similar manner. Again the numbers are written by the observer. If it is thought desirable to test the enhancement to bone acuity which is brought about by occlusion of the external canals, the ears are closed with plastocene and the test repeated the third time.

The advantage of employing a single receiver to accomplish both purposes is obvious. Further the test is sufficiently interesting to help rivet the attention on the problem; eliminates the personal equation of the examiner; and is both rapid and satisfactory. A demonstration of the apparatus was made to prove the surprisingly good quality of reproduction both through air and through bone vibrations.

4302

Effect of Amylase on Formation of Hexose-phosphate by Muscle Extract.

ETHEL RONZONI. (Introduced by P. A. Shaffer.)

From the Department of Internal Medicine, Washington University, St. Louis.

The mechanism by which the pancreatic factor[1] inhibits the formation of hexose-phosphate[2] and therefore glycolysis in muscle extract has been shown by Case and McCullagh[3] to be a direct action on the starch or glycogen used as the source of carbohydrate. Hydrolysis of these carbohydrates before the esterification of their mono-saccharid derivatives with phosphate seems a necessary assumption. Complete inhibition is difficult to explain since always there remains a residue of carbohydrate with but slight reducing value. We are, however, able to confirm these observations and find also that muscle amylase added in sufficient quantities produces marked inhibition of glycolysis.

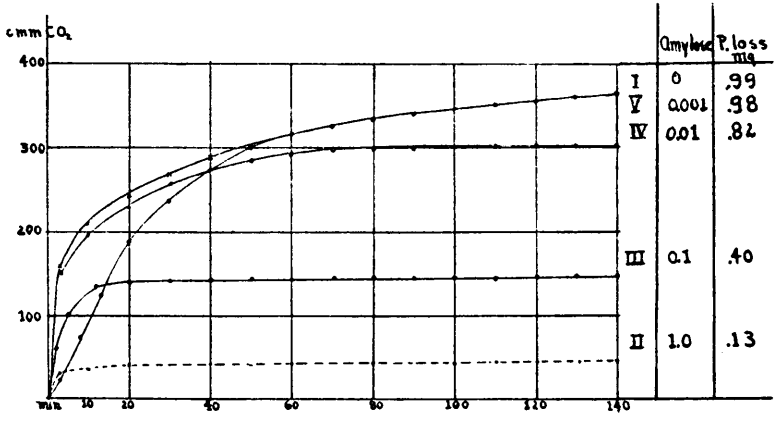
We report here a study of the action of pancreatic diastase on the formation of hexose-phosphate by muscle extract, undertaken with a view to establishing a possible rôle of amylase in muscle extract glycolysis. If hydrolysis of starch or glycogen is a necessary step before its esterification with phosphate, it should be possible to increase the rate of esterification by accelerating the hydrolysis of the carbohydrate, unless muscle extract contains an optimum amount

[1] Winfield, G., and Hopkins, F. G., *J. Physiol.*, 1915, l, 5.

[2] Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 178.

[3] Case, E. M., and McCullagh, D. R., *Biochem. J.*, 1928, xxii, 1060.

of amylase for this purpose. Muscle extract prepared by extraction with salt solution for one-half hour has an almost optimum amylolytic activity and the addition of small quantities of amylase has no effect or slightly inhibits the formation of hexose phosphate. However, muscle extracts prepared by brief extraction with water have a lower amylolytic activity and on these extracts the acceleration in the rate of formation of hexose-phosphate by added amylase is shown in the following chart. To the water extract of muscle Na_2HPO_4 was added to give a concentration of 200 mg. P. per 100 cc. The concentration of $NaHCO_2$ was .025 M. and NaF .02 M. To equal quantities of this extract equal amounts of water or amylase solution were added. After the addition of starch to give a concentration of 500 mg. %, the rate of formation of hexose-phosphate in 2 cc. of such an extract was followed by manometric determination of CO_2 liberated from $NaHCO_3$ by the formation of the more acid hexose-phosphate.[4] Phosphate changes were determined at the end of the reaction period. This curve would be influenced by small accumulation of acids other than lactic, since the latter was determined chemically and found not to increase.



The chart shows that even when marked inhibition in the total production of CO_2 occurs the rate of formation is accelerated. With smaller amounts of amylase although the total amount formed in 140 minutes is not increased the initial rate of its production is accelerated.

Changes in free reducing substance and in phosphates were followed chemically in samples of the same extract. These cannot be compared directly with manometric determinations since they

[4] Meyerhof, O., *Biochem. Z.*, 1926, clxxviii, 427.

were kept at room temperature about 3° C. below that of the water bath and were not shaken. The following Table I shows the increase in reducing substance expressed as glucose in mg. % formed from a total carbohydrate concentration of 500 mg. %.

TABLE I.

	I	II	III	IV	V	
Time Min.	0	1	.1	.01	.001	Amylase added
10	0	42	4	0	0	} increase in reducing substance Mg. %
15	0	60	6	6	0	
30	4	94	20	8	6	
140	10	112	50	16	14	
	Phosphate decrease (mg. 10 min.) per 10 cc. muscle extract.					
10	1.2	.55	1.3	2.11	2.20	

From this and other experiments of the same type, it is evident that muscle extract may be prepared with less than the optimum concentration of amylase, and that in such cases the rate of formation of hexose-phosphate may be accelerated by the addition of solutions containing amylase, thus establishing the fact that hydrolysis of starch is a necessary step in glycolysis of muscle extract. A further search for the changes in carbohydrates which prevents esterification with phosphate will require an investigation of the earlier changes taking place when massive doses of amylase are added to relatively small amounts of starch or glycogen.