A STUDY OF OXYGEN DEBT IN THE ALBINO RAT: THE INFLUENCE OF TRAINING ON THE DISTRIBUTION OF PHOSPHATES AND OTHER CHEMICAL COMPONENTS OF THE BLOOD AND MUSCLE OF RATS.

by

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INTRODUCTION

The search for the chemical substance which is initially exploded during excitation and which frees the energy responsible for the series of events leading to contraction has been complicated by the fact that its products exist for so brief a period. The earliest experiments by Regnault and Reiset (1850) demonstrated that increased oxygen consumption and CO_2 liberation occur during muscular contraction. Combustion is, therefore, intimately associated with muscular contraction. The composition of muscle naturally led Liebig to suggest that protein is the fuel burned, but the observations of Pettenkofer and Voit that nitrogen excretion is not increased significantly during strenuous exercise definitely proved the fallacy of this conception (78). In 1896 Chauveau found an increase in respiratory quotient from 0.75 during rest to 0.95 during exercise and formulated the theory that energy is derived entirely from carbohydrates, a conclusion supported by Hill (37) and his associates. However, abundant evidence has been accumulated by Rapport (56) and Lundsgaard (43) indicating that muscle, like other tissues, oxidizes either carbohydrate or fat, or both, depending upon the relative quantities available. The necessity of carbohydrate during performance of hard work seems to have been demonstrated by Dill, Edwards and Talbott (44). Lundsgaard (43) found that fats seems to require partial oxidation in the liver before they can be utilized by muscles. Also, according to Lundsgaard acids and ketone bodies are readily

oxidized by muscle tissue.

The modern viewpoint may be said to have begun with the discovery by Fletcher and Hopkins (24) in 1907 that lactic acid increases during contraction. This, and the thermodynamic studies of A.V. Hill (38) Meyerhoff (49) and their respective pupils which followed, led to the hypothesis that an anaerobic breakdown of glycogen to lactic acid furnishes the initial energy of contraction. In accordance with their hypothesis about one-quarter to one-sixth of the lactic acid is oxidized and the remainder reconverted to glycogen within the muscles concerned.

After the cessation of totally or partially anaerobic chemical processes in muscle, oxygen consumption remains increased for some time until the oxidation of accumulated products of the anaerobic reactions has been completed and the muscle has been recharged. This period of recharging is called the recovery period and as A.V. Hill (37) has said - "the subject is paying his oxygen debt".

While the lactic acid theory of muscular contraction held sway, it was supposed that the first rapid repayment lasting for only a few minutes, was due to oxidation of lactic acid in muscles; and that delayed repayment, extending over an hour or more, was due to oxidation of lactic acid that has diffused into the blood. Evidence has accumulated, however, that blood lactic acid increases only during the most strenuous exercise. In moderate work, accompanied by an oxygen debt, blood lactic acid is not increased. It appears, therefore,

that the extent of oxygen debt is not a criterion of lactic acid concentration in the tissues according to D.B. Dill (13). According to Margaria, Edwards and Dill (45,47) the current trend of opinion is that in humans the excess oxygen taken in during recovery is only exceptionally used to oxidize lactic acid, as was originally believed and in fact, such usage occurs only when the oxygen debt exceeds 3-5 liters. When it is less than this, the increased oxygen consumed after exercise is used (a) for oxidation of alactacid substances, i.e., any available foodstuff that can yield energy for resynthesis and (b) to satisfy increased basal metabolism which persists for several hours after exercise.

Minor variations of this concept developed. Thus, Lusk (44) continued to believe that lactic acid as such cannot be oxidized. Shorr, Barker and Malam (66) held that it is the main avenue of glucose combustion. All that can be said is that experimental work has given no decisive answer.

The extensive investigations of the Embden School made it evident that phosphoric acid is intimately associated with muscular contraction. They believed that lactic acid cannot be formed directly from glycogen but only through a precursor, hexose phosphate (lactacidogen). The source of the phosphate, however, remained obscure until the Eggletons (19) in 1927, discovered the existence in muscle of a labile organic phosphorus compound that they called phosphagen, and which Fiske and Subbarow (21), also in 1927, identified it as phosphocreatine. These discoveries were rapidly followed by the de-

monstrations (a) that phosphocreatine is broken down during contraction and is resynthesized during recovery, (b) that lactic acid formation does not begin until contraction is well under way, and (c) the primary shift in reaction of muscle during contraction is toward the alkaline, not toward the acid side.

Muscle contraction theoretically is a purely anaerobic process, and oxidation occurs only during recovery. This theory (usually referred to as the Hill-Meyerhoff theory) has been questioned by several workers. Since 1945, Sacks and Sacks (59,60) have presented a series of papers supporting the conception that when the circulation is adequate, the fundamental reactions producing energy for contraction are oxidative. They grant that anaerobic sources are emergency mechanisms that operate (a) at the onset of exercise and before vascular adjustments are completed, (b) during high rates of stimulation and perhaps in severe prolonged exercise. However, as long as the oxygen supply is adequate, energy is released solely by oxidation of some substance. They believe with Fiske that hydrolysis of phosphocreatine is not a source of energy but that it acts to buffer lactic acid formed during anaerobic contraction. Substantial support for this view was produced by Millikan (50) who studied the time course of the reduction of myohemoglobin during contraction in the soleus muscle of the cat by means of an ingenious photo-electric arrangement.

Finally, some recent work by Flock, Ingle and Bolhman (25)

indicated that the major changes in glycogen and in the phosphorous compounds occur during the first minute of muscle contraction. This would suggest that the chain of reactions in the Hill-Meyerhoff scheme may occur only at the beginning of muscular exercise when the oxygen supply to contracting muscles is apt to be inadequate. When circulatory adjustments have brought about a steady state (a condition in which oxygen supply meets the oxygen requirements) other sources of energy are made available, - perhaps the oxidation of glucose supplied to the muscle by the blood.

Gemmill (28) has demonstrated that fat may be used indirectly as a source of energy for muscle contraction, especially when the carbohydrate supply is low.

Evidence has been accumulating that the liver is the chief site of ketogenesis and that destruction of ketone bodies occurs mainly in the extrahepatic tissues. Embden and Kalberlak (20) and later Snapper, Grunbaum and Neuberg (67) using the perfusion technique of Embden, offered experimental evidence that liver is the site of ketone body formation. The results of in vitro studies of Quastel and Wheatley (55), Jowett and Quastel (40), and Edson (18) have all supported this hypothesis. Evidence that destruction of ketone bodies is mainly the function of the extrahepatic tissues has been furnished by Snapper, Mirsky, Grunbaum (68,69).

The present investigation was undertaken to study simultaneously in the intact animal the production and distribution of phosphates and other components of the muscle and blood of the normal and trained rat at rest and under the influence

of complete fatigue. This included the determination of the concentration of such compounds as phosphocreatine, glycogen, lactic acid, glucose, and ketone bodies. In addition, the alkaline reserve of the blood was also determined in order to evaluate conflicting data on the concentration of blood buffers in trained and untrained individuals.

EXPERIMENTAL METHODS

Forty-six albino male rats weighing between 220-350 gm. and from a single colony were used. The control and experimental rats, matched for age and weight, were kept for similar periods of time on the same diet. The experimental rats, twenty-three in number, were progressively conditioned on a battery of exercise wheels (see Plate I) for a period of one month until they were able to run rapidly for ten minutes without difficulty. They were then kept in this condition, up to the time of sacrifice by running in the exercise wheel for ten minutes, every other day. Normal resting metabolism, as well as oxygen debt capacity were determined on all animals before the training of the experimental rats was undertaken. The oxygen consumption at rest and after cessation of severe exercise was measured in a closed circuit metabolism chamber. The principle which reflects the 100 year old method of Regnault and Reiset (57), depends upon measurement of the rate of change in volume in a closed circuit metabolism chamber kept at constant temperature and pressure. The metabolar was modified to meet the needs of the experiment (see Plate II and Figure I). The modification consisted of an exercise wheel built within the chamber, and an attached small electrified grill which was used as an inducer if, and when the animal refused to run.

A typical determination was made as follows: the animal was placed in the metabolar and the entire chamber submerged in a constant temperature water bath set to 28.0°C. The cham-

ber was then connected to a micro spirometer (Phipps & Bird) which contained the oxygen supply. At least 30 minutes were allowed for thermal equilibration and to permit the animal to become quiet. Carbon dioxide evolved by the animal was absorbed by soda lime contained in a well in the metabolar. During equilibration the animal was allowed to breath atmospheric air. When equilibration was complete, as signalled by constancy of the chamber temperature, the oxygen inlet valve was opened and oxygen consumption recorded for one hour. At the end of the hour the oxygen inlet was closed and the chamber again opened to atmospheric air. The exercise wheel was set into motion, with the speed being built up gradually so that the resting animal could adapt itself to the change. The electrical stimulation aided in this process. The animal was exercised until signs of exhaustion, such as, inability to maintain proper position, little or no response to electrical shock, and severe hyperventilation, were noticed. The exercise wheel was then stopped and the chamber immediately flushed out with a fresh supply of oxygen. Oxygen consumption was again recorded until normal resting level was reached. The difference between the oxygen consumed during rest and that consumed immediately after exercise up to the time the normal resting level was reached was considered to be the oxygen debt. Thus the O_2 debt was measured by determining the O_2 consumption during each of 3 definite time periods, one immediately following the other. These periods were as follows: (a) rest (b) the time the animal was run to

exhaustion on an exercise wheel built within the metabolar, and (c) immediately after the cessation of exercise and continued up to the time recovery or normal resting metabolism was resumed.

After the training period of one month the normal resting metabolism and oxygen debt were again determined on all animals, both trained and untrained. Thus, two groups of animals, twenty-three animals in each, were available for the experiment. Group I - the control or untrained laboratory rat and Group II - the trained or conditioned animal. For blood and tissue analysis, the groups were further divided into two groups of thirteen animals each. Group I(a) - in which analysis was carried out with the animal at rest, and Group I(b) - in which analysis was carried out after complete exhaustion of the animal (maximum oxygen debt attained) on the exercise wheel. The same procedure was followed with the trained animals, or Group II. The groupings of the experimental animals, as described above, can be best summarized by the following table:

	Rest	Exhausted
Trained	II(a)	II(b)
Untrained	1(a)	1(b)

Blood analysis included the determination of total ketone bodies, lactic acid, glucose, and alkaline reserve. Tissue analysis included the determination of phosphocreatine, glycogen, lactic acid, and total ketone bodies.

The animals were sacrificed by decapitation for the col-

lection of blood and tissue samples after the experimental conditions were satisfied. The blood was collected in a small beaker containing heparin and immediately placed in a tray of crushed ice.

The muscles of the hind legs of the animal, which included the adductor brevis, biceps femoris, vastus lateralis, gluteus maximus, semitendinosus, and gastrocnemuis were quickly and carefully excised and dropped immediately into liquid air. Thus samples of muscle were immediately frozen upon removal from the animal and kept in a frozen state until analysis was possible. Samples of tissue were weighed while in the frozen state.

