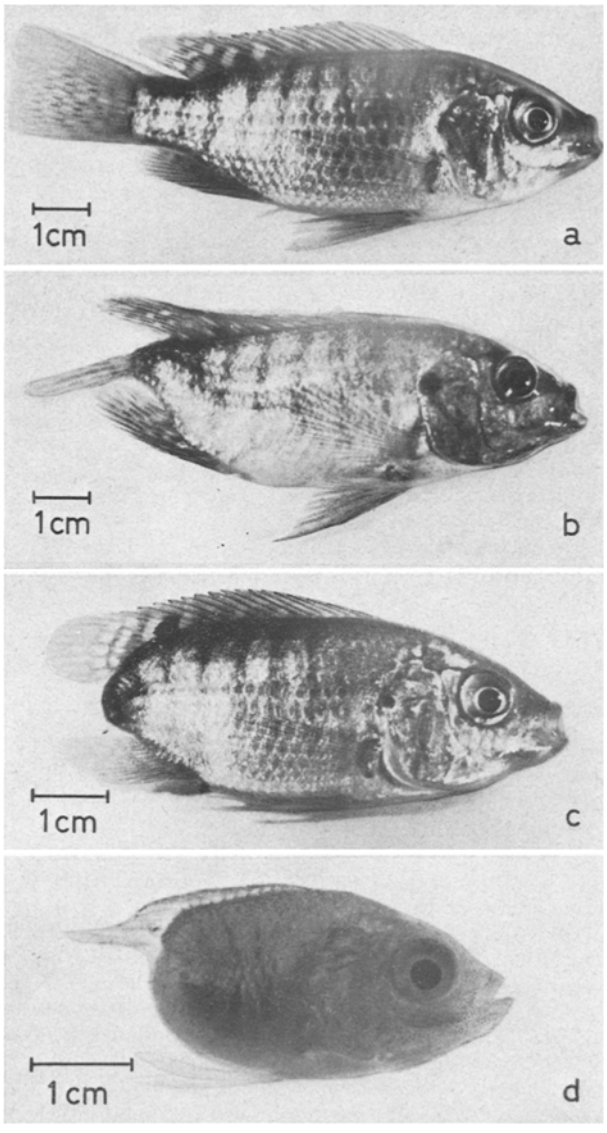


Fig. 2. *a* Regular development; *b–d* progressive malformation.

crippled individuals counted. To evaluate the results the LC-50 and LC-100 methods were used respectively. The toxic effect is represented in Figure 1. It was seen that the fertilized eggs (as long as the egg membrane is intact) are relatively insensitive; 3-day-old eggs showed LC-50 values between 8.4 and 11.4 ppm and LC-100 values between 12 and 15.3 ppm. As development progresses, the sensitivity increases as early as the 4th day (LC-50 7.3–11.4 ppm, LC-100 10.1–14.5 ppm). The toxic effect shows a sharp increase on the 5th day (day of hatching), when concentrations as low as 3–3.9 ppm are sufficient to kill 50% of the yolk-sac larvae within 15 days. In the course of the early development of the yolk-sac-larvae, the sensivity increases further and reaches a maximum (LC-50 1.5 ppm, LC-100 2.0 ppm) at about the 14th day with the end of the yolk-sac stage.

In all experiments, different degrees of deformity appeared in the course of development, which in serious cases resulted in death even before the end of the sac-fry stage. The developmental abnormalities appeared in the region of the abdominal vertebrae (curvature, coalescence of vertebrae, absence of entire groups of vertebrae) and in the region of the anal and caudal fins (Figure 2). From the eggs or yolk-sac larvae dipped on the 3rd, 4th and 5th days of development, up to 37, 31 and 69% of deformed fish respectively emerged, depending on the concentration of the molluscicide. In some cases, even among these deformed fishes, breeding and reproduction was successful.

