RECENT INVESTIGATIONS ON THE AEROBIC AND ANAEROBIC METABOLISM OF CARBOHYDRATES.

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I believe it would be in accordance with the wishes of our mourned colleague if I developed here the idea set forth in my book Chemical dynamics of life phænomena, and add to it from more recent work. At the wish of Jacques Loeb, this book was published as one of his series of monographs (1). These studies confirm the unity of chemical processes throughout the organic world, a unity which Loeb, in his pioneer researches, brought always afresh to light. The American reader will, at the same time, be told the state of research in our laboratory. This will give him the opportunity to take into account both the work already published on this subject, and that which is still under way. All these investigations are connected with the problem of energy transformation in muscle, which, to begin with, we should review briefly.

I.

The chemical processes which supply the energy for muscular work fall in two sharply delimited phases. The first is anaerobic, and consists of the cleavage of the glycogen present in the muscle to lactic acid. This phase coincides with the work yielded. The second phase, however, requires oxygen. The lactic acid disappears during oxidation, although it is not completely burned, but, chiefly at least, is reconverted; while (on the basis of experiments on excised cold-blooded muscle) only a certain proportion or carbohydrate equivalent is oxidized. The ratio: $\frac{\text{Total loss of lactic acid}}{\text{Oxidized lactic acid equivalent}}$ which represents the "oxidation quotient of lactic acid," is usually 4–5, and varies in

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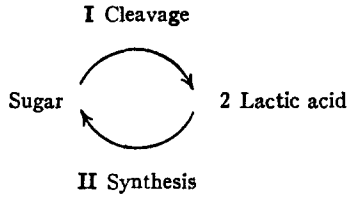
general only between 3 and 6. This result, which follows from chemical, calorimetric, and manometric experiments, also affords a quantitative explanation for the course of heat-change during muscle contraction, analyzed by Hill and Hartree (2). Although this explanation is in good correspondence with all exact data relating to muscle activity, it has quite recently become the subject of controversy.

(*a*). Embden (3) thought he had observed that the lactic acid formation in the anaerobic phase was without time relationship to muscle contraction, because the former set in to a considerable degree first after relaxation, and consequently was not to be regarded as the direct source of energy for the mechanical work. Repetition showed (4, 5) that such secondary formation of lactic acid took place only after excessive direct stimulation of the muscle, and was connected with the after-contracture to be seen in this case. With stimulation within physiological limits, especially with nervous stimulation, lactic acid formation is coincident with mechanical change. Besides, a comparison of the lactic acid formation with the work yielded shows that the lactic acid formed after excessive stimulation is unavailable as a source of mechanical energy. Thus it may be stated with even more certainty than heretofore that the initial heat developed at the time of contraction, measured by Hill and Hartree, is due to the formation of lactic acid from glycogen, and the accompanying removal of alkali from the proteins. This removal results in a "heat of deionization." New evidence is also furnished for the deionization of proteins during muscle fatigue. With increasing muscular exhaustion, the alcohol-soluble basic ash (calculated against potassium) increases proportionately to the lactic acid formation, while the alcohol-insoluble base decreases to the same extent (or to about 2 per cent of the dry weight of the muscle). Fully half of this base which becomes alcohol-soluble was previously, in the unfatigued muscle, linked with protein (5). In fatigue, the protein has surrendered this alkali to the lactic acid.

(*b*). The significance of the theory of the process of oxidative restitution has been to a certain degree misunderstood. It was not meant that the burning of part of the lactic acid was requisite as the exclusive source of energy for resynthesis of the remaining lactic acid (6). As a matter of fact, analysis of the muscle after restitution shows that one carbohydrate equivalent is wanting, corresponding to the oxygen

consumption. Whether lactic acid or carbohydrate is here oxidized one cannot tell. At any rate, the preferable picture is that which assumes a closed circle for the lactic acid:



while the cleavage phase I takes place anaerobically and spontaneously, in phase II all the lactic acid is reconverted to sugar, that is to glycogen, by means of energy furnished by the accompanying oxidation of carbohydrate. It has not yet been experimentally shown, that other foodstuffs such as fats and proteins may furnish directly the energy for the resynthesis in isolated muscle, but this is possible.[1] In the entire organism, such a connection with the burning of fat could be achieved through the resynthesis of lactic acid in the liver and other organs. Doubtless in various organs the reconversion of lactic acid can be associated with various substances.