The alkaline reserve was determined by the method of Van Slyke and Neil (75) on the Van Slyke Manometric gas analysis apparatus. Total ketone bodies (acetone, acetoacetic and Beta-hydroxybutyric acids) of blood was determined by the Gruenberg and Lester method (32). The total ketone bodies of the tissue were determined by a slight modification of the Van Slyke method (74), the difference being in the method of precipitation of proteins and other interferring substances as suggested by Harrison and Long (34).

Van Slyke's method is based on a combination of Shaffer's oxidation of Beta-hydroxybutyric acid to acetone and Deniges precipitation of acetone as a basic mecuric sulfate compound. Oxidation and precipitation are carried out simultaneously in the same solution, so that the technique is simplified to boiling the mixture for an hour and a half under a reflux condenser,

and weighing the precipitate which forms. The same process may be used for the smallest significant amounts of acetone bodies and likewise for the largest that are encountered. The precipitate is crystalline and beautifully adapted to quick drying and accurate weighing.

Lactic acid determinations were carried out on the blood and tissue samples by a method patterned after that of Barker and Summerson (2), but modified by Umbreit, Burris and Stauffer (75). Lactic acid is converted into acetaldehyde by treatment with concentrated sulfuric acid and the acetaldehyde determined by its color reactions with p-hydroxydiphenyl in the presence of cupric ions. The color was read in a photoelectric colorimeter with a filter having a peak transmission of 565 mu.

Muscle glycogen was determined by a method patterned after that of Good, et al. (30) in which glycogen is precipitated out of the tissue, and hydrolysed for two hours in 1N HCl at 100°C. The hydrolysate is neutralized and the reducing sugar measured with a copper reagent, such as that of Shaffer and Somogyi (65).

Glucose of the blood was determined by the method of Shaffer, Hartman and Somogyi as outlined by Hawk, Oser, and Summerson (35).

The determination of phosphocreatine was carried out by the calcium precipitation method of Fiske and Subbarow (22).

The data obtained during the experimental period were not checked by duplicate determinations in all cases because of

the economy which had to be exercised with the samples of blood and of the unavailability of extra samples. Checks were made only on data which seemed unusual or markedly different from those already obtained. Also, spot checks were made at intervals during the entire experimental period to enhance the reliability of the data and to test the accuracy of the apparatus.

EXPERIMENTAL RESULTS

I Normal Resting Metabolism and Oxygen Debt.

Table I shows normal resting metabolism, oxygen debt and weight of the experimental animals. These figures represent data collected on all animals before training was started, from which group a certain number of animals were to be selected at random, for conditioning. Resting metabolism is recorded in ml. of oxygen consumed per hour. It is to be noted in all determinations of metabolism, oxygen consumption represents raw data reduced to standard temperature and pressure, but it is not expressed as oxygen consumed per square meter of body surface per hour as is usually done in recording basal metabolic rates for humans.

After the initial determination of oxygen consumption and oxygen debt, the animals were divided into two equal groups. One group was trained or conditioned by running in an exercise wheel daily for a period of one month as described previously in the experimental methods. Following the training period of one month, normal resting metabolism and oxygen debt were again determined. Table II records these results on all of the animals now designated as Group I, the control or normal laboratory rats; and Group II, the trained or conditioned animals.

Differences between weights, metabolic rates, and oxygen debts of the untrained group before and after a period of one month, during which time animals in Group II were being trained, appear in Table III. Differences are recorded as percentages, plus or minus, indicating an increase or decrease respec-

tively.

Table IV presents data comparing the weights, resting oxygen consumption and oxygen debt of Group II animals, i.e., the trained or conditioned group after a training period of one month. Averages are recorded of all data as well as the range and standard deviation.

Data collected on the group of rats after one month of progressive rapid running on the exercise wheel and that, collected on the untrained group after a lapse of time of one month, are compared in Table V. Thus Table V demonstrates the effects of training on weight, normal resting metabolism, and oxygen debt. Since two different groups of animals were being compared, individual differences were not recorded. Delta represents the average percentage increase or decrease for the total number of animals in either Group I or Group II.

Although weights were recorded for all animals, they were not considered pertinent to the particular problem under consideration, and were recorded merely as routine procedure. Previous correlation studies of weight and oxygen consumption, carried out on the same group of animals used for this experiment, indicated a very poor correlation.

II Blood Analysis

Table VI shows data collected on nine untrained rats under condition of rest. Analysis included determination for alkaline reserve, lactic acid, glucose, and total ketone bodies. Individual determinations on all animals, as well as the mean, the range, and standard deviation from the mean are listed in

Table VI. In Table VIII may be found data from the blood analysis of untrained animals after complete exhaustion and maximum oxygen debt was attained.

A comparison of the above data is shown in Table X. The alkaline reserve, lactic acid, glucose, and total ketone body content of blood, of the untrained group, are reported under conditions of rest and after exhaustive exercise. The lactic acid concentration of the blood increases in the exhausted animals to an amount of 805 per cent, while the glucose content of the blood decreased an average of 62 per cent. In the exhausted animals there was an average decrease in the alkaline reserve of 30 per cent. The total ketone body concentration of the blood of exhausted untrained animals increased an average of 178 per cent over the normal resting animal.

Data accumulated from the blood analysis of trained animals under conditions of rest and after strenuous exercise are reported in Tables VI and VIII. Table VI records the data collected from animals that were trained or conditioned on a treadmill for a period of one month. A total of eleven animals comprised this group and data represents analysis made for blood components when the animals were in the resting state. In turn, Table VIII shows the response of trained animals, through their blood picture, to exhaustive exercise and when maximum oxygen debt was developed. A total of twelve animals were used for these determinations.

A comparison of similar data, between controls and trained animals at rest and after exhaustion is recorded in Tables XIV

and XV. These data offer some insight into the effect of training on the alkaline reserve, lactic acid, glucose and total ketone body concentration of blood. Differences between the blood concentrations of the various components mentioned above of trained and untrained animals at rest are shown in Table XIV, while Table XV shows the difference after the animals had been run to complete exhaustion.

The effect of training on the oxygen debt and the various chemical components of the blood is shown in Table XIX. Data found in this table represent the means of the determinations. Figures for the chemical components labeled "Before" represent untrained animals, while those labeled "After" represent trained animals.

III Tissue Analysis

Tissue for analysis was carefully dissected from the hind leg of the animal and immediately immersed in liquid air. Determinations were made for glycogen, lactic acid, total ketone bodies, and phosphocreatine by methods previously described or referred to in the experimental methods. Data from the analysis of hind leg muscle protein-free filtrates of untrained rats at rest is recorded in Table VII. No ketone bodies could be found in any of the tissue of animals under resting conditions and were reported as zero values in the tables. This was done instead of using blank spaces to indicate negative results, because blank spaces in the tables were otherwise used to indicate loss of an animal due to death or other causes during the experimental period.

Results of the tissue analysis of untrained animals after complete exhaustion by forced running in an exercise wheel are listed in Table IX. A total of eight animals were used in these analysis. In the ketone body determinations of this group, two out of the eight animals analyzed showed no ketone body production. The average ketone body content of all eight animals amounted to 1.38 mgm. per cent.

A comparison of the data described above, i.e., results of tissue analysis of the untrained animals before and after exhaustion may be found in Table XI. Since no ketone bodies could be found in resting muscle, and an average of 1.38 mgm. per cent in exhausted tissue of untrained animals, a comparison represents an infinite change and is recorded as ∞ in the tables.

Table VII, in addition to showing data collected from tissue analysis on untrained animals under conditions of rest, also records the data collected from animals that were conditioned in an exercise wheel. As in the case of the untrained resting animals, no ketone bodies could be found in the tissue of the trained animals under conditions of rest. The effect of exhaustive exercise on the various chemical components of the tissue of trained animals is shown in Table IX.

A comparison of the tissue data of trained animals at rest and after exhaustion is recorded in Table XIII. A shown, there was a decrease of 74.5 per cent in the glycogen content of the muscle of exhausted animals. The lactic acid concentration of the muscle tissue of exhausted trained animals was 243 per cent

higher than the lactic acid concentration in the resting tissue, indicating an approximate 54 fold change. Changes in ketone body concentration were quite marked in the trained group of animals since at rest no ketone bodies could be found in the tissue, but after exhaustive exercise an average of 8.65 mgm. per cent was found. Phosphocreatine decreases were also quite definite, as a comparison of the average phosphocreatine content of muscle of trained animals in the resting condition and after exhaustion showed an average decrease of 87 per cent.

A comparison of similar data between controls and conditioned animals at rest and after exhaustion is recorded in Tables XVI and XVII. This data gives some idea of the effect of training on the various chemical components of muscle tissue. Under resting conditions, i.e., the normal state, the animals that were trained showed an average muscle glycogen content that was 35 per cent greater than those animals which were considered normal laboratory animals. The conditioned animal also showed a lactic acid content which was 28.3 per cent higher than the untrained animal. No ketone bodies could be found in either the trained or untrained animals' muscle at rest. The phosphocreatine content of the muscle tissue of the trained animals was 74 per cent greater on the average than the phosphocreatine content of the untrained animals.

A comparison of the tissue data of the conditioned and normal animals, as shown in Table XVII, indicates that after exhaustive exercise, the trained animal is capable of utilizing

more of its glycogen stores since the trained group was able to use on the average of 38.1 per cent more as shown by calculation. In addition, the trained group was able to build up a lactic acid concentration which was 27.2 per cent greater than the untrained animals. Training also had some beneficial effects upon ketone body tolerance and phosphocreatine utilization.

Table XVIII compares the average of all data on tissue before and after training, including oxygen debt and weights of animals.

TABLE I

Normal Oxygen Consumption and Oxygen Debt
of all Experimental Animals Before Training.

Rat No.	Weight in gms.	O_2 Consumption at Rest cc/hr	O_2 Debt in ml.	Rat No.	Weight in gms.	O_2 Consumption at Rest cc/hr	O_2 Debt in ml.
*1	235	380	75	26	320	400	135
*2	245	380	80	*27	325	400	75
*3	257	360	70	*28	315	380	120
*4	275	430	125	*29	319	415	125
*5	237	300	85	*30	320	490	135
*6	255	370	85	31	348	375	85
*7	240	350	90	32	285	475	125
*8	310	480	130	33	255	355	97
*9	285	460	100	34	348	485	80
*10	265	490	85	35	350	355	110
*11	246	365	75	36	325	410	85
*12	285	500	120	37	---	---	---
*13	305	460	70	38	330	450	135
*14	290	560	90	39	310	450	85
*15	298	470	125	40	293	425	105
*16	265	480	80	41	230	380	80
17	287	375	105	42	285	430	120
18	290	430	100	43	299	465	125
*19	255	350	85	44	310	480	130
*20	273	510	120	45	325	405	90
21	330	385	90	46	277	370	75
22	318	360	80	*47	300	375	70
*23	320	490	---	48	305	380	75
24	340	500	75	49	---	---	---
*25	320	400	135	50	325	420	110
Average	291	428	89.7				

* Animals selected for training

TABLE II

Normal Resting Oxygen Consumption and Oxygen Debt of the Trained Animals and Untrained After one Month.