The young (F1 and F2 generation) developed in a completely normal way. The growth of the fish with serious abnormalities lagged significantly ($p < 0.0002$) behind that of the control fish. The experiments show that there is a high mortality risk for Tilapia immediately after hatching and during the following days, if they come into contact with the molluscicide. The egg membranes prove at first to be a protective barrier. Embryos artificially removed from the egg membranes and exposed to the molluscicide, showed an increased mortality compared with dipped eggs of the same age and with the controls (untreated, normal eggs and embryos removed from the egg membranes). The application of the molluscicide should not, therefore, take place during the early development of the fry in regions where the breeding of *Tilapia* is carried out on a commercial basis.

The Influence of Early Nutrition and Environmental Rearing on Brain Growth and Behaviour[1]

V. R. SARA, T. L. KING and L. LAZARUS[2]

Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney (N. S. W. 2010, Australia), 31 May 1976.

Summary. Preweaning malnutrition permanently reduced brain size and cellular content but in spite of changes in the adrenocortical stress response no learning deficit was observed. Differential rearing environments did not influence the effects of malnutrition.

Malnutrition retards brain growth and produces behavioural abnormalities[3–7]. In man undernutrition is inevitably accompanied by poor environmental conditions which may be detrimental to future development. Animal studies have shown that environmental factors induce biochemical changes in the brain and alterations in learning ability[8]. FRAŇKOVÁ[9], LEVITSKY and BARNES[10] and BLIZARD and RANDT[4] have demonstrated that such variables influence activity and exploration in malnourished rats. We therefore decided to examine the interaction of malnutrition and environment on brain growth and learning ability. The long-term effects of early malnutrition in rats and subsequent rehabilitation by refeeding and environmental manipulation are reported.

Pregnant Wistar rats whose time of conception was determined by the examination of daily vaginal smears were randomly allocated to control and experimental conditions and placed on a diet containing 23% protein, 56% carbohydrate, 11% fat, together with salts and vitamins, and with a calculated caloric value of 4.1 calories per gram[11]. Malnutrition, consisting of 50% food restriction established by pair-feeding techniques, was imposed from

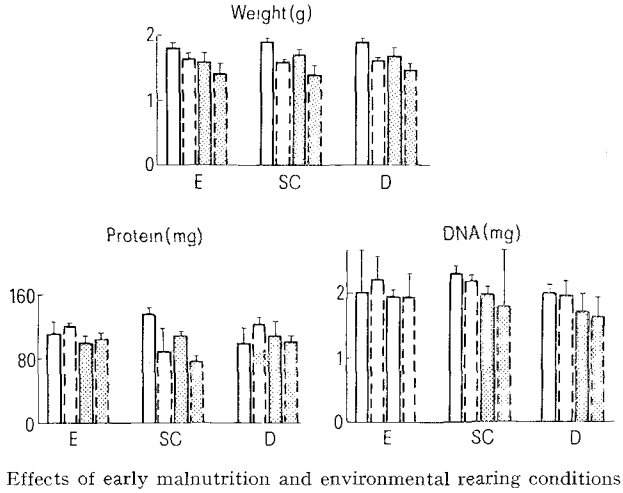
The effect of early undernutrition and environmental rearing on the number of trials to obtain criterion performance

	50% Food restriction (31)[a]						Control diet (35)					
	(14) E.C.		(7) S.C.		(10) D.C.		(14) E.C.		(10) S.C.		(11) D.C.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Trials to criterion	24.67 ±7.80	23.63 ±6.04	20.00 ±4.32	26.50 ±9.49	26.00 ±13.93	35.67 ±9.81	23.86 ±7.64	24.86 ±7.14	18.33 ±4.19	26.50 ±2.60	20.40 ±2.33	33.50 ±5.91

Results expressed as mean ± SD. E.C., enriched condition; S.C., standard colony; D.C., deprived condition.
[a] Numbers in parenthesis refer to the number of animals in each group.

