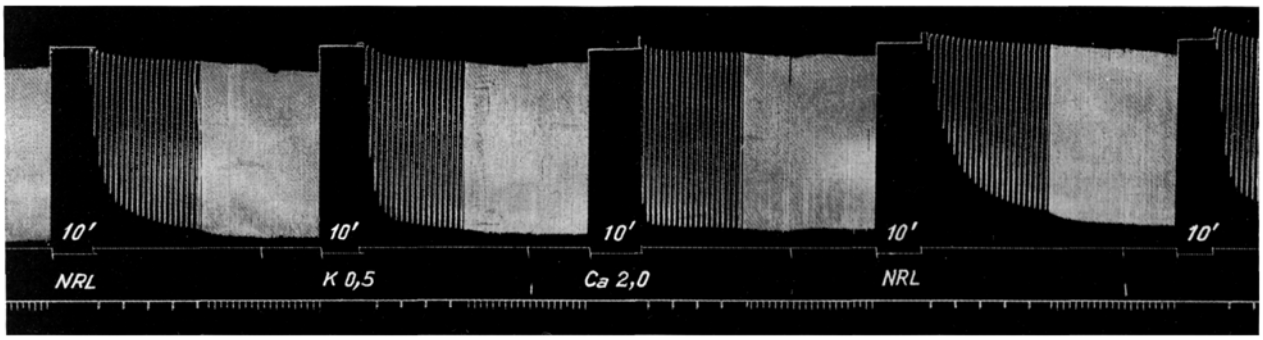

Abb. 1. Registrierkurven des Schlagvolumens am Froschventrikelpräparat bei elektrischer Reizung (alle drei Sekunden). Vergleich des Treppenphänomens (nach Reizpausen von 10 min) in Ringerlösungen normaler Zusammensetzung, mit halbem Kaliumgehalt und mit doppeltem Calciumgehalt. Die Treppe wird durch Erhöhung des Calciumgehalts stärker gehemmt als durch Verminderung des Kaliumgehalts.

durch die Vorgänge in der Erholungsphase, T'_{1-7} ausserdem auch durch die Erregungsphasen der ersten Kontraktionen nach der Reizpause beeinflusst ist, deutet das Resultat offenbar darauf hin, dass Kalium (relativ zu Calcium) in der Erholung weniger stark auf die für die Treppe bestimmenden Vorgänge wirken muss als in der Erregung.

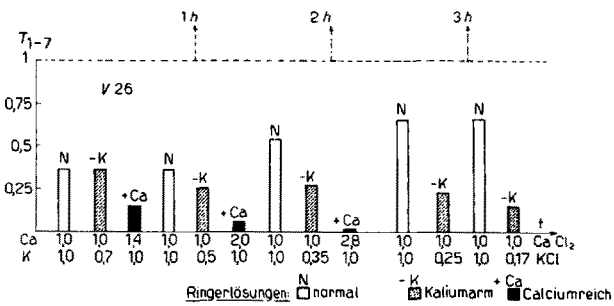

Abb. 2. Quantitative Bestimmung der Treppe mit Hilfe des Treppenquotienten T_{1-7} (Ordinate) am elektrisch gereizten Froschventrikelpräparat unter Variation der Zusammensetzung der Ringerlösung in bezug auf Kalium- und Calciumkonzentration. Abszisse: Zeit in Stunden. Unter jeder Messung ist die Zusammensetzung der verwendeten Ringerlösung angegeben (1,0 = normaler Calcium- bzw. normaler Kaliumgehalt).

Trifft unsere Annahme zu, dass Calciumionen in Erregung und Erholung gleichsinnig mit Natrium durch die Membran transportiert werden und dass die Treppe durch die Calciuminnenkonzentration am Ende der Reizpause bestimmt wird, so würde in der Erholung in erster Linie der Auswärtsflux des Calciums, in der Erregungsphase der Einwärtsflux als Angriffspunkt solcher Wirkungen zu betrachten sein. Verhältniszahlen wie die gefundenen, dürften mit einiger Wahrscheinlichkeit mit abgestuften Affinitäten zu Trägermolekülen in Beziehung stehen. Für detailliertere Deutungen scheint aber gegenwärtig die experimentelle Basis noch zu schmal.

