healthy coat over areas that appeared miserably "moth eaten" at the time the treatment started. The changes in this animal served as a dramatic illustration of the importance of administering the entire B complex which is to be found only in natural sources.

Although vitamin B_6 therapy in the case of a dog with vitamin B_6 deficiency anemia produces a dramatic and apparently specific effect, yet it has been observed that B_6 alone will not bring the hemoglobin back to normal. Borson and Mettier[5] found that some of the filtrate factors were needed for more complete blood regeneration in their B_6 deficient dogs, and McKibbin and his associates[6] found that certain factors present in liver were needed before normal hemoglobin values ("13–14" grams) could be reached in their deficient animals. This was also borne out in Wintrobe's study[8] with vitamin B_6 deficient swine. In the present study the giving of all the other synthetic B-complex factors in adequate amounts was sufficient to enable the addition of vitamin B_6 alone to bring the hemoglobin back to 15.5 grams but not to maintain it at this level. Brewers' yeast at a level of 10 per cent. not only maintained the hemoglobin level but kept it at a constant value considerably higher than the accepted normal of 14.0 grams.[9] These extremely high values in the yeast-treated dogs, *i.e.*, 18–20 grams, as compared with 14 grams were viewed with some scepticism at first, but they have been repeatedly confirmed when checked in other laboratories of this hospital. The observance of hemoglobin values from 4–5 grams higher than those usually found in dogs probably reflects the fact that dogs under natural conditions of living never receive an optimum supply of the vitamin-B complex. It is not urged that this is essential, but it is probably of value in situations not fully understood at the present moment.

From these data it is concluded that even though a diet lacking vitamin B_6 results in a typical hypochromic anemia in dogs which responds specifically to vitamin B_6 treatment, vitamin B_6 alone or even combined with the known synthetic factors of the B complex is not sufficient to maintain the hemoglobin at optimum levels. There is at least one factor, possibly more, in brewers' yeast in addition to vitamin B_6 which serves to stimulate hemoglobin production in the dog.

ACKNOWLEDGMENTS

We are indebted to Merck and Company, Rahway, N. J., for the Vitamin B_6 and calcium pantothenate used in these studies, to the Fleishmann Laboratories, Standard Brands, Inc., New York City, for the brewers' yeast and to Lederle Laboratories for the material used in immunizing the puppies against distemper.

SUSAN GOWER SMITH
ROBERT CURRY
HAROLD HAWFIELD

DEPARTMENT OF MEDICINE,
DUKE UNIVERSITY SCHOOL OF MEDICINE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

TECHNIQUE FOR STROBOSCOPIC STUDIES OF INSECT FLIGHT[1]

SINCE the application of the Edgerton stroboscope[2] to studies of insect flight,[3, 4] it has become clear that the frequency of wing-beat of standardized insects offers a rigorous technique in quantitative biology. There is reason to believe that the usefulness of such measurements will not be limited to studies of insect physiology, but may extend into other fields such as genetics and experimental pharmacology. It is therefore our purpose to describe the apparatus and technique that we have found most suitable for determining wing-beat frequency.

A survey of the flight of numerous insects has demonstrated that *Drosophila* is a most favorable experimental animal. Advantages offered by this genus include the year-round availability of numerous species and varieties and the generally consistent response under the experimental conditions, as well as the large body of information which already exists in regard to their structure and physiology. Furthermore, wing-beat frequency varies not only with the age and sex of the individual[5] but also with the temperature at which they are reared and flown,[6] so that these factors must be carefully controlled. The use of homogeneous, inbred strains is also highly desirable.[6] Procedures for regulating these details are better established and more easily applied for *Drosophila* than for most other insects. While the small size of *Drosophila* may be a hindrance in some types of work, as in analysis of chemical changes during flight, it simplifies the problem of mounting specimens for observation of wing movement. The relatively stiff

[8] M. M. Wintrobe, R. H. Follis, Jr., M. H. Miller, H. J. Stein, R. Alcayaga, S. Humphreys, A. Suksta and G. E. Cartwright, *Bull. Johns Hopkins Hosp.*, 72: 1, 1943.

[9] R. F. Scarborough, *Yale Jour. Biol. and Med.*, 3: 359, 1931.

[1] This study was aided by a grant from the Josiah Macy Junior Foundation.

[2] K. J. Germeshausen and H. E. Edgerton, *Electronics*, 10: 2, February, 1937.

[3] L. E. Chadwick, *Psyche*, 46: 1–8, 1939.

[4] L. E. Chadwick, *Physiol. Zool.*, 12: 151–160, 1939.

[5] C. M. Williams, L. A. Barness and W. H. Sawyer, *Biol. Bull.*, 84: 263–272, 1943.

[6] S. C. Reed, C. M. Williams and L. E. Chadwick, *Genetics*, 27: 349–361, 1942.

articulation between abdomen and thorax in this genus is also helpful in this respect. *Drosophila* thus seems particularly suitable for measurement of wing-beat frequency; however, the technique described below can be applied with minor modifications to a variety of other insects.

