WORK AND CHEMICAL CHANGE IN ISOTONIC MUSCULAR CONTRACTIONS

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SUMMARY

Under the conditions where the metabolism of frog sartorius muscle was restricted to a splitting of phosphocreatine, a study has been made of the extent of this splitting as related to activation (maintenance), work, and shortening in 400 msec tetani at 0°.

The maintenance metabolism amounted to 0.45 μmole/g per tetanus, compatible with an assumed heat of hydrolysis and neutralization of 9600 cal/mole, but without availability of a direct myothermal comparison on the same material.

Work done by the muscle mobilized extra metabolism, amounting to 9100 cal of work/mole, so that the biochemical basis of the FENN effect has been demonstrated.

A correlation of increased metabolism with shortening was not established, although not excluded. It was shown that, because of the dependence of activation metabolism upon length, a direct demonstration of shortening metabolism in free isotonic contractions is not to be expected.

INTRODUCTION

The extent to which exergonic chemical reactions are called forth in connection with contractile activity depends, under constant environmental conditions, upon the amount of work executed and the degree of shortening. This insight is based upon heat measurements, which have revealed that besides the activation or maintenance heat, the muscle liberates shortening energy in proportion to the amount it shortens[1], and mobilizes additional energy equal to the work done[1–3]. These phenomena have undoubtedly a chemical basis but, apart from certain incomplete approaches to this problem which will be mentioned in the DISCUSSION, no conclusive work seems to have been devoted to this relation**. Yet, it is obvious that only a direct demonstration of the associated chemical reactions, and of their quantitative correspondence to energetic quantities, will permit an understanding of the nature of the mechano-chemical linkage. From the viewpoint of the theory that muscular energy arises directly or indirectly from the fission of adenosinetriphosphate and indirectly of

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** We are aware that, parallel to our work, similar efforts have been made by F. D. CARLSON at Johns Hopkins University. The two investigations have been pursued independently and appear to agree on many experimental points.

creatine phosphate*, it appears evident that various amounts of these substances must be dephosphorylated to account for the several categories of mechanical activity. However, such fundamental relations must not be assumed, but demonstrated.

In the present work, we have attempted a first approach to this problem, which has brought evidence of the biochemical counterpart of the FENN effect, but which is inconclusive with regard to that of the shortening heat.

Plan of physiological experimentation

In general terms, the aim is to establish whether the chemical change in contracting muscle depends on the shortening and the work in such a manner that we can speak, in analogy with the myothermic terminology, of metabolism of activation, of shortening, and of work. In isotonic contractions, as studied in this investigation, these quantities are varied by changing the magnitude of the load. Each experimental datum was obtained from one pair of iodoacetate poisoned muscles (see below), one member of which was caused to perform a number of contractions with such a load, to give information as to the amount of phosphocreatine breakdown in this experiment; this is the sum of the activation, shortening and work metabolism in this one experiment, in which the shortening and work were also measured. The way in which the three categories of energy are segregated from the data will be described furtheron.

The metabolism was measured in terms of phosphocreatine breakdown in iodoacetate poisoned muscle. No adenosinetriphosphate decomposed under our conditions. The overall reaction is not necessarily hydrolytic, but may be accompanied by esterification; once this occurs, there may be formation of fructose diphosphate at the expense of additional phosphocreatine in reactions presumably not connected with activity. The experiments were performed at 0° for three reasons: (a) to reduce this esterification, and in most later experiments this was largely successful, (b) to reduce the maintenance metabolism, especially in tetanic stimulation, so that the contribution of the shortening and work metabolism would stand out more prominently, and (c) to permit closer comparison with myothermic investigations which are usually done at low temperature. In the later course of the work, it was found that even unpoisoned anaerobic muscles under our conditions often form no lactate, so that the iodoacetate might be omitted (which would probably improve the reproducibility of the experiments); however, since absence of lactate does not exclude formation of high-energy phosphate, this was not yet attempted.