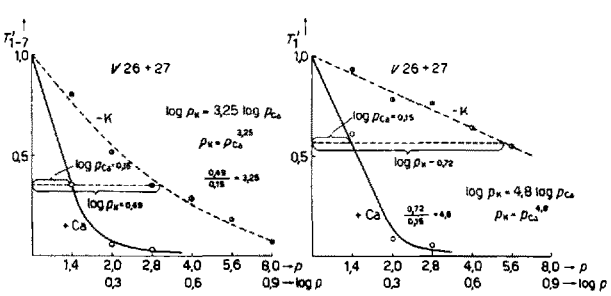

Abb. 3. Vergleich der Wirkung von Calciumüberschuss (+ Ca) und Kaliummangel (— K) auf das Treppenphänomen, das quantitativ mit Hilfe der relativen Treppenquotienten T' (nähere Definition siehe im Text) charakterisiert wird. Abszisse: Verhältniszahl p für die Veränderung der Calciumkonzentration (Erhöhung) bzw. der Kaliumkonzentration (Verminderung). Näheres im Text.

M. Moulin und W. Wilbrandt

Pharmakologisches Institut der Universität Bern, den 19. August 1954.

Summary

The stair-case phenomenon of Bowditch was studied on the electrically stimulated frog ventricle preparation. It depends not only on the concentration of potassium, as shown by Szent-Györgyi, but also on that of calcium. Excess of calcium as well as lack of potassium depress it (Fig. 1). A change of the calcium concentration in the ratio p yields a considerably stronger effect than a change of the potassium concentration in the ratio $1/p$ (Fig. 2 and 3). It is assumed that the stair case depends mainly on the concentration of calcium ions in the interior of the muscle fibers and that this concentration is affected not only by the outside concentration of calcium ions but (due to common affinities to carrier molecules) also by that of the potassium ions.

Some Metabolic Changes Occurring in Skeletal and Heart Muscles of Guinea Pigs Treated with Tetanus Toxin

The information available about the metabolic changes which occur in tetanized muscles is scarce. This paper reports the results of an investigation on the oxidative and phosphorylative activities of skeletal and heart muscles of guinea pigs after injection with tetanus toxin.

When the toxin of *Clostridium tetani* is injected intramuscularly in the guinea pig, tetanus is produced first in the muscles into which the toxin was injected (local tetanus). In a further stage, the animals develop tetanus also in the other, non-injected muscles (generalized tetanus). In this research, both stages were considered for the study of possible metabolic changes.

Guinea pigs weighing 230–300 g and fed with a standard diet were used. Tetanus toxin (a purified preparation from a culture filtrate of *Cl. tetani*, obtained by treatment with saturated ammonium sulphate) was injected

Table I.–Analysis of phosphorus fractions. (The data are referred to 1 g of fresh tissue and are given as mg ± σ.)

