THE HEAT OF COMBUSTION OF GLYCOGEN IN RELATION TO MUSCULAR CONTRACTION.

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HARTREE and HILL(1) in a recent paper have given a full analysis of the heat evolved in the various stages of isometric muscular contraction, and applying Meyerhof's(2) figure of 370 cals. per gm. of lactic acid formed under anaerobic conditions were able to give absolute values for the different phases of activity. The following figures are pertinent to the investigation described below.

Total anaerobic heat	370	cals. per gm.	of lactic acid
Total initial heat	296	,,	,,
Delayed anaerobic heat	74	,,	,,

It is of interest to compare these figures with those calculated from the heats of combustion of glycogen and lactic acid, and the heat of neutralisation of lactic acid by salts such as phosphates and carbonates or by alkali-protein[2].

If the accepted chemical view be correct, it should be possible to account for the initial and delayed anaerobic heats with considerable accuracy by the heat changes taking place in the decomposition of glycogen and the neutralisation of the resulting lactic acid.

For the purposes of the calculation there are available:

(*a*) the heat of combustion of lactic acid in dilute solution, determined by Meyerhof(3) to be 3601 cals. per gm.;

(*b*) the heats of neutralisation of lactic acid by salts and alkali-protein, shown by the same worker(3) to be 19 cals. and 138 cals. per gm. respectively; and

(*c*) the heat of combustion of glycogen for the solid anhydrous material, which had been determined by Stohmann and Schmidt(4) who obtained the value 4190 cals. per gm.

Since 0·9 gm. of anhydrous glycogen yields 1 gm. of lactic acid, the

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[1] Working for the Medical Research Council.

[2] The term alkali-protein is used throughout the paper to signify the sodium and potassium salts of protein.

heat of combustion of the glycogen forming 1 gm. should be 0·9 × 4190 cals., *i.e.* 3771 cals. As the resulting gram of lactic acid has a heat of combustion of 3601 cals. the heat liberated in its formation from glycogen could only have a maximum value of 170 cals. That this value is a maximum must follow unless the hydration and solution of glycogen are endothermic processes; from analogy, however, it is probable that the value would actually be too large, since in similar substances the hydration and solution are definitely exothermic.

If it be assumed that the lactic acid is neutralised immediately on formation by the alkali-protein, a value is obtained of (170 + 138), *i.e.* 308 cals. for each 1 gm. of lactic acid formed. This value would explain the initial heat, but would leave the delayed anaerobic heat entirely unaccounted for. It is necessary, therefore, if the figures on which the calculation is based be assumed to be correct, to postulate some further process so far unknown, which takes place in anaerobically contracting muscle at a comparatively slow rate for some time after the formation and neutralisation of the lactic acid. It is difficult in view of the chemical evidence to imagine what form such a reaction would take.

It was suggested to the author by Mr A. D. Ritchie that an investigation of the heat of wetting and solution of dry glycogen might throw some light on this discrepancy. The work was undertaken, and ultimately developed into a redetermination of the heat of combustion of glycogen. Those details of the investigation which are purely chemical will be given in another place, after some further points of interest in connection with the chemistry of glycogen have been elucidated. The present outline is intended merely to indicate the points of physiological interest.

The glycogen was prepared from sea water mussels (*Mytilus edulis*) which provide an easily purified and plentiful source of the material. The purification process consisted of heating on a water bath with 60 p.c. potash, and repeated precipitation with alcohol. The final product was ash free, fat free, and contained only minute traces of nitrogen. When the product was dried in an air oven at 110° C., a method generally adopted by previous workers, it was found impossible to reach a stage where the preparation ceased to lose weight. As an alternative, the glycogen wet with water and alcohol was dried by distilling off the constant boiling mixtures of alcohol-benzene-water and alcohol-benzene or water-benzene as the case might be, after the manner described by Atkins and Wilson(5). The resulting product after the benzene had been removed in a current of air, was an amorphous white solid stable in air, which on ultimate analysis proved to be the hydrated form $(C_6H_{10}O_5.H_2O)_n$ and not the anhydrous substance $(C_6H_{10}O_5)_n$.

Considerable interest attached to the further drying of the hydrate. When it was dried over calcium chloride *in vacuo*, the half hydrate $(C_6H_{10}O_5.\frac{1}{2}H_2O)_n$ resulted. A similar observation was made over fifty years ago by Bizio(6). Drying in an air oven at 110° C. does not give complete dehydration. After ten days' continuous drying the glycogen still contained 5 p.c. of its water of hydration, and the rate of drying had become almost negligible. The product was hygroscopic, picking up water rapidly on exposure to air. These observations are in agreement with those made by Harden and Young(7), who showed that only by heating to 100° C. over phosphorus pentoxide *in vacuo* could glycogen be rendered completely anhydrous.

