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.

[17] J. DOBBING, *Scientific Foundations of Paediatrics* (Eds. J. A. DAVIS and J. DOBBING, (Heineman, London, 1974), p. 565.
[18] S. FRAŇKOVÁ and R. H. BARNES, J. Nutr. *96*, 485 (1968).
[19] S. L. GIURINTANO, Physiol. Behav. *12*, 55 (1974).
[20] P. D. LEATHWOOD, M. S. BUSH, C. D. BERENT and J. MAURON, Life Sci. *14*, 157 (1974).
[21] B. P. F. ADLARD and J. L. SMART, J. Endocr. *54*, 99 (1972).
[22] D. A. LEVITSKY and R. H. BARNES, Nature *225*, 468 (1970).

Synaptosomal Adenosine Triphosphatase (ATPase) Inhibition by Organophosphates

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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].

[1] L. E. HOKIN and A. YODA, Proc. natn. Acad. Sci. USA *52*, 454 (1964).
[2] G. SACHS, E. Z. FINLEY, T. TSUJI and B. I. HIRSCHOWITZ, Arch. Biochem. Biophys. *134*, 497 (1969).
[3] A. K. LAHIRI and I. B. WILSON, Molec. Pharmac. *7*, 46 (1971).
[4] P. H. GLOW, L. H. OPITT and J. S. CHARNOCK, Archs Int. Pharmacodyn. Thér. *198*, 22 (1972).
[5] R. JOVIĆ, H. S. BACHELARD, A. G. CLARK and P. C. NICHOLAS, Biochem. Pharmac. *20*, 519 (1971).
[6] J. M. BARNES and F. A. DENZ, J. Path. Bact. *65* ,597 (1953).

Forty White Leghorn adult laying chickens of approximately 1.5 kg body weight were sacrificed and spinal cord sections were immediately removed from the cervical-thoracic vertebrae, frozen on dry ice and stored at − 90 °C, for a period not exceeding 4 months, before use. The specific activity of the preparation remained essentially unaltered over this period. Approximately 5 g of spinal cord were removed from storage 24–48 h before the ATPase assay was conducted and 10% (w/v) homogenate was prepared using a solution of 0.32 *M* sucrose, 0.94 m*M* *tris* and 1.28 m*M* ethylenediaminetetraacetate (EDTA). Homogenization was carried out in an ice bath using a Talboys stirrer with teflon pestle and a chamber clearance of 0.010–0.015 mm. The synaptosomal pellet was isolated from this homogenate by the method of KUROKAWA et al.[7], suspended in a solution of ice cold 0.32 *M* sucrose-*tris*-EDTA, and quickly frozen in a dry ice-acetone bath after dividing into 3 ml aliquots containing 2 to 3 mg/ml protein. These were stored at − 20 °C overnight. The following day the aliquots were thawed to room temperature and 0.1 ml added to tubes containing reagents to provide 100 m*M* NaCl, 20 m*M* KCl, 50 m*M* *tris*-HCl buffer (pH 7.5) and 24 m*M* $MgCl_2$ in a final 2.5 ml volume. For determining the ouabain-insensitive ATPase activity NaCl and KCl were omitted and 1 m*M* ouabain (G-strophanthin) was added to the solutions.

For the purpose of preincubation with the inhibitor, solutions of TOTP in 70% ethanol, or mevinphos in water were freshly prepared and added to the incubation mixtures 5, 15, 30, 60 and 90 min before starting the reaction. All incubations were carried out in a Dubnoff metabolic shaker bath at 41 °C. Control tubes were treated in the above manner except that 0.1 ml of respective solvent was added in place of the inhibitor solution. In all experiments the reaction was initiated by the addition of 6 m*M* *tris*-ATP that had been prepared from the disodium salt by the method of JÄRNEFELT[8]. The reaction was terminated by addition of 1.25 ml of ice cold trichloroacetic acid after 15 min. The tubes were immediately transferred to an ice bath for a period of 20 min, centrifuged at 1700 *g* for 10 min, then returned to ice bath. 1 ml of clear supernatant was drawn off from each of these tubes and inorganic phosphate determined[9]. Protein determinations were performed by the method of LOWRY et al.[10]. Specific activities of the control preparations varied from 5 to 6 μmoles PO_4/mg protein/h for the Na^+, K^+-stimulated ATPase and from 6 to 8 μmoles PO_4/mg protein/h for the Mg^{++}-dependent ATPase. Percentage of control activity was determined on the basis of control and treated samples tested on the same day. Experimental values represent averages obtained from 2 or more experiments. The Na^+, K^+-dependent enzyme activity was obtained by the subtraction of Mg^{++}-dependent, ouabain-insensitive enzyme activity from the total ATPase activity.