Phosphorus Fractions	Normal animals (4 experiments)		Animals with local tetanus (5 experiments)			Animals with generalized tetanus (3 experiments)		
	Leg muscle	Heart	Injected leg muscle	Controlateral leg muscle	Heart	Injected leg muscle	Controlateral leg muscle	Heart
Total P	1·788 ± 0·212	0·942 ± 0·008	1·404 ± 0·115	1·597 ± 0·208	0·891 ± 0·08	1·201 ± 0·200	1·148 ± 0·200	0·980 ± 0·04
"True" inorganic P	0·907 ± 0·252	0·431 ± 0·001	0·705 ± 0·096	0·901 ± 0·154	0·434 ± 0·135	0·605 ± 0·142	0·540 ± 0·02	0·540 ± 0·02
Phosphocreatine P	0·085 ± 0·04	0·038 ± 0·013	0·065 ± 0·009	0·063 ± 0·029	0·049 ± 0·014	0·104 ± 0·005	0·108 ± 0·005	0·014 ± 0·001
7′ P*	0·373 ± 0·061	0·124 ± 0·028	0·199 ± 0·079	0·243 ± 0·06	0·096 ± 0·023	0·149 ± 0·001	0·156 ± 0·04	0·260 ± 0·02
Ribose in this fraction	0·986 ± 0·162	0·390 ± 0·09	0·565 ± 0·184	0·680 ± 0·120	0·278 ± 0·038	0·450 ± 0·07	0·500 ± 0·06	0·085 ± 0·007
Molar ratio 7′P/ribose	1·83	1·54	1·70	1·73	1·61	1·60	1·52	1·71
ATP	1·687	0·365	0·759	0·967	0·319	0·459	0·442	0·329
ADP	0·436	0·393	0·411	0·629	0·259	0·410	0·515	0·169
HDP P	0·028 ± 0·014	0·088 ± 0·001	0·089 ± 0·048	0·075 ± 0·022	0·072 ± 0·006	0·048 ± 0·001	0·036 ± 0·012	0·063 ± 0·005
PPA P	0·135 ± 0·061	0·033 ± 0·017	0·094 ± 0·045	0·075 ± 0·014	0·056 ± 0·024	0·032 ± 0·04	0·045 ± 0·01	0·124 ± 0·004
Ba-soluble alcohol insoluble P	0·133 ± 0·07	0·136 ± 0·012	0·207 ± 0·023	0·216 ± 0·02	0·127 ± 0·01	0·250 ± 0·04	0·200 ± 0·02	0·105 ± 0·005
AMP	1·488	1·530	2·317	2·417	1·421	2·798	2·238	1·175
Ba-soluble alcohol soluble P	0·051 ± 0·007	0·096 ± 0·001	0·049 ± 0·016	0·040 ± 0·0	0·040 ± 0·019	0·050 ± 0·02	0·056 ± 0·01	0·052 ± 0·004
Adenine**	1·164	0·816	1·249	1·395	0·719	1·340	1·150	0·594
Total Recovery	1·712	0·946	1·408	1·613	0·874	1·238	1·141	0·983
Recovery %	95·6	100·4	100·2	101·0	98·1	103·0	99·3	100·3

* values corrected for 22·6% content of HDP P

** spectrophotometrically, 260 mμ

into the muscles of one leg. The injected amount was 10 L.D. pro animal. The animals were killed by bleeding and the leg muscles of both the injected and non-injected side, and the heart, were immediately removed and frozen with fluid carbonium dioxide. The amount to be used for analysis was then rapidly weighed and homogenized. The analysis of phosphorus (P) fractions was made on homogenates prepared by grinding the tissue (2 g of skeletal muscle or 1 heart deprived of the large vessels and of the fat) in a mortar and by suspending it in 7% trichloroacetic acid. The suspension was then homogenized in a Waring blendor by running for 30″ at 3000 R.P.M. The homogenate was then made up to 40 ml with 7% trichloroacetic acid, filtered and analysed. In one group of experiments, the animals were killed under magnesium sulphate anaesthesia, induced by intraperitoneal inoculation (PINCHOT and BLOOM[1]). Treatment with $MgSO_4$ was used by these authors in order to inhibit the splitting of ATP through ATPase, during the preparation of the extracts.