GROUP I UNTRAINED				GROUP II TRAINED			
Rat No.	Weight in gms.	O_2 Consumption at Rest ml./hr	O_2 Debt ml.	Rat No.	Weight in gms.	O_2 Consumption at Rest ml./hr	O_2 Debt ml.
17	430	440	140	1	340	384	296
18	418	430	115	2	435	266	416
21	390	408	79	3	430	382	200
22	375	383	85	4	340	250	258
24	422	505	80	5	415	316	291
26	---	---	---	6	375	400	419
31	415	466	90	7	375	365	305
32	450	336	100	8	410	490	410
33	436	470	85	9	465	450	250
34	417	425	75	10	420	490	325
35	---	---	---	11	440	395	375
36	447	425	100	12	420	334	307
37	---	---	---	13	460	499	226
38	405	468	73	14	485	293	490
39	365	456	94	15	480	350	258
40	400	430	110	16	420	366	233
41	430	385	90	19	355	334	299
42	385	460	130	20	405	332	206
43	419	460	115	23	485	482	262
44	410	475	120	25	470	353	438
45	445	500	99	27	460	499	375
46	405	395	108	28	445	350	350
48	---	---	---	29	415	358	267
49	---	---	---	30	446	358	250
50	455	492	100	47	465	305	275
Average	415	440	99.4		430	376	310

TABLE III

Normal Oxygen Consumption and Oxygen Debt of Group I (Untrained) Before and After a Period of one Month.

Rat No.	Weight in gms.			O_2 Consumption at Rest in ml./hr			O_2 Debt in ml.		
	Before	After	%change	Before	After	%change	Before	After	%change
17	287	430	+ 49	375	440	+ 14	105	140	+ 33
18	290	418	+ 44	430	430	0	100	115	+ 15
21	330	390	+ 18	385	408	+ 6	90	79	- 13
22	318	375	+ 18	360	383	+ 6	80	85	+ 6
24	340	422	+ 24	500	505	+ 1	75	80	+ 6
26	320	---	---	400	---	---	135	85	- 37
31	348	415	+ 11	375	466	+ 24	85	90	+ 5
32	285	450	+ 57	475	336	- 29	125	100	- 20
33	330	436	+ 32	450	470	+ 4	135	---	---
34	348	417	+ 19	485	425	- 13	80	75	- 6
35	350	---	---	355	---	---	110	---	---
36	325	447	+ 37	410	425	+ 6	85	100	+ 17
37	---	---	---	---	---	---	---	---	---
38	305	405	+ 32	380	468	+ 23	75	73	- 3
39	310	365	+ 17	450	456	+ 1	85	94	+ 10
40	293	400	+ 35	425	430	+ 1	105	110	+ 5
41	230	330	+ 43	380	385	+ 1	80	90	+ 12
42	285	385	+ 35	430	460	+ 7	120	130	+ 8
43	299	419	+ 40	465	460	- 1	125	115	- 8
44	310	410	+ 32	480	475	- 1	130	120	- 8
45	325	445	+ 36	405	500	+ 23	90	99	+ 10
46	277	405	+ 46	370	395	+ 7	75	108	+ 44
48	---	---	---	---	---	---	---	---	---
49	---	---	---	---	---	---	---	---	---
50	325	455	+ 40	420	492	+ 17	110	100	- 8
Average	310	415	+ 33	418	440	+ 5	100	99.4	- .6

TABLE IV

Normal Oxygen Consumption and Oxygen Debt of Group II (Trained Animals) Before and After a Period of one Month's Trai ning on Exercise Wheel.

Rat No.	Weight in gms.			O_2 Consumption at Rest ml./hr			O_2 Debt ml.		
	Before	After	%change	Before	After	%change	Before	After	%change
1	235	340	+ 44	380	384	+ 1	75	296	+ 279
2	245	435	+ 77	380	266	- 30	80	416	+ 420
3	257	430	+ 67	360	382	+ 6	70	200	+ 185
4	275	340	+ 23	430	250	- 42	125	258	+ 106
5	237	415	+ 75	300	316	+ 5	85	291	+ 242
6	255	375	+ 47	370	400	+ 8	85	419	+ 392
7	240	375	+ 56	350	365	+ 4	90	305	+ 238
8	310	410	+ 32	480	490	+ 2	130	410	+ 215
9	285	465	+ 63	460	450	- 2	100	250	+ 150
10	265	420	+ 58	490	490	0	85	325	+ 282
11	246	440	+ 78	365	395	+ 8	75	375	+ 400
12	285	420	+ 47	500	334	- 33	120	307	+ 155
13	305	460	+ 50	460	499	+ 8	70	226	+ 222
14	290	485	+ 67	560	293	- 48	90	490	+ 444
15	298	480	+ 61	470	350	- 26	125	258	+ 106
16	265	420	+ 58	480	366	- 24	80	233	+ 191
19	255	355	+ 39	350	334	- 5	85	299	+ 251
20	273	405	+ 48	510	332	- 35	120	206	+ 71
23	320	485	+ 51	490	482	- 2	---	262	---
25	320	470	+ 46	400	353	- 12	135	438	+ 224
27	325	460	+ 41	400	499	+ 24	75	375	+ 400
28	315	445	+ 42	380	350	- 8	120	350	+ 191
29	319	415	+ 30	415	358	- 14	125	267	+ 113
30	320	446	+ 39	490	358	- 27	135	250	+ 85
47	300	465	+ 55	375	305	- 19	70	275	+ 292
Average	282	430	+ 53	426	376	- 12.3	98	311	+ 217

TABLE V

Effect of Training on Weight, Normal Resting Metabolism, and Oxygen Debt.

No. of Animals		Weight in Gms.			O_2 Consumption ml./hr			O_2 Debt ml.		
Untrained	Trained	Untrained	Trained	Δ	Untrained	Trained	Δ	Untrained	Trained	Δ
20	25	430	340		440	384		140	296	
		418	435		430	266		115	416	
		390	430		408	382		79	200	
		375	340		383	250		85	258	
		422	415		505	316		80	291	
		415	375		466	400		85	419	
		450	375		336	365		90	305	
		436	410		470	490		100	410	
		417	465		425	450		75	250	
		447	420		425	490		100	325	
		405	440		468	395		73	375	
		365	420		456	334		94	307	
		400	460		430	499		110	226	
		330	485		385	293		90	490	
		385	480		460	350		130	258	
		419	420		460	366		115	233	
		410	355		475	334		120	299	
		445	405		500	332		99	206	
		405	485		395	482		108	262	
		455	470		492	353		100	438	
			460			499			375	
			445			350			350	
			415			358			267	
			446			358			250	
			465			305			275	
	Average	415	430	+ 3	440	376	- 14.6	99.4	311	+ 213

TABLE VI

Blood Analysis of Trained and Untrained Group During Resting State.

TRAINED					UNTRAINED				
Rat No.	Alkaline Reserve Vols.%	Lactic Acid mgm.%	Glucose mgm.%	Total Ketone mgm.%	Rat No.	Alkaline Reserve Vols.%	Lactic Acid mgm.%	Glucose mgm.%	Total Ketone mgm.%
47	63.0	18	83	2.64	50	53	16.5	87	1.75
23	67.0	24	76	3.50	33	48	18.7	74	2.40
27	61.0	13	92	1.76	40	50	19.2	80	1.80
2	58.0	10	51	1.10	34	52	14.0	72	.209
3	56.0	12	55	1.30	39	48	15.0	48	1.56
30	65.0	18	104	1.26	38	43	26.0	50	1.80
15	76.0	18	130	2.38	42	57	16.0	86	1.94
9	77.0	24	89	2.60	22	51	15.8	69	1.74
28	75.0	22	77	0.984	24	47	20.6	63	0.780
20	53.0	20	55	1.08					
5	74.0	15	52	0.897					
Aver.	65.9	17.6	78.5	1.773		49.8	17.9	69	1.55
Range	21	14	79	2.6		10	12	37	2.19
SD	± 6.12	± 4.65	± 23.21	± 2.44		± 11.35	± 10.34	± 14.24	±2.24

TABLE VII

Analysis of Muscle Tissue of Trained and Untrained Rats Under Experimental Conditions of Rest.

Rat No.	TRAINED Glycogen mgm.%	Lactic Acid mgm.%	Total Ketone mgm.%	Phospho-creatine mgm.%	Rat No.	UNTRAINED Glycogen mgm.%	Lactic Acid mgm.%	Total Ketone mgm.%	Phospho-creatine mgm.%
47	176	24.4	0	75	50	104.7	26.4	0	52
23	130	22.0	0	70	33	94.82	20.2	0	49
27	120	30.0	0	72	41	87.13	25.0	0	54
2	93.5	18.0	0	62	34	145.0	18.0	0	48
3	104.7	15.0	0	68	43	99.10	20.0	0	55
30	168.0	22.5	0	64	38	98.70	21.0	0	51
15	156.0	26.0	0	69	42	133.0	21.0	0	48
9	104.5	28.0	0	73	22	126.0	23.6	0	57
28	194.0	28.6	0	72	24	97.64	19.8	0	52
20	85.0	18.7	0	74					
5	130.0	22.0	0	72					
Aver.	133.4	27.7	0	70.0		98.4	21.6	0	51.6
Range	109	15	0	13		58	6	0	9
SD	± 12.36	± 6.78	0	± 4.12		± 20.73	± 2.64	0	± 3.16

TABLE VIII

Blood Analysis of Trained Animals and Untrained Animals After Complete Exhaustion.

TRAINED					UNTRAINED				
Rat No.	Alkaline Reserve Vols.%	Lactic Acid mgm/ 100ml.	Glucose mgm/ 100ml.	Ketone mgm/ 100ml.	Rat No.	Alkaline Reserve Vols.%	Lactic Acid mgm/ 100ml.	Glucose mgm/ 100ml.	Ketone mgm/ 100ml.
19	31	189	64	9.87	44	16	205	36	2.68
25	28	210	25	14.8	41	43	155	53	2.64
6	41	185	35	6.83	32	33	180	55	1.926
29	29	205	38	11.40	31	28	95	48	6.17
1	33	208	43	8.43	45	46	200	42	3.64
10	25	235	39	6.93	43	32	150	37	5.83
14	38	205	26	6.73	46	44	176	39	4.76
12	22	187	39	10.50	21	37	135	30	6.93
4	29	226	34	10.385					
8	24	164	33	5.98					
7	28	213	33	10.480					
11	33	208	28	5.432					
Aver.	30.0	203	36.4	8.980		34.9	162	42.5	4.32
Range	19	71	39	9.4		30	110	25	5
SD	± 5.56	± 19.15	± 10.29	± 2.74		± 9.94	± 44	± 8.77	± 1.84

TABLE IX

Analysis of Muscle Tissue of Trained and Untrained Rats After Complete Exhaustion.

