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Influence of Repeated Contractions of Muscle on its Lipid Content.

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Whereas muscular activity is accompanied by striking changes in carbohydrate content, most attempts to demonstrate changes in the lipid content of muscle have failed. Leathes[1] found no difference in the amount of fat in corresponding muscles of the 2 legs of frogs, after one leg had been stimulated for several hours by a tetanizing current. Winfield[2] obtained equally negative results on excised frog muscle. Since the respiratory quotient of isolated, glycogen-rich amphibian muscle is generally close to unity, indicating a predominant use of carbohydrate, there was the possibility that utilization of fat occurred only in a muscle with low glycogen content. In support of this idea may be cited the observation of Niernierko[3] that stimulation of the isolated gastrocnemius of frogs in winter had no effect on fat content, while in spring, when the glycogen content is known to be low, stimulation resulted in a decrease in fat content of 12 to 31%.

After a fasting period of 24 hours close to 90% of the heat production of rats is derived from fat oxidation, as indicated by a non-protein R. Q. of 0.71. Obviously the muscles of such animals are using chiefly fat (either directly or indirectly) and it therefore seemed worth while to investigate whether the marked increase in oxygen consumption brought about by activity, would result in a demonstrable change in lipid content.

The gastrocnemius of the rat (weighing 1 to 1.5 gm.) was thoroughly ground with sand, transferred to a 100 cc. volumetric flask and boiled for 15 minutes with 75 cc. of a 3 to 1 alcohol-ether mixture on a water bath. After making up to the mark, aliquot portions of the filtered extract were analyzed for free and total cholesterol, using Okey's[4] digitonide method and for phospholipids and total fatty acids, following Bloor's[5] procedure. Oxidation was carried out with standard dichromate-sulfuric acid mixture as recommended by Bloor.[5] The methods were tried out on

[1] Leathes, J. P., Problems of Animal Metabolism, London, 1906.

[2] Winfield, G., *J. Physiol.*, 1914, **49**, 171.

[3] Niernierko, W., *Acta Biol. Exp.*, 1929, **3**, 143.

[4] Okey, R., *J. Biol. Chem.*, 1930, **88**, 367.

[5] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53; 1929, **82**, 273.

known mixtures of cholesterol, lecithin and fatty acids before being used for the experiments.

First, 2 control experiments were performed in which one gastrocnemius was removed from a rat immediately after the induction of amytal anesthesia and the second 1 hour later. The cholesterol and phospholipid content of the muscles of the 2 sides showed good agreement, whereas in one case there was an appreciable difference in the total fatty acid content of the right and left side (Table I).

TABLE I.
Stimulation of rat gastrocnemius.
All values are given in gm. per 100 gm. fresh muscle.

Free cholesterol	Total cholesterol	Phospholipids	Total fatty acids	Free cholesterol	Total cholesterol	Phospholipids	Total fatty acids	Remarks
Right				Left				
0.148	0.159	1.40	2.67	0.137	0.151	1.35	2.69	No stimulation
0.143	0.146	1.56	2.42	0.133	0.136	1.52	2.09	" "
Resting				Stimulated				
0.157	0.167	1.12	2.12	0.136	0.151	1.10	2.05	36 times per min. 1 hr.
0.164	0.165	1.13	2.83	**0.162**	0.165	0.99	2.11	68 " " " 1 "
0.126	0.127	1.25	2.51	0.100	0.125	1.18	2.48	68 " " " 2 " load 10 gm.
0.122	0.125	1.36	2.88	0.119	0.124	1.45	3.08	68 times per min. 2 hrs. load 50 gm.
0.142	0.146	1.21	2.58	0.129	0.141	1.18	2.43	Average of last 4 exp.

In 4 experiments the cut sciatic nerve on one side (after severing branches to muscles other than the gastrocnemius) was stimulated with single induction shocks, the rate being regulated by a metronome. Though in 2 cases the muscle was made to lift a load, signs of fatigue did not set in during the 2 hours of stimulation. The unstimulated gastrocnemius of the other side (with its nerve cut at the start of the experiment) was removed at the same time that the stimulated muscle was taken for analysis. In 3 experiments there was no significant difference in the lipid fractions of the 2 sides. Experiment 2 cannot be regarded as indicating a disappearance of fatty acids during work, since a similar difference in total fatty acids was encountered in one control experiment. Since even the large amount of work performed in the last experiment (8160 twitches with a load of 50 gm.) had failed to cause a diminution in the lipid content, it seemed unprofitable to continue this type of experiment.

