O T T O F . M E Y E R H O F

Energy conversions in muscle

Nobel Lecture, December 12, 1923

This highest scientific honour, in the form of the Nobel Prize, which has been awarded to me for my investigations into the conversions of energy in muscle, gives me the pleasant duty of reporting to you on this problem and upon the results which my work has achieved. It is especially gratifying to me that this recognition is in part shared with me by my distinguished friend, the previous speaker, Professor A.V. Hill from London, with whom my work has had so many close points of contact and with whom, in spite of the present political unrest, I have worked in cooperation towards the mutual goal of explaining the process of muscle contraction.
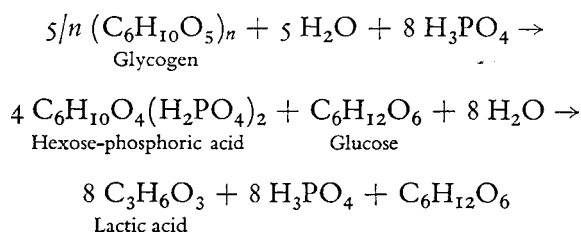
The fact that chemical processes must be involved as a source of energy for muscle performance was already accepted as a necessary deduction from their own thesis by the discoverers of the law of the conservation of energy. In fact, the young Helmholtz had already made certain observations concerning the conversion of matter in muscles during activity which in themselves were correct but, on account of their incompleteness and the lack of knowledge concerning the chemical nature of the relevant substances, served no useful purpose. The previous speaker has already told you about the considerable progress achieved by the English scientists Fletcher and Hopkins by their recognition of the fact that lactic acid formation in the muscle is closely connected with the contraction process. These investigations were the first to throw light upon the highly paradoxical fact, already established by the physiologist Hermann, that the muscle can perform a considerable part of its external function in the complete absence of oxygen. As, on the other hand, it was indisputable that in the last resort the energy for muscle activity comes from the oxidation of nutriment, the connection between activity and combustion clearly had to be an indirect one. In fact, Fletcher and Hopkins observed that in the absence of oxygen in the muscle, lactic acid appears, slowly in the relaxed state and rapidly in the active state, and that this lactic acid disappears again in the presence of oxygen. Obviously, then, oxygen is involved not while the muscle is active, but only when it is in the relaxed state, and this assumption has been supported by further

research on the part of Parnas and Verzar. In what relation the lactic acid stands to muscle performance, where it comes from and what becomes of it when it disappears in the presence of oxygen, was completely obscure. In fact, there were several different, irreconcilable interpretations current, all of which appeared nevertheless to be supported by experiment. It was at this point that I started to work on the problem. A bright light in the midst of this obscurity appeared when Professor Hill made the important discovery, about which he has just spoken to you, that the contraction heat of the muscle occurs in two distinct phases of approximately the same extent - one phase which is directly connected with the work and is the same in presence or absence of oxygen, which he called the "initial heat"; and a second phase, which basically only occurs in the presence of oxygen, and which he called "delayed heat" and quite rightly connected with the - disappearance of the lactic acid. Apart from the pioneer work of Fletcher and Hopkins, it was this discovery above all which, shining out like a beacon light through a sea mist, made it possible for me to steer a safe course through the shallows.
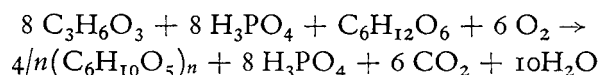
If we now observe an excised frog muscle operating under maximum oxygen supply, chemical analysis will only prove that a certain quantity of glycogen in the muscle disappears, whereas an exactly sufficient quantity of oxygen necessary for its oxidation is assimilated, and the corresponding amount of carbon dioxide is given off. The connection between these processes can be more exactly analysed if the muscle is first allowed to work under anaerobic conditions, and subsequently brought out into oxygen. During the anaerobic phase, in fact, lactic acid accumulates in the muscle approximately in proportion to the amount of work performed. At the same time a corresponding quantity of glycogen disappears, while the quantity of lower carbohydrates, particularly free glucose and the hexose-phosphoric acid discovered in the muscle by Embden, is not noticeably altered. In the second, oxidative, phase the lactic acid which has formed disappears, while a specific quantity of extra oxygen is assimilated. In fact, the disappearance of lactic acid during this period is in exact proportion to the increased consumption of oxygen. However, the oxygen is only sufficient to oxidize a fraction of the disappearing lactic acid; the remainder, which in the case of complete fatigue is about three-quarters of the total lactic acid, is quantitatively reconverted into glycogen. I must state already here that this ratio of the lactic acid which disappears altogether to that burnt is not always constant under all conditions, and from the energetic point of view this is important, to which I must return later. To start with, however, we