TRAINED					UNTRAINED				
Rat No.	Glycogen mgm.%	Lactic Acid mgm.%	Total Ketone mgm.%	Phosphocreatine mgm.%	Rat No.	Glycogen mgm.%	Lactic Acid mgm.%	Total Ketone mgm.%	Phosphocreatine mgm.%
19	18.5	296	3.50	10	44	64	302	1.235	18
25	24.0	325	3.65	0	41	68	201	.987	29
6	34.0	315	18.50	16	32	60	215	4.88	12
29	42.0	296	16.80	5	31	54.3	108	1.507	15
1	54.0	297	5.63	2	45	23.0	302	0	22
10	62.0	315	3.92	12	43	48	293	1.48	15
14	35.0	340	2.156	10	46	65	275	0	26
12	24.4	258	19.60	14	21	55	245	.987	38
4	14.20	350	2.716	5					
8	32.50	316	10.830	22					
7	28.76	310	3.897	14					
11	36.8	286	12.685	0					
Aver.	33.85	308.6	8.657	9.16		54.66	242.6	1.384	21.8
Range	48	92	16.7	20		45	194	4.8	26
SD	± 13.85	± 24.55	± 6.71	± 6.16		± 3.31	± 66.85	±1.73	± 8.71

TABLE X

A comparison of Blood Data of Untrained Animals at Rest and After Exhaustion.

Total No. of Animals		Alkaline Reserve Vols.%			Lactic Acid mgm/100ml.			Glucose mgm/100ml.			Total Ketone mgm/100ml.		
Rested	Exhausted	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ
9	8	53	16		16.5	205		87	36		1.75	2.68	
		48	43		18.7	155		74	53		2.40	2.64	
		50	33		19.2	180		80	55		1.80	1.926	
		52	28		14.0	95		72	48		.209	6.17	
		48	46		15.0	200		48	42		1.56	3.64	
		43	32		26.0	150		50	37		1.80	5.83	
		57	44		16.0	176		86	39		1.94	4.76	
		51	37		15.8	135		69	30		1.74	6.93	
		47			20.6			63			.780		
Average		49.8	34.9	-30	17.9	162	+805	69.0	42.5	-62	1.55	4.32	+ 178

Δ = % increase or decrease

TABLE XI

A Comparison of Tissue Data of Untrained Animals at Rest and After Exhaustion.

Total No. of Animals		Glycogen mgm.%			Lactic Acid mgm.%			Total Ketone mgm.%			Phosphocreatine mgm.%		
Rested	Exhausted	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ
9	8	104.7	64		26.4	302		0	1.235		52	18	
		94.82	68		20.2	201		0	.987		49	29	
		87.13	60		25.0	215		0	4.88		54	12	
		145.0	54.3		18.0	108		0	1.507		48	15	
		99.10	23.0		20.0	302		0	0		55	22	
		98.70	48.0		21.0	293		0	1.48		51	15	
		133.0	65		21.0	275		0	0		48	26	
		126	55		23.6	245		0	.987		57	38	
		97.64			19.8			0			52		
Average		98.4	54.66	-44	21.6	242.6	+1004	0	1.384	∞	51.6	21.8	-58

Δ = % increase or decrease

TABLE XII

A Comparison of Blood Data of Trained Animals at Rest and After Exhaustion.

Total No. of Animals		Alkaline Reserve Vols.%			Lactic Acid mgm/100ml.			Glucose mgm/100ml.			Total Ketone mgm/100ml.		
Rested	Exhausted	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ
11	12	63	31		18	189		83	64		2.64	9.87	
		67	28		24	210		76	25		3.50	14.8	
		61	41		13	185		92	35		1.76	6.83	
		58	29		10	205		51	38		1.10	11.40	
		56	33		12	208		55	43		1.30	8.43	
		65	25		18	235		104	39		2.38	6.93	
		76	38		18	205		130	26		2.60	6.73	
		77	22		24	187		89	39		.984	10.50	
		75	29		22	226		77	34		1.08	10.385	
		53	24		20	164		55	33		.897	5.98	
		74	28		15	213		52	33		1.26	10.480	
			33			208			28			5.432	
Average		65.9	30	-54.5	17.6	203	+ 1054	78.5	36.4	-53.7	1.773	8.980	+406

TABLE XIII

A Comparison of Tissue of Trained Animals at Rest and After Exhaustion.

Total No. of Animals		Glycogen mgm.%			Lactic Acid mgm.%			Total Ketone mgm.%			Phosphocreatine mgm.%		
Rested	Exhausted	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ	Rested	Exhausted	Δ
11	12	176	18.5		24.4	296		0	3.50		75	10	
		130	24.0		22.0	325		0	3.65		70	0	
		120	34.0		30.0	315		0	18.50		72	16	
		93.5	42.0		18.0	296		0	16.80		62	5	
		104.7	54.0		15.0	297		0	5.63		68	2	
		168.0	62.0		22.5	315		0	3.92		64	12	
		156.0	35.0		26.0	340		0	2.156		69	10	
		104.5	24.4		28.0	258		0	19.60		73	14	
		194.0	14.2		28.6	350		0	2.716		72	5	
		85.0	32.5		18.7	316		0	10.830		74	22	
		130.0	28.76		22.0	310		0	3.897		72	14	
			36.8			286			12.685			0	
Average		133.4	33.85	-74.5	22.7	308.6	+1243	0	8.657	∞	70	9.16	-87

TABLE XIV

A Comparison of Blood Data Between Trained and Untrained Animals at Rest.

No.of Animals Untrained	Trained	Alkaline Reserve Vols.% Untrained	Trained	Δ	Lactic Acid mgm/100ml. Untrained	Trained	Δ	Glucose mgm/100ml. Untrained	Trained	Δ	Total Ketone mgm/100ml. Untrained	Trained	Δ
9	11	53	63		16.5	18		87	83		1.75	2.64	
		48	67		18.7	24		74	76		2.40	3.50	
		50	61		19.2	13		80	92		1.80	1.76	
		52	58		14.0	10		72	51		0.290	1.10	
		48	56		15.0	12		48	55		1.56	1.30	
		43	65		26.0	18		50	104		1.80	1.26	
		57	76		16.0	18		86	130		1.94	2.38	
		51	77		15.8	24		69	89		1.74	2.60	
		47	75		20.6	22		63	77		0.780	0.984	
			53			20			55			1.08	
			74			15			52			0.897	
Average		49.8	65.9	+32	17.9	17.6	-1.7	69.0	78.5	+13.7	1.550	1.773	+ 17

TABLE XV

A Comparison of Blood Data Between Trained and Untrained Animals After Exhaustion.

No.of Animals Untrained	Trained	Alkaline Reserve Vols.% Untrained	Trained	Δ	Lactic Acid mgm/100ml. Untrained	Trained	Δ	Glucose mgm/100ml. Untrained	Trained	Δ	Total Ketone Bodies mgm/100ml. Untrained	Trained	Δ
8	12	16	31		205	189		36	64		2.68	9.87	
		43	28		155	210		53	25		2.64	14.8	
		33	41		180	185		55	35		1.926	6.83	
		28	29		95	205		48	38		6.17	11.40	
		46	33		200	208		42	43		3.64	8.43	
		32	25		150	235		37	39		5.83	6.93	
		44	38		176	205		39	26		4.76	6.73	
		37	22		135	187		30	39		6.93	10.50	
			29			226			34			10.385	
			24			164			33			5.98	
			28			213			33			10.480	
			33			208			28			5.432	
Average		34.9	30.0	-14.1	162	203	+25.3	42.5	36.4	-14.4	4.32	8.980	+107

TABLE XVI

A Comparison of Tissue Analysis Data Between Trained and Untrained Animals at Rest.

Total No. of Animals		Glycogen mgm.%			Lactic Acid mgm.%			Total Ketone Bodies mgm.%			Phospho-creatine mgm.%		
Un-trained	Trained	Un-trained	Trained	Δ	Un-trained	Trained	Δ	Un-trained	Trained	Δ	Un-trained	Trained	Δ
9	11	104.70	176		26.4	24.4		0	0		52	75	
		94.82	130		20.2	22.0		0	0		49	70	
		87.13	120		25.0	30.0		0	0		54	72	
		145.0	93.5		18.0	18.0		0	0		48	62	
		99.10	104.7		20.0	15.0		0	0		55	68	
		98.70	168.0		21.0	22.5		0	0		51	64	
		133.0	156.0		21.0	26.0		0	0		48	69	
		126.0	104.5		23.6	28.0		0	0		57	73	
		97.64	194.0		19.8	28.6		0	0		52	72	
			85.0			18.7			0			74	
			130.0			22.0			0			72	
Average		98.4	133.4	±35	21.6	27.7	±28.3	0	0		51.6	70.0	±74

TABLE XVII

A Comparison of Tissue Analysis Data Between Trained and Untrained Animals After Exhaustion.

Total No. of Animals		Glycogen mgm.%			Lactic Acid mgm.%			Total Ketone Bodies mgm.%			Phospho-creatine mgm.%		
Un-trained	Trained	Un-trained	Trained	Δ	Un-trained	Trained	Δ	Un-trained	Trained	Δ	Un-trained	Trained	Δ
8	12	64	18.5		302	296		1.235	3.50		18	10	
		68	24.0		201	325		0.987	3.65		29	0	
		60	34.0		215	315		4.88	18.50		12	16	
		54.3	42.0		108	296		1.507	16.80		15	5	
		23.0	54.0		302	297		0	5.63		22	2	
		48	62.0		293	315		1.48	3.92		15	12	
		65	35.0		275	340		0	2.156		26	10	
		55	24.40		245	258		.987	19.60		38	14	
			14.20			350			2.716			5	
			32.50			316			10.830			22	
			28.76			310			3.897			14	
			36.80			286			12.685			0	
Average		54.66	33.85	-38.1	242.6	308.6	+27.2	1.384	8.657	+525	21.8	9.16	-58

Δ = % increase or decrease

TABLE XVIII

The Effect of Training on the Oxygen Debt and the Various Chemical Components of Muscle Tissue.

Condition	Weight in gms. Before	After	Δ	O_2 Debt ml. Before	After	Δ	Total Ketone mgm.% Before	After	Δ	Lactic Acid mgm.% Before	After	Δ	Phosphocreatine mgm.% Before	After	Δ	Glycogen mgm.% Before	After	Δ
	291	430	+53	99.4	311	+213												
Resting							0	0		21.6	21.7	+28.3	51.6	70.0	+74	98.4	133.4	+35
Exhaustion							1.384	8.657	+525	242.6	308.6	+27.2	21.8	9.16	-58	54.6	33.85	-38.1
Δ										+1023	+1243		-57.8	-87		-45	-75	

Note: Figures represent averages. "Before" figures for chemical componers represent untrained animals, while the "After" figures represent trained animls.

