TABLE II.

Aertrycke extract	Precipitin titer. Dilutions of extract 5,000	50,000	500,000	5,000,000	Tested with antibody derived from:
Untreated	+++	++±	+±	±	Rabbit serum
	±*	+	+	tr	Horse "
90 min. in N/15 acid	±	tr	0	0	Rabbit "
	+±	+±	±	0	Horse "

*Zone effect.

Precipitin reactions recorded were made after the tests stood 2 hours at room temperature and over night in the ice box.

view of the characteristic behavior of the acid-treated solutions when tested with antibodies derived from rabbit and horse, namely better precipitin reactions with the anti-*B. paratyphosus B* horse serum (Table II). The assumption that the observations made are due to the appearance of hydrolytic products is further suggested by a similar finding of Heidelberger and Kendall,[6] made with isolated hydrolytic products of the specific soluble substance of Pneumococcus type III, which gave precipitin reactions with specific antibodies obtained only from the horse and not from the rabbit.

The lack of parallelism between the precipitin reaction and phage inhibition may be understood if one assumes that for a precipitin reaction, as a rule, substances of higher molecular weight are required; whereas, specific inhibition reactions in general may be brought about by simpler chemical substances. (Landsteiner.)

Conclusive tests to determine whether the phage inactivating solutions obtained by mild acid treatment inhibit the precipitin reaction with rabbit antibodies, must await studies on the isolation and purification of the active phage inhibiting substances.

7673 C

Inactivation and Regeneration of the Glycolytic Enzyme System of Muscle Extract.

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An aqueous extract of muscle tissue, prepared according to Meyerhof,[1] contains the glycolytic enzyme which produces lactic acid

[6] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

[1] Meyerhof, O., *Biochem. Z.*, 1926, **178**, 395.

from carbohydrates. This enzyme is very labile and loses its activity quickly on standing. Recently Lipmann[2] advanced the idea that this enzyme is easily oxidized, is inactive in its oxidized form, and can be reactivated by reduction. This idea led him to a theory of what has been called the Pasteur reaction. The fact that a cell produces lactic acid as end product when utilizing carbohydrate anaerobically, but much less or no lactic acid under aerobic conditions, has led Meyerhof to assume that lactic acid is also produced aerobically, but resynthesized to glycogen, the energy of the synthesis being furnished by the respiration process. Lipmann, on the other hand, assumes that lactic acid is not produced aerobically, or to a smaller extent, owing to the fact that aerobically the lactic acid enzyme is present in its oxidized inactive state. Though Lipmann's theory is still open to question, one part seems certain, namely that the lactic acid enzyme is easily oxidized and thereby loses its activity. Holmberg[3] tries to explain Lipmann's results on the following basis: When an oxidant is added to the system the enzyme is not oxidized, but the presence of the oxidant prevents the reduction processes necessary for the formation of lactic acid (formation of glycerol phosphoric acid and reduction of pyruvic acid). Thus only the oxidation products such as phospho-glyceric acid can be formed. This interpretation, however, cannot be accepted, because the formation of this acid also would be recorded by the manometric method used by Lipmann.

There is also evidence from Lipmann's experiments that the inactive enzyme might be reactivated by reduction, but this evidence is, as yet, not very strong. He succeeded in restoring the previously oxidized enzyme to a certain extent by ascorbic acid, but the effect is poor. In his experiments the reactivation does not raise the effect of the enzyme to its original value. This original value, however, is very likely smaller than the maximum value that would be obtained if all the enzyme were in the active form. A regenerating agent should at least restore the original activity of the fresh muscle extract and even raise it above this level, since, even in a fresh extract a part of the enzyme might have already undergone inactivation.

This paper shows that the glycolytic enzyme of muscle extract, even after it has become entirely inactive by standing for several days, can be reactivated by thioglycolic acid to an extent exceeding the activity in the freshly prepared extract.

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[2] Lipmann, F., *Biochem. Z.*, 1933, **265**, 133.