will concern ourselves with this figure obtained under suitable conditions of extreme anaerobic fatigue, and subsequent recuperation in oxygen. Of four molecules of lactic acid which disappear, three are then converted back into glycogen and one is oxidized. To be exact, we cannot even maintain with certainty that the lactic acid itself is burnt. We find only an oxidized carbohydrate-equivalent with the respiratory quotient 1. Whether this is sugar or lactic acid we cannot be certain. I have, therefore, chosen the formulation for the two phases which you can see on this board.

Anaerobic fatigue phase

$$5/n \, (C_6H_{10}O_5)_n + 5 \, H_2O + 8 \, H_3PO_4 \rightarrow$$

Glycogen

$$4 \, C_6H_{10}O_4(H_2PO_4)_2 + C_6H_{12}O_6 + 8 \, H_2O \rightarrow$$

Hexose-phosphoric acid   Glucose

$$8 \, C_3H_6O_3 + 8 \, H_3PO_4 + C_6H_{12}O_6$$

Lactic acid

Oxidative recovery phase

$$8 \, C_3H_6O_3 + 8 \, H_3PO_4 + C_6H_{12}O_6 + 6 \, O_2 \rightarrow$$
$$4/n(C_6H_{10}O_5)_n + 8 \, H_3PO_4 + 6 \, CO_2 + 10 H_2O$$

In the anaerobic, active phase the glycogen is broken down into lactic acid via glucose and, I assume in agreement with Embden, by way of hexose-di-phosphoric acid. On the board the decomposition of five sugar-equivalents of glycogen is assumed, of which four are esterified with phosphoric acid and form eight molecules of lactic acid.

In the second, aerobic phase these eight molecules of lactic acid disappear, while two of them, or alternatively, as we might equally well assume, one molecule of sugar, are burnt. The importance of this strangely coupled re-action can only be understood after a study of energetics. But before I turn to this, it is important to stress that this activity metabolism in the muscle is not a separate phenomenon, but is no more than an increase of the metab-olism in the resting state. For even in the resting state the glycogen in an isolated muscle in oxygen disappears directly by way of oxidation into car-bon dioxide and water. If, however, we keep a resting muscle in nitrogen for a considerable time, lactic acid is constantly accumulating in it during the

anaerobiosis. If we now compare this lactic acid accumulation with the quantity of oxygen which the muscle would have assimilated in the same time under aerobic conditions, we find that approximately three times the amount of lactic acid has accumulated as could have been consumed by the oxygen in the same amount of time. Here also, then, the lactic acid is not just a simple intermediate product of the decomposition of the sugar. In fact, if we bring the muscle back into the air after extended anaerobiosis, it will assimilate a certain quantity of extra oxygen, approximately equivalent to the amount previously lost. At the same time, the lactic acid disappears once again in such a way that most of it is reconverted into glycogen, whereas only a fraction, or the corresponding quantity, of carbohydrate is consumed. The process is, therefore, exactly the same as when the muscle is active, only the accumulation of lactic acid progresses much more slowly. We can directly see from this the importance of muscle respiration in the resting state, in that it maintains a labile condition of lactic acid production and removal, which can be accelerated instantly on stimulation. Probably this explosive release of lactic acid during contraction occurs, because stimulation suddenly increases the permeability of membranes which have previously to a certain extent acted as a barrier between the participants in the reaction. Respiration in the muscle in the resting state can, therefore, be said to keep them in a state of readiness for activity.

We can establish that the lactic acid is directly associated with muscle contraction by an exact comparison of the work performed under anaerobic conditions with the formation of lactic acid. As the best expression of the activity potential of muscle we may choose here, following Fick and Professor Hill, the tension which the muscle develops on stimulation when prevented from shortening, i.e. the so-called isometric contraction. If we allow the muscle to go on working under anaerobic conditions until it is exhausted it produces a certain quantity of lactic acid and develops a degree of tension in proportion to this quantity. This total anaerobic work can be very considerable - for instance, a frog muscle of 1 g in weight in N_2 can produce 160 kg of tension in 1000 contractions.

