to the second (convalescent) serum specimen. On the other hand, in complement-fixation tests with ethylene-glycol extracts of leptospirae, titers reached a maximum early in the disease. Thus a decline in complement-fixing titer from the first to the second specimen would not necessarily obviate a diagnosis of leptospirosis.

Extracts of leptospirae made with desoxycholate solution in a manner similar to that reported for the preparation of *Treponema pallidum* complement-fixation test antigen by Portnoy and Magnuson(18) proved reactive with rabbit antiserum but poorly reactive with serum from human leptospirosis.

Summary. A colloidal aqueous solution obtained by ethylene-glycol extraction of leptospirae appears to offer a complement-fixing antigen of considerable promise. Extract pools have broad valency and high sensitivity in the detection of leptospiral antibodies.

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Mechanism of Oxygen Deficit.* (22451)

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After studying the origin of oxygen debt during human exercise, Hill, Long, and Lupton(6) concluded that it resulted entirely from accumulation of lactic acid. Margaria and his co-workers(10) divided the oxygen debt repayment curve into two components, a rapid one with no significant decreases in lactic acid, and a slower one during which accumulated lactic acid was removed. These workers observed no accumulation of lactic acid during exercise producing oxygen debts of less than 2.5 l in man. They postulated that the component of the debt which is rapidly repaid results from a depletion of phosphocreatine in the muscles. A number of observations seem to support this theory. Lundsgard(9) showed that in anaerobic conditions, lactic acid accumulates and organic phosphate dissipates in muscle in tetanus, and that phosphorylation follows a short tetanus. Tiegs(15) found much more creatine, a fission product of phosphocreatine, in fatigued than in rested muscle, a finding confirmed by Eggleton(2) who observed three times the

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normal amount of creatine in fatigued muscle. When Tiegs placed fatigued muscle in an oxygen stream, the creatine level decreased. Grollman(3) reported a decrease in phosphocreatine in rat muscle during exercise and noted interesting correlations between phosphocreatine levels and breakdown in conditioning. Hansen(4) also found that a debt incurred at low exercise levels apparently is not accompanied by accumulation of lactic acid. In discussing the mechanism of this debt, he showed that it was not due to either increase in arteriovenous oxygen difference during exercise or circulatory deficiency. He also concluded that a drain on oxygen stored in the muscle and bound to myoglobin is not the responsible factor even though an oxygen debt of 1-5 cc per 100 g of muscle would occur if myoglobin were saturated at rest and completely unsaturated during exercise. Hansen felt that myoglobin serves as an oxygen supply during contraction when circulation is shut off, and is resaturated between each contraction. The myoglobin saturation curve lies at quite low oxygen tensions; that whatever the role played by myoglobin, it does not bind sufficient oxygen to account for the 2.5 l debt that can occur without lactate accumulation. Belitzer(1) found that adding creatine to pulped muscle increased its oxygen consumption while adding phosphocreatine had no effect. He concluded that respiration is increased by addition of a phosphate acceptor. Niemeyer, Crane, Kennedy and Lipmann(11) observed that respiration of liver mitochondria is increased up to 50% by addition of a phosphate acceptor system, namely hexokinase plus glucose. This removes phosphate from ATP, forming ADP which is a phosphate acceptor, and ATP is regenerated by oxidative processes. Controls with glucose-6-phosphate, hexokinase, or glucose alone were negative. It appears, then, that with muscular exercise, a considerable oxygen debt is incurred which is not explained by accumulation of lactic acid. This debt may result from depletion of high-energy phosphate in muscle during exercise and an increase in oxygen consumption caused by an increase in phosphate acceptor. In this complex equilibrium there may be a mass action effect—rate of oxidation, and thus of oxygen consumption, being regulated by rate of high-energy phosphate depletion and phosphate acceptor accumulation in the muscle. Henry (5) suggested that oxygen consumption during exercise cannot increase until there is a substrate to be oxidized, and that substrate is formed as a result of work. Thus, time would be required for accumulation of a substrate which would be responsible for the debt. If the word 'substrate' were replaced with the words 'phosphate acceptor' and the word 'oxidized' placed in quotes, his idea would resemble the hypothesis we are proposing. The systems would differ markedly if there were uncoupling of phosphorylation. This concept is consistent with the hypothesis that energy for contraction is stored in muscle structure, released during contraction, and restored from chemical stores during relaxation(13).

In this study, the debt calculated from change in lactic acid and inorganic phosphate concentration in the isolated canine gastrocnemius muscle was compared with the oxygen deficit calculated from oxygen consumption.