[3] Holmberg, C. G., *Skand. Arch. f. Physiol.*, 1934, **68**, 1.

Other sulphydril compounds could have been used, but thioglycolic acid has the great advantage of being rather stable when exposed to the air. The whole system, *viz.*, muscle extract + coenzyme + glycogen + buffer + reductant must be prepared in the microrespiration vessel exposed to the air. The pH of the system being 7 to 8, the conditions for autoxidation of sulphydril compounds such as cysteine are most favorable, and it is difficult to prevent the added reductant from being oxidized before the air in the chamber has been displaced by the N_2-CO_2 mixture. Thioglycolic acid is much more slowly oxidized under these conditions even in presence of a considerable amount of iron, and is therefore preferable as a reductant.

The problem arises as to the nature of the enzyme or at least of the oxidizable and reducible group of the enzyme. Among those substances which inactivate the enzyme is iodoacetic acid. This acid has been shown by Lundsgaard[4] to suppress the formation of lactic acid even in the living animal, and Lipmann finds it inactivates the enzyme contained in the muscle extract. Lipmann ascribes this property of iodoacetic acid to its oxidizing faculty. He simply enumerates this acid as one among the oxidants used. However, iodoacetic acid is no oxidant at all. Von Euler[5] advances the hypothesis that iodoacetic acid might act due to its reaction with copper. Such an interaction, however, can not be demonstrated experimentally. On the other hand, iodoacetic acid exhibits an almost specific reaction to sulphydril compounds.[6] It exhibits another reaction towards amino groups.[7] These reactions consist in substituting the radical CH_2 COOH for H in the SH or in the NH_2 group. In general the reaction with SH groups is faster and goes on in less alkaline solutions than the reaction with NH_2 groups. A quick reaction in an approximately neutral solution may be taken as a fairly strong evidence that a SH group is concerned and may be taken as a full proof if corroborated by other evidence. We have the following facts pertaining to this problem: (1) The enzyme is, according to Lipmann, inactivated by oxidation (quinone, oxygen, suitable dye stuffs). (2) The enzyme is also inactivated by iodoacetic acid. (3) The enzyme inactivated by exposure to air is regenerated by thioglycolic acid.

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[4] Lundsgaard, E., *Biochem. Z.*, 1933, **27**, 1141.

[5] von Euler, H., *Ergebnisse der Enzyrnforschung* 111, Leipzig, 1934, edited by F. Nord and Weidenhagen.

[6] Dickens, F., *Biochem. J.*, 1933, **27**, 1141.

[7] Michaelis, L., and Schubert, M. P., *J. Biol. Chem.*, 1934, **106**, 331.

These facts appear to be fair evidence for the hypothesis that the enzyme is a sulphydril compound, active as an enzyme only in its sulphydril form, but inactive in its disulphide form.

This is another case where the activity of an enzyme is correlated with a sulphydril group which is obviously a constituent of the enzyme itself, and where the oxidation of this group is correlated with the inactivation of the enzyme.

Various enzymes have been known which can be activated by potassium cyanide or hydrogen sulphide [Papain, Kathepsin, Arginase, Urease (Grassmann[8] and Waldschmidt-Leitz[9]).] The mode of action of these activators was sometimes interpreted by the assumption that these substances were to eliminate traces of heavy metal salts which exhibit an inhibitory influence (Krebs[10]). Another interpretation was the assumption that glutathione, in its reduced form has the function of a coenzyme, and that oxidized glutathione is reduced by these agents (Perlzweig[11]). What seems to us the first appropriate interpretation of the nature of the sulphydril group concerned is the one given by Sumner and Poland,[12] who showed that the sulphydril group concerned with the action of urease is not that of glutathione but that the enzyme itself contains a sulphydril group. This idea has been more fully and very convincingly developed by Hellerman, Perkins, and Clark,[13] who gave evidence for the hypothesis that the activity of the enzyme is correlated with a sulphydril group of the enzyme molecule itself. At the same time Bersin and Logemann[14] developed the same idea for Papain. The case of the glycolytic enzyme, as here presented, can be added to that group of enzymes which contain a sulphydril group and are active only when this group is present in its reduced state.