The analysis of phosphorus fractions was made according to the procedure described by LE PAGE[2]. 3 fractions were collected: Ba-insoluble (precipitation with Ba acetate, pH 8·2, followed by removal of Ba with H_2SO_4), Ba soluble-alcohol insoluble (precipitation with 4 volumes of absolute ethanol), Ba soluble-alcohol soluble. Following determinations were made on each of 3 fractions and on the original unfractionated extract: total P, inorganic orthophosphate P (IP) adenine. "True" inorganic P (precipitation with $CaCl_2$, saturated with $Ca(OH)_2$, pH 8·8) was determined only in the unfractionated extract. Phosphocreatine P was then calculated by difference. The following determinations were made on Ba-insoluble fraction, in addition to those reported above: 7′ P, ribose, fructose. 7′ P was determined after hydrolysis at 100°C for 8′ with 1 N HCl. The values obtained were corrected for 26·5% of total hexosediphosphate P, t.i. the amount of this substance which is hydrolysed in 8′ by 1 N HCl at 100°C. Ribose was determined by the orcinol method of MEIJBAUM[3], the extinctions being read at 660 mμ. From the molar ratios of 7′ P to ribose, the actual amounts of ATP and ADP respectively present in this fraction were calculated, by assuming that ATP contains 2 easily hydrolysable P units per mole of ribose and ADP only 1.

Hexosediphosphate P was calculated from the values obtained for fructose. This was determined by the resorcinol test of ROE[4], the extinctions being read at 490 mμ. Since hexosediphosphate does not react as the theoretical amount of fructose (LE PAGE), the actual values were obtained by multiplying the fructose values by the factor 3·6.

When the sum of the values obtained for inorganic orthophosphate P,7′ P and hexosediphosphate (HDP) is subtracted from that of total P present in the Ba-insoluble fraction, a residual amount of P is obtained. This is stated by LE PAGE to consist mainly of phosphopyruvic acid (PPA). It has been reported in the table under this heading.

The amount of adenine present in each fraction was also checked by reading the extinction at 260 mμ.

Phosphorus determinations were made by the method of FISKE and SUBBAROW[5]. The colour (Mo_3O_8) was de-

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Table II.–Succinoxidase, d-amino acid oxidase and cytochrome oxidase activities in normal and tetanized skeletal muscles of guinea pigs. (The values are referred to 1 g of fresh tissue and 1 h; in the case of sarcosomes, they are referred to the material isolated from 1 g of tissue. The data are given as $\mu l0_2 \pm \sigma$. Temperature 38° C.)

Animals	Number of experiments	Homogenates						Sarcosomes					
		Succinoxidase		d-aminoacidoxidase		Cytochrome oxidase		Succinoxidase		d-amino acidoxidase		Cytochrome oxidase	
		$\mu l0_2$	$\mu l0_2/N$	$\mu l0_2$	$\mu l0_2/N$	$\mu l0_2$	$\mu l0_2/N$	$\mu l0_2$	$\mu l0_2/N$	$\mu l0_2$	$\mu l0_2/N$	$\mu l0_2$	$\mu l0_2/N$
Normal	5	3595±318	214	620±29	36·9	4354±427	259	1647±156	1372	371±13	308	2420± 67	2016
Tetanized	6	3820±156	226	664±43	39·3	4423±374	261	1783±145	1114	370± 6	218	2517±243	1573

Nitrogen content of homogenates was mg 16·8 ± 1·2 for normal muscles, 16·9 ± 0·8 for tetanized muscles. That of mitochondria was 1·2 ± 0·1 for normal muscles, 1·6 ± 0·2 for tetanized muscles.

veloped with 40% $SnCl_2$ in concentrated HCl. Readings were made at 730 mμ. Nitrogen was determined by usual microkjeldhal technique.

Table III.–Oxidative phosphorylation in skeletal muscles of normal and treated guinea pigs. (The data represent μatoms O_2 taken up in 10′ at 25°C and μatoms P disappeared from the incubation mixture during the same time. 30 μM substrate, 200 mg tissue, reaction mixture as indicated in the text).