Methods. The preparation used was similar to that of Kramer and Quensel(7). A long incision extending from the femoral triangle to the tendo calcaneus was made on the medial side of the hind limb. The long saphenus artery, vein, and nerve were cut between two ligatures and reflected from the medial aspect of the knee. The sartorius, gracilis, semitendinous, and semimembranosus muscles were separated, at their insertions, from the tibia and the gastrocnemius muscle. The femoral vein, the short saphenus vein, and the posterior femoral vein were cleaned. The short saphenus and posterior femoral veins were ligated below their last branch from the gastrocnemius muscle. All branches not from the gastrocnemius muscle were ligated and cannulae for collecting and returning venous blood were inserted into the femoral vein. The tibial nerve was cleaned, ligated, cut, and placed upon stimulating electrodes. Blood flow was determined at 5- or

15-second intervals with a direct volume collector recording via a spirometer on a kymograph. Oxygen saturation in venous blood was recorded continuously with a Kramer arterial oximeter. In each experiment, the apparatus was calibrated, and arterial saturation was determined by Roughton and Scholander's method(14). The muscle was stimulated for 1-second intervals with 1 second rest at 300 cycles per second (See 7 and 12). After an initial run, the muscle was rapidly excised during stimulation and placed in liquid air. The contralateral gastrocnemius was immediately removed as a control. Lactic acid was determined by the method of Barker and Sommerson(16), true inorganic phosphate by the method of Lowry and Lopez(8), and total phosphate (7-min. phosphate) by the method of Fiske and Subbarow(16). Oxygen deficit was calculated from the chemical determinations of high energy phosphate and lactate. Assume: 34 mol~P formed per mol glucose oxidized per 6 mol O_2 used $\therefore$ 0.1277 cc O_2/mg P. Assume from glucose 1 mol~P per mol lactate. Each mol lactate formed is equivalent to 1 mol of~P reconstituted. 1 mol~P equivalent

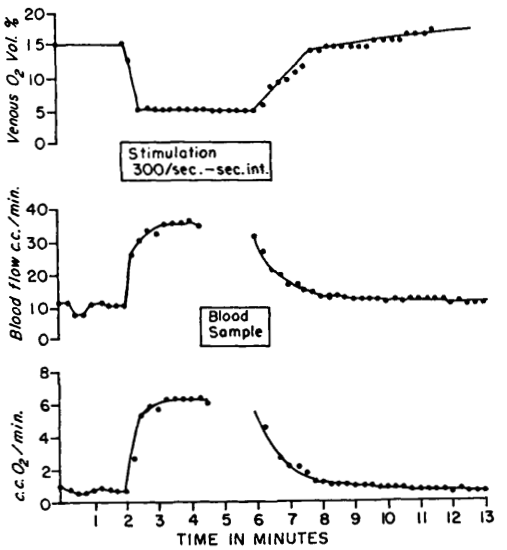

FIG. 1. Changes in oxygen uptake, blood flow and venous oxygen content with time in gastrocnemius muscle. Stimulation of cut tibial nerve indicated between 2 and 7 min. Blood sample taken during final minutes of exercise. During similar experiment the gastrocnemius was excised at end of 2 min. stimulation.

TABLE I.