I found that there is a very simple reason why there is any limit at all to this and why activity does not in fact continue until the available glycogen is used up. It was thought earlier, and in particular by Fletcher and Hopkins themselves, who were the first to become aware of the so-called fatigue-maximum, that this was conditioned by the exhaustion of an immediate preliminary stage of the lactic acid. This is, however, not the case - it stems

rather from the accumulation of the acid in the muscle itself. If we remove a large part of the acid from the muscle by placing it in a Ringer's solution particularly rich in bicarbonate, it produces before total exhaustion not only very much more lactic acid, but also correspondingly more work. By the addition of various buffer mixtures to the muscle it was proved that the increase in performance due to this admixture corresponded almost exactly to the percentage of lactic acid which escaped from the muscle into the surrounding solution.

The significance of these chemical reactions only becomes clear when we consider the energetic conditions. In the anaerobic active phase lactic acid is formed from glycogen, at the rate of 1 g lactic acid from 0.9 g glycogen, since during the formation of every 180 g of lactic acid 18 g of water are absorbed

$$(C_6H_{10}O_5) \, (162) + H_2O \, (18) = C_6H_{12}O_6 \, (180) = 2 \, C_3H_6O_3 \, (2 \times 90)$$
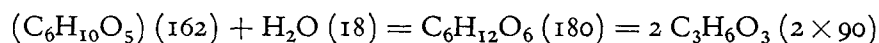
The combustion heat of glycogen, according to Stohmann's readings, is 4191 cal/g - that is, 3772 per 0.9 g. As these readings were made about thirty years ago with still somewhat primitive instruments, a revised determination seemed desirable, especially as the American scientists Emery and Benedict had found a rather higher value of 4227 cal/g. This new determination was made at my suggestion in Germany by a pupil of Professor Roth in Brunswick, and at the same time in Manchester by Mr. Slater. In the first case Stohmann's readings were completely confirmed, resulting in 4188 cal/g or 3769 cal/0.9 g. Slater, however, using a differently produced glycogen, obtained a very much higher value, i.e. in relation to the above glycogen formula he obtained 3883 cal/0.9 g. I will come later to the reasons which for the time being have caused me to regard the values of Stohmann and Roth to be more accurate. The combustion heat of lactic acid I determined anew, since the values given in the literature appeared unreliable, and I obtained 3601 cal for dilute lactic acid, a value which was confirmed in Roth's Institute and which agreed also with the Americans Emery and Benedict. If the chemical process during contraction turned out to be as one was only recently tempted to imagine it-that is to say, if during activity lactic acid is formed from glycogen and this is evenly consumed during relaxation-then only the difference in combustion heat between 0.9 g of glycogen and 1 g of lactic acid - i.e. 170 cal - would be released by activity in the muscle. On the other hand, the combustion of the lactic acid at 3601 cal would take

place in the oxidative recuperative period. Such a process, in which only 5% of the heat released would occur in the contractive phase, would appear to be extremely doubtful theoretically and would into the bargain contradict the fact established by Professor Hill that heat quantities in the active and recuperative phases are approximately the same. In fact, this consideration was really the beginning of my preoccupation with the problem of muscle. The process is actually quite different. It soon became evident to me from a great number of determinations that during the formation in the muscle of 1 g of lactic acid, not 170 cal but 380-390 cal were released, a figure which was not very far removed from older, slightly less accurate, results which Peters, a pupil of Professor Hill, had obtained. Before we discuss the reason for this very big divergence of the contraction heat from the difference between the combustion heats, we must first calculate the energy balance of the recovery phase. If, as is represented in the above equation and has on average been proved by my experiments, of a total of 4 molecules of lactic acid which disappear, one is burnt (or, which comes to the same thing, a carbohydrate equivalent of it), then altogether, for 1 g of sugar taking part in the reaction, or 0.9 g of glycogen, 3772/4 = 943 cal must be released. As we have measured 3 85 cal in the active phase, the remainder - i.e. approx. 560 cal - must be expected during the recuperative phase. According to this, 40% of the heat must occur in the active phase, 60% in the recovery phase. In fact this was very well confirmed, at least with regard to the order of magnitude, by measurement of the total heat production in the recovery phase and comparison with the oxygen consumption. According to this: (1) the increase in heat produced in the oxidative recuperative phase was approximately as great as, or only slightly greater than, the anaerobic heat of the exhaustion state of the muscle; (2) this heat, reckoned according to oxygen assimilation, was smaller than the corresponding carbohydrate consumption which took place simultaneously. For every 1 c.c. of oxygen during carbohydrate oxidation 5 cal should have appeared; but there were only 3.5 cal, and altogether had vanished about the same amount of heat in the recovery phase as had appeared in the anaerobic phase.