The extract was prepared from guinea pig muscles which immediately after the dissection were put on ice. The muscles were cut up in small pieces and extracted with 1.5 times their weight of water, the extract filtered and some drops of octyl alcohol added to keep it approximately sterile for several days. In some experiments fresh mutton meat was used, with similar results.

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[8] Grassmann, W., Schoenbeck, O., and Eibeler, H., *Z. Physiol. Chem.*, 1930, **194**, 124.

[9] Waldschmidt-Leitz, E., and Purr, A., *Z. Physiol. Chem.*, 1931, **198**, 260.

[10] Krebs, H. A., *Biochem. Z.*, 1930, **220**, 289.

[11] Perlzweig, W. A., *Science*, 1932, **76**, 435.

[12] Sumner, J. B., and Poland, L. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 553.

[13] Hellerman, L. H., Perkins, M. E., and Clark, W. M., *Proc. Nat. Acad. Sc.*, Washington, D. C., 1933, **19**, 855.

[14] Bersin, T., and Logemann, W., *Z. Physiol. Chem.*, 1933, **220**, 209.

The coenzyme used was prepared according to Lohmann.[15] We did not, however, attempt to purify it but used the crude preparation which turned out to have a high content of adenylpyrophosphate and to be very active.

The glycolysis was measured in the Warburg apparatus. 10 cc. of the muscle extract were mixed with 1 cc. of 0.85% $NaHCO_3$ solution, and 2 cc. of this mixture used for each Warburg vessel. In addition 0.2 cc. coenzyme and 0.1 to 0.3 cc. of an approximately molar neutralized solution of thioglycolic acid, or, for the control experiment, an equivalent amount of water, was added. The side arm of the vessel was filled with 0.2 cc. of a 0.2% solution of starch, or, in most experiments, glycogen. In some experiments, a 0.18 molar solution of hexosediphosphate was used as a substrate. The gas space of the apparatus was filled with a mixture of purified nitrogen with 5%, or in most experiments, 10% CO_2 (causing a pH of about 7.8 in the solution). After the first reading the substrate was tipped into the main compartment.

As the manometer readings were used only comparatively, no phosphorylation correction (Meyerhof) was applied. Only a "retention factor", R, amounting to 1.4 has been applied in the tables besides the immediate readings. The experiments recorded here are a few among a great number carried out.

The chemical determination of lactic acid was carried out essentially by the method of Friedemann, Cotonio and Shaffer, with the modification recommended by Wendel.[16] It may be mentioned that in presence of thioglycolic acid, special care should be taken that a sufficient amount of copper sulphate is used at that stage of the procedure, where the deproteinized filtrate is mixed with calcium hydroxide + $CuSO_4$ to eliminate those substances which might interfere with a sharp endpoint of the final iodine titration of the distillate.

A freshly prepared muscle extract is usually active. A mixture of such an extract + glycogen, without coenzyme or still better with coenzyme added, produces acid. On addition of thioglycolic acid this effect is distinctly increased. (Table I.)

TABLE I.

Muscle extract (cc.)	2	2	2	2
Coenzyme (cc.)	0	0.2	0	0.2
Thioglycolic acid (cc.)	0	0	0.3	0.3
Glycogen (cc.)	0.2	0.2	0.2	0.2
CO_2 (cbmm. in 40 min.)	119	126	272	297
R $\times$ CO_2	167	176	380	415

[15] Lohmann, K., *Biochem. Z.*, 1931, **233**, 460.

[16] Wendel, W. B., *J. Biol. Chem.*, 1933, **102**, 47.

The activity of this muscle extract gradually decreases and drops to zero within the first day. On the second or third day, there was never any activity with glycogen as substrate, but the activity could be entirely regenerated by thioglycolic acid (Table II). The activity towards hexose phosphate persisted to a certain extent to the second day but was always increased by addition of thioglycolate (Table III).