Frogs which had remained in the laboratory during the winter and spring and were in a poor nutritional condition, were anesthe-

tized with urethane and the muscles of one leg were fatigued by repeated stimulation of the sciatic with single induced shocks, while the muscles of the resting legs served as control. A decrease in the total fatty acid content of the fatigued muscle was observed in each of the 6 experiments recorded in Table II. Since the water content of the stimulated muscle was found to be higher than that of resting muscle, the average values were calculated in terms of dry weight. In 4 control experiments in which the muscles of the right and left side were compared without previous stimulation, the average values of the 2 sides showed good agreement.

TABLE II.

Stimulation of hind legs of frogs.

Values are given in gm. per 100 gm. fresh muscle. Experiments were made in July at a room temperature of about 25°C.

Total cholesterol	Phospholipids	Total fatty acids	Total cholesterol	Phospholipids	Total fatty acids	Remarks
	Right			Left		
0.061	0.38	0.73	0.062	0.35	0.76	No stimulation
0.069	0.31	0.65	0.060	0.31	0.72	" "
0.083	0.41	0.77	0.090	0.41	0.67	" "
0.106	0.51	0.68	0.10	0.45	0.67	" "
0.079	0.40	0.71	0.078	0.38	0.70	*Av. water content:* R 88.2; L 88.2%
0.67	3.39	6.01	0.66	3.22	5.94	Av. per 100 gm. dry weight
	Resting			Stimulated		
0.046	0.33	0.73	0.045	0.24	0.64	30 times per min. 3 hr.
0.036	0.30	0.75	0.039	0.18	0.52	30 " " " 2 "
0.050	0.39	0.61	0.043	0.29	0.51	22 " " " 2 "
0.045	0.39	0.85	0.049	0.28	0.52	30 " " " 2 "
0.047	0.47	0.79	0.040	0.45	0.51	30 " " " 2 "
0.059	0.27	0.77	0.049	0.27	0.53	30 " " " 2 "
0.047	0.36	0.75	0.044	0.28	0.54	*Av. water content:* R 86.8; St. 88.2%
0.356	2.72	5.69	0.373	2.37	4.57	Av. per 100 gm. dry weight

Cuthbertson[6] fatigued muscles of decerebrate cats by faradic stimulation but was unable to demonstrate a decrease in fat content. Similar experiments on rats recorded in this paper were also negative. The failure of contracting mammalian muscle to use its own fat, especially when (as in the present experiments) the respiratory quotient of the whole animal indicates predominantly fat combustion, makes it probable that the muscle derives its energy from blood fat. Determinations of the fat content of arterial and

[6] Cuthbertson, D. P., *Biochem. J.*, 1925, **19**, 896.

venous blood of muscle should demonstrate this, and Lafon[7] reported that the arteriovenous difference in fat content increases during activity of the muscles lifting the upper lip of the horse. The results obtained on frogs in summer indicate that muscle may use its own fat during prolonged activity. Conditions favoring this utilization seem to be a low glycogen content of muscle and possibly an insufficient supply of blood fat.

Summary. The cholesterol, phospholipid and total fatty acid content of the rat gastrocnemius remained unchanged during prolonged and severe muscular work. In frogs in summer the fatigued muscles of one leg contained on an average 19.7% (per dry weight) less fatty acids than the resting muscles of the other leg.

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Failure of Nicotine to Alter the Estrus Cycle in the White Rat.

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Ten white female rats of the Wistar strain were injected twice daily with nicotine hydrochloride in a dose of 2 to 3 mg. per kg. in 1 to 1,000 solution. This amount was sufficient to produce convulsions of varying degree, usually becoming less as the experiment progressed. Injections were begun at the age of 2 to 4 weeks, and an equal number of litter mates were injected with equal volumes of 0.85% NaCl solution as controls. Beginning at the age of 3 months in one set of 7 nicotine and 7 control rats, and at 5 months in a set of 3 nicotine and 3 control rats, vaginal smears were examined daily, between 4:30 P. M. and 6:00 P. M., for a period of approximately 3 months. During the course of the experiment, one of the control group was accidentally killed, and another became pregnant, and could not supply data for the test for the period of pregnancy, and until estrus began again after taking away the young. These accidents account for the discrepancy between the total rat days of nicotine and control groups. Comparison of the 2 groups of rats was made by 2 methods, as follows:

Method I. A value of 2 was assigned to each day of complete cornification of the vaginal epithelium, as indicated by the smear,

[7] Lafon, G., *Compt. rend. Acad. Sc.*, 1913, **156**, 1248.