day 7 of gestation through to 28 days postnatally when offspring were weaned and randomly allocated to one of three rearing conditions with food and water ad lib: enriched; standard colony; deprived. Littermates were allocated to all three conditions. The enriched condition consisted of an open wire mesh cage (41 × 24 × 55 cm) containing various manipulanda which was placed in a busy laboratory and housed 8 animals. The standard colony group was reared in normal wire cages (41 × 24 × 27 cm) in the animal holding room with 4 animals per cage. The deprived condition involved individual caging in flat black cylinders ((diam.) 22 × 30 cm) maintained in a darkened soundproofed room. At 60 days of age all animals were placed in standard colony conditions. On day 100 they were placed on a 23 h food deprivation schedule and after a 3 week adaptation period were tested for learning ability on a spatial conditional discrimination task as previously described[12]. A forced trial procedure was used to equate drive strength[13]. Contribution from incentive motivation was assessed by latency measures and the plasma corticosterone response to stress was used as a physiological index of activation. On day 170 animals were given a 2 mA shock for 2 sec and sacrificed by decapitation 30 min later. Blood was collected and the plasma corticosterone[14] levels determined. Brains were removed and the DNA and protein content determined[15]. Results were analyzed by a three-way analysis of variance for unequal group sizes. Post-hoc comparisons were performed according to the method of SCHEFFÉ[16]. Latency of responding was assessed by an analysis of variance of group averages.

Malnutrition resulted in a significant reduction in weight gain during pregnancy ($t = 3.6255$, $p < 0.005$) however there was no significant difference in litter size or mortality before weaning ($p > 0.05$). Development was retarded in malnourished offspring as determined by eye-opening time which occurred at 15 days in controls and 18 days in malnourished animals. Somatic growth was markedly reduced and body weight was still significantly less in malnourished animals at 170 days of age ($F = 62.23$, $df = 1, 69$; $p < 0.001$). The effect of malnutrition and environment on brain weight and DNA and protein content is shown in the Figure. All brain growth parameters measured in adult rats were significantly reduced by malnutrition prior to weaning ($F_{\text{weight}} = 52.38$, $df = 1, 58$; $F_{\text{DNA}} = 6.67$, $df = 1, 47$; $F_{\text{protein}} = 9.15$, $df = 1, 42$; $p < 0.05$). The concentration of DNA and protein per mg of brain tissue was unaffected ($p > 0.05$). Neither environmental rearing nor any interaction term significantly altered these brain parameters ($p > 0.05$). Sex differences were only found in brain weight which was significantly reduced in females as compared to males ($F = 32.91$, $df = 1, 58$; $p < 0.05$).



Effects of early malnutrition and environmental rearing conditions on mean adult brain weight, DNA and protein content. Error bars represents standard deviation. E, enriched environment; SC, standard colony; D, deprived environment. Clear histograms represents animals on control diet and cross-hatched histograms represent malnourished animals. Full lined histograms represent males and broken lined histograms represent females.

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The number of trials taken to obtain the learning criterion of 10 consecutive correct responses is shown in the Table. Malnutrition did not produce any significant effect on learning ability ($F = 0.53$, $df = 1\ 61$; $p > 0.05$). A significant sex ($F = 7.27$) and environment ($F = 3.66$) effect was observed but no interaction term was significant ($p > 0.05$). Females reached the learning criterion in a greater number of trials than males ($p < 0.05$). Although the learning ability of deprived animals was significantly reduced ($p < 0.05$), there was no significant difference between enriched and standard colony conditions ($p > 0.05$).

The lack of any significant change in the learning ability of malnourished rats cannot be attributed to motivational differences interfering with the task performance since no significant effect on latency of responding was observed ($F = 0.67$, $df = 1, 54$; $p > 0.05$). Plasma corticosterone levels in response to shock stress were significantly reduced in malnourished animals ($F = 9.73$, $df = 1, 69$; $p < 0.01$). Elevated plasma corticosterone levels were observed in females ($F = 29.75$, $df = 1, 69$; $p < 0.01$) however no significant environment or interaction effect was obtained ($p > 0.05$).