Two modes of contraction were studied: twitches and tetani (20 and 12 per experiment, respectively). Twitch experiments were first performed under preloaded conditions, but the results of these will not be discussed since they contain an additional variable: length. In a twitch, maintenance metabolism is at a minimum and this was originally felt to be advantageous until it was learned that considerably more work and shortening could be obtained in tetani without causing (at 0°) an undue increase in the maintenance metabolism; a tetanus of 400 msec duration was judged a fair compromise for this purpose. It is also believed that, the duration of the active

* We recall that in certain muscles contraction is observed without splitting of these two substances[4,5], which might be explained by an additional energy providing process. In the sartorius muscle of winter frogs, however, this situation does not prevail[6], so that no such additional reaction will be considered in this paper.

state being set by the stimulation period, the total maintenance energy is likely to be more constant than with single stimuli.

With sufficient reproducibility of the data, the analysis of the three forms of metabolism might be performed graphically, from a plot as presented in the DISCUSSION (Fig. 1). However, the scatter of the results was too great to permit this. It is likely that, on the basis of the experience now available, further efforts would lead to an improvement of the reproducibility.

METHODOLOGY

The muscle preparation

The sartorius muscles were dissected from specimens of *Rana Pipiens* (or, in some cases, *Rana Catesbiana*), obtained in large batches and kept in cooled tanks (about 4°) without food for several weeks before the beginning of an experiment. The reported data were obtained on muscles in the 30 to 35 mm length and 80–100 mg weight ranges. The animals were pithed, or were decapitated and then promptly transsected at the lumbar level; the muscles were then dissected with care, and inspected for damage prior to the experiment. A cotton thread was tied on the distal tendon, and a loop was formed by tying a square knot around a 1/16 in stainless steel probe. The proximal tendon was tied in like fashion, close to the pelvic bone without crushing any muscle fibers. By means of these cotton loops, the muscles could subsequently be mounted in the apparatus with minimal handling, whereas their lengths were small enough not to contribute unduly to the extensibility of the connections. In later parts of the work, the muscles remained on the pelvic bone. This is now the method of choice, since it guarantees a preservation of the original geometry of the fibers. When the pelvic tendons were tied, the muscles often folded sideways, which might have led to irregularities in the contributions by individual fibers to the support of the load; however, even the natural attachment did not assure even support of the load by all fibers, since in the material at our disposal a lateral segment of each muscle was attached more loosely to the bone than the larger part connected through the main tendinous sheet. Such irregularities are not believed to affect our analysis grossly, since the work done must be covered by chemical reactions regardless of its distribution over the muscle; but they do introduce an ill-analyzable feature, and may have been contributing to the irregular work output obtained from one muscle to the next. When the muscles were tied on both ends, they were weighed separately before the experiment on the parafilm-coated pan of a torsion balance; the threads and cut-off parts of the muscles were later weighed back. When the muscles were left on the bone, the wet weight of the sample analyzed was determined after freezing.

After dissection, the muscles were soaked in bicarbonate–Ringer of the following composition: NaCl 95 m*M*, KCl 2.5 m*M*, $CaCl_2$ 1.0 m*M*, $MgSO_4$ 1.0 m*M* and $NaHCO_3$ 20 m*M*, which is the solution of BOYLE AND CONWAY[7] but without phosphate, and were aerated with O_2–CO_2 (95:5) for 1 h at room temperature followed by 1 to 2 h at 0°. In the early parts of the investigation (mostly covering the series of twitches) only low-temperature recovery was applied; towards the end of the work they were permitted to recover in the cold (4°) overnight. These and other changes in technique over the years may have caused changed trends in the results, but no indications were seen that such changes exceeded the variability due to other reasons. After recovery,

the muscles were transferred into another batch of the same medium, containing in addition 0.001 *M* Na-iodoacetate and 0.002 *M* Na-lactate, for exactly 1 h at 0°, whereupon they were rinsed in Ringer for 5 min. Muscles so treated had their phosphoglyceraldehyde dehydrogenase completely poisoned, and formed no lactate; while their creatine phosphoryltransferase was not inhibited[8]. Since iodoacetate poisoning is irreversible, and might affect respiration, any subsequent stimulation of metabolism may leave an unreversed effect. It was to minimize this throughout the period of aerobic manipulation that lactate was added to the medium; still, the poisoning may have exaggerated the variability. We have also attempted to prevent glycolysis by applying an atmosphere of pure CO_2 (see refs. 9, 10), but in our material, this did not prevent lactate formation. In muscles where it does, this would be a preferable procedure because it can be applied to the muscles after mounting and because, if it acts by inhibiting phosphofructokinase[11], it would suppress the use of creatine phosphate for esterification.