As previous workers had prepared "anhydrous" glycogen by drying in an air oven, it seemed probable that their figures for the heat of combustion would be too low, owing to the unsuspected presence of water in the purified glycogen used. It was decided, therefore, to repeat the determination of the heat of combustion, in addition to the determination of the heat of wetting and solution. The total heat of wetting and solution of the hydrated glycogen was determined, the average value obtained in three experiments, which agreed within 1·5 cals., being 9 cals. per gm. For the heat of combustion a value of 3883 cals. per gm. as the average of five concordant experiments was obtained. Hence the value for the heat of combustion of hydrated glycogen in solution is 3874 cals. per gm. This is about 100 cals. higher than that deduced from the determination of Stohmann and Schmidt.

Employing Meyerhof's value for the heat of combustion of lactic acid the heat liberated on the conversion of 1 gm. of glycogen to 1 gm. of lactic acid works out at (3874–3601), *i.e.* 273 cals. If it be assumed that the neutralisation of lactic acid on formation is carried out entirely by salts, then the heat liberated during contraction and relaxation would be (273 + 19), *i.e.* 292 cals. per gm. of lactic acid formed. This figure is in remarkable agreement with the total initial heat as determined by Hartree and Hill, viz. 296 cals.

Meyerhof(2) and Hartree and Hill(1) have shown that salt buffering alone is inadequate to deal with the large amounts of lactic acid formed during continued muscular activity. It would seem reasonable therefore to assume that the immediate salt buffering is only temporary in character, and replaced by the more efficient and more thermostable buffering by alkali-protein as the opportunity offers. That it is this change over from one type of buffer to the other, which is the source of the delayed anaerobic heat, offers a simple explanation for this phenomenon. The complete change would produce a further 119 cals., so that, with only 74 cals. to account for, the reaction need not be assumed to proceed to completion, but only to the extent of about 60 p.c. The change is not likely to involve the lactic acid further, but to consist of a transfer of alkali from protein to acid phosphate and carbonic acid.

Hartree and Hill have further shown, that the velocity of the reaction responsible for the delayed anaerobic heat reaches a maximum about 2½ minutes from its commencement, and is independent of the time of stimulus and temperature. If the reaction responsible for the delayed anaerobic heat were purely chemical in character, the independence of its velocity of time of stimulus and temperature would be difficult to explain; this difference is typical rather of a physical process. The difficulty, however, is easily overcome if it be imagined that the controlling factor in the velocity of reaction is the physical one of the rate of diffusion of the acid phosphate and carbonic acid ions through the muscle tissue, until they come in contact with an alkali-protein molecule.

Such a theory seems to demand some special distribution of phosphate and carbonate molecules in the muscle substance. We may imagine the small carbonic acid and phosphate molecules as carriers, going out from the neighbourhood of the immediate contractile mechanism, where they were initially situated, and reacting with the general muscle tissue by combining with alkali again, depositing their acid character, so to speak, on the heavy, less mobile, and less acid protein molecules. Such a reaction would reach a maximum velocity after a short period of time. The concentrated layer of molecules of acid salts existing immediately after relaxation would have at first no alkali protein within range with which to react, but as they diffused apart, each would have a greater opportunity of meeting the required type of protein molecule. The following scheme may make the hypothesis clearer:

Contraction. (*a*) Glycogen → lactic acid; (*b*) lactic acid and contractile mechanism produce mechanical response.

Relaxation. (*a*) Lactic acid + $\left\{\begin{matrix}\mathrm{Na_2HPO_4}\\ \mathrm{NaHCO_3}\end{matrix}\right. \rightarrow$ Sodium lactate + $\left\{\begin{matrix}\mathrm{NaH_2PO_4}\\ \mathrm{H_2CO_3}\end{matrix}\right.$.

Anaerobic recovery. (*a*) $\left\{\begin{matrix}\mathrm{NaH_2PO_4} + \text{Na protein}\\ \mathrm{H_2CO_3}\end{matrix}\right.$

$$\rightarrow \left\{\begin{matrix}\mathrm{Na_2HPO_4} + \text{H protein.}\\ \mathrm{NaHCO_3}\end{matrix}\right.$$

SUMMARY.

1. A redetermination has been made of the heat of combustion of glycogen in dilute solution, and the value, 3874 cals. per gm., obtained for the hydrated material $(C_6H_{10}O_5 . H_2O)_n$. This value is about 100 cals. higher than that previously accepted.

2. It is possible, using this new figure, to explain the actual heat measurements on muscle entirely on chemical grounds, by assuming that the lactic acid formed from glycogen is first neutralised locally by alkaline salts, and that then more slowly these salts are restored at the expense of the alkali-protein of the general muscle tissue.

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