TABLE XIX

The Effect of Training on Oxygen Debt and the Various Chemical Components of Blood.

Condition	Weight in gms			O_2 Debt ml.			Total Ketone mgm/100ml.			Lactic Acid mgm/100ml.			Glucose mgm/100ml.			Alkaline Reserve mgm/100ml.		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
	291	430	+53	99.4	311	+213												
Resting							1.55	1.773	+14.3	17.9	17.6	-1.7	69.0	78.5	+13.7	49.8	65.9	+ 32.3
Exhaustion							4.32	8.980	+107.8	162	203	+25.3	42.5	36.4	-14.4	34.9	30.0	- 14.1
Δ							+178	+406		+805	+1054		-62	-53.7		-30	-54.5	

PLATE I

A photograph of the exercise apparatus on which the experimental animals were trained and all of the animals were run to exhaustion. "A" shows powerpac providing power source for electrical stimulator located inside exercise wheels "B".

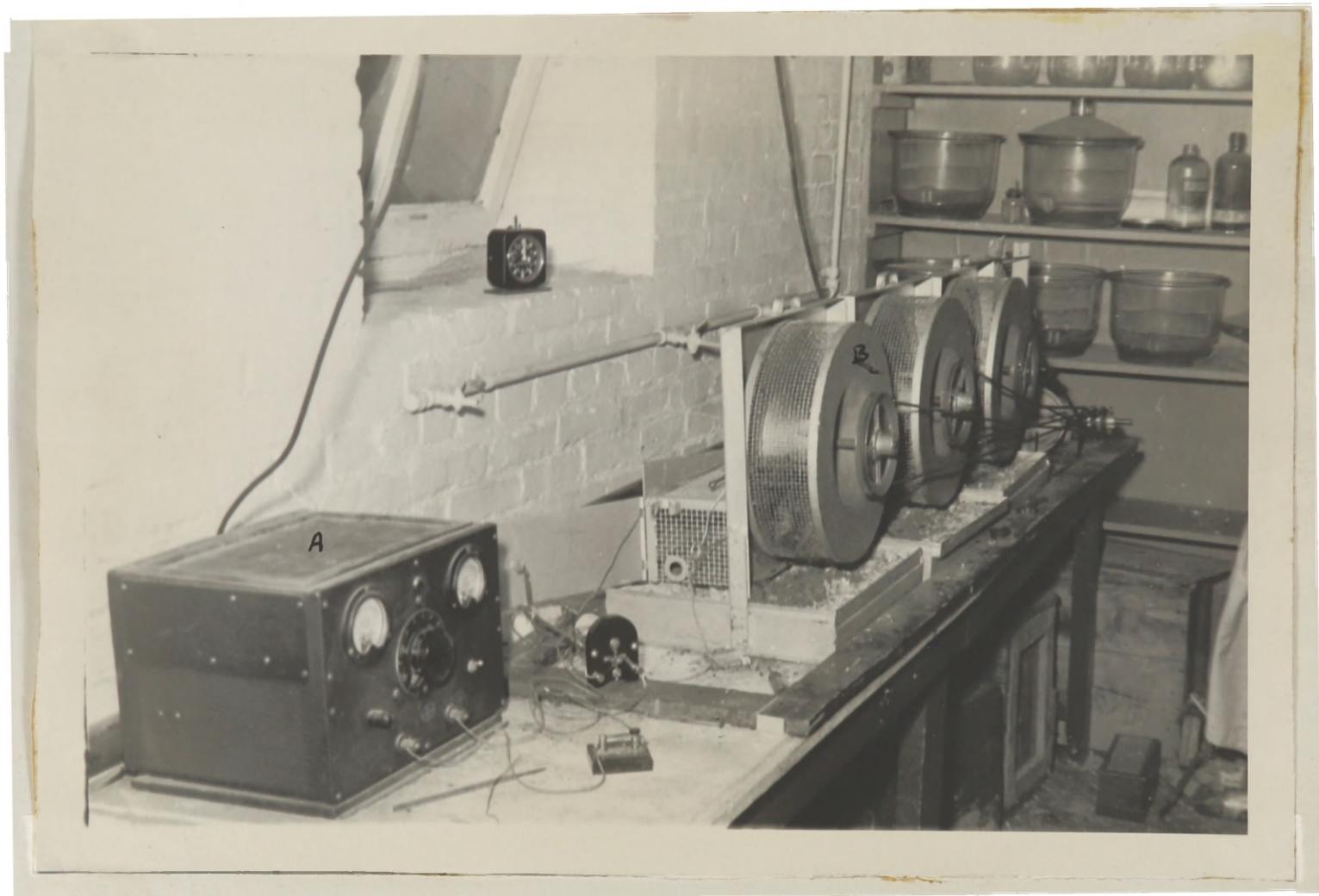

PLATE I

PLATE II

A photograph of the metabolar apparatus used to determine oxygen debt in rats showing "A" the motor which powers the exercise wheel built into the metabolar "B" "C" the constant temperature water bath, "D" the micro spirometer from which oxygen consumption was recorded, and "E" the oxygen source.