Animals Substrate	μAO	μAP	P:O
Normal glutamate	2·5	5·6	2·24
Normal glutamate	2·3	6·0	2·6
Normal α-ketoglutarate	2·0	7·6	3·7
Normal α-ketoglutarate	2·1	7·8	2·5
Local tetanus-glutamate.	2·4	6·2	2·4
Local tetanus-glutamate.	2·2	6·2	2·8
Local tetanus-α-ketoglutarate . .	2·1	7·9	3·7
Generalized tetanus-glutamate . .	2·0	4·0	2·0
Generalized tetanus-glutamate . .	2·2	4·2	1·9
Generalized tetanus-α-ketoglutarate	2·0	5·8	2·9

The homogenates for enzyme determinations were prepared in a Potter-Elvehjem glass apparatus with the technique described by SCHNEIDER[1]. The tissues were finely minced with scissors and ground in a mortar before submitting them to homogenization in the Potter-Elvehjem apparatus. Suspension fluid was 0·25 *M* sucrose. Succinoxidase was determined according to the method of POTTER and SCHNEIDER[2], d-amino acid oxidase with that of RODNEY and GARNER[3], with dl-alanine as a substrate. Cytochrome oxidase was determined according to the method of POTTER[4], ATPase activity according to that of POTTER and DUBOIS[5], with 0·01 N borate buffer, pH 7·0. Oxidative phosphorylation was studied at 25°C as previously reported (DIANZANI[6]), with 1 (+)-glutamate or with α-ketoglutarate as substrates, AMP as P acceptor, wheat germ hexokinase and fructose as a trapping system. P : O ratios were calculated by dividing the μatoms of inorganic orthophosphate P which disappeared from the incubation medium in 10′ by the μatoms of oxygen taken up by the system during the same time. Ratios very close to 3 were obtained for glutamate and to 4 for ketoglutarate in normal animals. The amount of tissue used corresponded to 200 mg of fresh tissue.

Table IV.–ATPase activity of homogenates, sarcosomes and myofibrils isolated from normal and from tetanized skeletal muscles of guinea pigs. (The values are given as mg inorganic orthophosphate P liberated in 1 h by 1 mg N of the enzymatic preparations. Standard deviations are also given.) Temperature 38° C, pH 7·0.

Animals	Number of experiments	Homogenates	Sarcosomes	Myofibrils
Normal . . .	6	37·5±5	128±37	49±15
Tetanized . .	5	39·5±5	147± 5	87±20

Nucleic acids determinations were made according to SCHMIDT and THANNHAUSER[1]. Myofibrils and muscle granules (sarcosomes) were isolated by using previously described methods (MOR[2]).

Each determination was made in duplicate. A BECKMAN spectrophotometer Mod. DU was used for the photometric determinations, a WARBURG conventional apparatus for the gasometric ones. The values obtained were studied statistically, standard deviation (σ), standard error of the standard deviation and the FISHER's "t" values being calculated for each average value[3].

Table I summarizes the results which were obtained in the experiments of fractionation of the P compounds. It is clear that the ATP content of tetanized skeletal muscles is strongly decreased. Animals with local tetanus showed a stronger decrease in the injected than in the controlateral leg. Such a difference disappeared in the animals showing generalized tetanus.

ADP concentration was slightly increased in tetanized muscles. This shows that the ADP synthesis through myokinase is probably unaffected. AMP, on the other hand, increased strongly. The total adenine content showed, therefore, only small modifications. An increase of HDP could be observed in the tetanized muscles. A certain degree of ATP decrease was found also in the heart of treated animals.

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[4] V. R. POTTER, *Manometric Techniques and Tissue Metabolism*, edited by W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER (Burgess Publ., Minneapolis, 1951).
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Table V.–Nucleic acids content of normal and tetanized skeletal muscles. The values are referred to 1 g of fresh tissue and are given as μg of NA, DNA or RNA phosphorus.

Animals	Number of experiments	Homogenates			Sarcosomes				
		Total NA	NA/N	DNA	DNA/N	RNA	RNA/N	RNA	RNA/N
Normal . . .	7	179 ± 14	10·6	44 ± 8	2·6	135 ± 7	8·0	20 ± 4	16·6
Tetanized . .	7	142 ± 12	8·4	31 ± 8	1·8	112 ± 11	6·6	24 ± 1	15·0

In the experiments which were made on animals killed under Mg sulphate anaesthesia, no difference was found between normal and tetanized muscles. The values found for ATP were about 20% higher, those for AMP about 20% lower, than those obtained in the non-anaesthesized guinea pigs. This is probably due to the fact that Mg sulphate produces inhibition of ATP splitting, while ATP synthesis is not greatly affected.