Apparatus

The experiments were done with a rapid-freezing assembly as developed by MOMMAERTS AND SCHILLING[12] but considerably modified. Among the various technical improvements, which will be communicated in due time, the most relevant were better assurance of anaerobiosis, and the fact that the distance of movement of the cooling medium was shortened so that the connection from muscle to lever was now only 27 cm; this connection was made with 0.016 inch cold-stretched stainless steel wire, deemed inextensible (12 μ extension/100 g load) in comparison to the characteristics of the muscle. The electrodes were of a multi-polar design to assure simultaneous activation of most of the muscle length. The shortening gages were originally photoelectric, later the variable capacitance gage of SCHILLING[13] was used, with a lever designed for minimal inertia and maximal rigidity. Isometric experiments were likewise done with the corresponding capitance gage of SCHILLING[13]. The muscles were stretched to an initial tension of about 200 mg/mm^2, and in the case of isotonic experiments this represented the preloaded weights, to which the remainder of the weight was added under after-loading conditions.

After mounting, the muscles were first kept for 5–10 min in a flow of O_2–CO_2 (95:5), moistened with Ringer's solution at the temperature of the experiment; then they were kept anaerobically for 15 min. Originally, the anoxic gas phase was CO_2–helium (5:95), led through a tall tower of cuprous chloride in ammonium chloride solution. Later, it was CO_2–H_2–N_2 (5:1:94) led over a catalytic deoxygenation cartridge. All gas conduits were either glass or copper, with use of metal and glass semi-ball joints for connections, and corrugated brass tubing for flexibility at points of stress.

The experiment was then performed, consisting of electrical stimulations of the one muscle slightly above threshold with square pulses of 1 millisecond duration, singly, or as tetani at a rate of 20/sec, followed by rapid freezing after the last contraction. No distinction was made, in the handling of the muscles, between right and left, and their placement in the apparatus was randomized in this fashion. The stimulus was applied to one or the other position in the apparatus, not systematically randomized, but without correlation to any of the other experimental parameters. The loading conditions were varied arbitrarily throughout the series.

Biochemical analyses

In all communicated experiments, the frozen muscles were extracted with 0.3 N perchloric acid, with the technique developed in this laboratory[14]. The analytical methods used were those of previous investigations[4,14]. ATP was determined with the firefly method according to STREHLER AND TOTTER[15] and MCELROY AND STREHLER[16] All analyses are expressed in micromoles per gram muscle.

RESULTS

End products of phosphocreatine splitting

While presumably the splitting of phosphocreatine occurs *via* ATP $\rightleftharpoons$ ADP (LOHMANN[17]), no variations in ATP content were ever encountered in the course of our experiments, in agreement with recent experiences documented by CARLSON AND SIGER[18]. We shall, therefore, discuss all our results in terms of phosphocreatine breakdown. First, because these are the experimentally obtained data, beyond which we have no direct information. Second, because these data represent the overall reaction, whose enthalpy change is to be compared with the heat produced, regardless of the specific pathways followed.

The following reactions (1 and 3) can lead to phosphocreatine breakdown in our experiments:

$$PC + H_2O \rightarrow P + C \quad (1)$$

$$P + \text{glycogen} \rightarrow \text{hexose-phosphate} \quad (2)$$

$$\text{hexose-phosphate} + PC \rightarrow \text{fructose-diphosphate} + C \quad (3)$$

Of these, reaction 1 is to be correlated with mechanical activity, while reaction 3, to our knowledge, is not (although it would, of course, contribute to the heat evolved).

In an earlier phase of the work, the extent to which reaction 2 occurred, as evidenced by the equivalence of the liberated phosphate and creatine, was variable, but this was not correlated with any characteristic of the mechanical activity. During the larger part of the investigation, correlated with the employ of a longer recovery phase after dissection, the correspondence between phosphate and creatine liberation was close, and reaction 3 was therefore suppressed, because reaction 2 was suppressed. This will not be further discussed in the present paper, except to say that to a minor but variable extent reaction 3 may yet have occurred and, inasmuch as it is not held to be connected with contractility, may have been one of the contributory causes to the variability of the data.

