It seemed desirable to attempt to find out whether some monatomic ion having a suitable absorption spectrum could be found which would sensitize invertase to the action of light; for here, presumably, the first of the above possibilities would not exist. In this paper are reported the results of a preliminary investigation of the effect of certain inorganic ions on the photo-inactivation of invertase.

The enzyme used in this study was a commercial preparation[4] labeled "invertase scales"; it had been obtained from bottom fermenting yeast and contained some melibiase. For each experiment 0.03 gm of this preparation was dissolved in 4 cc of distilled water containing 1 drop of 0.1 N HCl and the desired concentration of the inorganic salt. After the enzyme had completely dissolved, 2 cc of this solution were placed in a quartz test-tube for irradiation and 2 cc reserved as a control in a glass test-tube of the same dimensions, but wrapped in black paper. Both tubes were then placed at a distance of 10 cm from a glass enclosed Type H-3 General Electric mercury vapor lamp for a measured length of time. The solution in the quartz tube thus received the full radiation from the lamp, while the control in the glass tube was affected only by the heat radiated from the lamp.

Following irradiation, the enzyme solutions were brought to 25° C. and each thoroughly mixed with 10 cc of a 20 per cent. sucrose solution. The rates of inversion of sucrose at 25° C. were then followed simultaneously in the 20 centimeter tubes of two polarimeters.

TABLE I

EFFECT OF COLORED IONS ON THE PHOTO-SENSITIVITY OF INVERTASE

Experiment number	Ion concentration (Mg/cc)	Length of time irradiated (min.)	Degrees of rotation			
			Original	20 min.	40 min.	60 min.
1.	None	0	12.5	8.0	4.6	2.4
		15	12.5	8.0	4.8	2.5
2.	0.1 mg Cu^{++}/cc	0	12.5	6.0	1.25	0.8
		15	12.3	12.0	12.0	12.0
3.	0.1 mg Cu^{++}/cc	0	12.33	7.48	4.15	2.1
		8	12.35	8.12	5.93	3.75
4.	0.4 mg Cu^{++}/cc	0	11.5	9.0	8.4	....
		15	11.5	11.5	11.5	....
5.	0.1 mg Fe^{++}/cc	0	12.3	7.45	3.75	1.25
		15	12.3	7.45	3.80	1.25
6.	0.1 mg Ni^{++}/cc	0	12.1	7.48	3.75	1.20
		15	12.1	7.48	3.98	1.98
7.	0.1 mg Co^{++}/cc	0	12.0	7.47	3.80	2.0
		15	12.0	7.47	3.83	2.38
8.	0.11 mg UO_2^{++}/cc	0	11.25	10.25	10.18	....
		15	11.30	10.31	10.31	....

The results are shown in Table I, which contains in successive columns the number of the experiment, the ion concentration, the length of time of irradiation and degrees of rotation in the polarimeter at various times after mixing the enzyme solutions with sucrose. From the results of Experiment 1 in which no salts were added to the enzyme, it is seen that 15 minutes irradiation of the enzyme alone had no effect on the activity. Experiment 2 shows that, while 0.1 mg Cu^{++} (as $CuSO_4$) per cc has a negligible direct effect on invertase activity, 15 minutes irradiation of a mixture containing this concentration of Cu^{++} results in almost complete inactivation of the invertase. In a supplementary experiment similar inactivation was found to result from 15 minutes irradiation of a solution containing only 0.05 mg Cu^{++} per cc. Experiment 3 shows that an 8-minute period of irradiation of invertase in the presence of 0.1 mg Cu^{++} per cc results in only partial inactivation of the enzyme. Experiment 4 shows that 0.4 mg Cu^{++} per cc partially inactivates the enzyme even without radiation, while irradiation increases the extent of inactivation by this concentration of Cu^{++}.

Experiments 5, 6 and 7 show that the colored ions Fe^{+++}, Ni^{++} and Co^{+++} in concentrations of 0.1 mg per cc bring about negligible inactivations of invertase even when the solutions were irradiated. Uranyl ion as $UO_2(NO_3)_2$ containing 0.1 mg of uranium per cc almost completely inactivates the enzyme; irradiation in the presence of uranyl ion causes still further inactivation. In another experiment Ca^{++} was found to have no effect.

The results suggest that, in order to bring about a photo-inactivation, a monatomic ion must have first, a suitable absorption spectrum, and second, a means of transferring the absorbed energy to the enzyme molecule. It seems probable that the cupric and uranyl ions form complexes with the invertase which, themselves, are capable of absorbing energy and decomposing to form inactive material. It would appear that the other colored ions used were incapable of forming such complexes and were therefore ineffective. Further work will have to be done to elucidate further the mechanism of the reaction.