	Date	Lactate, mg/100 g			~P, mg/100 g			O_2 deficient, cc/O_2/100 g		Blood O_2			O_2 cons/100 g & muscle, cc/min.		
		Rest	Stim	Δ	Rest	Stim	Δ	Chem	Blood	Ven. rest	Ven. stim	Arterial	Ex. A-V diff.	Rest	Exercise	Δ
1.	7/20	27.2	54.9	27.7	85.1	65.1	20.0	1.88	—	14.2	3.1	17.4	14.3			
2.	25	—	—	—	—	—	—	—	4.13	15.5	5.5	22.3	16.8	1.73	13.50	11.77
3.	27	9.8	21.9	12.1	55.4	41.6	13.8	2.29	2.94	10.8	5.0	15.5	10.5	.89	6.67	5.78
4.	8/ 1	20.7	56.5	35.8	84.5	57.8	26.7	4.98	1.59	15.8	2.5	18.4	15.9	.68	9.75	9.07
5.	3	8.1	14.3	6.2	80.9	67.5	13.4	1.98	2.80	13.2	5.2	18.2	13.0	.80	5.15	4.35
6.	9	13.3	35.6	22.3	—	—	—	—	3.14	14.0	6.4	20.0	13.6	.98	8.58	7.60
7.	11	21.9	32.0	10.1	—	—	—	—	2.69	17.2	7.5	19.2	11.7	.23	4.19	3.96
8.	16	10.7	19.2	8.5	67.9	55.0	12.9	2.01	—	11.0	4.0	16.5	12.5	.38	3.40	3.02
9.	18	8.6	11.0	2.4	61.4	53.4	8.0	1.13	.91	14.0	4.6	19.2	14.6	.80	3.49	2.69
10.	19	20.0	29.9	9.9	65.3	53.3	12.0	1.97	1.03	15.0	6.0	18.8	12.8	1.35	7.53	6.18
11.	22	—	—	—	—	—	—	—	.78 per muscle	16.2	8.8	18.1	9.3	—	—	—
12.	23	8.8	19.2	10.4	71.6	52.9	18.7	2.84	1.26	11.8	4.7	16.3	11.6	1.11	8.36	7.25
13.	29*	12.3	21.6	9.3	67.0	62.7	4.3	0.91	1.60	13.7	4.0	18.1	14.1	2.40	10.68	8.28
14.	31*	9.5	33.8	24.3	63.6	50.4	13.2	2.76	2.10	12.5	5.0	14.1	9.1	1.31	11.60	10.29
Avg		14.2	29.2	14.9	70.3	56.0	14.3	2.28	2.20	13.9	5.2	18.0	12.8	1.06	7.74	6.68
Surgical control†		9.1	7.9	1.2	62.5	62.4	0.1	.04 on control (rt.) leg								

* Muscle taken after 45 sec stimulation; all others after 2 min. † Muscle taken without stimulation.

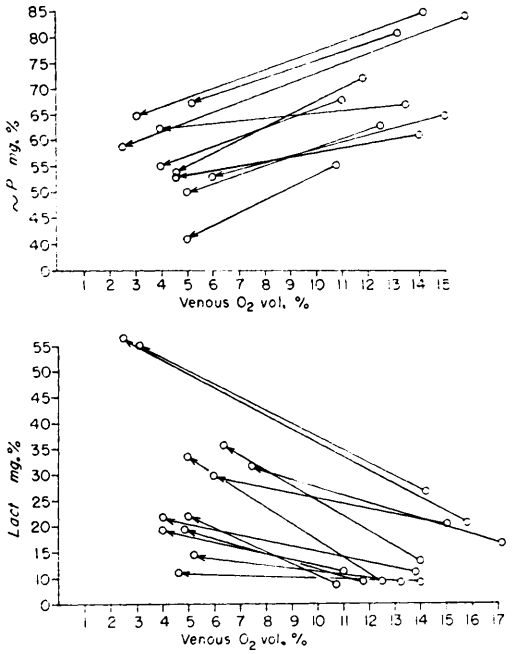

FIG. 2. Changes in organic phosphate (upper) and lactic acid (lower) with exercise plotted *vs* venous oxygen content. Individual points at rest and exercise are connected by arrows indicating shift with exercise.

to 3.96 LO_2. 1 mol lactate formed is equivalent to 3.96 LO_2. ∴ .044 cc O_2 deficit per mg lactate formed. The actual oxygen deficit was obtained from the oxygen consumption data by determining the difference between the oxygen consumption during the initial period and the steady state oxygen consumption.

Results. A typical experiment is shown in Fig. 1, and the data from all experiments appear in Table I.

In every case, the high energy phosphate decreased during exercise, and the muscle lactate increased. These differences are significant at the 0.1% level. The relationship to venous oxygen tension is shown in Fig. 2.

Assumption that oxygen debt is dependent on the change in the level of high-energy phosphate and calculation of "lactate debt" on the basis of high-energy phosphate replacement would indicate a relationship between depletion of high-energy phosphate and the increase in lactate during exercise. Fig. 3 illustrates this relationship in these experiments. Although the absolute levels of oxygen consumption are not directly related to the level of phosphate acceptor, the ratios of change are proportional (Fig. 4).