Relation to contractile activity: orientation

Originally, it was hoped that arrangement of the data according to Fig. 1 in the DISCUSSION would lead to all the desired conclusions. When the variability of the results clearly made this impossible, this approach was abandoned, and recourse was taken to the analyses presented in the next section.

A complete listing of all data is given in Table I for 93 experiments on tetani. These will be utilized as obtained, without corrections or adjustments.

Analysis of the results

Our starting point is the specification of the energy liberated in muscle by the HILL equation:

$$E = A_{t,l} + aS + W \quad (4)$$

TABLE I

SUMMARIZED RESULTS OF FROG MUSCLE CHEMISTRY CONTRACTION AND WORK

All analyses are given in μmoles/g.

Date	Free Creatine		Free Creatine		Shortening cm/cm length/contraction	Total w g cm
	Control	Stim.	Control	Stim.		
	F_C	F_S	T_C	T_S		
2/16/59	6.16	20.20	27.80	28.60	0.43	101
2/16/59	5.25	11.80	22.90	20.50	0.065	50
2/17/59	7.50	22.80	28.40	27.60	0.40	88
2/18/59	9.80	16.40	30.60	29.20	0.42	68
2/18/59	9.43	17.45	30.20	29.20	0.29	167
2/19/59	10.30	18.40	28.20	30.20	0.22	112
2/19/59	9.21	12.45	28.00	26.00	0.065	57
2/23/59	5.22	13.38	25.30	26.20	0.28	140
2/23/59	5.55	13.90	19.50	20.40	0.19	1
2/23/59	7.12	12.20	29.10	28.30	0.018	14
2/23/59	5.94	14.65	20.80	21.60	0.273	101
2/24/59	8.75	16.70	27.00	28.10	0.094	62
2/24/59	9.85	11.75	27.50	26.90	0.299	81
3/18/59	5.65	10.40	25.00	23.90	0.35	87
3/18/59	7.70	9.00	22.50	21.20	0.226	107
3/19/59	4.92	13.80	27.50	28.20	0.334	120
3/19/59	4.46	8.69	21.90	20.20	0.151	90
3/23/59	7.45	14.70	26.50	27.60	0.344	65
3/23/59	6.75	16.40	28.80	29.20	0.263	131
3/24/59	5.62	15.30	29.80	30.60	0.225	126
3/24/59	5.50	14.65	28.40	27.10	0.156	136
4/22/59	6.63	15.70	25.30	26.50	0.283	119
4/22/59	6.61	11.80	25.90	24.60	0.293	146
4/23/59	6.41	17.00	29.90	29.10	0.293	91
4/23/59	7.05	15.35	28.40	29.10	0.422	105
4/24/59	6.52	15.60	24.70	25.60	0.41	93
4/24/59	4.03	8.80	19.80	20.40	0.40	53
4/28/59	9.05	18.90	29.50	30.20	0.351	147
4/28/59	4.25	11.10	23.60	22.20	0.133	100
4/29/59	4.91	12.79	25.00	26.60	0.103	93
4/29/59	4.57	7.55	20.10	18.80	0.034	29
5/26/59	11.30	21.60	31.20	32.80	0.128	105
5/26/59	8.05	18.25	27.90	27.90	0.267	72
5/28/59	10.42	20.20	31.70	32.50	0.289	103
5/28/59	7.03	16.50	25.60	25.30	0.174	206
6/ 2/59	9.25	15.80	29.40	30.20	0.301	70
6/ 2/59	11.90	16.40	29.20	28.30	0.330	18
6/ 4/59	8.14	17.75	30.40	30.40	0.238	65
6/ 4/59	9.66	19.10	30.30	29.40	0.257	72
6/ 5/59	10.35	18.25	32.00	30.65	*	
6/ 5/59	9.92	16.05	32.05	32.60	*	
6/ 8/59	8.60	16.40	30.80	31.00	*	
6/ 8/59	10.32	14.95	28.30	28.80	*	
6/ 9/59	11.88	16.13	30.60	28.60	*	
6/24/59	7.64	14.85	36.20	35.20	0.176	71
6/24/59	6.35	13.40	32.40	33.60	0.148	66
6/25/59	9.54	16.45	34.10	35.00	0.313	34
6/25/59	8.55	13.40	32.40	32.80	0.129	50
7/13/59	8.55	12.41	28.70	27.80	0.254	96
7/13/59	6.07	9.35	25.10	25.90	0.108	41
7/14/59	10.35	14.25	31.95	31.40	0.064	46
7/14/59	9.75	18.30	29.40	29.80	0.189	143
7/15/59	5.94	15.10	24.40	25.20	0.388	99
7/15/59	5.67	13.20	27.20	27.90	0.329	75

