subinoculations from this animal have produced orchitis in all injected rabbits, and the testicular suspension injected into guinea pigs has caused the disease described above. Intraperitoneal injection into 2 cats has been negative.

The agent causing this infection is in suspensions of various organs, in tracheal washings, and in the blood serum. Titrations of sera from infected guinea pigs have shown that from 1×10^{-3} to 1×10^{-5} cc will cause disease. Heating of infective serum for 20 minutes at 50°C has not destroyed its activity. Serum heated at 55°C for the same time gave a prolonged incubation period, while serum heated for 20 minutes at 60°C was inactive.

Cultures of infective organ suspensions or serum show no growth on ordinary media. The agent readily passes through tested Berkefeld N filters, and Berkefeld W filtrates cause disease but death occurs 2 to 3 days later than in the controls. The agent has been propagated on the chorioallantoic membrane of embryonated chicken eggs, and suspensions of membranes from the 18th serial transfer have produced the characteristic disease in guinea pigs.

The source of this infection has not been determined. It is probably not of human origin, for the sera of the 2 caretakers and of 4 of us who have been working with the disease all fail to neutralize the agent.

This agent appears to be a filtrable virus capable of producing disease by itself as well as in association with the common guinea-pig pathogens.

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Metabolism of the Alcoholate of the Trimer of Hydroxypyruvic Aldehyde.

WILLIAM E. EVANS, JR., C. JELLEFF CARR AND JOHN C. KRANTZ, JR.

From the Department of Pharmacology, School of Medicine, University of Maryland, Baltimore, Md.

Hydroxypyruvic aldehyde has been prepared previously by Evans and Waring[1] and by Hynd.[2] Hynd also studied its toxicity and action in insulin shock. Elsewhere the authors[3] have described a

1 Evans, W. L., and Waring, C. E., *J. A. C. S.*, 1926, **48**, 2678.

2 Hynd, A., *Biochem. J.*, 1931, **25**, 11.

3 Evans, W. E., Jr., Carr, C. J., and Krantz, J. C., Jr., *J. A. C. S.*, 1938, **60**, 1628.

new method for the purification and isolation of the alcoholate of its trimer [$(C_3H_4O_3)_3.C_2H_5OH$].

Glycogen Storage in the Livers of White Rats. Nine male animals fasted for 48 hours were given 0.4 g of hydroxypyruvic aldehyde per 100 g of animal. The compound was dissolved in 3 cc of water and administered by stomach tube. After 3 hours the rats were anesthetized with sodium amytal, the livers extirpated and the glycogen determined by the Good[4] modification of Pflüger's method. The dextrose was determined by the Shaffer and Hartmann[5] method. The low value was 0.11%, high 0.62%, and the average 0.29% liver glycogen. Four control animals which received 3 cc of water showed an average liver-glycogen content of 0.28%.

Under the same experimental conditions 6 additional animals were given 0.4 g of dihydroxyacetone per 100 g of animal. The average liver-glycogen content was 1.01%.

In order to eliminate the possibility of slow absorption of hydroxypyruvic aldehyde from the alimentary tract and consequent lack of effect on liver-glycogen content, doses of 0.25 g per 100 g and 0.025 g per 100 g were administered intraperitoneally to 9 and 10 rats respectively. Six animals which had received 1 cc of Ringer's solution intraperitoneally served as controls. Thirty minutes after injection, the liver-glycogen content was determined. The controls averaged 0.30% glycogen. Those which had received 0.25 g of hydroxypyruvic aldehyde per 100 g averaged 0.19% glycogen, and the livers of the rats which were injected with 0.025 g of the osone per 100 g averaged 0.11%. Under these conditions hydroxypyruvic aldehyde does not serve as a precursor of glycogen.

