up to 250 cc by adding a Ringer-glucose solution, filtered through a Berkefeld N filter and placed in the perfusion apparatus.

In another series of experiments, the crude hemocyanin was dissolved in 60 cc of Ringer's solution which had been brought to a pH of 8.4. This fluid was centrifuged at a speed of 40,000 r.p.m. in a centrifuge constructed by Chiles. After centrifugation for one hour, the hemocyanin at the bottom of each tube was dissolved in plasma at a pH of 8.4.

The purpose of the experiments was to determine: First, whether or not the oxygen capacity of the perfusion fluid can be considerably increased by the addition of the respiratory pigment. Second, whether or not the isolated mammalian organ is able to reduce the hemocyanin and use its liberated oxygen for respiration. Third, whether or not the organs perfused with a fluid containing hemocyanin survive in better condition than control organs cultivated in serum and Ringer's solution alone.

By the addition of hemocyanin to the perfusion fluid, as described above, the oxygen capacity was brought up to 2.5 volumes per cent. Still higher values are possible, as the solubility of hemocyanin is high in the presence of sufficient electrolytes. The experiments have shown that oxyhemocyanin is reduced in mammalian tissues. This is evident by comparing the color of the arterial with that of the venous blood. Oxygenated hemocyanin, flowing into the organs, is blue. Reduced hemocyanin, pouring out of the veins, is colorless. The oxygen removed from each cubic centimeter of the perfusion fluid, calculated merely as the difference between arterial and venous O_2 contents, amounts to 0.10 cc for an adult cat's kidney and 0.001 cc for an adult cat's thyroid. These figures correspond with those obtained on the kidney by Van Slyke and Hiller in their heart-liver-kidney preparation.[4] The experiments herein described were undertaken at a temperature of 27° C., since the affinity of hemocyanin for oxygen decreases with increasing temperature.[5] The cultivation of organs, such as thyroid gland, skeletal muscle and intestine, do not, however, require hemocyanin in the perfusion fluid. The oxygen consumption of these organs is low, and their call for oxygen can be satisfied with the oxygen dissolved in a fluid containing 40 per cent. serum and 60 per cent. Ringer's solution (0.25 volumes per cent.). Other organs, however, such as kidney, nerve tissue and pancreas, with high oxygen requirements can not be successfully cultivated without the presence of an oxygen carrier in the perfusion fluid.

Organs have been perfused with hemocyanin solution for more than four days. A comparison of the histological pictures of these organs with those of organs from the same animal kept as controls in serum and Ringer's solution alone demonstrates that the hemocyanin is superior for perfusion over long periods. Experiments with hemocyanin in tissue culture showed a slight toxicity beginning at the concentration of 3.5 per cent. (unpublished observations). The toxicity of hemocyanin in tissue cultures does not, however, prove that it is toxic when perfused through whole organs, since the size of the hemocyanin molecule prevents it from passing through the capillary wall.

No trace of hemocyanin could be found in the urine of isolated kidneys kept alive for four days in the Lindbergh apparatus. Bayliss, Kerridge and Russell[6] have also demonstrated that hemocyanin is unable to pass through the glomerular capillary wall.

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[5] A. C. Redfield, *Biol. Rev.*, 9: 175, 1934.

[6] L. E. Bayliss, P. N. P. Kerridge and C. S. Russell, *Jour. Physiol.*, 77: 386, 1933.

ESTIMATION OF FIBER, FAT CELLS AND CONNECTIVE TISSUE IN MUSCLE

THIS paper presents a very brief account of a simple analytical technique for the separate estimation of the three main morphological elements of muscle. A representative portion of the muscle to be studied is cut into longitudinal strips about 50 mm long, 20 mm wide and 2 mm thick, and a 6–8-gm sample of the strips is employed for a test. To each sample, in a 125-ml Erlenmeyer flask, is added 30 ml of 5-N aqueous HNO_3 and maceration is allowed to proceed, at about 25°, for about 36 hours—or until fibers and fat cells have become disconnected and free; gentle agitation during the last two hours is desirable. The acid action is then stopped by dilution, and the liquid level is brought into the flask neck, by the addition of 90 ml of 0.01-N HNO_3. The upper portion of the liquid, with the floating fat cells, is next decanted off, and those cells are collected on Whatman filter paper No. 1, by means of Hirsch funnel and aspirator pump, the resulting filtrate being returned to the flask. After being dried to constant weight at 40°, the weight of the fat cells is recorded as a percentage (a) of the original weight of the muscle sample. The tangled mass of fibers is next separated from the liquid by filtering, as above, after which it is dried at 92°–95° and its weight is recorded as a percentage (b) of the original weight of the sample.

To the filtrate from the fibers is gradually added just enough aqueous solution of phosphotungstic acid (Merck "Reagent," 5 gm in each 100 ml) to complete the resulting precipitation. The precipitate, which represents a combination of phosphotungstic acid with material extracted from the muscle during maceration,

is removed by filtration, as above, and dried at 92°–95°, after which it is weighed and then incinerated (at about 550°) in a porcelain crucible. From the dry weight is subtracted the weight of the residual ash and the difference, expressed as a percentage (c) of the original weight of the sample, is taken as an approximate measure of the connective tissue originally present. The sum of the three weight percentages ($a+b+c$) is taken to represent the dry weight of the sample, and the difference between that sum and 100 is taken to represent the original water-content percentage (w).