[7] M. KUROKAWA, T. SAKAMOTO and M. KATO, Biochem. J. *97*, 833 (1965).
[8] J. JÄRNEFELT, Biochim. Biophys. Acta *48*, 104 (1961).
[9] J. B. MARTIN and D. M. DOTY, Analyt. Chem. *21*, 965 (1949).
[10] O. H. LOWRY, N. T. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

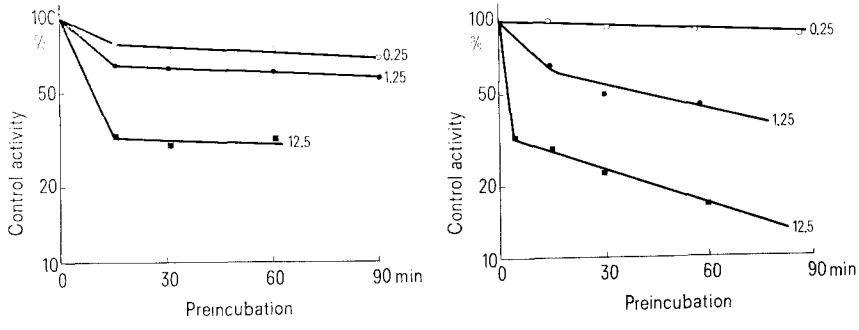

Fig. 1. Inhibition of Mg^{++}-dependent ouabain-insensitive (left) and Na^+, K^+-stimulated ATPase (right) activity in chicken spinal cord synaptosomal fraction by Tri-*o*-tolyl phosphate (TOTP). All values are means of 2 or more determinations. Numbers against the plots indicate millimolar concentration of the inhibitor.

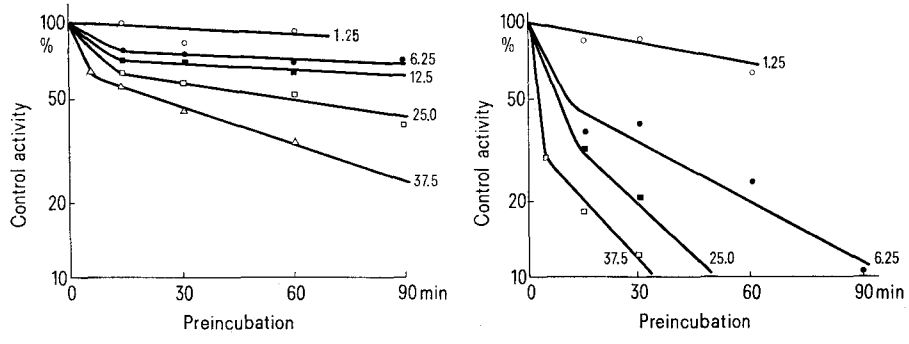

Fig. 2. Inhibition of Mg^{++}-dependent ouabain-insensitive (left) and Na^+, K^+-stimulated ATPase (right) activity in chick spinal cord synaptosomal fraction by mevinphos. All values are means of 2 or more determinations. Numbers against the plots indicate millimolar concentration of the inhibitor. Additional points involving less than 10% of control activity are not illustrated.

TOTP demonstrated a marked ability for time dependent inhibition of both Na^+, K^+-dependent and Mg^{++}-dependent ATPases (Figure 1). At the lowest concentrations used (0.25 m*M*) Mg^{++}-dependent ATPase activity was depressed to a much greater extent than the Na^+, K^+-dependent component. At higher concentrations, however, the Na^+, K^+-dependent enzyme activity was more susceptible.

Mevinphos uniformly inhibited Na^+, K^+-activated ATPase to a greater extent than Mg^{++}-dependent enzyme (Figure 2). In all cases after the addition of inhibitor there was at first a rapid decline of activity for a short period, followed by a gradual inhibition which appeared to be exponential with time.

The observed initial drop in ATPase activity produced by mevinphos may represent a rearrangement of the ATPase protein in the lipid matrix of the membrane. The extent of this initial depression of activity is greater for the Na^+, K^+-stimulated activity, which may coincide with the possible location of the Na^+, K^+-stimulated enzyme in the external plasma membrane and therefore its ready availablility to the medium[11].