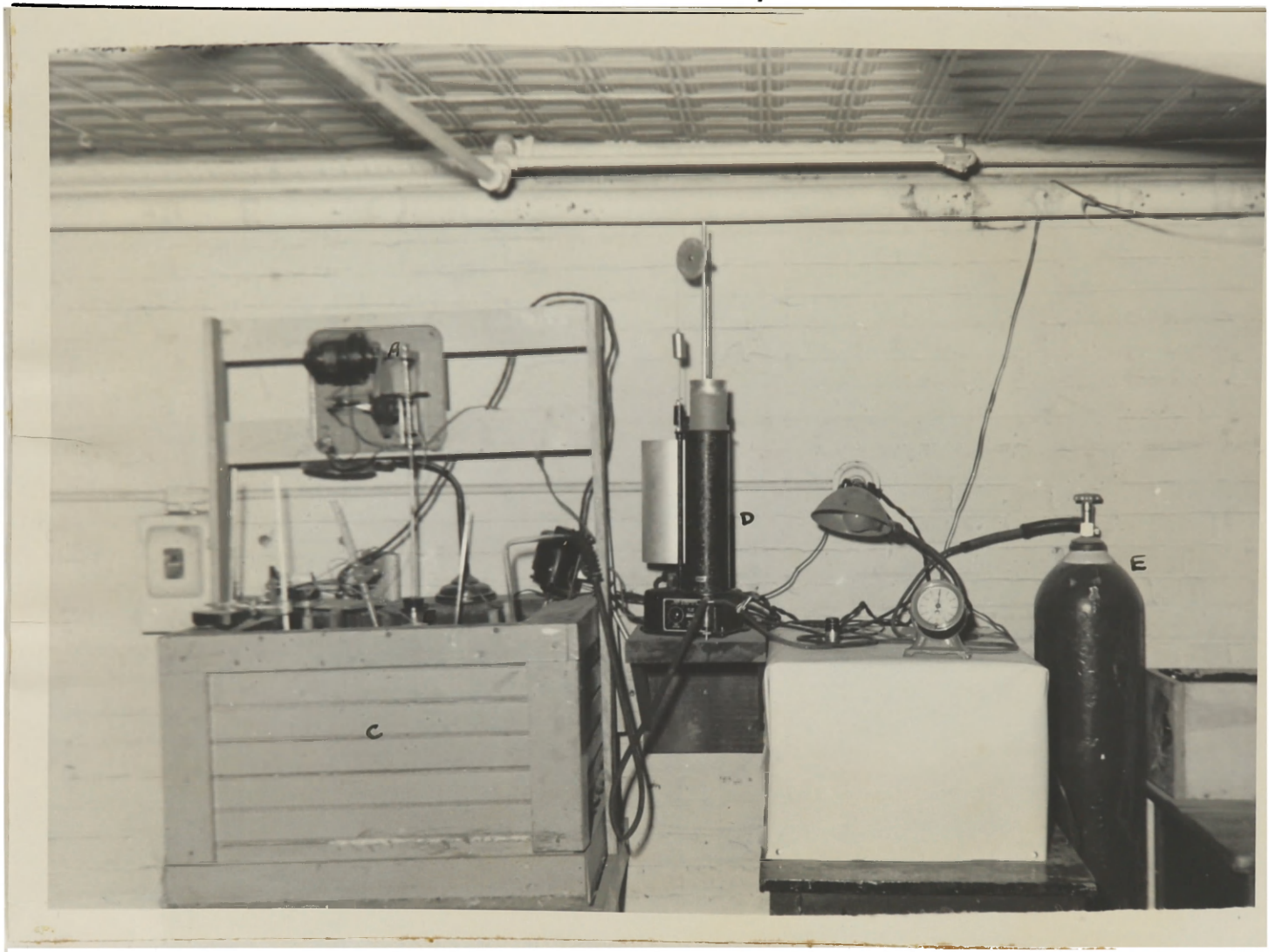

PLATE II

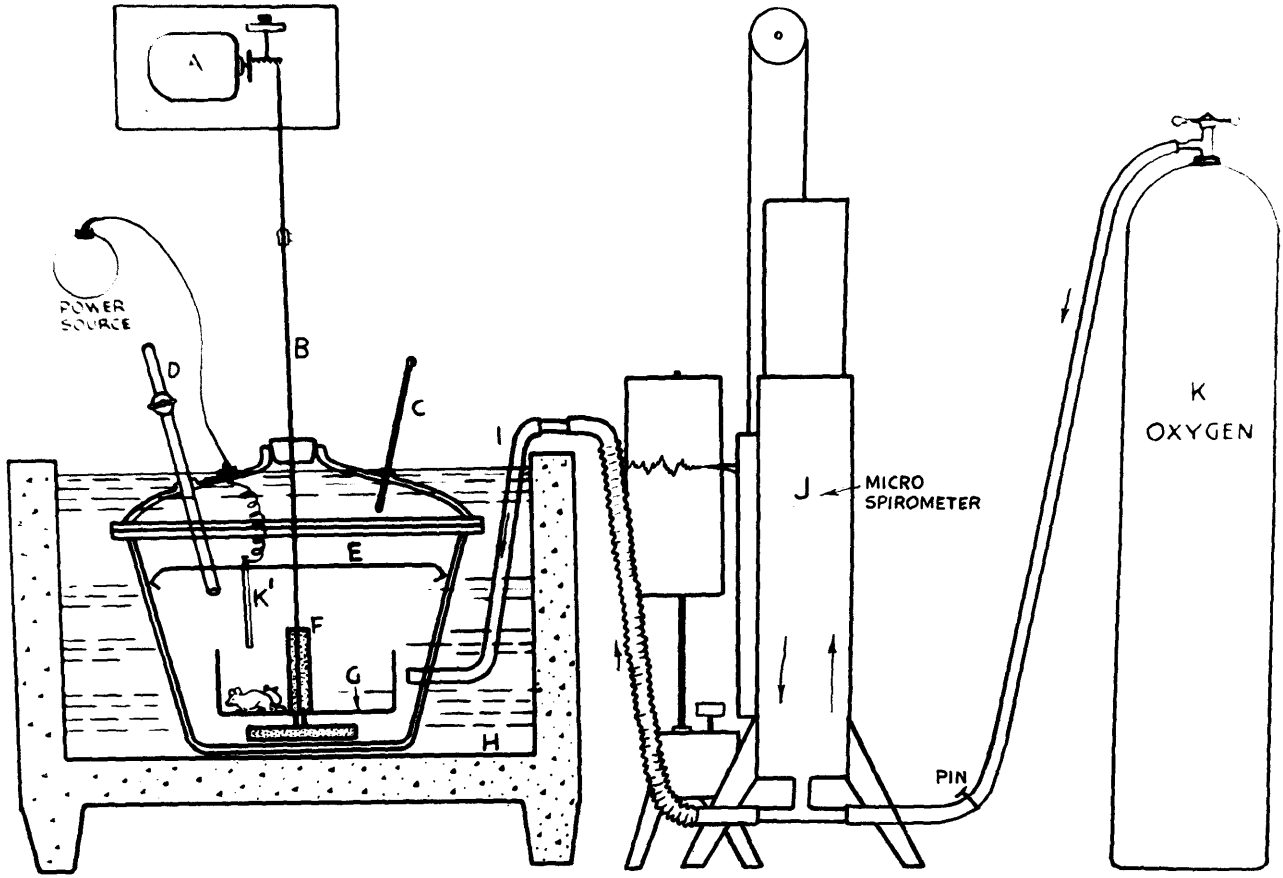

FIG. 1 DIAGRAM OF METABOLAR ILLUSTRATING THE MODIFICATION FOR DETERMINING OXYGEN DEBT. A REPRESENTS MOTOR FOR TURNING EXERCISE WHEEL. B SHAFT FROM MOTOR TO TURN TABLE G. C THERMOMETER. D INLET OR OUTLET FOR GASES E CHAMBER OF THE METABOLAR. F WELL FOR CO_2 ABSORBENT. H CONSTANT TEMPERATURE WATER BATH. I OXYGEN INLET TUBE. J MICROSPIROMETER AND RECORDER. K REPRESENTS OXYGEN SUPPLY.

DISCUSSION

OXYGEN DEBT

The amount of oxygen used, in excess of the resting level, during the period commencing at the moment exercise ends and ending when recovery is complete, for example, when oxygen intake has returned to its resting level, may be considered to be the oxygen debt which was incurred during the period of exercise. Thus, the extend of debt is determined by measuring the total amount of oxygen consumed during the period of recovery, and subtracting from it the amount of oxygen which would have been normally consumed during the same period if the subject had remained at rest. After the cessation of totally or partially anaerobic chemical processes in muscle, oxygen consumption remains increased for some time until the oxidation of accumulated products of the anaerobic reactions has been completed, and the muscle has been recharged. This period of recharging is called the recovery period and, as A.V. Hill (37) has said, "the subject is paying his oxygen debt."

It is possible for a person to take muscular exercise that requires far more oxygen than can conceivably be supplied by respiration and circulation during the period of exercise. In the severest forms of exercise something over 22 liters of oxygen may be required to provide the energy used in one minute. To deliver to the tissue this amount of O_2 is an impossible task for the respiratory and circulatory systems,

which even in a well developed athlete may supply only 4 or 5 liters a minute. By contracting an oxygen debt of 17-18 liters the demand for 22 liters a minute can be met.

If exercise is moderate and uniform the oxygen intake rises gradually and then in a minute or two levels off and remains at this level for the duration of the exercise. Since the other bodily functions such as respiration, heart beat, and lactic acid production also maintain a steady level, this is called the "steady state". Oxygen intake is equal to oxygen expenditure. When intensity of work rises to such an extent that a steady state cannot be maintained because more oxygen is required then can be supplied, it is obvious that additional work has to depend entirely on anaerobic chemical processes in the muscles. The amount of additional work which is dependent upon the amount of oxygen debt the individual is capable of accumulating will be limited by the degree of body tolerance for the accumulation of products of anaerobic decomposition, chief among which is lactic acid. When the concentration of lactic acid in muscle tissue reaches 2.8 per cent the muscle cannot contract. However, this level is probably seldom reached. Muscular activity ends when the concentration of lactic acid in the blood is between 32 and 140 mg/100 cc. of blood.

Formerly, it was believed that there was a quantitative relationship between oxygen debt and lactic acid production. This belief led to the statement that lactic acid is the security given for the payment of the oxygen debt. Now it is

known that this theory is only partially correct. Margaria, Edwards and Dill (47) have shown that no extra lactic acid appears in the blood during or after an exercise involving an oxygen debt of less than 2.5 liters. In an untrained person this quantity will be decidely less. When exercise requires a larger amount of oxygen than this, lactic acid accumulates in the blood to the extent of 7 mgm. for each liter of additional oxygen debt.

Thus oxygen debt consists of two parts: alactacid and lactacid. The alactacid debt is paid at a much faster rate than the lactacid debt. While the identity of the substances oxidized is not known, the hypothesis has been advanced that resynthesis of phosphocreatine is the process which absorbs the energy developed in the oxidation that occurs as the alactacid oxygen debt is paid.

The factors determining the rate at which oxygen can be delivered to the tissues are:

A - The partial pressure of oxygen in the lungs. By raising the partial pressure, the diffusion of oxygen through the lung membranes into the capillaries is hastened.

B - Degree of saturation of blood in the lungs. In normal individuals 100 cc. of blood ordinarily holds when fully saturated 18.5 cc. of oxygen combined with Hgb. In men in training this value tends to be somewhat higher.

C - The coefficient of oxygen utilization. The utilization coefficient of the oxygen in the blood never rises above about 75%. During rest muscles take 4 cc. of oxygen from the

20 cc. present in each 100 cc. of blood - hence muscles consume $\frac{4}{20}$ = .2 of the available oxygen. This is called the coefficient of oxygen utilization.

D - Cardiac Output. The minute volume of the heart, i.e., the amount of blood circulating per minute determines the amount of O_2 delivered to the tissues.

The maximum oxygen debt to which the body can submit depends upon various factors. It really is a measure of the maximum amount of lactic acid which the tissues can tolerate and the factors involved are:

(1) The amount of alkali available in the protein buffers of tissue and blood to neutralize the acids formed during metabolic processes.

(2) The rate at which lactic acid is liberated. If this liberation is great, the hydrogen ion concentrations in the blood will not rise immediately as high as in the muscles, and consequently respiratory distress will not occur for some time after the acid has been liberated because this distress is due largely to acid acting on the nervous system.

(3) The resolution of the subject in pressing his body to exhaustion.

In one unusual case, a trained athelete was capable of incurring an oxygen debt of 18.6 liters with a lactic acid concentration in the muscles close to .30 per cent. In less able bodied persons, laboratory workers, etc., the greatest oxygen debt tends to be about 12.5 liters in a man weighing about 154 pounds, and a concentration of about .21 per cent lactic acid in

his muscles.

This paper concerns itself with a study of oxygen debt in which albino rats were used as experimental animals in an attempt to find some experimental evidence which will explain why a trained individual is capable of incurring a greater oxygen debt than an untrained individual. Table I shows the normal resting metabolism and oxygen debt of all animals before training was started. The normal resting oxygen consumption ranged from 300 to 560 ml. of oxygen per hour, the average hourly oxygen consumption at rest being 428 ml. Oxygen debts ranged from 70 to 135 ml., the average being 89.7 ml.

As the results of this experiment indicate, Table V, the oxygen debt of rats was increased over 200 per cent above the level of untrained animals as a result of progressive training on an exercise wheel.

Effect of Training on Basal Metabolism.

Oxygen consumption of the trained group in comparison with the untrained group showed an average decrease of 14.6 per cent.

There is much uncertainty regarding changes in basal metabolism brought about by training. Observations made by Steinhaus (70) on dogs, indicate a slight decrease in the basal metabolic rate. In his review of the literature dealing with this problem, Steinhaus (71) calls attention to numerous experimental errors that might have affected the many studies of basal metabolism and thus have been responsible for contradictory reports. Most of the confusion regarding the effect of training upon the basal metabolic rate has resulted from an insufficient number of tests of

basal metabolic rate on each subject.

Schneider (62) in his observations on ten athletes found that eight had a drop in their basal metabolic rate, one had an increase, and one had no change. Of seven non-athletes, three had a fall in their basal metabolic rate, two had a rise, and two had no change.

Morehouse (53) carried out investigations on twenty athletes in and out of training and concluded that metabolism was not altered by training. Although the basal metabolism was 2 per cent higher after training, the difference was well within the normal range of fluctuation of metabolism.

One can observe from the data presented in this paper, Table V, that longer and probably more strenuous training coincides with a fall in the basal metabolism.

Training increases the ease with which work is performed. It is difficult to determine accurately the relative importance of increased skill and of increased mechanical efficiency of the muscles. Improvement in skill and consequent reduction in oxygen requirements are often noted in army recruits learning to march. Schneider (63) points out, improvement in the mechanical efficiency of muscles is due largely to the increase in diameter of each muscle fiber which results from continued usage. As a result, fewer fibers must contract for the development of a given amount of tension. Thus, the oxygen requirement for a given task is diminished as a result of more efficient use of muscles and elimination of extraneous movements, and of greater mechanical efficiency of the muscles themselves. Perhaps, this

greater efficiency and skill acquired by training accounted for the lowered basal metabolic rate.

Lactic Acid.

The maximum oxygen debt which can be tolerated by a subject is usually increased by training. This means primarily, that the trained individual is able to buffer a larger amount of lactic acid since there is no evidence of any difference in ability to tolerate a higher hydrogen ion concentration in the muscle tissue.

This experiment showed that the trained group of animals had a slightly smaller lactic acid concentration in the blood at rest in comparison with the untrained group to the extent of 1.7 per cent. This is not a statistically important change so that actually it is possible to say the trained and untrained groups had practically the same concentration of lactic acid at rest. After exhaustive exercise a comparison of the blood data between the trained and untrained animals as tabulated in Tables XIV and XV may be summarized as follows:

Average Lactic Acid Concentration of Blood in mgms. per cent

	Rest	Exhausted	Change
Untrained	17.6	162	+ 144
Trained	17.9	203	+ 185

This shows that the trained group was capable of producing and withstanding 185 mgm. per cent of lactic acid while the untrained group was capable of producing and withstanding only 144 mgm. per cent of lactic acid, a difference of 41 mgm. per cent.