TABLE I (*continued*)

Date	Free Creatine		Total Creatine		Shortening cm/cm length/contraction	Total work g cm/g
	Control	Stim	Control	Stim.		
	F_C	F_S	T_C	T_S		
7/17/59	7.14	14.00	27.40	28.80	0.170	1072
7/17/59	8.05	16.19	25.40	26.80	0.231	1220
7/28/59	9.84	17.34	28.20	29.60	0.257	1218
7/28/59	8.15	13.85	29.50	28.30	0.282	950
7/29/59	6.00	16.25	27.30	28.60	0.281	1405
7/29/59	7.75	13.70	27.20	28.10	0.175	850
7/30/59	8.20	15.15	26.80	27.20	0.322	977
7/31/59	7.11	10.93	26.40	26.50	0.357	552
7/31/59	6.37	9.72	25.00	27.10	0.378	323
8/11/59	4.10	13.85	26.40	27.20	0.317	1055
8/12/59	5.12	9.15	23.20	22.90	0.031	281
8/13/59	7.45	13.35	27.70	28.90	0.395	358
8/13/59	4.48	10.30	23.30	23.90	0.382	465
8/14/59	9.00	21.80	27.60	26.60	0.403	702
8/14/59	6.32	13.00	27.20	27.60	0.368	622
9/10/59	7.45	15.00	27.10	27.80	0.415	552
9/10/59	7.55	11.10	25.20	24.30	0.427	440
9/11/59	7.78	15.90	28.90	29.00	0.337	692
9/11/59	5.58	10.25	26.80	26.50	0.295	551
9/14/59	6.67	13.65	26.60	26.80	0.333	850
9/14/59	7.65	16.50	25.40	26.60	0.303	1360
9/15/59	8.20	12.20	26.60	26.70	*	*
9/15/59	8.50	13.22	27.60	27.40	*	*
2/12/60	7.45	9.39	26.50	26.20	0.324	103
2/12/60	9.59	12.10	28.50	27.90	0.324	703
2/15/60	6.24	12.25	22.40	23.40	0.235	998
2/15/60	5.83	13.70	24.60	25.60	0.220	1275
2/16/60	6.90	12.63	22.10	23.30	0.197	900
2/16/60	5.84	17.10	25.80	27.00	0.186	855
2/17/60	9.55	14.70	28.10	28.30	0.069	819
2/17/60	5.55	17.70	26.10	26.00	0.242	1900
2/17/60	10.00	18.29	30.15	30.00	0.081	1335
9/17/60	6.83	18.10	27.30	29.10	0.309	1030
3/22/60	3.45	11.20	29.00	28.90	0.390	366
3/22/60	7.70	20.30	32.00	32.60	0.030	640
?	6.75	12.50	34.60	33.00	0.348	292
?	8.54	19.20	26.90	34.60	0.027	567
?	5.20	11.80	31.60	31.80	0.310	248
?	6.34	14.20	31.40	30.30	*	*

* Isometric

and it is to be investigated whether this corresponds to an equation for chemical change:

$$\Delta PC = B_{t,l} + b_s S + b_w W \qquad (5)$$

In these equations, $A_{t,l}$ is the activation (maintenance) heat as a function of the time of maintenance and the length (tension) of the muscle* (AUBERT[19]), aS is the shortening heat proportional to the shortening S, and W is the work while $B_{t,l}$ is the activation metabolism, b_s the chemical coefficient of shortening corresponding to a,

* The quoted paper by HILL shows clearly that A was not considered constant; in the context of that work, its variability was not emphasized but it would be incorrect to deduce from this that HILL ever considered it to be invariable. (see DISCUSSION).

and b_w the chemical coefficient of work. The dimensions of the terms in Eqn. 5 are μmoles/g.