The splitting of sugar to lactic acid and the oxidation are two distinct processes, and the formation of lactic acid is not to be regarded as the introductory stage in carbohydrate combustion. An observation of R. Loebel (7), gives an especially clear example of this: Cerebral cortex oxidizes fructose as easily as glucose. Under anaerobic conditions, however, the cortex forms lactic acid only from glucose, and furthermore it forms about three times as much as the oxygen consumed in respiration would be able to oxidize. From fructose, on the contrary, no lactic acid is formed; hence the latter cannot be an intermediate stage in the oxidation of fructose.

[1] The other alternative, namely that fat supplying energy for muscular work may first be converted to carbohydrate, is touched upon in the work of Furusawa (*Proc. Roy. Soc. London, Series B*, 1925, xcviii, 65). In very short periods of activity, the respiratory quotient of the human body is 1,0, and it is the same during fat consumption. Attention should also be called to the observations of Calvo-Criado (with Asher, *Biochem. Z.*, 1925, clxiv, 76), according to which a conversion of fat to carbohydrate is possible in warm-blooded animals under certain conditions.

II.

But there is in muscle a further relationship between the lactic acid formed during activity and the oxidation process of restitution. This is seen in the closely coordinated rise in resting respiration during recovery. This rise causes the lactic acid formed during activity to disappear, by synthesis to glycogen. The cause of this rise in respiration is nothing else but the appearing of the lactate itself. For if one places resting muscle in Ringer solution containing lactate, the respiration rises just as during restitution. Here the lactic acid added from without is also built up to glycogen. In this process the proportion of synthesized lactic acid to consumed oxygen is equal to that during recovery (oxidation quotient 4–5 (8)). Thus living liver tissue also shows, on addition of lactate, the ability to synthesize glycogen, together with a rise in respiration similar to that seen in muscle (9). That this rise in respiration during restitution is really due to the lactate ion, and not the hydrogen ion, the concentration of which rises during fatigue, is shown by the fact that raising only the hydrogen ion concentration even reduces respiration. The synthesis of glycogen from externally added lactate by resting muscle as well as by liver is of importance in the recovery of the entire organism after the activity of isolated muscle groups. Lactic acid given up to the bloodstream is synthesized to glycogen in resting parts of the body (10).

At the same time this mechanism permits the determination of what substances added to the muscle from without may be built up into carbohydrates. While amino acids are without effect, *one* substance, namely pyruvic acid, can completely replace lactic acid. By reduction this is synthesized to glycogen, with a corresponding rise in respiration. Here also, a comparison of the increase in carbohydrate with the extra oxygen intake shows that for the oxidation of one sugar molecule about four molecules of sugar are synthesized from pyruvic acid.

III.

The closed cycle of lactic acid is not limited to muscle. O. Warburg and his coworkers found that various mammalian tissues in Ringer solution containing sugar formed lactic acid at a certain rate under anaerobic conditions ("glycolysis") but that in the presence of

oxygen this lactic acid formation ceased or became much slower (11). Here also, the oxygen causes more lactic acid to disappear than it could oxidize, namely three to six times as much. The "oxidation quotient" is the same. Note that the ratio of the absolute value of cleavage and respiration vary in different tissues, and whether lactic acid formation disappears completely, or whether a certain part remains, depends on the ratio of respiration velocity to glycolysis velocity. According to Warburg, remaining of glycolysis in oxygen is certainly the case with malignant tumors, which can live with the lactic acid fermentation as energy source in presence and in absence of O_2. The question is whether also in other glycolyzing tissues the effect of oxygen is to be attributed to a true lactic acid cycle as in muscle, inasmuch as here also three to six times as many lactic acid molecules are formed as could be oxidized. The following shows the probability that this is the case. All glycolyzing tissues are able to use lactic acid so that in its metabolism the respiration is either maintained as in the presence of glucose, or, in most cases, rises above that in sugar solution (9). Thus an oxidative consumption of lactic acid takes place; and in those cells which may store carbohydrates, in muscle, liver, and also in yeast cells, this is connected with a measurable synthesis of carbohydrate.

