(see Fig. 3). During centrifugation at 28°C, the growth constant drops in a more or less linear manner as the field increases beyond 500 G's(6). Such linearity is not demonstrable for I. In all cases, there was little mortality after centrifugation. For both control and experimental cultures, 90% of animals surviving after centrifugation had ceased, were alive 24 hours later.

Discussion. Prolonged centrifugation causes fruit fly larvae to develop altered metabolism. This is evidenced by the fact that, upon return to control conditions, these animals can accomplish more than the normal amount of physical growth with a given quantity of oxygen. The mechanism of this alteration is unknown. It is tempting to interpret this as an increased efficiency of growth processes.

The validity of the preceding suggestion could well depend upon how broadly we wish to define "efficiency" and "energy for growth." Let us define efficiency in terms of the ratio of growth accomplished to food metabolized. Then, assuming a constant, caloric equivalent for oxygen, efficiency has increased.

One would not necessarily expect changes in chemical production of protoplasm to greatly alter the overall oxygen requirements. Processes other than production of protoplasm could be altered. These other processes might include various movements, digestion, and maintenance. Should any change cause a greater efficiency, then, once gravitational stress is removed, a larger fraction of food would be available for growth processes.

After centrifugation, larvae do develop into pupae. However, unlike controls, a sizable percentage of these individuals do not emerge as adults. Perhaps, food materials can be conserved by sacrificing structures.

Summary. 1. Prolonged centrifugation can alter an organism's growth. 2. After centrifugation at 20 and 28°C, fruit fly larvae do not require as much oxygen for growth. 3. It is suggested that the animal has adapted by developing a greater efficiency for growth.

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Adaptation of Orbital Bleeding Technic to Rapid Serial Blood Studies. (25975)

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During investigations requiring serial blood samples on experimental animals our attention was drawn to the ingenious but apparently little known eye bleeding procedure (1-5). Tail bleeding is difficult, time consuming and unsatisfactory from the standpoint of contamination of the collected blood. Other methods have obvious drawbacks.

Methods. Since enzyme studies usually require plasma rather than whole blood it is desirable to centrifuge directly in the original blood collection tube. The technic requires a

* With assistance of David Bardell and Elizabeth Booth.

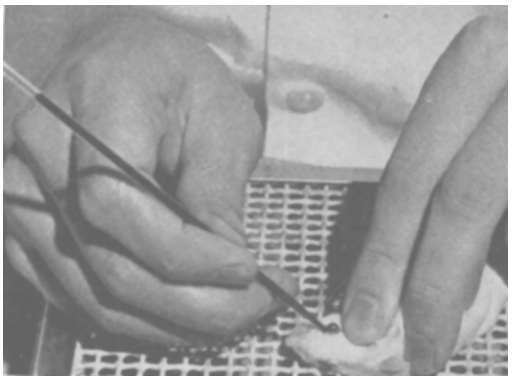


FIG. 1. Proper position of fingers, animal, and pipette during bleeding procedure.