We now find that this result agrees very well with Professor Hill's findings, about which he has just spoken to you: the heats of the active and the recovery phases are equal. But, as he went on to establish, this result can be confirmed even more exactly by the more accurate analysis of heat formation which is made possible by the myothermic method developed by Hill and Hartree, also by the study of the oxygen consumption in relation

to the disappearance of lactic acid during recovery under various conditions. The result of these experiments shows that the quotient

$$\frac{\text{total disappearing lactic acid}}{\text{lactic acid burnt}}$$

is not constant - it is greater in completely fresh muscles, and can in fact amount to as much as 5:1 - 6:1, and it is of approximately the same size in live humans as in live frogs. In the case of humans this was proved indirectly in Professor Hill's laboratory. I myself obtained a similar result on the whole frog by using the same direct methods as would have been used on the isolated muscle.

Such an increase in the quotient means, however, that a smaller part of the heat occurs in the recovery phase. For if out of six molecules, for instance, only one is burnt then there must arose from the conversion of 1 g of sugar 3772/6 = 630 cal, of which 385 are in the fatigue phase, so that 245 must be in the recovery phase. In this case 60% must already be released in the active phase, and only 40% in the recovery phase. The quotient appears to lie between these two amounts according to the degree of exhaustion and the condition of the muscle - between 6:1 and 4:1; and in unfavourable conditions it is even smaller. The muscular mechanism operates so much the more economically the more lactic acid molecules can be transformed back into glycogen through the oxidation of one of them. Thus this figure represents the efficiency of the recovery process. It is an expression of how much of the oxidative energy is used in endothermal processes for the conversion of the material in the preliminary stages. In the case of the above equation the efficiency would be 40% - under the more favourable conditions of a completely fresh frog muscle or of a living animal it would be 50-60%. Curiously enough, the ratio is smaller in the case of respiration in rest, i.e. of 2-3 molecules lactic acid which disappear 1 is burnt. My more recent experiments have, in fact, shown that many poisons, and also traumatic damages, experienced by the animal before death, will cause the ratio to deteriorate still further. All these circumstances result in a squandering of energy.

There is a very important problem connected with the size of the anaerobic contraction heat itself, which, as we have seen, is in the region of 385 cal, whereas thermochemical data only give a difference in the combustion heat of 170 cal for the conversion of glycogen into dilute lactic acid. How does this difference arise? The following has been established: if the forma-

tion of heat and of lactic acid are compared, not in the working muscle, but in crushed muscular tissue suspended in phosphate solution, we then obtain about 200 cal/g instead of 385 cal, and at the same time the lactic acid passes into the phosphate solution. The heat of neutralization of lactic acid with biphosphate is, however, 19 cal/g. Added to this is the heat of the cleavage of glycogen into lactic acid, 170 cal, and these taken together amount to 190 cal, which agrees, allowing a margin of error, with our measured value of 200 cal.

Similarly, the heat even in an intact muscle can be reduced if a considerable amount of the lactic acid passes into the surrounding solution. This can be brought about if lactic acid is allowed to form in a resting muscle suspended in a carbonated Ringer's solution. Half of the lactic acid can escape into the surrounding solution, and the ratio will be 280 cal per g lactic acid instead of 385 cal, and finally only 230 cal.

This particular heat formation which lactic acid produces in the living muscle is, however, bound up with the hydrogen ion. In fact, other acids which we allow to penetrate from outside into the frog muscle cause considerable heat production which is independent of lactic acid formation within the muscle. In this way I observed that the penetration of valeric acid into the muscle caused the release of up to 11,000 cal/mol of acid assimilated by the muscle. Reckoned in terms of lactic acid this corresponds to a heat production of 120 cal/g. This heat, as close analysis shows, is dependent upon the reaction of the acid with the tissue protein. This tissue protein acts as a buffer substance and keeps the reaction within the muscle always more or less constant, even during heavy lactic acid production. Even in the case of maximum exhaustion, when approx. 0.4% of lactic acid is produced in the muscle, the index of the hydrogen-ion concentration (pH) is only displaced from 7.5 to 6.8. With this buffer reaction there is a characteristic heat formation - an inverse protein dissociation heat - which is bound up with the deionization of the protein.