IV.

The relationships here described are by no means restricted to the tissue metabolism of the higher animals. We know that Pasteur described fermentation as a *"vie sans air,"* and assumed that it replaced respiration as an energy source in the absence of oxygen, while in the presence of oxygen the oxidation as alternative process took place. Until recently this doctrine has been disputed, principally because alcoholic fermentation by yeast proceeds also in oxygen, apparently with the same intensity, so that the energy rôle of fermentation in the presence of oxygen would be quite inexplicable. Recent experiments show, however, that the Pasteur theory is correct in principle, and that the same relationships exist in alcoholic fermentation as in the glycolytic cleavage in muscle and other tissues of higher animals, discussed above. For not only does the metabolism of nearly obligatory anaerobic lactic acid bacteria, and other lactic acid-forming

organisms show the same reaction to oxygen, indicated by their oxidation quotients (12), but alcoholic fermentation of yeast shows it as well (13). The fact that the brewer's yeast generally used for these experiments respires exceptionally slowly in proportion to its fermentation has hindered, until recently, the elucidation of these relationships. But respiration differs among different kinds of yeasts; and this depends on the great variety of sugar effects on the respiration. In sugar-free solutions, the respiration of brewer's yeast and baker's yeast is almost the same; in sugar, on the other hand, the respiration of the former increases only slightly, perhaps doubles, while with baker's yeast it rises to ten times the previous value. In oxygen brewer's yeast ferments about fifty molecules of sugar for each one oxidized, baker's yeast about three or four for each one oxidized. *But the effect of oxygen is the same on all kinds of yeast.* Respired oxygen causes a decrease in fermentation to the extent of about one sugar molecule per mol of consumed oxygen (corresponding to $\frac{1}{6}$ of a molecule of oxidized sugar). Hence the oxidation of one molecule of sugar will interfere with the fermentation of about six. The oxidation quotient: $\frac{\text{decrease in cleavage per mol}}{\text{oxidation per mol}}$ is precisely the same as in the case of lactic acid formation. The effect of oxygen on baker's yeast is so great, on account of its rapid respiration, that fermentation falls to $\frac{1}{3}$ or $\frac{1}{4}$. That respiration, not the presence of oxygen, is here the deciding factor is shown by the fact that fermentation increases to several times its former value either after the removal of oxygen or after the addition of prussic acid (N/1000) in the presence of oxygen. N/1000 prussic acid practically stops respiration, while on fermentation (measured in nitrogen) it has very little effect. This checking of respiration causes fermentation to increase, even in oxygen. The wild yeasts, such as Torula, give the key to the type of metabolism in yeasts. In Torula, respiration is considerably greater than in baker's yeast, fermentation about the same. Here the respiration stops fermentation completely in oxygen, and with approximately the same oxidation quotient, as found in culture yeasts. This behavior corresponds exactly to the respiration of resting muscle, or, on the basis of O. Warburg's work, to the behavior of embryonic tissue. A large anaerobic cleavage metabolism will be completely

stopped by rapid respiration in oxygen. The erstwhile inexplicable behavior of domestic yeast is merely the result of cultivation, which brings about a decrease in respiration, fermentation remaining constant. Hence the persistence of fermentation in oxygen, the most extreme case of which is seen in brewer's yeast.

