8954 C

A Stable and Potent Lactic Dehydrogenase Preparation.*

CHARLES GURCHOT AND ARNOLD LOWMAN.

From the Pharmacological Laboratory, University of California Medical School.

In investigating some of the biological effects of oxidases, it was found desirable to prepare active enzymes stable enough to keep for some time. Stephenson,[1] in England, prepared a cell-free extract of *B. coli,* containing fairly active dehydrogenases, by lysing a concentrated suspension of the organism in hypertonic buffer solution. Bernheim[2] has also reported the preparation of lactic dehydrogenase from acetone yeast. The product, called "Zymin", gives a fairly active extract and will keep for some time. Gurchot[3] recently described a simple method of extracting lactic dehydrogenase from *Prunus* seeds. This was published without the knowledge that Thunberg[4] had done the same things previously for other seeds.

We have now found a method for making a stable and potent dry preparation of lactic dehydrogenase, 100 mg. of which will decolorize 0.8 mg. of methylene blue per minute in the presence of lactate. This product was obtained by washing ordinary untreated baker's yeast with saline, and then grinding it with phosphate buffer solution saturated with ether, in a ball mill for 8 to 15 hours. This lysate was centrifuged, cooled in the refrigerator, and shaken with one-half its volume of ether. It was then allowed to stand overnight in the refrigerator. A stable ether gel formed, which had no activity, and which could be separated from the remainder of the solution. This served to remove foreign proteins, and cell debris. After 3 such treatments the liquid was filtered and the solution, which was clear and yellow, was saturated with ammonium sulphate. This was allowed to stand in the refrigerator for one hour and then centrifuged. The precipitate so obtained was dried in a vacuum desiccator.

A second extract of enzyme was obtained by grinding the yeast residue with phosphate buffer for 6 hours and allowing it to stand overnight in the refrigerator. This was then centrifuged and treated exactly as the first extract.

The activity of the preparation was estimated by the Thunberg

* Supported in part by the Christine Breon Fund for Medical Research.

1 Stephenson, M., *Biochem. J.*, 1928, **22**, 605.

2 Bernheim, F., *Biochem. J.*, 1929, **22**, 1178.

3 Gurchot, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 285.

4 Thunberg, T., *Skand. Arch. Physiol.*, 1925, **46**, 339.

method, using 1 cc. M/20 sodium lactate, 1 cc. M/2 phosphate buffer at pH 7.6, 2 cc. 1/5000 methylene blue, 100 mg. enzyme powder and 6 cc. of water, making a total volume of 10 cc. The tubes were evacuated for 2 minutes by a Hyvac pump. The reduction time for such tests varied from 30 to 60 seconds, or from 0.8 to 0.4 mg. methylene blue per minute. This dehydrogenase preparation is free from sulphydryl groups as shown by a negative nitroprusside reaction. It has no reducing action on methylene blue when sodium succinate or formate are substituted for sodium lactate. Differences in various other methods of preparation used by other workers result in different final concentrations of enzyme and make a comparison of potency difficult. However, the extract described is probably twice as active as the best previously reported upon by Ogston and Green.[5]

8955 P

Comparative Toxicity of Some Powerful Drugs for the Cat.

DAVID I. MACHT.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore.

While making a pharmacological and therapeutic study of snake venoms,[1] the writer deemed it desirable to compare their toxicity with that of some other powerful drugs and poisons. To render such a comparative study effective a uniform method of experimentation was required for all the chemicals to be examined. Solutions of the respective substances were accordingly tested for their toxicity by a uniform technique similar to that employed in ouabain and digitalis assay.[2] Healthy cats, weighing from 2 to 3 kg. and kept under light ether anesthesia, were used in these experiments. A cannula was introduced into the femoral vein and a dilute solution of the drug to be tested was injected at the rate of one cc. per 30 seconds until the heart stopped. The drugs examined were atropine sulphate, strychnine sulphate, cocaine hydrochloride, coniine hydrochloride, nicotine alkaloid, aconitine hydrochloride, potassium cyanide, sodium arsenate, cobra venom, rattlesnake venom, ouabain, ricin and abrin. The average lethal dosage of each substance per kg.

5 Ogston, F. J., and Green, D. E., *Biochem. J.*, 1935, **29**, 1983.

1 Macht, *Proc. Nat. Acad. Sc.*, 1936, **61**, 22.

2 Rowntree and Macht, *J. A. M. A.*, 1916, **66**, 870.