In the light of recent findings it appears that several apparently unrelated reports may well involve ATPase inhibition. BULLOCK et al.[12] reported the inhibitory effects of organophosphates on axonal conduction to be either reversible or irreversible depending on the duration of exposure and concentration of inhibitor. HOSKIN et al.[13] used 3 potent organophosphate acetylcholinesterase inhibitors to examine nerve conduction, acetylcholinesterase inhibition, and inhibitor penetration. The authors noted, as observed previously[12], that irreversible inhibition of axonal conduction was concentration and time dependent, and that the concentrations of inhibitor needed were far above those needed for acetylcholinesterase inhibition. These workers proposed that the observed effects possibly occurred due to the binding of the compounds to an unspecified membrane component. The possibility of a mechanism of nerve conduction inhibition which is irreversible, slower, and not related to acetylcholinesterase inhibition was, therefore, broached.

Because of the role of Na^+, K^+-ATPase in the maintainance of transmembrane ionic gradients[14] and the secondary involvement of this gradient in amino acid and sugar transport[15], its inhibition by organophosphates could result in extensive neuronal damage. Also, the disruption of utilization of ATP by ATPase within the synaptic area could alter energy metabolism of the nerve terminal by secondarily altering the activities of other enzymes for which ATP or ADP may be allosteric effectors. The inhibition observed for the neurotoxic compound TOTP and previously reported for DFP, therefore, could conceivably provide a plausable explanation for the delayed neurotoxicity found in association with exposure to these compounds. The lack of such neurotoxicity of mevinphos may be explained on the basis that this compound does not accumulate in the nervous system[16]. TOTP, on the other hand has been shown to persist in the nervous system[17]. In our studies reported previously, TOTP accumulated in brain and spinal cord of chicken after a single effective dose to the extent of 90 µg/g tissue (ca. 0.25 m*M*), a concentration that has been shown to be inhibitory to ATPases in this report.

[11] J. C. SKOU, Physiol. Rev. *45*, 596 (1965).
[12] T. H. BULLOCK, H. GRUNDFEST, D. NACHMANSOHN and M. A. ROTHENBERG, Neurophysiology *10*, 63 (1947).
[13] F. C. G. HOSKIN, L. J. KREMZNER and P. ROSENBERG, Biochem. Pharmac. *18*, 1727 (1969).
[14] J. C. SKOU, Biochim. biophys. Acta *23*, 394 (1957).
[15] A. A. EDDY, Biochem. J. *108*, 489 (1968).
[16] R. P. SHARMA, J. L. SHUPE and J. R. POTTER, Toxic. appl. Pharmac. *24*, 645 (1973).
[17] P. G. WATANABE and R. P. SHARMA, in *Pesticides and the Environment* (Intercontinental Medical Book Corp., New York 1973), p. 503.

The Glycocalyx of the Epithelial Cells of the Colon, Observed in Normal and Ulcerous Colitic Conditions

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Summary. Biopsies of subjects affected by ulcerous colitis were stained with ruthenium Red. Alterations of the cellular coat and glycocalyx of the epithelial cells of the colon were identified.

The importance of the 'cell coat' in many functions of cellular cycle has been well established[2-4]. JOHNSON[5] showed that maltase and invertase enzymes are located in the brush-border's glycocalyx of the small intestine's absorbing epithelium. This coat probably takes a prominent part during the process of absorption of several substances and is also considered to be the site of a number of antigenical cellular receptors. The digestive enzymes seem to be strictly related to the basal areas of the glycocalyx and represent the so-called 'coat strictly attached'. WILLIAMS and MCKENZIE[6] described significant cell coat's variations in the small and large intestine of mice. In fact, the epithelial cells of jejunum-ileum and colon regions showed a remarkable structural likeness, and a great difference was also noticed between the two superficial glucide-coats.

Further, MORGAN[7], DULBECCO and STOKER[8], MARTINEZ-PALOMO and WIRBR[9] observed significant variations of the polysaccharidic substance at the surface of cells infected by virus. These variations might be related to the phenomena showed from infected cells, as, for in-

[1] The Authors are indebted to Prof. PIETRO MOTTA for criticism and revision of the manuscript.
[2] A. MARTINEZ-PALOMO, Int. Rev. Cytol. *29*, 29 (1970).
[3] A. RAMBOURG, Int. Rev. Cytol. *31*, 57 (1971).
[4] R. I. WINZLER, Int. Rev. Cytol. *29*, 77 (1970).
[5] F. C. JOHNSON, Fed. Proc. *28*, 26 (1969).
[6] G. WILLIAMS and S.MCKENZIE, J. Cell Biol. *34*, 447 (1967).
[7] H. R. MORGAN, J. Virol. *2*, 133 (1968).
[8] R. DULBECCO and M. G. STOKER, Proc. nat. Acad. Sci. *66*, 204 (1970).
[9] A. MARTINEZ-PALOMO and T. WIRBR, Mosc. Sci. *171*, 905 (1971).