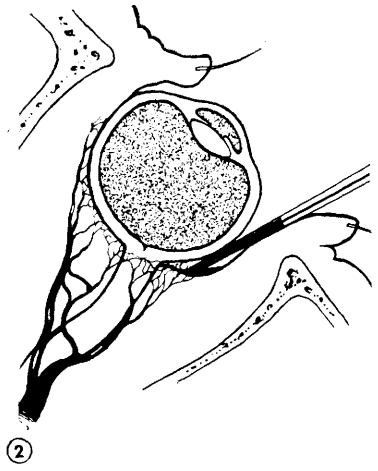
small, disposable, heparinized pipette with polished tip for non-traumatic entry into the orbit alongside the eye. Such a pipette is now commercially available.† Utilizing the technics described here, a technician can bleed 100 mice in about an hour and a half. Our standard procedure is as follows: 1. Test tubes, 13 x 100 mm, are labelled with appropriate experimental numbers and are set up in a rack to receive the blood filled capillary tubes. 2. As illustrated in Fig. 1, the donor animal is held by the back of the neck with the left hand, and the loose skin of the head is tightened with thumb and middle finger. With the aid of the index finger, the eye is made to bulge slightly by further traction of the skin adjacent to the eye. The tip of the pipette is then placed at the lower or inner corner of the eye and gently but firmly slid alongside of the eyeball to the ophthalmic venous plexus which lines the back of the orbit (Fig. 2). The venous capillaries forming this network are so fragile that they rupture upon contact with the tip of the pipette and resulting hemorrhage fills the orbital cavity which serves as a useful reservoir. A slight withdrawal of the pipette frees the tip so that the accumulated blood is immediately drawn into the tube by capillary action. The actual bleeding part of the procedure, while the pipette is in position, takes about 2 seconds. Residual blood around the eye is swabbed clear with a soft absorbent tissue to avoid clot formation. Bleeding usually stops immediately upon withdrawal of the pipette and re-establishment of normal ocular pressure on the venous network. By appropriate change in positioning of the animal, either eye may be bled, although the right eye is more convenient for a right-handed technician. 3. If blood is to be centrifuged, it is necessary to seal the upper end of the collection tube, by thrusting the blunt end of the tube through a thin wall of softened clay‡ which forces 3 to 5 mm of the plastic material into the bore of the tube. This is held in place by a tight fitting serum ampule stopper.§ 4. The sealed blood pipettes are placed in their previously labelled test tubes, with the stopper end down, for centrifugation. For most purposes, 2,000 rpm (approx. 1,000 x G) for 20 minutes‖ provides adequate separation of plasma and red cells. Either a horizontal or angle head may be employed, although traces of red cells tend to cling to the sides of the tubes with the latter. 5. If the hematocrit (packed red cell volume) is desired as an additional parameter, it may be obtained at this stage by holding the capillary tube against the inclined scale previously described(6). This gives the hematocrit directly in percent. 6. To obtain the plasma, the tube is nicked with a file just above the plasma-erythrocyte interface and is readily snapped in two. The plasma is recovered and measured by touching the tip of a 0.1 ml or 0.025 ml pipette to the open end of the severed tube. Exact adjustment of plasma volume is accomplished by applying a wiping tissue to the tip of the measuring pipette. The collection tube holds approximately 0.2 ml of blood which yields about 0.1 ml of plasma. This is ample for most enzymes determined spectrophotometrically when employing a 1.0 ml microcuvette. If more plasma or blood is required, additional tubes may be obtained from the same donor.

† Micro Blood Collection Tubes, Heparinized with Ammonium Heparinate, Scientific Products, Div. of Am. Hospital Supply Corp., Evanston, Ill.

‡ Children's plastic modeling clay, blue, or green color to contrast with blood cells.

§ No-2319-B Rubber Stoppers, Arthur H. Thomas Co., Philadelphia, Pa.

‖ International Refrigerated Centrifuge, Model PR-2 Head No. 253.



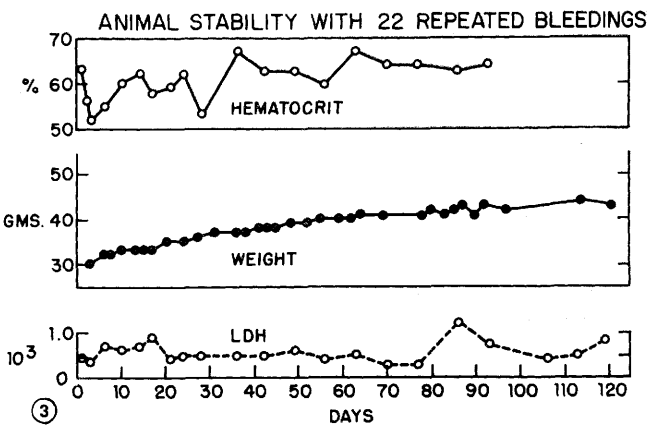


FIG. 2. Diagrammatic illustration of location of ophthalmic venous plexus and relationship of pipette entry into the orbit.

FIG. 3. Lack of significant influence of repeated 0.2 ml ocular bleedings of mice over a period of 4 mo in respect to hematocrit, mouse wt, and plasma lactic dehydrogenase activity (LDH).

However, it is necessary to bear in mind the results of excessive bleeding on the hematopoietic system, if this is of pertinence to the experiment, since collection tube volume of 0.2 ml may represent 5 to 10% of total mouse blood volume. If large volumes of blood are required, the mouse can be exsanguinated by this technic.