Representative percentages obtained by means of this technique for samples of longissimus dorsi muscle from two hothouse-grown lambs are shown in the accompanying table, along with some additional percentage values derived from Barbella, Hankins and Alexander's analyses[1] of similar samples from the same individual muscles. The additional values are: P, total protein percentage from Kjeldahl decomposition (corresponding approximately to $b+c$); F, total fat percentage from ether extraction (corresponding approximately to a); W, total water percentage from acetone extraction (corresponding approximately to w); D, dry-weight percentage derived by subtracting W from 100 (corresponding approximately to $a+b+c$). Values secured by means of the new technique are shown in bold-face type.

	Lamb no. 423	Lamb no. 517
Fat cells (a)	**4.3**	**6.2**
Total fat (F)	3.1	4.8
Fiber (b)	**17.1**	**19.1**
Connective tissue (c)	**3.5**	**2.7**
Fiber and connective tissue ($b+c$)	**20.6**	**21.8**
Total protein (P)	21.8	21.2
Fat cells, fiber and connective tissue ($a+b+c$)	**24.9**	**23.0**
Total fat and protein ($F+P$)	24.9	26.0
Dry weight (D)	26.5	27.2
Water, by subtraction (w)	**75.1**	**72.1**
Water, by extraction (W)	73.6	72.8

These lambs were of different breeds, but both had received the same liberal ration. When killed, No. 423 was 117 da. old and No. 517 was 122 da. old, but their dressed weights were alike (29.5 lbs.). The fat-cell percentage (a) was 31 per cent. less for the first sample than for the other, while the fat percentage (F) was 35 per cent. less for the first; the two methods of analysis thus show essential agreement in this respect. But in each case a is greater than F, as might be expected, since a represents intact fat cells, while F represents only extracted fat. For each sample, $b+c$ is in essential agreement with P, $a+b+c$ is in essential agreement with $F+P$ and with D, and w and W are in essential agreement. Finally, it is to be noted that the new technique furnished values for fiber alone (b) and for connective tissue alone (c), for which no estimates can be derived from F, P, D and W.

The new procedures described above were developed partly in the U. S. Bureau of Animal Industry and partly in the laboratories of zoology and plant physiology of the Johns Hopkins University. For practical advice and criticism the writer is indebted to Dr. E. A. Andrews, Dr. R. P. Cowles, Dr. Hugh C. McPhee, Dr. Paul E. Howe and Mr. O. G. Hankins. Financial aid was received from the U. S. Works Progress Administration.[2] Dr. Burton E. Livingston has helped a great deal in the preparation of this paper.

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1 *Proc. Am. Soc. Animal Prod.*, 1936, pp. 289–294.

A PHOTODYNAMICAL BIOELECTRICAL POTENTIAL

ALTHOUGH a great deal of valuable empirical work is now being done on the clinical and morphological aspect of electrical phenomena in animals, there is need of more information concerning the intrinsic mechanism involved comparable to what is known about the potentials in plant cells.

In the course of an extended study of the effect of temperature, oxygen, ions and heavy water on the potential of frog skin an interesting photodynamical effect has come to light. If a frog skin stained in 0.1 per cent. eosin is exposed to strong light from a carbon arc a striking and sudden increase in potential results. The skins were from the belly region tied in holders of 2 to 5 cc of stained Ringer's solution leading through

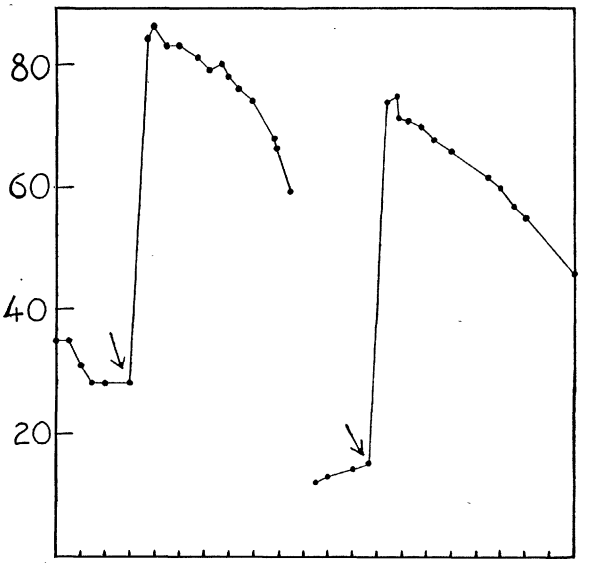

FIG. 1. Increase in potential of two frog skins transferred to Ringer's solution containing eosin and radiated (at arrows). Ordinates: e.m.f. in millivolts (outside surface is negative). Abscissae: time in ten-minute intervals.

2 W. P. A., Maryland Project No. 47, 1936.