Metabolism Cages

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In laboratories which only occasionally engage in metabolism experiments with rats, it may be difficult to obtain quickly a suitable arrangement of cages. The unit to be described is efficient, easily constructed, and requires few items not usually stocked. The complete unit has gradually evolved over a period of years, and therefore proper credit cannot be given to individuals who have contributed ideas.

The ordinary round cage used in nutrition experiments may be used (Fig. 1). This cage, as shown, has an efficient

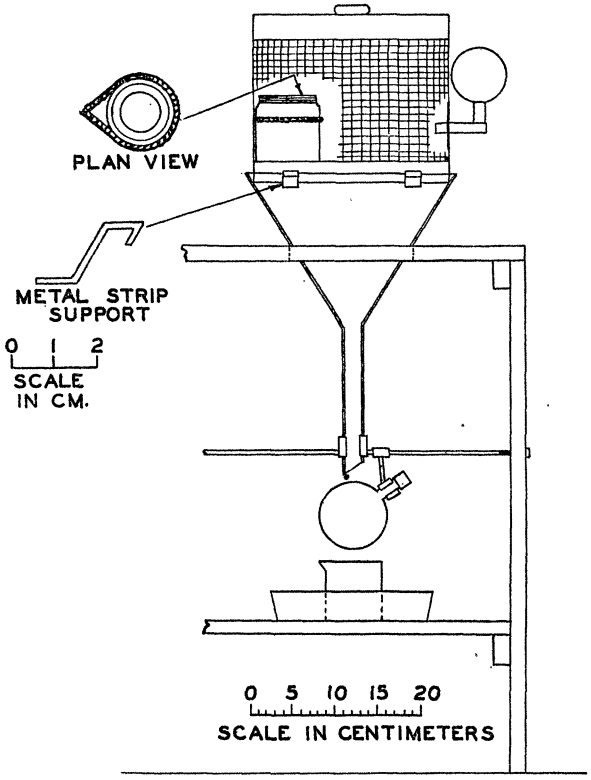

FIG. 1. Arrangement of metabolism cage and stand.

watering device, but the trough, unless cleaned often, tends to accumulate particles of food from the mouth of the rat. If this is objectionable, a drinking tube may be pushed between the wire meshes in the top of the cage, with an inverted bottle outside the cage. The food receptacle is held in place against the cage by a weak spring, which encircles the upper part of the jar. The spring also permits easy removal or replacement of the food jar. For most of our work we use half-pint Mason jars, with Kerr screw caps, a circular opening, 3.5–4 cm. in diameter, being cut through the center of each lid. Although the lids may be dispensed with, their use often helps prevent the animals from throwing out and wasting food.

The cage rests upon four strips of stainless steel or other metal (each about 12 mm. wide), bent on one end to fit over the edge of a funnel, 250 mm. in diameter, and bent up on the other end to form a firm ledge for the cage bottom. These strips may be lifted off for cleaning. The glass funnel fits into a hole in the wooden stand and is gripped by a burette clamp near the lower end of the stem. A 200-ml., round-bottom, short-ring flask (balloon flask) is held by a burette clamp beneath the stem of the funnel. Feces drop between the meshes of the cage and, falling through the stem of the funnel, are deflected by the rounded surface of the flask into a pan or large evaporating dish. Urine passes down the funnel onto the surface of the flask and follows the curve of the flask to the lowest portion, whence it drips into a beaker, or Erlenmeyer flask, placed directly beneath the balloon flask.

The wooden stand, 109 x 63 x 30.5 cm., has three openings, 15.2 cm. in diameter, to support the funnels, and a shelf 19 cm. from the floor to support the receptacles for the urine and feces. A metal rod, 0.9 cm. in diameter and 38 cm. from the floor, runs the entire length of the stand. To this rod are fastened the various burette clamps.

The stand described is designed for three cages, which provides a unit easily moved by one person and conveniently stored.

Sharp Interfacial Precipitin Reactions in Capillary Pipettes[1]

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The capillary pipette precipitin technique, which was developed for grouping and typing hemolytic streptococci (*2*), has proven adaptable to other investigations in which precipitin tests are required. The obvious advantages lie in the great saving of both precipitating serum and bacterial extracts or other antigenic substances. With moderately to strongly reacting reagents, easily detectable precipitates are formed in capillary pipettes having an external diameter of 1.0 ± 0.02 mm., in which the two reagents readily mix. With weaker precipitating sera, or with sera in which prozoning is liable to occur, particularly with those which react with the group-specific carbohydrates of streptococci, it is often necessary to employ larger capillary pipettes (*i.e.* 1.5 ± 0.02 mm.). With such pipettes, however, it is frequently difficult to obtain as clear-cut reactions as occur in small test tubes in which the antigen is carefully layered over the serum so that a sharp ring of precipitate forms at the interface between the two reagents (*1*). It has long been recognized that ring, or interface precipitin reactions are at times convenient in developing satisfactory precipitin tests. A difficulty encountered in trying to obtain sharp layering in capillary pipettes is the tendency for the two reagents to mix—a tendency roughly proportional to the movement of the interface. This move-

[1] This investigation was carried out under a contract between the Rockefeller Institute for Medical Research and the Commission on Hemolytic Streptococcal Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army.

ment is held to a minimum in the larger pipettes by the following modifications:

