

At the same time an animal that had been used as a control for this group responded to the second injection of nucleic acid in the same manner as it did to the original injection which had been given 28 days earlier. Similar inhibition was obtained after the administration of antipyrin, phenylhydrazine hydrochloride, o-quinone and catechol. The reaction to *a*-dinitrophenol differed slightly in that the quantity (20 mg./kg.) which was required to produce the inhibition of the leucogenic tissues, produced a preliminary stimulation which was characterized by an increase in the percentage of juvenile cells in the circulation without a concomitant leucocytosis. After the drug had been administered for a few days this reaction was lost and the proportions of the cellular elements in the peripheral circulation returned to normal. Shortly after this return to normal, the administration of nucleic acid failed to evoke a response.

The bone marrows of the experimental animals showed a marked degree of hyperplasia, with an increase in the number of primitive haemocytoblasts.

7873 C

The Influence of Sodium Thioglycollate on the Glycolytic Enzyme System of Muscle Extract.

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It has been suggested by Lipmann¹ that the enzyme (or one component of the enzyme system) of muscle extract which is capable of converting glycogen into lactic acid is reversibly oxidizable and reducible and is active as an enzyme only in its reduced state. This hypothesis was seemingly confirmed by Michaelis and Runnström.² The evidence presented by the latter authors for the reactivation of a muscle extract which had become partially or completely inactive by standing exposed to the air was as follows. If such an extract was treated with a neutralized solution of thioglycollic acid and then subjected to a manometric experiment, with the addition of sodium bicarbonate and a suitable partial pressure of CO₂, a definite and sus-

¹ Lipmann, F., *Biochem. Z.*, 1933, **265**, 133.

² Michaelis, L., and Runnström, J., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 343.

tained positive pressure was recorded on the manometer. If the thioglycollate was replaced by water a much smaller pressure or no pressure was recorded. This evidence was strengthened by the finding of a small but definite increase in lactic acid in the thioglycollate experiment as compared with the control.

Certain difficulties in preparing the mixtures containing thioglycollate for the application of the Friedemann, Cotonio and Shaffer³ method for lactic acid were mentioned in the earlier paper, but it was believed that they had been overcome. We have now to report that this increase in lactic acid cannot be confirmed.

The method used by us is as follows: The contents of a respiration vessel amounting to about 2.5 cc.* is diluted to 20.0 cc. with H₂O, 10.0 cc. of a 20% CuSO₄ solution is added gradually with constant shaking, and 10.0 cc. of a 10% suspension of calcium hydroxide is then added. After thorough mixing the solution should be definitely alkaline to litmus. The volume is made up to 50.0 cc. with water and it is then allowed to stand for 10-18 hours. Upon filtering, from 28.0 to 33.0 cc. of clear filtrate are obtained. This is sufficiently free from protein that it gives no precipitate with sulfosalicylic acid and gives a negative Molisch test for carbohydrate.† This filtrate is used directly for the lactic acid determination. It shows no foaming and remains entirely clear so that the end point of the permanganate addition, the separation of MnO₂, is clearly discernible. The end point of the subsequent iodine titration is as sharp as with a determination on pure lactic acid.

By the use of this method we have found that the positive pressure developed in excess of the control in a manometric experiment with the addition of thioglycollate is not due to, or accompanied by, the formation of lactic acid. This positive pressure, which is readily

³ Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335. Wendel, W. B., *Ibid.*, 1933, **102**, 47.

* This 2.5 cc. contains 1.8 cc. of the muscle extract,⁴ 0.2 cc. of a 5% solution of coenzyme,⁵ sufficient NaHCO₃ to make the final concentration 0.025 m, (a gas mixture of 5% CO₂ and 95% N₂ was used), sufficient glycogen to give a final concentration of 0.2% and the thioglycollate (usually 0.3 cc. of a freshly neutralized 1.0 m. solution of sodium thioglycollate).

⁴ Meyerhof, O., *Biochem. Z.*, 1926, **178**, 395.

⁵ Lohmann, K., *Biochem. Z.*, 1931, **233**, 460.

† This is essentially the method used by Van Slyke⁶ for the removal of sugars. With our material, under these conditions, it also removes the protein. A similar observation has been made and utilized by Elliott and Schroeder.⁷

⁶ Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chem.*, Vol. II, p. 626.

⁷ Elliott, K. A. C., and Schroeder, E. F., *Biochem. J.*, 1934, **28**, 1920.

obtained, is, according to our experience, due to the thioglycollate itself. An equally great, or even greater pressure, which continues for some hours at a decreasing rate, is obtained if the thioglycollate is added to pure sodium bicarbonate (*e. g.* 0.3 cc. of a 1.0 m. solution gives a pressure equal to 275 cmm. in 2 hours at 37°). The rate at which the pressure develops varies considerably with temperature. We have carried out experiments at 20°, 25°, 30°, and 37°. The results differ only quantitatively, being greater at the higher temperatures. This pressure is probably due to the fact that the thioglycollic acid contains one or more anhydrides which are slowly hydrolyzed during the experiment. This is in agreement with the finding that if an approximately 1.0 m. solution of our thioglycollic acid, which had been distilled 3 weeks previously, was refluxed for 2 hours, with volume control to see that no water was lost, it increased its titratable acidity by 14.3% as judged by titrating with NaOH using methyl red as indicator. This solution then developed much less pressure in a manometric experiment.

On the basis of the above findings we believe it is no longer justifiable to speak of a regeneration of this inactivated enzyme system by thioglycollate. We herewith withdraw the statement, and any conclusions based on the statement, that the inactivated glycolytic enzyme system of muscle can be regenerated by thioglycollate.

7874 P

Effect of Certain Agents on Cochlear Effect and Hearing.*

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There has been some question as to whether the Wever and Bray¹ phenomenon will prove to be an accurate tool for the study of hearing in animals. These authors showed that electrical currents could be picked up from the eighth nerve of the cat with a suitable amplifier. With telephone receiver or cathode ray oscillograph such cur-

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¹ Wever, E. G., and Bray, C. W., *J. Exp. Psychol.*, 1930, **5**, 373.