A comparison of lactic acid content of muscle between untrained and trained animals at rest and after exhaustion is recorded in Tables XVI and XVII. This data in concise form appears as follows:

Average Lactic Acid Concentration of Muscle in mgm. per cent

	Rest	Exhausted	Change
Untrained	21.6	242.6	+ 222
Trained	27.7	308.6	+ 281

Under resting conditions, the animals that were trained and those that were untrained showed an average lactic acid content that was practically in the same range. After exhaustive exercise the trained group was able to build up a lactic acid concentration in the muscle which was 60 mgm. per cent greater than the untrained animals.

One of the many factors which may determine the lactate response of an individual to a given exercise is the individual's exercise tolerance (physical fitness). Several reports made by Bock (6) and Owles (54) allude to the higher blood lactate following a submaximal exercise in persons considered unfit or untrained. The lower blood lactate response to a submaximal exercise after a training program was also observed by Robinson and Harmon (58) who reported a higher blood lactate response to exhausting exercise after a training period. This latter finding has been confirmed by Knehr, Dill, and Neufeld (42). The present report indicates that insofar as exhaustive exercise or work is concerned, the blood lactate re-

sponse is higher in the trained animal. This increased level of lactate noted in the trained group may be looked upon as one of the most significant effects of training.

According to Margaria, Edwards and Dill (47) the oxygen consumption curve during recovery may be considered as being madeup of (a) an oxygen consumption attributable to oxidation of the lactic acid. This process is a very slow one. Another process (b) which also has an exponental time function occurring at a much faster rate. The mechanism (a) is looked upon by Margaria, Edwards, and Dill as the lactic acid mechanism, described by A.V. Hill. The mechanism (b) is independent of any lactic acid formation or removal, and therefore, has been termed the alactacid debt. The alactacid oxygen debt is approximately a linear function of the oxygen intake in exercise. It is supposed to be related to the oxidation of substances furnishing the energy for the resynthesis of phosphocreatine split down during muscular contraction. There are reasons to believe that the lactacid oxygen debt comes into play only when work is carried on in anaerobic conditions. Its amount, according to the authors cited above, increases rapidly at the maximum rates of work. The maximum absolute amount is about 5 liters. It may be increased by increasing the capacity of the body to accumulate lactic acid, as for example, after ingestion of alkali or due to the increase in alkaline reserve acquired by training.

Alkaline Reserve

As has been stated previously, increased oxygen debt due to training is probably due to the ability of trained individuals

to buffer a larger amount of lactic acid. It might be expected then, that training would increase the body's supply of buffer alkali, and this seems to be true. Full and Herxheimer (27) reported an average alkaline reserve of 72.12 volumes per cent in 13 highly trained athletes as compared with 65.15 volumes per cent in 18 normal but untrained men. Davis and Brewer (12) observed a steady rise in the alkaline reserve of dogs subjected to regular exercise on a treadmill for a period of 7 to 9 weeks. Walinski (77) has reported an increased alkaline reserve in man after physical training but an increased alkali reserve in trained rats has not, to the knowledge of the author, been reported previously. At rest, the results of this experiment indicate, Table XIV, that the 11 trained animals showed an average alkaline reserve which was 32 per cent greater than the group of 9 animals which were untrained. After exhaustive exercise a comparison of the blood data between the trained and untrained animals, Table XV, shows that even though the trained group had a greater alkaline reserve to begin with, it was also depleted to a greater extent. The untrained group had an average alkaline reserve of 34.9 volumes per cent after exhaustive exercise, while the trained group had an average alkaline reserve of 30 volumes per cent. For a clearer picture of the results, the data is presented in summary as follows:

Alkaline Reserve of Blood
in Volumes per cent

	Rest	Exhausted	Change
Untrained	49.8	34.9	- 14.9
Trained	65.9	30.0	- 35.9

Thus it can be seen the trained group was capable of using 21 volumes per cent more buffer substance than the untrained group after exhaustive exercise. Exercise it seems, "chemically trains" the blood by increasing its content of available base for the neutralization of acids.

It is well known that during exercise carbonic, lactic, and other acids are formed in the muscles. Carbon dioxide at all times, and lactic acid sometimes, appear in the blood during exercise. Since these must be buffered, their very presence in excess in the blood may induce the formation of a greater alkali reserve.

Glucose and Glycogen

The exponents of the newer conception of the nature of muscular contraction maintain that the primary fuel of muscle is carbohydrates, namely, glucose and glycogen, but Lusk (44) and Henderson (36) have shown that the energy expended by athletes may also be derived from fats. However, all students of the problem admit that sugar is the best fuel for intense exertion.

During the period of work lactic acid loss occurs in two ways. The first loss occurs in the reconversion of lactic acid to lactic acid precursor. The second source of loss is found in the kidneys. When exercise is violent, the blood is quickly loaded with acid; some of which the kidneys eliminate. According to Schneider (54) in long periods of work, this loss may be pronounced and the food which makes good this loss to the muscles must first be converted into glycogen. Sugar is the best

food for this purpose.

D - glucose as stated by Wiggers (78) is the chief carbohydrate constituent of blood and the only known form concerned in energy metabolism. It is distributed equally between blood cells and plasma in anthropoids, including humans, but according to Klinghoffer (41) in other mammals red corpuscles are less permeable or wholly impermeable to glucose.

Variations in the sugar content of the blood are not great, but they may be large enough after violent exertion to account for certain disturbances. Various forms of mild exercise do not produce any significant changes in blood sugar, but as the intensity of the exertion increases the sugar content may show a marked decrease if the exertion is maximum. As we demonstrated in the present experiment, Table VI, in the untrained group of animals, the resting glucose level ranged from 48 to 87 mgm./ 100 ml. with an average for nine animals of 69/100 ml. After exhaustive exercise glucose concentrations as shown in Table VIII ranged from 30 to 55 mgm/100 ml. of blood with an average of 42.5. This indicates an average decrease of the sugar content to be 62 per cent. In the group of animals that were trained, the blood sugar level at rest was an average of 78.5 mgm/100 ml. as is recorded in Table XII, while after exhaustive exercise, the mean value for twelve animals was 36.4 milligrams. This demonstrates an average decrease in the blood sugar level of 53.7 per cent. Thus, it seems training increased the amount of sugar normally present in the blood and, therefore, made it

possible for the animal to continue exhaustive exercise longer; as the trained animals in their exhausted condition were also able to withstand a lower blood sugar level than the untrained animals. The above data is summarized in Tables XIV and XV and is presented here in simplified form, as follows:

Glucose Content of Blood in mgm. per cent

	Rest	Exhausted	Change
Untrained	69.0	42.5	- 26.5
Trained	78.5	36.4	- 42.1

Whether the sugar content of the blood falls below normal or not will be dependent upon the amount of fuel required. Hence, various investigators have reported different findings. Sheunert and Bartsch (61) in a study of the influence of normal amounts of work on the composition of the blood of the horse, reported that work did not affect the percentage of sugar. Burger and Martens (8) in seven fasting subjects found immediately after an hour of gymnastics and boxing that in four there was no change, in two a definite fall, and in one a slight rise in blood sugar. Scott and Hastings (64) and Brosamlen and Sterkel (7) found that the concentration of sugar fell steadily throughout a prolonged period of fatiguing work.

The observations in this experiment were, of course, carried out on animals which were exercised to complete exhaustion, in which the animal was unable to run any further or some were unconscious. The present study indicates that training increases the resting blood sugar level of rats, - perhaps due to a more

efficient or stimulated hepatic glycolysis.

In addition, the present study indicates that the muscle glycogen of rats is also increased by training. The average glycogen content, Table VII, of the muscle of untrained animals at rest was on the average, for nine animals 98.4 mgm. while the trained group, at rest, consisting of eleven rats, had a mean of 133.4 milligrams. It was also shown that training enabled the animals to utilize more glycogen, as the results recorded in Table XVIII show that the trained group in the exhausted state had a glycogen concentration of 38.1 per cent less than the untrained. It can be seen from a survey of the literature that during light exercise, the ordinary rate of delivery of glucose to the blood from the storage depot is adequate to balance the rate of glucose utilization by the muscles, and the blood sugar level is unchanged. As exercise increases in intensity, especially if accompanied by emotional excitement, the secretion of adrenalin becomes excessive insofar as blood sugar regulation is concerned and glucose is added to the blood from the glycogen storage reservoirs at a rate faster than the metabolic activities of the contracting muscles require. The result is a rise in the blood sugar concentration. This effect is more pronounced in intermittent than in continuous exertion. The results of the present experiment indicates that if the exercise is both strenuous and prolonged, the blood sugar level as well as muscle glycogen show a fall - sometimes to less than half the normal value. This is interpreted as indicating exhaustion of available glycogen stores.

Phosphocreatine

The oxygen debt gives an estimate of the reserve energy that can be derived from anaerobic processes in excess of the immediate available oxygen supply. The maximum reserve energy that can be furnished in severe exercise to the point of exhaustion is limited by the creatine phosphate reserve in muscle and by the amount of lactic acid which the body can tolerate.

The results obtained in the present experiment on the phosphocreatine content of muscle tissue of trained and untrained animals under conditions of rest and exhaustion is recorded in Tables XVI and XVII. A summary of the average data is as follows:

Phosphocreatine Content of Muscle Tissue recorded in mgm. per cent

	Rest	Exhausted	Change
Untrained	51.6	21.8	29.8
Trained	70.0	9.1	60.9

From the above it can be seen that the phosphocreatine content of the muscle of the trained animals was 74 per cent greater than in the untrained group. The trained group of animals showed an average phosphocreatine content of 70 milligrams per cent while the untrained had an average of 51.6 milligrams per cent, indicating an average of 21.6 mgm. more phosphocreatine. In addition, it can be seen that not only did the trained animals have a greater phosphocreatine concentration in their muscle tissue, but they were also able to utilize a greater amount. As the table above indicates, the trained animals in contrast to the untrained ani-

mals were able to use 31.1 mgm. per cent more phosphocreatine than the untrained animals. Thus, it would seem that training increased the phosphocreatine reserve of muscle, thereby enabling the trained animal to acquire a greater oxygen debt than the untrained, due to the fact there is a greater range of energy reserve for anaerobic processes.

Fiske and Subbarow (21) found the dissociation constant of phosphocreatine was roughly 250 times as great as the constant of ortho phosphoric acid at the same ionic strength. This result, which is presumably to be attributed to the unmasking of the carboxyl group, to mention one function, since there may be others, is that of neutralizing a considerable part of the lactic acid formed during muscular contraction. The above authors think this is the primary purpose of the hydrolysis of phosphocreatine instead of the liberation of energy for muscular contraction.

Calculations by Fiske and Subbarow have shown that the hydrolysis of phosphocreatine liberates sufficient base under optimum conditions (i.e., the maximum amount of base is released at pH 6, which is roughly the acidity of completely fatigued muscle) to neutralize the lactic acid formed up to a concentration of about 0.23 per cent. Approximately half of this amount of lactic acid, moreover, can be neutralized at pH 7, i.e., without the development of any acidity at all.