The specimens are fastened as indicated in Fig. 1. A series of mounts are prepared, each of which consists of a short length of copper wire soldered at one end to a pin (head removed) and glued at the other end to a narrow strip of paraffined paper. The insects are lightly etherized and further manipulations carried out under a dissecting microscope. By means of a scalpel the hind pair of legs are removed at the femoro-tibial joint in order to prevent their touching the mount and inhibiting flight. This operation has no demonstrable effect on wing movement. Next the specimen is placed belly-down on a piece of cork and the wings spread by means of a forked pin placed behind their margins. A dissecting needle with a short piece of resistance wire wrapped around its shaft is warmed electrically and used to fuse the paraffined tip of the mount carefully to the dorsal end of the abdomen. The insect is not damaged by this process and regains its flight abilities within a few minutes. After half an hour it is fully recovered from the anesthetic and ready for testing.

As diagrammed in Fig. 1, the mounted insects are fastened to a brass fitting that slides snugly into the Pyrex tube which serves as an experimental chamber. It is advantageous to enclose a number of individuals in the chamber in order to equilibrate them simultaneously with the conditions that are being tested. Each mount is fastened in place with a set screw and adjusted, by bending the copper wire so that the specimen stands on the corresponding spring platform. These springs consist of phosphor-bronze, 0.15 mm thick, cut to appropriate shape and soldered at their free ends to thin squares of soft iron. After the chamber has been sealed, the springs may be depressed by an electro-magnet placed outside the apparatus. Due to the tarsal reflex,[7] flight is stimulated by withdrawal of the platform and inhibited by its return, so that it may be controlled at the will of the operator.

The chamber, which consists of a length of Pyrex combustion tubing, is sealed by clamping it between brass end-pieces connected by three rods. The triangular end-pieces are machined to receive the ends of the chamber and fitted with rubber gaskets. With this arrangement the chamber may be made tight for pressures as high as ten atmospheres. The desired gas mixtures are supplied and pressures controlled through copper tubing provided with suitable valves and sealed into the central openings of the brass end-pieces.

Since accurate control of the temperature of the chamber is essential, the apparatus is most advantageously immersed in a water bath that is thermostatically regulated. We have found most convenient for this purpose a bath equipped with a glass-bottom offset. A mirror placed outside the bath and beneath the offset permits the animals to be studied in silhouette against the stroboscopic lamp clamped above the water surface. An extension cord leads from the lamp and reflector unit to the control box on the table below. The flight chamber is supported by a clamp so that it can be lifted from the water or moved longitudinally in front of the water-tight electromagnet, and is so oriented that the dorso-ventral axis of the animals lies in the horizontal plane. Thus, by moving the chamber, each animal can be brought opposite the magnet and tested in turn, while it is viewed through the glass offset without obstruction. The flight response of the animals does not seem to be influenced in any way by their static orientation in respect to the field of gravity. If the animals are to be studied during continuous flight, a permanent magnet may be substituted in place of the electromagnet.

By means of an apparatus thus arranged, it has been possible to measure precisely the frequency of

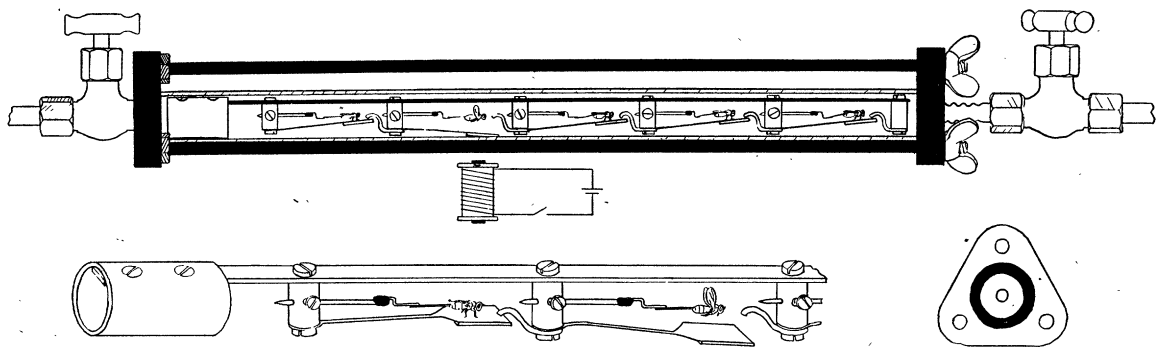

Fig. 1. Profile view of flight chamber (× ⅓), details of brass fitting and internal view of brass end-piece.