The following additional notations will be used: F_S, free creatine in the stimulated muscle after contractile activity is over; F'_C, free creatine in this muscle before stimulation; F_C, free creatine in the unstimulated control muscle; T_S, T'_C, and T_C are the corresponding quantities for total creatine.

In Eqn. 5, $\Delta PC = F_S - F'_C$, but F'_C is not accessible; instead it is evaluated from the analysis of the control muscle: $F'_C - \bar{F}'_C = b_C(F_C - \bar{F}_C)$.

The analysis with respect to the effect of the independent physiological variables required a multiple linear regression[20] of the form:

$$F_S = \bar{F}_S + b_C(F_C - \bar{F}_C) + b_w(W - \bar{W}) + b_s(S - \bar{S}) \qquad (6)$$

(in which the symbols with a bar denote the mean values of the corresponding quantities). The means, variances, covariances, and analysis of variance, are given in Tables II, III and IV.

TABLE II

MEANS

$\bar{F}_S$ in μmoles/g	$\bar{F}_C$ in μmoles/g	$\bar{W}$ in gcm/g	$\bar{S}$ in cm/cm
14.62 ± 0.34	7.42 ± 0.20	772 ± 47	0.233 ± 0.013
$\bar{T}_c$ in μmoles/g	$\bar{T}_s$ in μmoles/g		
27.50 ± 0.34	27.74 ± 0.35		

TABLE III

VARIANCES AND COVARIANCES

	F_S	F_C	W	S
F_S	10 349			
F_C	3 198	3.653		
W	435.27	— 113.26	205 635.905	
S	0.0089	— 0.0541	17.986	0.01686

Where variance $= \Sigma(x - \bar{x})^2/n\text{-}1$ and covar $= \Sigma(x - \bar{x})\ (y - \bar{y})/n\text{-}1$

TABLE IV

ANALYSIS OF VARIANCES

Variation	Degree of freedom	Sum of Square	Variance	P
Regression F_S on F_C	1	285.161	285.161	< 0.001
Regression F_S on W	1	102.915	102.915	< 0.001
Regression F_S on S	1	0.734	0.734	NS
Sum of above	3	388.810		
Unaccounted	89	563.325	6.329	
Total	92	952.135		

First regression: There was a strict correlation between F_S and F_C; $r_{F_S F_C} = 0.519$ ($P < 0.01$, $n = 93$); $b_C = 0.969 \pm 0.141$; confidence limits 1.246 and 0.682 ($P = 0.05$, $n = 93$); hence $b_C = 1$ is a good estimate.

It is interesting to note that T_C, T_S and F_C were all well correlated: $r_{T_C T_S} = 0.92$, $r_{T_C F_C} = 0.47$, $P < 0.01$, $n = 93$; thus, F_C could well be estimated from T_S. The amount of variation removed by the regression of F_S on F_C was 285.16 (Table IV), whereas that in the regression of F_S on T_S was 258.16. While this means that the determination of T_S provides as good a control as that of F_C, no use was made of this in the present work, because this relation had to be proven first, and because it would eliminate the determination of $B_{t,l}$. These correlations further show that the concentration of PC, equal $(T - F)$, is more constant than that of F, and that no difference suggested itself between T_S and T_C. In other words, there is no destruction of phosphocreatine other than dephosphorylation*.

Second regression: There was a good correlation between F_S and W: $r_{F_S W} = 0.298$ ($P < 0.01$, $n = 93$), and $b_w = 0.00257 \pm 0.00068$.

Third regression: No correlation was assured between F_S and S: $r_{F_S S} = 0.021$; b_s was 0.896, but with a standard error of 2.16.

Conclusions

The variability of F_S accounted for by the three regressions is 388.81, or about 40% of the total variability. The sources of the total variability, apart from analytical errors, (including extraction error, minimized but not excluded by the technique of SERAYDARIAN, MOMMAERTS AND WALLNER[14]), were numerous. They include the variabilities of each of the terms, notably of the activation metabolism $A_{t,l}$ or $B_{t,l}$ (intrinsically, and with respect to the parameters t and l) and of the shortening and work coefficients b_s and b_w, and also such circumstances as a variable amount of fructose diphosphate formation from PC.