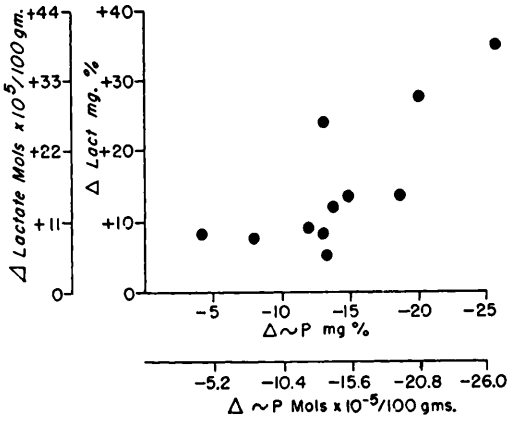

FIG. 3. Change in lactic acid with exercise *vs* change in organic phosphate.

The calculated oxygen deficit (Table I) agrees well with the measured deficit.

Discussion. The results of the experiments are compatible with the assumption that oxygen deficit is related to depletion of high-energy phosphate (increase in phosphate acceptor) and accumulation of lactic acid. Clearly, the role of myoglobin is small.

It is of interest to note that lactic acid appeared in the muscle during these experi-

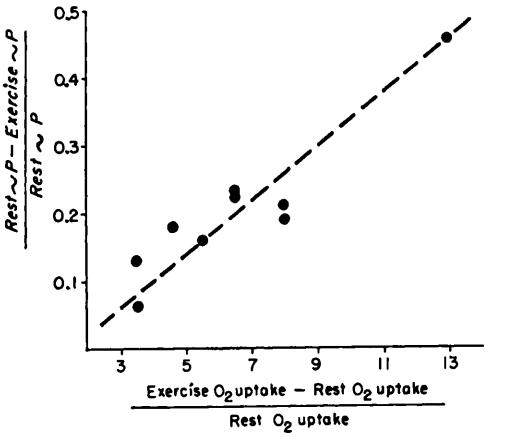

FIG. 4. Relation of change in phosphate acceptor to oxygen consumption expressed as ratio of change in organic phosphate to resting level of phosphate on ordinate, and ratio of increase in oxygen consumption to resting oxygen consumption on abscissa.

ments. The oxygen consumption of exercise (7.7 cc/100 g muscle) is not large (about 1 l/min in a man with 14 kg of muscle active). From these experiments it seems reasonable to suggest that lactic acid is formed in the muscle at any level of work, and that the lactate relationship to oxygen debt observed by Margaria *et al.*(10) indicates a threshold mechanism in the whole-body metabolism of lactate. The direct relationship between the change in high-energy phosphate and the amount of lactate produced, as well as the relationship of the amounts to the work done, is evident in Lundsgard's experiments(9).

Further, it is assumed that lactate is produced to provide high-energy phosphate and the oxygen deficit is calculated on a high energy phosphate equivalent, then the final oxidation of lactate (1/5 oxidized to reconvert 4/5) would involve a greater oxygen uptake, and the oxygen debt would exceed the oxygen deficit, *i.e.*, in terms of high energy phosphate formed 1 mol lactate formed is equivalent to 3.96 LO_2. 1 mol lactate requires 67.2 LO_2 for oxidation. If 1/5 oxidized to reconvert 4/5, then debt for 1 mol of lactate is 13.4 LO_2 or $\frac{13.4}{3.96}$; 3 times the calculated oxygen deficit.

During the time of stimulation before sacrifice, some lactic acid undoubtedly was carried away in the blood. Assuming a change in the concentration in the blood equal to that in muscle (14.9 mg/100 g) the error would be of the order of 5%.

The blood flows with exercise are similar to those reported by Kramer and Quensel(7) and illustrate the rapid increase in blood flow through the muscle with exercise concurrent with the widening A-V difference. The return of the blood flow and the A-V difference to resting levels follows a prolonged but related time course. This would seem to emphasize the role of the muscle pump in exercise blood flow and separate local effects on blood vessels from the central nervous control.

Summary. In muscular exercise, a considerable oxygen deficit is incurred which cannot be explained by the accumulation of lactic acid. The hypothesis that this deficit is due to depletion of high energy phosphate in muscle and that the oxygen consumption is increased by an increase in phosphate acceptor was tested in the perfused dog gastrocnemius. Oxygen deficit was calculated from change in lactic acid and high energy phosphate after 2 minutes contraction. Oxygen deficit was calculated from oxygen consumption determined at 5-second intervals by measurement of blood flow and arteriovenous difference. For exercise, the muscle was stimulated at 300 cycles/second for 1-second intervals with 1 second rest. In 14 experiments, the average values of lactic acid were 14.2 mg % at rest, and 29.2 mg % during stimulation. The calculated deficit was 2.3 cc O_2/100 g muscle, and the measured deficit was 2.2 cc O_2/100 g muscle.

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