These conditions are very clearly seen in relation to the amino acids, which behave in principle in the same way as protein, which is in fact composed of amino acids. If we start, for instance, with a solution of glycine with the addition of caustic soda solution there will be in the solution, apart from other substances, the salt sodium glycine, which we can regard as being completely dissociated, and which will be abbreviated here as NaG.
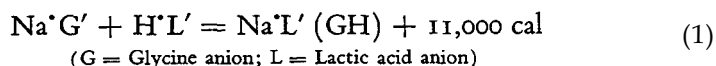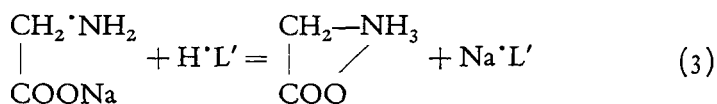
$$Na^{\cdot}G' + H^{\cdot}L' = Na^{\cdot}L' \, (GH) + 11{,}000 \, cal \qquad (1)$$
$$(G = Glycine \; anion; \; L = Lactic \; acid \; anion)$$

If we now add hydrochloric acid or lactic acid or another not too weak acid, then a reaction will take place which is shown here in Formula (1). Out of the totally dissociated glycine-acid sodium there is formed the weak, non-dissociated glycine acid, and in this reaction a positive heat of approx. 11,000 cal can be measured. This is nothing else than the inverse heat from the electrolytic dissociation of the glycine. If a buffer solution is made of concentrated protein solution, free from basic salts, by the addition of caustic soda solution at about pH 8, and if lactic acid is now added in such a quantity that the H-ion concentration is barely altered, a corresponding reaction will obviously take place, which is shown here in the following equation:

$$Na^\cdot P' + H^\cdot L' = Na^\cdot L' + (HP) + 12,650 \text{ cal} \qquad (2)$$
$$(P = Protein \ anion)$$
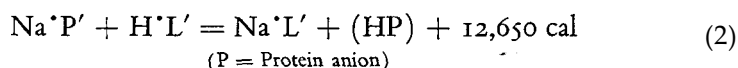
Here we find an even greater heat production - with muscle proteinin the presence of ammonium salt it will be 12,650 cal. This dissociation heat of protein is the largest known dissociation heat of any acid. The reason for this may be connected with the fact that the deionization of amino acids and protein causes the production of internal ammonium salt, as shown in the following diagram:

$$\begin{matrix} CH_2{}^\cdot NH_2 \\ | \\ COONa \end{matrix} + H^\cdot L' = \begin{matrix} CH_2{-}NH_3 \\ | \quad \diagup \\ COO \end{matrix} + Na^\cdot L' \qquad (3)$$

We can check this supposition by means of formaldehyde. Formaldehyde causes the formation of a methylene compound from the amino acids with stronger acid properties. At the same time as the addition of acids the very high dissociation heat disappears almost completely,

Prom the dissociation heat of 12,600 cal per equivalent can be calculated a heat production of 140 cal per g lactic acid. As, however, the lactic acid in the muscle to a certain reacts with phosphate and carbonate this figure should be reduced a little. I have already shown that of the 385 cal formed per 1 g of lactic acid in the anaerobic contraction phase 170 are due to the splitting of the glycogen into dilute lactic acid. There remain 215 cal. Of these, up to 140 can be explained by the dissociation heat of the protein. There remain, over and above the margin of possible error, 70-80 cal for which up till now we can only provide a hypothetical explanation. At first

