and this may counteract the effects of undernutrition[11].

The dietary rehabilitation for a period of 5 weeks failed to bring about a total 'catching up' in the body and brain weights. These observations are in agreement with Telang[18] and Adlard and Dobbing[19–21]. The concentration of ACh in the brains of both the rehabilitated groups showed no significant deficits as compared to the controls. Thus it is quite evident that the earlier deficits could be reversed by the dietary rehabilitation.

In conclusion, early undernutrition, induced by feeding mothers a low protein diet during gestation and/or lactation, resulted in significant decrease in the concentration of ACh in the rat brain, and this can be reversed by subsequent dietary rehabilitation.

Effects of early undernutrition and subsequent dietary rehabilitation on ACh concentration in rat brain*

Dietary regimen	Body weight (g)	Brain weight (g)	Brain acetylcholine (μg per g)
Undernutrition			
G^+L^+	45.0 (6) ± 1.3	1.391 ± 0.008	1.60 ± 0.05
G^+L^-	13.0 (6)[a] ± 1.0	1.167[a] ± 0.035	1.37[a] ± 0.08
G^-L^-	12.0 (6)[a] ± 0.8	1.033[a,b] ± 0.032	1.22[a] ± 0.09
Rehabilitation			
$G^+L^+R^+$	134.0 (4) ± 3.0	1.510 ± 0.042	2.40 ± 0.20
$G^+L^-R^+$	74.0 (4)[a] ± 1.4	1.350[a] ± 0.022	2.05 ± 0.30
$G^-L^-R^+$	65.0 (4)[a,b] ± 1.5	1.290[a] ± 0.040	1.95 ± 0.40

* Female rats were either fed 5% (−) or 20% (+) protein diet ad libitum during gestation (G) and/or lactation (L). Pups were fed ad libitum 20% protein diet from 22nd day for 5 weeks for rehabilitation study (R). The number in parenthesis represents number of observations. Values are expressed as mean ± SEM. a–b Values different at the $p < 0.05$ level as determined by one way analysis, F and Studentized range tests; [a]values different from the control group and [b]values different from G^+L^- or $G^+L^-R^+$ group in undernutrition or rehabilitation studies, respectively.

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The prevention of alloxan-induced diabetes in mice by the iron-chelator detapac: Suggestion of a role for iron in the cytotoxic process

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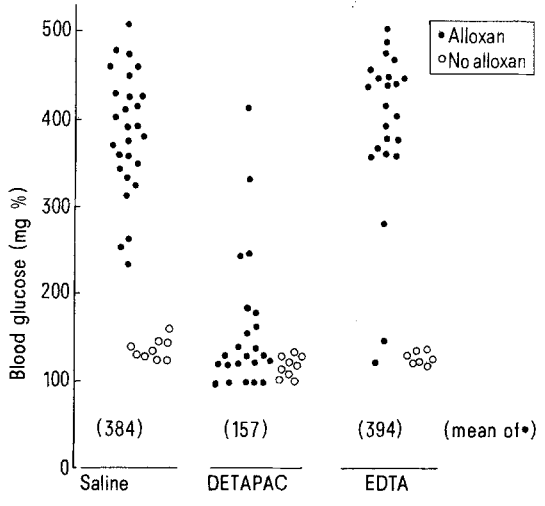
Summary. DETAPAC, an iron-chelating agent, given to male Swiss-Webster mice prior to alloxan, was able to protect the mice from the diabetogenic actions of alloxan. In contrast EDTA, another chelating agent, offered no protection. Possible mechanisms for these effects, including inhibition of hydroxyl radical formation, will be discussed.

The diabetogenic agent alloxan is a widely used tool in diabetes research. It has recently been reported that diethylenetriaminepentaacetic acid (DETAPAC) could prevent alloxan-induced damage in isolated islet cell preparations. It was suggested[2,3] that DETAPAC protection was due to its prevention of hydroxyl radical formation[4–7]. With the above knowledge, we speculated that DETAPAC administration might also prevent alloxan-induced diabetes in vivo. The data in the present report will show that this is indeed the case.

Materials and methods. Male Swiss-Webster mice (Perfection Breeders) were used in all experiments. Food was routinely withheld from the mice for 3–4 h. At this time the mice received an i.p. injection of DETAPAC (Sigma) or EDTA (Baker) (100–250 mg/kg, dissolved in distilled water) or a saline vehicle. 1 h later, the mice received an i.v. (tail) injection of 75 mg/kg alloxan monohydrate (Calbiochem). Food was returned to the mice approximately 1 h post-alloxan. Blood sugar was determined 48 h later by a glucose oxidase assay (Trinder method, Boehringer-Mannheim) after deproteinization of the blood with barium hydroxide and zinc sulfate.

Results. In saline-pretreated mice, alloxan at 75 mg/kg caused a large increase in blood glucose (fig. 1). Most of the mice had values greater than 300 mg%, which is generally considered diabetic. Mice that had been pretreated with 250 mg/kg of DETAPAC prior to alloxan were largely protected against the actions of alloxan. Only 2 of the 23 mice had blood glucose greater than 300 mg%. The non-alloxan treated mice (saline, DETAPAC or EDTA alone) had blood glucose values ranging from 105 to 158 mg%. Pretreatment with EDTA failed to protect against alloxan. Most animals (20 of 23) had glucose values above 300 mg%. The effects with DETAPAC showed a clear dose response relationship. In experiments not presented, mice pretreated with saline prior to alloxan had a mean blood glucose ± SD of 507 ± 178 mg%. In contrast, mice pretreated with 100 mg/kg of DETAPAC prior to alloxan had a blood glucose of 349 ± 108 mg% and mice pretreated with 200 mg/kg of DETAPAC prior to alloxan had a blood glucose of 229 ± 165 mg% (n = 8 to 10 mice in each group).

Discussion. Alloxan can be readily reduced to dialuric acid in vitro by standard reducing agents including ascorbic acid[8]. Dialuric acid is extremely unstable and rapidly reacts with oxygen (autoxidizes). Products of dialuric acid autoxidation include alloxan itself as well as several very reactive



The effects of DETAPAC or EDTA on alloxan-induced diabetes. Mice were pretreated with saline, or DETAPAC or EDTA at 250 mg/kg 1 h prior to 75 mg/kg of alloxan. Other mice received only the pretreatment (no alloxan). Blood glucose was measured 48 h later. Data represent individual values. Mean values for the alloxan-treated mice are in parentheses.