and Psychology, on April 9, 1938. More than two hundred members attended.

The morning session was devoted to papers of a more general interest, papers in the Jefferson Medal competition, and the address, "Lightning and Lightning Protection," of the retiring president, Professor A. C. Carson, of the University of South Carolina. The afternoon session was divided into sections of Biology, Chemistry, Mathematics, Philosophy-Psychology, Geology and Physics.

At the business session the following officers for 1938–39 were elected:

President: Dr. G. G. Naudain, Winthrop College, Rock Hill, S. C.

Vice-president: E. B. Chamberlain, Charleston Museum, Charleston, S. C.

Secretary-Treasurer: Dr. G. N. Collings, Clemson College, Clemson, S. C.

Curator: Dr. J. E. Copenhaver, University of South Carolina, Columbia, S. C.

Editor: To be appointed.

Executive Committee: Dr. J. E. Mills, Sonoco Products Company; Professor A. C. Carson, University of South Carolina; Dr. C. D. Riddle, Furman University; Dr. R. M. Byrd, The Citadel; Professor J. J. Petty, University of South Carolina.

The Jefferson Medal for the outstanding paper was awarded to Dr. H. D. Bruner, of the Medical College of the State of South Carolina, for a paper entitled "The Blood Picture of Rats from Birth to Twenty-Four Days of Age." The 1938 Research Fund was granted to Dr. Jessie Reed Cockrill, of the Medical College of the State of South Carolina.

The next meeting will be held in the spring of 1939 in Columbia, South Carolina.

F. W. KINARD,
Retiring Secretary

MINNESOTA ACADEMY OF SCIENCE

THE sixth annual meeting of the reorganized Minnesota Academy of Science was held at St. John's University, Collegeville, Minnesota, on Saturday, April 23. More than 200 people attended the programs of the various sections. Five papers were read to the entire group in the morning. In the afternoon 25 papers were presented in the Biological and Physical Sciences and Science Education sections. For the first time, a Junior Academy program was given. Representatives from four high school science clubs gave papers. Several club exhibits were arranged by the Junior group. In the evening, at an open meeting Dr. William Carpenter MacCarty, of the Mayo Foundation, gave an address entitled, "Individualism and Collectivism in Nature." At the business session nearly 200 members were taken into the academy, making a total membership of well over 600.

American Association for the Advancement of Science Research grants of $55 were made to Dr. Alfred M. Elliott, of Bemidji Teachers College, and to Dr. John W. Moore, of the University of Minnesota.

Officers for 1938–39 are: *President,* L. M. Gould, Carleton College; *Vice-president,* R. B. Harvey, University of Minnesota; *Secretary-Treasurer,* H. K. Wilson, University of Minnesota. The councilors are: E. M. Freeman, University of Minnesota; E. T. Tufte, St. Olaf College; H. E. Essex, Mayo Foundation; and L. H. Powell, St. Paul Institute. The officers of the Junior Academy are: *President,* M. H. Kuhlman, Stillwater, and *Secretary-Treasurer,* Lewis L. Barrett, of Edison High School, Minneapolis.

The 1939 meeting will be held on Saturday, April 22, at Macalester College, St. Paul.

H. K. WILSON,
Secretary

SPECIAL ARTICLES

THE PERFUSION OF WHOLE ORGANS IN THE LINDBERGH APPARATUS WITH FLUIDS CONTAINING HEMOCYANIN AS RESPIRATORY PIGMENT[1]

ORGANS perfused in the Lindbergh apparatus[2] are ordinarily supplied only with dissolved oxygen. The use of red blood cells or hemolyzed blood leads to the formation of methemoglobin after six to eight hours, making perfusion for several days impossible. Attempts to prevent the formation of methemoglobin by adding glutathione or ascorbic acid were unsuccessful.

In order to provide more oxygen for the organs it was considered preferable to replace hemoglobin by another respiratory pigment, hemocyanin, rather than to increase the dissolved oxygen by increasing the oxygen tension. Hemocyanin was collected from *Limulus polyphemus* (about 100 cc of blood per crab). The blood was centrifuged to remove mucus and then dialyzed in Cellophane against running tap water until the conductivity showed that most of the electrolytes had dialyzed out. After removal of the proteins precipitated by the dialysis, crude hemocyanin was precipitated by adding N/25 HCl, until the pH was about 6.4, and the solution was again centrifuged. The supernatant fluid was removed and the precipitate dissolved in 50 cc of cat plasma[3] which had been adjusted to a pH of 8.4. This mixture was then dialyzed against distilled water. The resulting solution, containing 4.5 per cent. hemocyanin, was made

[1] From the Department of Surgery of the College of Physicians and Surgeons, Columbia University, and the Department of Surgery of the Presbyterian Hospital, New York City.

[2] A. Carrel and C. A. Lindbergh, SCIENCE, 81: 621, 1935.

[3] Cat organs were used for the perfusion.

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.

RICHARD BING

[4] D. D. Van Slye, C. P. Rhoads, Alma Hiller and Alf S. Alving, *Am. Jour. Physiol.*, 109: 324, 1934.

[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,