Table II shows that the oxidative capacities of tetanized muscles against succinate, dl-alanine and cytochrome c are practically not modified. Oxidative phosphorylation (Table III) is also quite normal in the earlier stage of tetanus, but decreases slightly with the progress of time. ATPase activity (Table IV) of both homogenates and sarcosomes does not show any significant modification. A striking difference was observed, however, in ATPase activity of myofibrils, which increased strongly in those isolated from tetanized muscles.

Nucleic acids (Table V) are decreased in tetanized muscles. The decrease affects both desoxyribo- and ribonucleic acids.

From this rapid survey of the results obtained, one may conclude that (1) ATP is strongly decreased in tetanized muscles; (2) ATP formation through the processes of oxidative phosphorylation is at least normal; (3) ATPase activity of myofibrils is strongly increased. (4) Nucleic acids are decreased. The most probable phenomenon responsible for the decrease of ATP thus resides in the activation of ATPase located within myofibrils.

ATP is known to exert in the muscle 2 fundamental functions: (1) it acts as a plasticizing (relaxing agent), which conditions the thread disposition of the polymeric actomyosine molecules; (2) it acts as the physiological contracting agent, its splitting being responsible for the shortening of the actomyosine threads. It has been shown (BATHE-SMITH[1], BENDALL[2], WEBER and PORTZEHL[3]) that skeletal muscle in state of rigor mortis contains a very low amount of ATP and that rigor itself is probably due to the absence of the plasticizing action of ATP. Whether a mechanism, in some manner similar to that which occurs in rigor mortis, exists in the tetanized muscle is under investigation in this laboratory.

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Riassunto

Gli Autori hanno studiato il metabolismo ossidativo e le frazioni fosforilate dell'omogenato di muscolo e di cuore in cavie normali e in cavie trattate con tossina tetanica. Nel muscolo tetanizzato si produce una forte diminuzione di ATP, insieme con un forte aumento di AMP e con un lieve aumento di ADP. La fosforilazione ossidativa è normale, la succinossidasi, la d-aminoacidossidasi e la citocromossidasi non subiscono modificazioni, la ATPasi dell'omogenato totale e quella mitocondriale non presentano differenze significative dal normale, ma la ATPasi presente nelle miofibrille è fortemente aumentata. Anche il contenuto di acidi ribo- e desossiribonucleinico è diminuito. Gli autori concludono che la diminuzione dell'ATP è dovuta ad una aumentata demolizione piuttosto che ad una diminuita sintesi.

Acetylcholine, Cholinergic Drugs, and Cortical Electrical Activity

It appears certain that acetylcholine (Ac) has some part to play at the level of the central nervous system, but this role has by no means been entirely clarified or put in its true context (FELDBERG[1]).

The conclusions which may be drawn after analysis of the results obtained in the series of investigations, which have appeared on this subject since 1937, cannot be definite. A certain number of facts are, however, available for discussion.

It is possible to influence cortical electrical activity either by cholinergic or anti-cholinergic drugs; and the modifications obtained are very similar to functional physiological states which can be induced in the normal animal. In fact, the modifications induced by anticholinesterasic drugs, such as eserine and DFP, closely resemble the state of activation of the cortex (WESCOE and co-workers[2], FUNDERBURK and CASE[3], BRADLEY and ELKES[4]), whereas those induced by atropine and other anticholinergic drugs resemble the state of rest or sleep (FUNDERBURK and CASE[3], LONGO and coworkers[5]).

On the other hand, BONNET and BREMER[6] have been able since 1937 to demonstrate a cortical action of Ac when injected by the carotid route, which was described as an increase in the amplitude and frequency of the

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