The data are described by the relation:

$$F_S = 14.615 + 0.969(F_c - 7.415) + 0.00257(W - 772.25) + 0.896(S - 0.232) \quad (7)$$

which reduces to:

$$F_S = 5.42 + 0.969\,F_C + 0.00257W + 0.896S \quad (8)$$

DISCUSSION

Continuing the evaluation of data in the previous sections, it will first be observed that the constant term 5.42 represents the splitting of phosphocreatine in 12 tetani without work and shortening, and corresponds therefore to the term $B_{t,l}$ in Eqn. 5, or 0.45 μmole/400 msec tetanus at 0°. This is approximately as could be expected, *e.g.* in comparison with the recent figure of 0.286 μmole per isometric twitch[18]. A further specification may be attempted as follows: as the mean work in our muscles was 772 g·cm and the mean shortening 0.234 cm/cm, or 0.82 cm/3.5 cm length, the mean load on the muscles was 78.5 g. We compare this to AUBERT's data on the maintenance heat in *Rana temporaria* at this tension, and find 0.0034 cal/g/contraction, to correspond to our 0.45 μmole, or 7600 cal/mole. From the work of GELLERT AND STURTEVANT[21], and of BERNHARD[22], it may be estimated that the total heat of hydrolysis of

* There could, *e.g.*, have been a formation of creatinine connected with some aspects of activity.

phosphocreatine in muscle may be 9600 cal (with uncertainty as to the heat of neutralization), and in view of the species difference, the agreement is satisfactory.

The multiple regression analysis has revealed a highly significant connection between extra metabolism and work, the inverse slope corresponding to 390 g·cm or 9.1 mcal/μmole. The variation of the data was still appreciable, but if credence is given to the quoted figure which is closely equal to the estimated reaction heat of phosphocreatine hydrolysis, it evolves that a direct chemical demonstration has been given of the FENN effect. We note that, descriptively, this effect means that when work is done, additional chemical reactions are mobilized of which the total enthalpy change under the circumstances is numerically equal to the work; by coincidence, or for fundamental reasons unrevealed as yet.

On the other hand, no demonstration has been brought of a statistically significant correlation between metabolism and shortening. It is true that the regression suggests a consumption of 0.26 μmole of phosphocreatine/g/contraction/cm of shortening; which would correspond well to the value of a as determined myothermally. But, inasmuch as this dependence is not assured, we do not wish to elaborate upon this. In fact, we can only state that in this work the existence of shortening metabolism has not been established.

Actually, it must be emphasized that it is not evident *a priori* that investigations of the type presented here can be expected to demonstrate shortening metabolism, for this it would imply that the activation or maintenance energy A or B (Eqn. 4 or 5) were independent of shortening. In that case only would the total metabolism in isotonic contractions depend on shortening as indicated in curve I of Fig. 1. But as it is known that A depends on length (or tension; AUBERT[23]), it is evident that such a plot is to be expected only in ranges where the various final lengths cause no great change in A. Now it appears that oftentimes in twitches or brief tetani the diminution of A with shortening is approximately equal to the shortening heat, in which case curve II of Fig. 1 would result; while, with an even stronger dependence of A upon S, a maximally shortening muscle would actually mobilize less energy than an isometrically contracting one, instead of more (curve III in Fig. 1). Measurements by FENN[2, 3]

