EFFECT OF ISATIN ON CHICK BRAIN ACID AND ALKALINE PHOSPHATASES

In brain several vitamin-B₉ dependent enzymes influence the level of γ-amino butyric acid (GABA) whose deficiency has been implicated as a possible causative factor of convulsive seizures. Since Turner has postulated that phosphatases may be involved in the regulation of vitamin-B₉ requiring enzymes, it was thought desirable to investigate the effect of isatin, a promising anticonvulsant agent on brain phosphatases.

Roberts et al. as well as Begum and Bachhawat have demonstrated the presence of pyridoxal phosphate phosphatase. They found this enzyme to be an acid phosphatase with broad specificity. An increase in the activity of this enzyme may lead to deficiency of pyridoxal phosphate which in turn leads to deficiency of GABA and hence convulsions. In the present study, although isatin administration produces an increase in acid phosphatase activity, yet it does not cause convulsions (rather it prevents them) showing that this increase is not associated with pyridoxal phosphate phosphatase. At present we are unable to fathom the full implication of the increase of acid phosphatase activity by isatin.

In vitro inhibition and in vivo activation of the acid phosphatase by isatin may be explained on the basis of its reported breakdown in the body.

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A REVERSIBLE SPERMIOSTATIC FACTOR PRESENT IN BUFFALO SEMINAL PLASMA

The occurrence of initially non-motile but revivable spermatozoa in occasional buffalo semen samples was reported earlier. The preliminary findings on the possible basis of this peculiar phenomenon of buffalo semen are briefly presented here. pH, tonicity, fructose level and cation distribution (Na, K and Ca) in the seminal plasma did not appear to have any direct implication in the occurrence of this phenomenon since the non-motile and motile ejaculates were indistinguishable in respect of these variables. It was found that a factor located in the seminal plasma was responsible for the reversible inhibition of spermatozoan motility since washed motile spermatozoa from normal ejaculates were found to be reversibly immobilised when resuspended in cell free seminal plasma obtained from non-motile ejaculates and vice versa. On fractionation of the seminal plasma from non-motile ejaculates by dialysis in a cellophane tube at 2°C against three changes of distilled water every 48 h and redialysis of the non-dialysable portion against isotonic phosphate buffer (pH 7-4), it was found that the motility-inhibitory factor was located in the non-dialysable fraction. The factor was fairly heat-stable (90°F for 1 min.) and possibly, therefore, non-enzymic in nature.

In the presence of cysteine, glycine and citrate-which share the common property of chelating heavy metals (Cu, Zn, etc.), the inhibition of motility in revivable non-motile ejaculates was found to be instantaneously released indicating the reversible nature of the phenomenon. Addition of glucose or fructose did not appear to erewice the motility of non-motile ejaculates. It is difficult to say if the motility-inhibitory factor of buffalo seminal plasma and the so-called toxic factor claimed to be present in bull seminal plasma adversely affecting sperm viability during in-vitro storage are chemically identical, though there are a few points of apparent similarities between the two. The instantaneous revival of motility in initially non-motile ejaculates by chelating agents is suggestive that the inhibition may be operative at the level of ATP-ase enzyme which is involved in the enzymic breakdown of ATP providing immediate energy for sperm movement. It is conceivable that the motility-inhibitory factor may, in fact, be an organo-metallic compound which acts as a potent ATP-ase inhibitor and is neutralised in the presence of metal-binding agents. Further work on chemical characterisation of the factor is in progress.

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PROTECTIVE EFFECT OF CHLORPROMAZINE ON TISSUE CULTURE AGAINST POLIOVIRUS

Many antihistaminic drugs protect tissues against damaging effects of viruses due to stabilization of the lysosomal membranes. Damage to different subcellular organelles of tissue occurs during poliovirus infection. The present experiments demonstrate that chlorpromazine stabilizes membranes of different subcellular organelles besides lysosomes, of monkey kidney tissue culture and consequently protects it against poliovirus infection.

Monkey kidney tissue cultures were prepared in bottles. To one group of culture bottles, poliovirus type I was inoculated in doses of 1 ml of about 100 TCID₅₀ per 0.1 ml. In the second group chlorpromazine (Largactil—May and Baker) was mixed with the tissue culture fluid so that its concentration was 10⁻³ M. The third group had poliovirus and chlorpromazine while the fourth group was used as untreated control.

All the bottles were examined for morphological changes after incubating at 37°C for 24 hours when the experiment was terminated. The tissue culture cells were prepared and acid and alkaline phosphatase and adenosine triphosphatase were estimated as described before. For the estimation of 5'-nucleotidase, the test reaction mixture consisted of 1·0 ml tris buffer (pH 7·5) and 0·1 ml of manganese sulfate. The control reaction mixture consisted of 1 ml of tris buffer (pH 7·5) and 0·1 ml manganese sulfate and 0·2 ml of nickel chloride. To both tubes, 0·2 ml of cell homogenate was added and these were placed in a water bath at 37°C for 3 minutes. Then 0·2 ml of adenosine-5'-phosphate was added and after 30 minutes the reaction was stopped by the addition of 2 ml of 10 per cent trichloracetic acid. The tubes were centrifuged for 5 minutes and suitable