Finally it was shown that the acid formed was essentially lactic acid (Table IV).

The amount of lactic acid actually produced is smaller than the acid produced according to calculation from the manometric results. This may be accounted for by the fact that the phosphorylation which causes a shift to the acid side (Meyerhof[1]) is faster than the formation of lactic acid. This is shown by Table V.

TABLE II.

Muscle extract (cc.)	2	2	2	2
Coenzyme (cc.)	0	0.2	0	0.2
Thioglycolic acid (cc.)	0	0	0.3	0.3
Glycogen (cc.)	0.2	0.2	0.2	0.2
CO_2 (cbmm. in 60 min.)	2	17	159	204
R × CO_2	3	24	223	286

TABLE III.

Muscle extract (cc.)	2	2	2	2
Coenzyme (cc.)	0	0.2	0	0.2
Thioglycolic acid	0	0	0.2	0.2
Glycogen (cc.)	0.2	0.2	0	0
Hexosediphosphate (cc.)	0	0	0.2	0.2
CO_2 (cbmm. in 60 min.)	0	4	62.5	129.5
R × CO_2	0	6	87.5	182

TABLE IV.

Muscle extract (cc.)	10	10	10	10
Glycogen (cc.)	1	1	1	1
Coenzyme (cc.)	1	1	1	1
Thioglycolic acid (cc.)	0	0	1	1
Duration of exp. (min.)	0	105	0	105
Lactic acid (mg.)	3.5	4.2	3.4	5.2
Increase of lactic acid, %		20		53

TABLE V.

Muscle extract (cc.)	2	2	2	2
Glycogen (cc.)	0.2	0.2	0	0
Hexosediphosphate (cc.)	0	0	0.2	0.2
Coenzyme (cc.)	0.2	0.2	0.2	0.2
Thioglycolic acid (cc.)	0.3	0.3	0.3	0.3
Duration of exp. (min.)	0	120	0	120
Mg. inorganic P/1cc. extract	0.257	0.068	0.247	0.318

In the course of the experiment with glycogen as substrate almost 75% of the inorganic phosphate initially present has been esterified. Table V shows also that the content of inorganic phosphate increases, when hexose phosphate is used as substrate, in accordance with expectation.

Summary. The glycolytic enzyme of muscle extract after becoming inactive on standing can be reactivated by thioglycolic acid to a higher state of activity than that originally present. Lipmann's hypothesis of the enzyme being inactivated by oxidation and reactivated by reduction is corroborated and specified by the evidence that this oxidation-reduction is concerned with a sulphydril group of the enzyme. There is evidence for the assumption that glycolytic enzyme of muscle seems to be a sulphydril compound which loses its enzymatic property by oxidation to the disulphide state and is reactivated by reduction to the sulphydril state.

7674 P

Pharmacological Studies on the Blood of Trachoma.

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The writer has reported his phytopharmacological studies on blood sera from various clinical conditions and described characteristic phytotoxic reactions produced by such specimens from certain diseases. These reactions were found useful in establishing a differential diagnosis and also in evaluating the results of therapeutic procedures.[1] Such specific phytotoxic reactions were particularly noted in pernicious anemia,[2] leprosy,[3] and pemphigus.[4, 5] This method of investigation has been repeatedly employed by Professor Leon Tscherkes of Odessa, who has confirmed Macht's findings. Some time ago, in a personal communication to the author, Professor Tscherkes wrote that he had discovered that blood sera from still another pathological condition exhibited a marked phytotoxic property for the seedlings of *Lupinus albus*—namely, trachoma. Since

[1] Macht, *Science*, 1930, **71**, 302.

[2] Macht and Anderson, *J. Pharmacol. and Exp. Therap.*, 1928, **34**, 365.

[3] Macht, *Acta Dermat.*, 1932, **18**, 126.

[4] Pels and Macht, *Arch. Dermat. and Syph.*, 1929, **19**, 640.

[5] Pels and Macht, *Arch. Dermat. and Syph.*, 1931, **23**, 601.