It can be shown that in alcoholic fermentation as well the disappearance of fermentation is connected with a true carbohydrate cycle. The most important cleavage products, just as the substances formed from them by dismutation, methyl glyoxal, lactic acid, pyruvic acid, acetaldehyde, alcohol, and acetic acid, affect respiration as does sugar: they multiply the respiration of baker's yeast and wild yeast eight to ten times, but leave the respiration of brewer's yeast almost unaffected. It can be shown that this sugar-like effect is connected with the building up of these materials to carbohydrates. During the absorption of the above substances while respiration is going on, carbohydrates are assimilated, as Fürth and his students (14) have already shown with some of them. However, the simultaneous disappearance of alcohol and CO_2 of fermentation under the influence of respiration, shows that the cycle runs through a tricarbon-product, as with the lactic acid cleavage of sugar; that is, either lactic acid itself, or pyruvic acid, as in muscle. Therefore, the cycle is the same throughout the organic world. Respiration serves throughout to conserve material; for it reconverts, at the expense of energy, the major part of the intermediate product formed anaerobically to supply the needs of vital functions, only a relatively small part being used up in this process. The conversion of sugar to lactic acid sets free the same amount of energy as does alcoholic fermentation, as follows from the heat of combustion (15); and if pyruvic acid is assumed the intermediate product, the same is true *mutatis mutandis*, for the difference between the heats of combustion of lactic and pyruvic acids is precisely compensated by the corresponding difference between alcohol and acetaldehyde. Thus in every case all the energy of fermentation available may be utilized during the cleavage phase of the carbohydrate cycle.

V.

The circumstance that lactic acid added to the cells from without can be built up just as that formed in metabolism makes it possible

to decide whether *d*-lactic acid, which the higher animals form exclusively, differs in action from its optical isomer. A single experiment along this line has already been carried out by Dakin and Dudley (16). They found in one case that the administration of *l*-lactate to a phlorhizin-diabetic dog caused a considerable elimination of excess sugar. However, our experiments (17) show that noticeable quantitative differences exist between the actions of *d*- and *l*-lactic acid. Although, to be sure, the rise in yeast respiration brought about by the two optical isomers differs only by ten to twenty per cent, and the corresponding carbon assimilation shows no measurable variation, yet in cold-blooded muscle and various mammalian tissues which consume lactic acid, the difference is considerable. The velocity of lactate consumption and sugar formation in intact frog muscle is about $\frac{1}{4}$ as great with *l*-lactate as with *d*-lactate, or with racemic lactate. (Between the last two, of course, there is no difference to be found at higher concentrations.) The rise in respiration behaves similarly. With *l*-lactate it often falls within the limits of variation of the control. Still more divergent are the effects of the two acids on various mammalian tissues. In liver, the rise in respiration caused by *d*-lactic acid is not found at all with *l*-lactic acid, and with the latter no true increase in carbohydrate content can be demonstrated. The loss of carbohydrate is only somewhat slower than in the control. Nor is the lactate consumption measurable; hence it is at most $\frac{1}{10}$ as great as in the case of *d*-lactate. Cerebral cortex behaves similarly, for *l*-lactate is not measurably consumed, nor can it maintain the normal respiration rate as can glucose or racemic lactate. Only in kidney tissue does *l*-lactate cause a slight rise in respiration, and is gradually used up. Thus not only in muscle and liver is the ability to convert lactic acid dependent on its optical configuration; the same holds true for all organs of warm-blooded animals so far tested, at least in respect to velocity. Here again, the rule that animal tissues much prefer one optical isomer to another is unbroken.

VI.

Up to this point the discussion has centered around the bearing of oxidation on the cleavage of lactic acid to sugar, through which the latter process is reversed. The cleavage itself is interesting especially

on account of the mechanism, and the way in which lactic acid is formed from carbohydrate. The author showed earlier 1. that in both rest and activity of intact muscle as well as in anaerobiosis of cut muscle, glycogen disappears to the same extent that lactic acid is formed, 2. that minced muscle in phosphate solution is able, after splitting the preformed glycogen already present, to split externally added glycogen as well as hexoses such as glucose and fructose, and finally also hexosediphosphoric acid, to lactic acid. Embden (18) found longer ago that muscle press juice, which was without effect on externally added glucose and glycogen, formed lactic acid from the hexosediphosphoric acid of alcoholic fermentation, discovered by Harden and Young. Hence he regarded this sugar-phosphoric-acid ester, which he could demonstrate in muscle, as the precursor of lactic acid. It was further found by Laquer (19) that minced frog muscle showed great differences in effect on different carbohydrates, when the lactic acid formation was studied at high temperature (45°), or when the structure of the muscle mass was destroyed by repeated freezing in liquid air. In both cases the ability to form lactic acid from glycogen and hexosephosphate remained, while all simple sugars, including glucose, were no longer attacked.