[7] G. Fraenkel, *Ztschr. f. Vergleich. Physiol.*, 16: 370–393, 1932.

wing-beat under a variety of experimental conditions. Many hundreds of reliable measurements may be accomplished on each individual during intermittent flights having durations of 3 to 5 seconds, separated by adequate periods of rest (about 20 seconds). Under carefully controlled conditions the variability of such determinations on a single individual is generally less than one per cent. For 24 strains of *Drosophila* the coefficient of variability among different individuals of single strains was found to average 3.10 per cent.[6] Useful information in regard to metabolism and fatigue can be obtained in terms of the progressive changes in wing-beat frequency during continuous flight.[5] The principle of the apparatus has also been adapted for measurements correlating wing-beat frequency with respiratory exchange.[8]

LEIGH E. CHADWICK
ANESTHESIA LABORATORY OF THE HARVARD MEDICAL SCHOOL AT THE MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MASS.

LEIGH E. CHADWICK
DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY

THE COAGULUM-CONTACT TECHNIC IN TRAUMATIC RUPTURE OF THE LIVER IN DOG AND MAN

AT the suggestion of Dr. W. Wayne Babcock, it was decided to extend the principles involved in the coagulum-contact technic of skin grafting[1] to the control of bleeding and the promotion of healing in traumatic injuries of the liver and spleen. In the past it has been customary to sew the opposing surfaces of the liver together. This frequently promotes rather than controls bleeding. Likewise the packing of the injured field is often complicated by bleeding at the time the packing is removed. The new method presents neither of these difficulties.

Equal quantities of plasma and cell extract (autogenous, homologous or heterogenous) are mixed together just before using. The bleeding surfaces of the liver which are to be joined are exposed and the mixture is rapidly brushed over both surfaces with a sterile camel hair brush. The surfaces are firmly held together for about three minutes. They are then released and will adhere firmly to one another. If the bleeding is still present in poorly apposed parts, some of the mixture is brushed over these areas and left to coagulate. If there is profuse bleeding the brush may be held over the site of bleeding for one to two minutes. On microscopic examination, three days later, fibroblasts are seen to have proliferated in the coagulum formed. Five days after operation, small sinuses appear in certain sections. By the end of ten days, it is often difficult to find the line of incision on gross inspection of the specimen. Microscopically, one finds these areas well organized with very little evidence of damage to the adjacent liver cells.

Damaged surfaces of liver will adhere to one another on pressure without the interposition of plasma and extract. However, oozing does not stop so readily and when profuse bleeding occurs at the inner angle of two opposed surfaces where it is difficult to exert pressure, it is almost impossible to stop the bleeding by pressure alone. While adhesion has been 100 per cent. when using the plasma-extract mixture on fifteen dog livers and one human liver, the liver in three (or 20 per cent. of the cases) had to be restuck when no plasma-extract was used. In no case did the dog bleed to death with either method.

Similar experiments have been carried out on the spleens of dogs. Here the plasma extract gives definitely superior results but neither method is as satisfactory as in the liver. Due to the intrinsic structure of the spleen itself, infarctions are apt to occur. Again, no death actually occurred due to hemorrhage but healing was slow and unsatisfactory.

This new method is extremely simple and uses the physiologic principles of blood clotting and wound healing thus eliminating any extraneous factors which might complicate and endanger the individual's life in some other way. It is hoped that this method may be of use in the treatment of war wounds.

MACHTELD E. SANO
CLARENCE A. HOLLAND
DEPARTMENTS OF PATHOLOGY AND SURGERY, TEMPLE UNIVERSITY HOSPITAL AND SCHOOL OF MEDICINE

[8] L. E. Chadwick and D. Gilmour, *Physiol. Zool.*, 13: 398–410, 1940.

[1] M. E. Sano, *Am. Jour. Surg.*, lxi, 105–106, 1943. This new method of skin grafting using plasma and cell extract to form an adhesive and growth-stimulating coagulum is being used not only at Temple University Hospital but at other hospitals with very good results.

BOOKS RECEIVED

EDDY, SAMUEL and THADDEUS SURBER. *Northern Fishes with Special Reference to the Upper Mississippi Valley.* Illustrated. Pp. xi + 252. The University of Minnesota Press. $4.00.

JAMIESON, GEORGE S. *Vegetable Fats and Oils.* Pp. 508. Reinhold Publishing Corporation. $6.75.

MACDUFFEE, CYRUS COLTON. *Vectors and Matrices.* Pp. xi + 192. Open Court Publishing Co., La Salle, Ill. $2.00.

Pain. Research Publication of the Association for Research in Nervous and Mental Disease. Illustrated. Pp. xii + 468. Williams and Wilkins Company. $7.50.

RAINVILLE, EARL D. *Intermediate Differential Equations.* Illustrated. Pp. vi + 211. John Wiley and Sons. $2.75.

SPICER, WILLIAM M., WILLIAM S. TAYLOR and JOE D. CLARY. *General Chemistry Problems.* Illustrated. Pp. v + 120. John Wiley and Sons. $1.25.