Discussion. Moderate use of this procedure, such as 1 or 2 bleedings per week, seems to be harmless to the animal, and even daily bleedings have been imposed for limited periods with a lowered hematocrit being the only adverse effect noticed. No anesthetic is required with this procedure and, although the technic carries a formidable psychological barrier for an identifying human when it is first observed, neither the eye nor the health of the mouse appears to be damaged. The same mice have been bled repeatedly from both orbits for periods of several months without evidence of blindness or other serious damage. Occasionally accidental trauma or infection may require the bleeding to be carried out on the opposite eye or the animal to be sacrificed. As indices of animal stability, Fig. 3 illustrates uniform level of weight, hematocrit, and a blood enzyme (LDH) in normal mice bled repeatedly during an experiment lasting several months. For further data see(7,8).

While our experience has been mostly with mice, the same technic has been used successfully with a variety of other small laboratory animals. Chickens seem to be an exception, however, since the structure surrounding the eye is too rigid to permit passage of the pipette to the posterior vascular area.

For enzyme and chemical determinations requiring plasma free of hemoglobin and other erythrocyte contents, we have found it desirable to keep the blood cool at least until plasma has been separated from red cells. The rack of collection tubes is therefore placed in a refrigerator or a portable ice box during bleeding and while waiting centrifugation. This seems to minimize interfering subliminal hemolysis. The procedure described here permits daily following of the course of a disease or effects of treatment on individual laboratory animals, as well as groups, similarly to the close clinical observations on a human patient. With this single bleeding operation requiring about 30 seconds, one can obtain a smear for differential cell counts or cytological study, erythrocyte sedimentation rate, hematocrit, and plasma for biochemical determinations or enzyme studies.

Summary. Adaptation of orbital bleeding procedure to rapid, serial blood sampling of mice and other small laboratory animals has been made. Blood (0.2 ml) is collected in disposable, heparinized micropipettes by rup-

turing the fragile opthalmic venous plexus with tip of blood collection tube. The tube may be sealed and centrifuged for hematocrit determination and plasma separation for enzyme or other biochemical studies. Bleeding procedure for a mouse requires less than 30 seconds, and 100 mice can be bled and processed by a technician in about 1½ hours. Appropriate illustrations demonstrate technical details of procedure.

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Rapidity of Breakdown of Nucleotides in Different Tissues.* (25976)

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Mammalian blood can be incubated *in vitro* for several hours without affecting distribution and amounts of nucleotides in the formed elements of the blood. This has allowed us to perform incorporation studies under essentially *in vivo* conditions. In attempting to perform similar incorporation studies on other mammalian tissues we discovered that after incubation, the major nucleotide, ATP, had largely disappeared and that AMP was now present in considerable quantities. The present paper describes our attempt to determine *in vivo* distribution of nucleotides in certain tissues and the rapidity of change in this distribution after removal of the tissue from the animal.

Materials and methods. Dog muscle and dog and rabbit intestinal mucosa were originally selected because they might be expected to contain large amounts of nucleotides that would be turning over fairly rapidly. The muscle was minced with scissors while the mucosa was merely scraped off the intestine with a glass microscope slide. The nucleotides were extracted from either preparation with cold trichloroacetic acid (TCA) and fractionated on Dowex-1 formate columns according to our previous procedure for blood (1). In the first experiments a dog was given a lethal dose of Nembutal intravenously and the hind legs rapidly skinned. One portion of leg muscle was dropped into ice cold 10% TCA and minced with scissors. A second portion was excised and allowed to stand at room temperature for 20 minutes. It was then treated like the first. A third portion was minced with scissors in calcium-free Krebs-Ringer-Bicarbonate medium(2) containing 200 mg % glucose. Ice cold TCA was then added to the mince. A fourth portion was prepared like the third except that it was incubated 2 hours at 37° under 95%O_2–5%CO_2 before TCA was added. No attempt was made to weigh portions of muscle accurately, because this would have slowed down handling of the tissue and because only distribution of nucleotides was of interest at this point. A second type of experiment was designed to ascertain if nucleotides in intestinal mucosa were as labile upon incubation as nucleotides in dog muscle. For this purpose a dog was injected intravenously with lethal dose of Nembutal and a length of small intestine removed as quickly as possible. This was slit open, rinsed briefly with cold saline, and

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