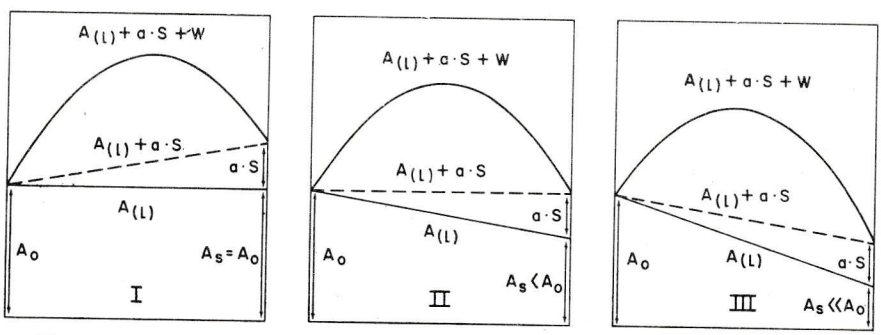

Fig. 1. Three schemes of the relation between total energy liberated, or metabolism (ordinate), and the shortening (abscissa) in free isotonic contractions. Fig. 1, part I, is drawn on the assumption that A (B) is not a function of the length during and after shortening; this represents the HILL Equation (4 or 5) in a special case, which should not be mistaken for the general. In Fig. 1, part II, the reduction of A with shortening is set equal to the shortening heat (metabolism) $a \cdot S$, an assumption which may approximately apply to this investigation. In Fig. 1, part III, the reduction of A with S is even greater than $a \cdot S$, and the total metabolism in maximal shortening is less than in isometric activity.

(when extrapolated to zero load), by HILL (quoted by BROWN[24]) and by TIGYI[25], indicate that this may occur. Likewise, there are chemical measurements showing this possibility, namely those of NACHMANSOHN[26] and of ROTHSCHILD[27] on the production of lactate, and of FISCHER[28] on oxygen consumption, isometrically and isotonically. These effects, furthermore, may depend on the initial length of the muscle as shown by HILL[1] and especially AUBERT[19], and also on the pretreatment of the muscle as indicated by preliminary results in this laboratory. Thus, special investigations in which the several variables are controlled independently will be required. We shall, therefore, refrain from further discussions at this time, referring to another paper[32] for an earlier recognition of these interrelations, and a discussion of their bearing upon cardiac physiology (touched upon by SARNOFF *et al.*[30]). At any rate, the results are in full accord with what could be expected from the investigations of HILL and AUBERT; they also indicate, however, the limits of the information obtainable from experiments with free isotonic shortening, even though with further technical improvements a somewhat greater reproducibility might be obtainable.

There is one more point on which we wish to elaborate, in relation to the recent discussion by WILKIE[31] on the thermodynamics and the interpretation of heat measurements. Writing Q for the heat evolved, his Eqn. 4* becomes:

$$Q = -T\Delta S + (-\Delta F - W) \tag{9}$$

In the case of a constant quantity of metabolites being transformed, any work done would diminish the heat developed by the equivalent amount. The fact that this is not so in muscle has, as also in the present study, been considered to indicate that with shortening and work more metabolism is activated. This can be specified by separating the quantities of heat Q_i associated, respectively, with activation (Q_A, used for the proper length or tension), shortening, and work, and ascribing each to the corresponding molar amount m_i of metabolic conversion.

$$A = Q_A = m_A\,[-T\Delta S - \Delta F] \tag{10a}$$

$$a\Delta L = Q_S = m_S\,[-T\Delta S - \Delta F] \tag{10b}$$

$$Q_W = m_W(-T\Delta S) + [m_W(-\Delta F) - W] \tag{10c}$$

$$Q = (m_A + m_S + m_W)\,[-T\Delta S - \Delta F] - W \tag{10}$$

The present investigation, then, gives no separate clues as to m_A and m_S, but does indicate the magnitude of m_W. Furthermore, it emerges, and follows with greater accuracy from the myothermal measurements of HILL[1], that the term specifically associated with work (Eqn. 10c) does not lead to additional heat, hence:

$$Q_W = m_W(-T\Delta S) + [m_W(-\Delta F) - W] \simeq 0 \tag{11}$$

$$m_W\,[-T\Delta S - \Delta F] \cong W \cong m_W(-\Delta H) \tag{12}$$

While we have no accurate information on the value of ΔS for phosphocreatine splitting, this may be sizable, in which case the apparent equality of W to $-m_W \Delta H$

* By this equation, WILKIE states explicitly that the heat produced has two sources: a part due to the entropy change, and a part due to the imperfection of the transformation of free energy into work. These two categories have previously[32] been called the obligatory and the dissipational heat production.

instead of $-m_W \Delta F$ might be a coincidental result of the summation of various processes. This would imply that the free energy efficiency were $\varepsilon \simeq -\Delta H/-\Delta F$, which might well be a coincidence, although its constancy, at different velocities of work performance, would be noteworthy.

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