Blood Sugar. All animals were fasted 24 hours prior to treatment. Doses of 4 g of dextrose per kg and 4 g of the glycerosone per kg were given by stomach tube to 7 and 10 rabbits respectively. The blood sugar was determined by the Folin[6] ferricyanide method. No differentiation was made between the blood dextrose and blood hydroxypyruvic aldehyde. The quantitative reducing power of the osone compound was 30% less than that of dextrose. The effects on blood-sugar level are summarized in Table I. After hydroxypyruvic aldehyde a mild glycemia existed for 6 or 7 hours; the value was normal the following day.

The effect on the blood sugar level of the intravenous administration of 0.25 g per kg of hydroxypyruvic aldehyde and dex-

[4] Good, C. A., Kraemer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

[5] Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1920-21, **45**, 349.

[6] Folin, O., *J. Biol. Chem.*, 1928, **77**, 421.

TABLE I.
Effect of Hydroxypyruvic Aldehyde on Blood-Sugar Level of Rabbits.

No. of Rabbits	Substance Administered Dose = 4 g per kg	Oral Administration Blood-Sugar Level (avg mg %) Time in Hours					
		Control	½ hr	1 hr	2 hr	3 hr	4 hr
7	Dextrose	120	177	205	164	145	128
10	Glycerosone	107	120	112	120	123	134

TABLE II.
Effect of Hydroxypyruvic Aldehyde on the Blood-Sugar Level of Rabbits.

No. of Rabbits	Substance Administered Dose = 0.25 g per kg	Intravenous Administration Blood-Sugar Level (avg mg %) Time in Min.													
		Control	3	6	9	12	15	18	21	24	27	30	60	90	120
4	Dextrose	107	205	190	199	188	168	172	168	168	153	158	128	116	116
10	Glycerosone	109	106	102	121	112	100	104	100	104	119	116	122	128	123

trose was determined. The results are summarized in Table II.

Intravenous injection of 0.5 to 1.0 g of hydroxypyruvic aldehyde per kg produced immediately the following symptoms: transient exophthalmus, respiratory stimulation both in rate and depth, and ischemia of the ears for varying periods of time. Clonic convulsions ensued, characterized by dorsiflexion of the head, with intervening periods of muscular weakness of the extremities especially the hind legs. Expressed urine samples did not reduce Fehling's solution. The foregoing symptoms were relieved or prevented by the subsequent or simultaneous administration respectively of 5 cc per kg of a 5% solution of disodium phosphate intravenously. Dextrose administered under the same conditions produced extreme dilatation of the ear veins. Urine expressed a short time after the injection reduced Fehling's solution strongly upon heating.

Insulin Shock. Thirty-five mice, weighing about 20 g each, were fasted for 24 hours. Each was then injected intraperitoneally with 1/6 unit of insulin and placed in a chamber heated to 29°. Ten of these animals served as controls. The intraperitoneal injection of 2.0 mg per gram of hydroxypyruvic aldehyde in 4% solution into each of 15 animals relieved the convulsions but this was succeeded by depression characterized by muscular weakness and labored respiration, followed by death. The insulin convulsions were relieved for the greatest period of time by the intraperitoneal injection of 1 mg per gram of the osone into each of 6 mice. These animals later died in convulsions which were typical of insulin. Hynd[7] reported that the injection of hydroxypyruvic aldehyde did not relieve insulin convulsions.

The injection of 0.25 g per kg of hydroxypyruvic aldehyde into a totally depancreatized dog caused temporary relief of convulsions produced by protamine zinc insulin.

Conclusions. 1. The oxidation of one primary alcoholic group in dihydroxyacetone with the production of hydroxypyruvic aldehyde destroys the capacity of the former compound to be stored as glycogen in the liver of the rat. 2. The oral administration or intravenous injection of the osone does not significantly affect the blood-sugar level of rabbits within 2 hours. 3. The intraperitoneal injection of hydroxypyruvic aldehyde into mice causes temporary recovery from insulin convulsions, although it is not a precursor of glucose.

[7] Hynd, A., *Proc. Roy. Soc. London*, 1927, B **101**, 244.