The present findings demonstrate that malnutrition prior to weaning produces a permanent and irreversible deficit in brain structure which cannot be reversed by later refeeding or environmental manipulations. This is in accordance with the concept that the critical period for brain growth in the rat occurs prior to weaning[17]. In spite of the brain deficits, no alteration in learning ability was observed. Although the use of food reward with previously malnourished animals is questionable, this does not appear to have influenced incentive motivation on the present task as determined by latency of responding. The learning performance of animals reared in deprived conditions was impaired but no interaction between nutrition and environment was observed. Whilst other studies have reported behavioural debilitation in malnourished rats[18–20] these are often based upon performance in aversive situations which depend strongly upon motivational variables. Both the present finding and that reported by ADLARD and SMART[21] show that malnutrition modifies the adrenocortical response to stress. Temporal data are necessary to define the form and peak of this response. Other studies[4,10,22] indicate that malnutrition results in heightened emotionality. Since the present task minimizes such influences, differences in behavioural procedures may occount for these discrepant findings. Malnutrition may affect performance, however its primary effect may be via activation rather than learning ability per se.

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Synaptosomal Adenosine Triphosphatase (ATPase) Inhibition by Organophosphates

H. R. BROWN and R. P. SHARMA

Toxicology Program, Department of Veterinary Science, Utah State University, Logan (Utah 84322, USA), 17 May 1976.

Summary. Chicken spinal cord adenosine triphosphatases (both Na^+, K^+ stimulated and ouabain insensitive) were inhibited by tri-*o*-tolyl phosphate (TOTP, a neurotoxic organophosphate which is not a cholinesterase inhibitor) and mevinphos (a non-neurotoxic compound but inhibitor of cholinesterases). The inhibition was concentration and time dependent, with an initial rapid drop in activity followed by a gradual exponential decline.

There is a notable lack of reports involving organophosphorous compounds and ATPase activity. The bulk of the reports that do exist relate to diisopropyl-fluorophosphate (DFP). The initial report of ATPase inhibition by this compound came in 1964 when HOKIN and YODA[1] reported the irreversible inhibition of renal Na^+, K^+-ATPase by phosphorylation of serine residue. SACHS et al.[2] confirmed these results using a Na^+, K^+, Mg^{++}-dependent ATPase prepared from pig brain. They also found similar effects for the compounds methanesulfonyl chloride and diethyl-*p*-nitrophenyl phosphate. LAHIRI and WILSON[3] questioned the efficacy of DFP itself in producing inhibition and suggested that the inhibition be ascribed instead to the fluoride released by hydrolysis from DFP. Critics of this theory point to the results of SACHS et al.[2] but the question largely remains unanswered.

In vivo work with DFP has produced apparently contradictory results. GLOW et al.[4] found that rats treated with sublethal doses of DFP developed an increase in the specific Na^+, K^+-ATPase activity from the heavy microsomal fraction of brain and kidney homogenates. Since the apparent increase in activity subsided after cessation of DFP treatment the authors concluded that microsomal enzyme induction produced the increased activity. In contrast, JOVIĆ et al.[5] reported significant inhibition of oxygen uptake of cerebral cortex from rats treated with phosphamidon and Soman, respectively.

These reports led us to examine the compounds Tri-*o*-tolyl phosphate (TOTP), a known neurotoxic organophosphate and mevinphos (2-carbomethoxy-1-methylvinyl dimethyl phosphate), a potent cholinesterase inhibitor. Both compounds were found to inhibit Na^+, K^+-dependent and also the ouabain-insensitive, Mg^{++}-dependent ATPase. Chicken spinal cord synaptosomal fraction was used since this species has been used in past for investigating the neurotoxic role of TOTP and the pathologic lesions are more pronounced in the spinal cord than in other parts of the central nervous system[6].

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