If it is true, as has been claimed by Meyerhoff and Lohmann (61) that the main restriction on muscular performance is the accumulation of acid in the cells, then the hydrolysis of phospho-

creatine as Fiske points out seems now to be the principal factor permitting contraction to take place without the appearance of fatigue.

It appears then that training in enabling the animal to build up a greater creatine phosphate reserve, makes it possible for the animal to acquire a greater oxygen debt in two ways: First, by providing the conditioned animal a greater store of reserve energy that can be derived from anaerobic processes, and secondly, by providing more base in the hydrolysis of more available phosphocreatine for the buffering of lactic acid.

Ketone Bodies

Abundant evidence has accumulated that ketone bodies are freely used by the normal muscle tissue. Experiments with perfused surviving limbs of experimental animals were done by Griesbach (31), Snapper and Grunbaum (69) and Toenniessen and Brinkman (72). Ketone utilization from calculations of blood and tissue ketone body levels following injection of acetoacetate or B-hydroxybutyrate in eviscerated preparations have been made by Friedman (26), and Chaikoff and Soskin (10). The evidence uniformly points to complete oxidation of the substrate. Careful measurements by Blixenkrone-Moeller (5) who determined both the ketone body utilization and oxygen consumption of perfused hind limbs of the cat in the resting state and when working (electrical stimulation), gave as the mean of 11 determinations a ratio of 3.6 moles of oxygen to one mole of acetoacetati consumed. This is remarkably close to the theoretical value of 3.5

and proves that the substrate is completely oxidized.

The arterio-venous ketone difference of certain organs has also been used to show ketone body utilization. Goldfarb and Himwich (29) in such studies reported that striated muscle and heart utilized ketones, but that the brain did not. Barnes and Drury (3) also demonstrated the utilization of ketones by peripheral tissues by this method.

Harrison and Long (34) employed a unique method for the determination of ketone utilization by the muscle. From blood level studies they calculated ketone body concentration in cellular water. Even when blood ketones were high following phlorhizin (phlorhizin is a bitter glucoside, C_2, H_{24} O_{10} + $2H_2O$, derived from the root bark of apple, cherry, plum and pear trees) or anterior pituitary injection, they could find no significant amount of ketone in muscle cell water except at high blood concentrations, thus indicating that muscle metabolism of ketones was sufficiently active to maintain them at practically zero level within the cell.

The experimental data obtained in the research under discussion show that no ketone bodies could be found in muscle tissue of either the trained group or the untrained group at rest, Table XVI, while the blood of the trained animals, Table XIV, had an amount slightly higher than the untrained. The former being 1.773 mgm/100 ml., the latter being 1.550 mgm/100 ml. of blood.

After exhaustive exercise to the point of losing consciousness the blood picture of the rats was quite different. As Table XV shows, the blood of the trained group contained much greater

concentrations of ketone bodies than the untrained; to the extent of 107 per cent more on the average. The analysis of muscle tissue, Table XVII, also gave a rather interesting picture, showing ketone bodies accumulated to the extent of 1.384 mgm. per cent in the untrained and 8.657 per cent in the trained, the mean difference being 525 per cent, indicating training also had some beneficial effects upon ketone body tolerance.

From results obtained in experiments on dogs by Mirsky and Broh-Kahn (52), it is evident that an increased metabolism elicits an increased rate of utilization of ketone bodies by the extra hepatic tissues. Implicit in Barker's (2) calculations is the assumption that ketones accumulate in the blood and urine of the depancreatized dog because they are incapable of further oxidation. However, as Chaikoff and Soskin (10)demonstrated, the diabetic organism utilizes ketones at a normal rate but these substances accumulate solely in amounts that represent the balance between their rate of formation by liver and their rate of utilization by the extra hepatic tissues.

Drury, Wick and MacKay (16) have demonstrated in experiments with rats in which a state of ketosis had been artificially produced, a short bout of heavy exercise causes an immediate drop in the blood ketone level. During a period of three to four hours thereafter there was a phase of over-production of ketone bodies, so that the blood values for exercised animals went to higher levels than in controls. The present work confirms this view in normal animals and even more so in the trained group of animals.

Results of experimental work supports the view that in ketosis states, exercise increases the oxidation of ketone bodies and also causes the liver to produce them at a higher rate. In man these changes were not demonstrated for light exercise. A drop during heavy exercise was obtained.

The possible mechanism of the action of exercise on ketone bodies may possibly be explained in the following way. The initial fall which has been observed by some workers in the ketone body level of the blood, which results from exercise is most reasonably explained by an increased rate of utilization. In severe exercise the increase in ketone body concentration may be explained in the following manner. Dill, Edwards and Mead (15) and Cannon and Britton (9) have demonstrated in severe exercise epinephrine secretion is abundant and causes an increase in glycogenolysis when carbohydrate for such is available. When there is a lack of carbohydrate, epinephrine increases ketone body formation. This fact was demonstrated by Anderson and Anderson (1) in their work on ketosis. This may well be the cause of the over-production of ketone bodies during and immediately following exhaustive exercise when there is a condition that may require a lot of them, such as in carbohydrate lack similar to the way in which it increases glucose production when there is need for it and an ample supply of glycogen is available.

Since in the condition of exhaustion there is more ketone bodies produced then can be utilized by the tissue, they will accumulate as does lactic acid, waiting for more favorable circumstances (an ample oxygen supply) so that they may be oxidized

releasing CO_2 and water and energy, the energy being used for resynthesis of more phosphocreatine.

From the results obtained in this experiment with the trained group of animals, it also appears that training makes the pathway for fat metabolism more efficient and more easily available to the animal for the acquisition of necessary energy.

Studies on R.Q. indicate that oxidation of fat may play a more important role in providing necessary energy for animal during severe exercise than was previously believed. Studies of the respiratory quotient of muscles in the fasting state either in vitro or in the intact state gives values close to theoretical value of 0.7 for oxidation of fat as has been demonstrated by Goldfarb and Himwich (30). Drury and McMaster (17) contributed valuable evidence by determining the R.Q. of rabbits deprived of their livers. Following fat feeding, the R.Q. was not significantly changed from the preliminary period before hepatectomy. Their average R.Q. value in the preliminary period was 0.73 ± 0.01, and remained unchanged even as late as 24 hours after the operation. Apparently the organism deprived of liver is still capable of burning fat independent of any possible formation of carbohydrates or ketones by prior action of the liver. In other words, the low R.Q. value is a true fat combustion R.Q. and not the algebraic sum of processes going on in the liver and muscles simultaneously. Cruickshank and Kosterlitz (11) in many experiments on dog hearts in which they measured the total initial and final phospho lipids, fatty

acids, and cholesterol; found when there was low cardiac glycogen to favor fat oxidation, they were able to demonstrate that the heart could and did utilize stored fatty acids per se for its metabolic needs. This confirms the older observations of Visscher (76) who, on the basis of oxygen and carbohydrate balance studies, had made the same conclusion.

Evidence accumulated by Bartlett(4) on R.Q. studies with rats and mice would support the contention of the author from evidence in this present investigation that oxidation of accumulated ketone bodies represented the alactacid O_2 debt. This was suggested to Bartlett by the author as an explanation for his findings of low R.Q. values characterizing the first time period of recovery for rats and mice in oxygen debt, since it indicated the oxidation of fats.

SUMMARY AND CONCLUSIONS

(1) An apparatus was designed for running animals to exhaustion and measuring their oxygen consumption and oxygen debt.

(2) Resting oxygen consumption as well as oxygen debt were determined on all animals before training was started.

(3) Blood analyses were made, both under the resting condition and after exhaustion, to determine the effect of exercise and training on the alkaline reserve, lactic acid, and ketone body content.

(4) Tissue analyses were made to determine the effect of training on the phosphocreatine, glycogen, lactic acid, and ketone body content of rat muscle.

(5) As a result of training, fundamental differences between trained animals and untrained animals was demonstrated. The resting oxygen consumption of trained animals was lower than the untrained, indicating that longer and probably more strenuous training brought about a fall in basal metabolism.

(6) Training definitely increased the ability of rats to acquire greater oxygen debts then their untrained kind. Data of the present experiment indicates that the oxygen debt of rats was increased over 200 per cent above the level of untrained animals after progressive training on an exercise wheel.

(7) Analysis of the blood of rats at rest conclusively showed that training increased the body's supply of buffer alkali. Exercise it seems, "chemically trained" the blood by increasing its content of available base for the neutralization

of acids.

(8) Lactic acid concentrations of the blood at rest in the trained animal were slightly lower then in the untrained. After exhaustive exercise a comparison of the blood data shows that the trained group was capable of producing and withstanding an amount of lactic acid 25.3 per cent greater than the untrained group.

(9) Training increased the amount of sugar normally present in the blood and, therefore, made it possible for the animal to continue exhaustive exercise longer. The trained animals in their exhausted condition were found to have lower blood sugar levels then the untrained, indicating the possibility of a greater efficiency in the use of available energy sources.

(10) Experimental data shows that no ketone bodies could be found in the muscle tissue of either the trained animal or untrained animal at rest.

(11) The blood of trained animals at rest had an amount of ketone bodies on the average slightly higher than the untrained. After exhaustive exercise the blood of the trained group contained much greater concentrations of ketone bodies then the untrained. The average ketone body concentration being 107 per cent greater.

(12) Ketone bodies accumulated in exhausted muscle to the extent of 1.384 mgm. per cent in the untrained and 8.657 mgm. per cent in the trained, the mean difference being 525 per cent.

(13) Results obtained in this experiment with the trained

group of animals, makes it appear that training made the pathway for fat metabolism more efficient and more easily available to the animal for the acquisition of necessary energy.

(14) In consideration of the low R.Q's demonstrated by other investigators in the first time period of recovery of animals in oxygen debt, and the fact that ketone bodies accumulate in muscle tissue of animals in states of extreme exhaustion, as demonstrated by experimental evidence of the author, it is suggested that the accumulated ketone bodies may represent the alactacid portion of the oxygen debt.

(15) It was shown that training definitely increased the glycogen content of muscle, and also, enabled the animals to utilize more glycogen.

(16) Under the conditions of the experiment the phosphocreatine content of the muscle of the trained animals was 74 per cent greater than in the untrained animals.

(17) It may be concluded:

A. That training in enabling the animal to build up a greater alkaline reserve, glucose, glycogen, and phosphocreatine content, made it possible for the animal to acquire a greater oxygen debt in two ways:

(a) by providing the conditioned animal with a greater store of reserve energy that can be derived from anaerobic processes, and

(b) by providing more base from the hydrolysis of a greater amount of available phosphocreatine and the greater alkali reserve for the buffering of metabolic acids.

B. That training increases the ease with which an animal may derive energy from fat sources, as is evidenced by an increase in ketone body concentration in the blood and muscle of trained animals, both at rest and in exhaustion.

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