sight three such explanations appear possible: *(1)* the combustion heat of the glycogen may be higher than has been supposed; *(2)* secondary reactions about which we as yet know nothing may take place; *(3)* the dissociation heat of the protein may be greater in living muscle than in solution. The first possibility seemed to have found strong support from Slater's experiments, according to which glycogen is supposed to have a combustion heat 100 cal greater than the value I have given it. However, I was able to prove that this was unlikely, by bringing about a splitting of the glycogen into maltose and dextrose by means of a diastatic ferment which engendered combustion heats of 3 752 and 3 748 cal. To my surprise the heat of the cleavage of dissolved glycogen into dissolved maltose and dextrose was only about 10 cal/g of glucose. But these values in fact agree approximately with the glycogen combustion heat determined by Stohmann, as the solution heat of dextrose amounts to minus 12.5 cal/g. For the conversion of 0.9 g of glycogen into 1 g of solid dextrose the figure of 10 + 12.5 = 22.5 cal was thus determined by experiment. From this the combustion heat of the dissolved glycogen could be calculated as about 3770 cal per 0.9 g. That of anhydrous glycogen is incidentally much higher, on account of a very considerable hydration heat, but this need not concern us here, since the glycogen in the muscle is in hydrated form. At the same time, there is a very interesting conclusion to be drawn in connection with the result of these readings. We have good reason to believe that the actual process of muscular work does not begin with the splitting of the glycogen but with that of the dextrose or the phosphoric acid hexose. The energy released by the conversion of the glycogen into hexose is then lost to the activity process. Up till now it has been possible to assume that more than 30 cal/g of sugar - that is to say, no less than 8% of the total energy - was squandered in this way. It is now apparent, however, that as a result of the negative solution heat of glucose, the conversion of dissolved glycogen into dissolved dextrose only requires approx. 10 cal - that is to say, barely 3% of the contraction heat. It is possibly even lower in the case of the conversion into phosphoric acid hexose. This result is in harmony with the splendid economy which is shown in the reactions of the living organisms.

Of the possibilities just mentioned for the explanation of the difference of about 70 cal there now remain, therefore, only the two last. Up till now there has been no reason to suppose that, side by side with the carbohydrate metabolism, a fat or protein metabolism also plays a part in the contraction mechanism, nor that inorganic compounds such as phosphoric acid undergo

permanent changes on account of anaerobic exhaustion. Therefore I incline to the third of the above hypotheses, namely that protein in living muscle has a higher dissociation heat than in solution: this supposition is supported by the consideration that the material of which the muscular machinery is composed is protein, whereas carbohydrates form the combustion material. Somehow the oxidation energy of the combustion material must play a part in the mechanism itself. This is in fact the case with the deionization of muscle protein. The oxidation energy first becomes active in the recovery phase; and then, as a result of the coupling of the oxidation with the re-synthesis of the lactic acid, not only is the endothermic process of rebuilding the glycogen brought about, but also the alkali is released from the vanishing alkali-lactate, and this leads to the endothermic, involuntary dissociation of the muscle protein. In this way the muscular apparatus is once again put into working order. We can compare this process to the charging of an accumulator, which Professor Hill has given as an image of the recovery reaction, or, if we prefer, to the winding-up of a watch, as I have more often described it. It would then appear theoretically quite logical to expect that a relatively large part of the total energy should be lost in this process. But we must leave the definitive explanation of this point to the future.

We can, however, say now that the deionization of the protein by the lactic acid produced by muscle activity plays, without any doubt, an important part in the contraction mechanism. It explains in the first place the flaccidity of the muscle which sets in after shortening under anaerobic conditions in spite of the presence of lactic acid. This flaccidity would be brought about by nothing more than the diminution of the lactic acid acidity, just as, conversely, we would hold the H-ions responsible for the release of the contraction. Certainly, the flaccidity heat discovered by Hill and Hartree, even though it must be considered to originate in the superimposition of various chemical and physical processes, is obviously the principal cause of the deionization heat of protein. On the other hand, the exhaustion maximum of lactic acid would be conditioned by the supply of alkali separable from the protein salt of the muscle.

I think, therefore, that in this way we have obtained a comparatively simple and satisfactory picture of energy conversions in muscle, the future shape of which will be of theoretical value and practical interest.

To remove the uncertainty concerning the combustion heat of glycogen mentioned in this lecture, I have since, with Dr. Meier, brought about the combustion of several glycogen preparations with various reservations. For

anhydrous glycogen ($C_6H_{10}O_5$) from frog muscles we obtained 3806 cal, for glycogen hydrate ($C_6H_{10}O_5 \cdot H_2O$), 3786 cal, from which, taking into account the solution heat of dissolved glycogen, 3775 cal was found, so that the value used above is almost exactly correct.

It also became clear that during deionization of saturated solutions of ammo acids in non-aqueous media (alcohol-water mixture) besides a deionization heat, a precipitation heat occurs, which amounts to just about 70 cal/mol - a pointer to the possible explanation for the cause of the unexpected remainder in the contraction heat.