Pointed capillary pipettes are made from tubing, 1.5 ± 0.2 mm. in external diameter, which is broken into lengths of about 13 cm.[2] The middle of each length is heated in a narrow flame, such as is made by a fishtail burner or the pilot of a Bunsen burner, and, when melted, drawn to a very fine point by quickly pulling on the two ends of the tubing. When cool, the very finely drawn glass is broken close to the conical tip; thus, two conically pointed pipettes are formed with fine openings about the diameter of a hair. To insure that these openings are patent, they may be inspected with the aid of a hand lens. With practice, three to four such pairs can be drawn at one time. The outer surface of these pipettes is cleaned with paper tissue, the pipettes then being placed in test tubes containing absorbent cotton on which the conical points rest. These containers are capped with unsized paper and then sterilized by dry heat, or by autoclaving followed by drying in an incubator.

For testing, the sterile pointed end of a pipette is dipped into the precipitating serum until a column of serum between 1.0 and 1.5 cm. long has been slowly drawn in by capillary action, then wiped with paper tissue and dipped into a drop of antigen solution until an equal amount has been drawn into the pipette. Air bubbles must not separate serum and antigen solution. This conical end is again wiped and plunged into a lump of plasticine in order to seal the hair-sized opening. The lower end of the pipette is pressed against a strip of plasticine, which has been previously placed on a block of wood, so that the pipette is held in a vertical position without being plunged into the plasticine. If it is mounted in this manner, the fluid in the upper part of the pipette will not be forced out by air pressure which would be developed were the lower open end forced directly into plasticine. The upper end should, however, be inspected to see whether any solution has been forced out, and if this has occurred, it should be wiped off so that no film will be deposited on the pipette.

If this procedure has been followed, the column of fluid will remain at the upper part of the pipette, the surface of which should be perfectly clean.

The above-outlined technique insures the minimal mixing of the underlying serum and overlying antigen solution; hence, there is a narrow zone in which the precipitate forms. When longer columns of serum and antigen are drawn into the capillary pipette, this interzone moves over a proportionally wider range, more mixing of the two reagents occurs, and the zone of precipitate is wider; hence, the reaction may be less intense. The same tendency to mixing of the two reagents exists when the reaction is set up, as previously advocated, in 1.5-cm. pipettes with both ends wide open: rapid mixing of the two reagents occurs, and the reactions may be so indeterminate that they often require confirmation in small test tubes.

Within 5 to 10 minutes a positive reaction is shown by a cloudy white disc of very fine precipitate at the junction of serum and antigen. With weak sera or antigens, a longer time may be required. When testing with streptococcal grouping sera, if the pipettes are placed in the incubator at 37° C. for an hour, weak cross-reactions with sera of other groups sometimes occur; hence, readings made within 5 to 10 minutes probably indicate more specifically the group to which the streptococci under examination belong. Upon standing, the precipitate formed early may redissolve, or it may clump, fall to the bottom of the serum, and be detectable the following morning after refrigeration.

Errors due to weak reactions may result from excessive mixing of the antigen and serum. Another source of error at times is to allow the test to stand too long before reading, because the precipitate formed early may redissolve. False readings may result from grease marks or other materials on the outer surface of the tubes over the zone of reaction. Antigens and sera should always be clear before preparing the test, since hazy sera, or sediment drawn into the pipettes with the reagents, may lead to false-positive readings.

[2] This capillary tubing is carried in stock by Arthur H. Thomas, Philadelphia.

References

1. LANCEFIELD, R. C. *Proc. Soc. exp. Biol. Med.*, 1938, **38**, 473.
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Warm Safranine for Plant Tissues

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In the course of recent botanical investigations conducted at the University of Michigan Biological Station, an attempt was made to decrease the length of time necessary for staining plant slide material with safranine. The slides and safranine were placed in a paraffin oven which was thermostatically controlled at 53° C., and a series of tests were made subjecting different materials of different thicknesses to varying lengths of time in the heated safranine. The procedure up to and following the safranine bath conformed to the norm.

The results indicated that completely satisfactory staining could be obtained in 15 minutes as compared with the 24- to 48-hour period necessary when the stain is used at room temperature. The color imparted by the warm stain is quite brilliant and remains fast in the tissue even through prolonged immersion in absolute alcohol. Young *Lycopodium* stem, ordinarily difficult to stain, showed excellent results after 15 minutes in the heated stain. Other material tested included hypocotyl of collard, stem of *Prenanthes alba*, and stem of *Diervilla Lonicera*.

Other tests showed that successful staining could be accomplished when the slides were left in the safranine for periods up to 3½ hours. However, material left in for 12 hours was overstained, resulting in a deep purple color which was not readily distinguishable, under low power, from the fast green used as a counterstain.

As the heat induces a rapid and thorough penetration of the dye, the excess stain is not as rapidly removed as under room temperature, and it is desirable to leave the slides in absolute alcohol for a somewhat longer time than is usual. For the same reason, the normal 1- to 2-minute immersion in fast green was increased to 3–10 minutes.

A brief examination of the literature indicates no previous mention of the possibilities of using warm safranine for botanical preparations, although Lee (*1*) mentions its use for zoological preparations by Griesbach in 1887.

Reference

1. LEE, B. *Microtomist's vade-mecum.* (9th ed.) Philadelphia: Blakiston, 1928.