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THE OCCURRENCE OF FITS IN PYRIDOXINE DEFICIENT RATS

CHICK *et al.*[1] described fits of an epileptic nature in rats maintained on pyridoxine deficient diets for a period of four months or more. No such fits have been reported in this country, although thousands of

[1] H. Chick, M. M. El Sadr and A. V. Worden, *Biochem. Jour.*, 34: 594, 1940.

pyridoxine deficient rats have been studied. In the laboratory of the Poultry Division, hundreds of pyridoxine deficient rats[2] were studied without the appearance of such epileptic-like fits. Recently, however, fits such as described[1] were observed. The fits occurred among pyridoxine deficient rats, each of which was fed 5 cc of water by stomach tube every 3 hours. After the second water feeding 5 out of 16 rats were observed to go into fits which lasted from a few seconds up to about 2 minutes. Others possibly went into fits but were not observed. Fits developed when the rats were startled by noise or were surprised by being suddenly picked up. Others were nervous but were not observed to go into convulsions. The rats had been on the pyridoxine deficient diet about 4 to 5 months.

Since there is a disturbance of water metabolism in human epilepsy[3] we repeated the experiment, but the fits did not recur. The rats were then exposed to the piercing sounds produced continuously by frequencies of 30 to 10,000 cycles after they had been given water by stomach tube, but the convulsions did not recur.

At about this time, fits were observed in pyridoxine deficient rats in the laboratory of the Vitab Corporation. They were few but in sufficient numbers to arouse interest. These rats were also given water by stomach tube without producing any fits.

After the rats were 6 to 8 months old, convulsions occurred spontaneously with increasing frequency. The convulsions were not alike, but they followed a definite pattern. They generally occurred when several rats were together in a can and frequently handled during the time when they were weighed or the vitamin doses fed. One rat in fits will generally set off other rats in convulsions. Without warning the rat will sometimes start convulsively forward in jerks similar to the hopping of a mechanical rat. Often the rat will paddle the air with its paws. The head is often jerked up into an upward tilt and the rats get up on their hind legs and paddle the air. Sometimes they get up so high on their hind legs that they lose their balance and tumble over backwards or on their sides. When paddling or fanning the air with their paws their ears will sometimes wiggle rapidly, appearing much like a fan without a handle. Sometimes their ears are pressed flat against the back of their necks. While rearing on their hind legs, they will often involuntarily hop in the air, sometimes jumping out of the can. The paddling of the air and the fanning of the ears seemed to be motivated from one source as though controllable by one switch. These involuntary hops gave the appearance of being motivated by a hidden spring in the rat resembling a hopping mechanical rat. Mild convulsions were generally limited to a few convulsive hops or a half minute's pawing of the air often accompanied by the wiggling of the ears. Severe convulsions lasted 2 to 3 minutes. A round drop of saliva sometimes involuntarily appeared at the mouth of the rat during a severe convulsion. After a severe convulsion, the rat would sit or lie perfectly still, sometimes holding the wire screen tightly in the front paws. During this period the rat seemed unconscious and did not react when touched. The only sign of life was the heaving in and out of the sides, the forward part of the rat's body slowly moving forward and backward in rhythm with the heaving in and out of the sides. Occasionally the rats lay on their sides with their legs outstretched as though dead. They came out of this coma-like condition without any apparent effort and scratched themselves behind the ears or washed their faces as though nothing had happened. Occasionally after they had regained consciousness they shook themselves, much like a dog just coming out of water.

Convulsions were never observed under $4\frac{1}{2}$ months, and then only rarely. When they reached the age of 6 to 8 months they occurred more frequently. Some rats had fits daily. The duration of the convulsions gradually increased. Out of 13 rats 7 months of age, 6 regularly had fits. Out of 3 rats 9 months of age receiving 5 micrograms daily of pyridoxine, one occasionally had a short mild convulsion.

We can, therefore, confirm the findings of Chick *et al.*[1] that fits will occur in pyridoxine deficient rats and we wish to suggest the possibility, remote though it may be, that disturbed water metabolism may in some way be involved in the production of these epileptic-like fits.

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[2] S. Lepkovsky, *Jour. Biol. Chem.*, 124: 125, 1938.

[3] C. H. Best and N. B. Taylor, "The Physiological Basis of Medical Practice," p. 1460. Baltimore: Williams and Wilkins Company. 1940.

EFFECT OF l-ASCORBIC ACID ON THE ISOLATED FROG HEART[1]

SIMPLE unsaturated lactones like βγ-angelicalactone, αβ-angelicalactone and crotonolactone γ-acetic acid and its esters have a characteristic digitalis-like action upon the frog heart isolated according to the method of Straub. If administered in an adequate concentration and replaced continuously (by means of a special cannula) at a steady rate for a suitable length of time,

[1] Carried out under the auspices of the University Committee on Pharmacotherapy.