It is easy (20) to separate completely the lactic acid ferment from frog or rabbit muscle, and to obtain it in aqueous solution free of carbohydrate. Indeed, following a method of Buchner, it is possible to obtain a dry enzyme preparation by precipitation with acetone, which when redissolved shows considerable activity. The ferment solution extracted from the muscle shows, on addition of glycogen, for several hours an activity about as great as the minced muscle at the same temperature, when calculated against muscle weight. But when calculated against the dry weight of the extract it is at least five times as great as in cut muscle. The coenzyme, which is dialyzable and stable to boiling, can be separated from the ferment mixture as earlier described (21). Now it has been found that the water-soluble lactic acid ferment splits hexoses only under special conditions, but it splits easily starch as well as glycogen, the starch components amylose and amylopectin, and their simple building stones trihexosan (Pictet, Pringsheim (22)) and dihexosan (Pringsheim (22)). Indeed, it splits all these compounds with about the same velocity. Besides these, the hexosediphosphoric

acid from yeast, the hexosemonophosphoric acid prepared by Neuberg (23), and the hexosemonophosphoric acid of Robison (24), are split to lactic acid, though in general more slowly.

The enzyme which splits hexosephosphoric acid is easy to separate from the ferment complex. Brief heating to 37° destroys the capacity of the extract to split glycogen and other polysaccharides, scarcely changing its capacity to split hexosephosphoric acid to lactic acid. Similarly, a separation is possible by removal of the coferment. The coferment-free enzyme solution still splits hexosephosphoric acid, but not glycogen or starch. For the latter, boiled extract is necessary. (Because it is itself free of carbohydrates, it shows no activity until after the addition of starch or glycogen.)[2] This varying behavior of glycogen and hexosephosphoric acid depends (as can be shown by direct experiment) on the fact that removal of the coferment, just as brief heating to 38°, stops the ability of the extract to esterify the cleavage products of polysaccharides with inorganic phosphate. Thus the earlier suggestion (25), which has in the meantime received support from H. von Euler (26) as well as Gottschalk and Neuberg (27), that the primary point of attack of the common coferment of anaerobic and aerobic carbohydrate cleavage should be sought in the esterification of sugar and phosphoric acid, is now a certainty. For, as I have suggested earlier, in my stoichiometric formulation of carbohydrate decomposition, made on the basis of Embden's lactacidogen hypothesis, the labile hexose formed from the cleavage of glycogen must first be esterified with phosphoric acid, before splitting can take place. The experiments at present under way on the mechanism of lactic acid formation in the presence of dissolved enzyme furnish considerable evidence for Embden's theory. One example may be given: if hexosediphosphoric acid is added to the carbohydrate-free enzyme solution,

[2] An earlier observation, according to which minced muscle tissue extracted with water was no longer able to split hexosephosphoric acid, should not be explained by lack of coferment, but to some other injury of the ferment. The above experiment shows further that there is present in the boiled extract a true coferment, at least for glycolysis, not, as the Hopkins school conceived, a mere mixture of nutrient substrates. For the carbohydrate-free boiled extract does not alone cause any lactic acid formation with the enzyme, but only after the addition of starch or glycogen.

lactic acid formation sets in at once, with an equivalent splitting off of inorganic phosphate (2 mols of lactic acid for 2 mols of phosphoric acid). If, under the same conditions, glycogen or starch is added, sometimes a good 2 minutes must pass before the beginning of lactic acid formation. During this time a certain amount of inorganic phosphate is esterified, and this esterification continues as time goes on, while the lactic acid formation sets in with increasing velocity. The formation of a phosphoric acid ester is necessary before lactic acid can be formed from polysaccharides. Furthermore, the quantity of the ester increases generally during lactic acid formation. It is here assumed that the velocity of esterification exceeds that of cleavage to lactic acid as a rule, so that the intermediate product or its stabilized form accumulates. Experience up to now shows that all influences which interfere with the formation of lactic acid from glycogen, also destroy the faculty to esterify the inorganic phosphate. Whether the same relationship holds for glycolysis in other organs, is at present undecided, because a similar separation of their lactic acid ferments has not yet been effected.

BIBLIOGRAPHY.

1. Meyerhof, O., Chemical dynamics of life phænomena, Monographs on experimental biology, J. B. Lippincott Company, Philadelphia and London, 1924, contains the literature of the subject until the end of 1923.
2. Hill, A. V., Summary in *Physiol. Rev.*, 1922, ii, 310.
3. Embden, G., *Ber. ges. Physiol.*, 1925, xxxii, 690; *Klin. Woch.*, 1924, iii, 1393. Embden, G., and Jost, H., *Deutsch. med. Woch.*, 1925, li, 636; in Handbuch der Physiologie, Berlin, Springer, 1925, viii, 401.
4. Meyerhof, O., and Lohmann, K., *Arch. ges. Physiol.*, 1925, ccx, 790.
5. Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1926, clxviii, 128. Suranyi, T., *Arch. ges. Physiol.*, 1926, ccxiv, 228.
6. Lusk, G., *Biochem. Z.*, 1925, clvi, 334. Meyerhof, O., *Biochem. Z.*, 1925, clviii, 218.
7. Loebel, R. O., *Biochem. Z.*, 1925, clxi, 219.
8. Meyerhof, O., Lohmann, K., and Meier, R., *Biochem. Z.*, 1925, clvii, 459.
9. Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1926, clxxi, 381. Takane, R., *Biochem. Z.*, 1926, clxxi, 403.
10. Barr, D. P., and Himwich, H. E., *J. Biol. Chem.*, 1923, lv, 525. Long, *Proc. Roy. Soc. London, Series B*, 1926, xcix, 167.
11. Warburg, O., Posener, K., and Negelein, E., *Biochem. Z.*, 1924, clii, 309.
12. Meyerhof, O., and Finkle, P., *Chem. Zelle u. Gewebe*, 1925, xii, 157.
13. Meyerhof, O., *Biochem.* Z., 1925, clxii, 43.

14. Fürth, O., and Lieben, F., *Biochem. Z.*, 1922, cxxviii, 144. Lieben, F., *Biochem. Z.*, 1923, cxxxv, 240. Lundin, H., *Biochem. Z.*, 1923, cxli, 310; 1923, cxlii, 454.
15. Meyerhof, O., *Biochem. Z.*, 1922, cxxix, 594. Meier, R., and Meyerhof, O., *Biochem. Z.*, 1924, cl, 233. Blaschko, H., *Biochem. Z.*, 1925, clviii, 428.
16. Dakin, H. D., and Dudley, H. W., *J. Biol. Chem.*, 1913, xv, 143.
17. Meyerhof, O., and Lohmann, K., *Biochem. Z.*, 1926, clxxi, 421.
18. Embden, G., Kalberlah, F., and Engel, H., *Biochem. Z.*, 1912, xlv, 45.
19. Laquer, F., *Z. physiol. Chem.*, 1922, cxxii, 26.
20. Meyerhof, O., Preliminary notes, *Naturwissenschaften*, 1926, xiv, 196, 756.
21. Meyerhof, O., *Z. physiol. Chem.*, 1918, ci, 165; 1918, cii, 1; *Arch. ges. Physiol.*, 1921, clxxxviii, 148.
22. Pringsheim, H., *Naturwissenschaften*, 1925, xiii, 1084; *Ber. chem. Ges.*, 1924, lvii, 1581.
23. Neuberg, C., *Biochem. Z.*, 1918, lxxxviii, 432.
24. Robison, R., *Biochem. J.*, 1922, xvi, 809; 1923, xvii, 286.
25. Meyerhof, O., *Arch. ges. Physiol.*, 1919, clxxv, 81.
26. von Euler, H., and Myrbäck, K., *Z. physiol. Chem.*, 1924, cxxxix, 15; *Ber. chem. Ges.*, 1924, lvii, 1073.
27. Gottschalk, A., and Neuberg, C., *Biochem. Z.*, 1924, cliv, 492.