large amounts of selenium in their fruit as a result of spraying with selenium dissolved in potassium ammonium sulfide solution for the control of red spider.

A complete account of this and other work on selenium carried out in the California Agricultural Experiment Station is in process of compilation.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

GROWTH SUBSTANCE DETERMINATIONS

THE Went *Avena*-coleoptile test and the *Cephalaria* test of Söding are the most sensitive ones that have been devised for quantitative hormone studies. These tests, however, are available only to a few laboratories which have facilities for the accurate regulation of temperature, humidity, etc.

The authors have developed a method of hormone determination which requires only the facilities available in every botanical laboratory. The manipulation is simple, no special apparatus is required, and the method is applicable over a wide range of growth substance concentrations. It may be used for detection of hormones at the low concentrations to which the *Avena* coleoptile responds, but so far as the method has been tested, does not appear to be available for the detection of minute differences within the low range of concentrations, as do the *Avena* and *Cephalaria* tests.

The new method depends on the fact that when etiolated seedlings of *Lupinus albus,* decapitated below the cotyledons, are exposed to light, growth ceases almost completely. However, when a growth substance is applied to the cut surface of the hypocotyl, elongation of the hypocotyl takes place in the presence of light, and this elongation is proportional to the concentration of the growth substance applied.

Some twelve or fourteen *Lupinus albus* seeds of approximately uniform size are planted in six-inch pots in sand. They are germinated in a dark room for six to seven days, or until the hypocotyls are seven to eight centimeters high. In the morning the pots are brought into the light, and the seedlings are selected for uniformity. The cotyledons are cut off with a razor blade, at the apex of the V-shaped notch which they make with the hypocotyl. A mark is made one centimeter below the cut, with India ink, and the growth substance is applied in an agar block, or in lanolin paste to the cut surface of the hypocotyl. The pots are placed under bell jars in order to insure high humidity and are exposed to full daylight. On the morning of the fourth day, the increase in length of the original centimeter segment is measured. Ten to fifteen test plants are used for each determination. With low concentrations of growth substances, control plants treated with lanolin or agar only are run in the same pot with the test plants. Indole-3-n-acetic acid in appropriate dilutions is used as a standard.

A straight-line relationship is obtained when high concentrations of heteroauxin ($100\,\gamma - 0.01\,\gamma$) are plotted logarithmically against increase in growth. However, in the lower range of heteroauxin concentration detected by the *Avena* test[1] ($0.01\,\gamma - 0.001\,\gamma$) the curve rounds off.

The wide range of concentrations to which the hypocotyls respond should make this test especially applicable to the study of the growth-promoting activities of various substances that are not inactivated by light. Moreover, a test object whose physiological make-up is somewhat different from that of *Avena*[2] should prove of value in attempting to understand the mechanism of growth reactions.

The authors have had the benefit of Professor F. G. Gustafson's interest and advice in the studies, which will be described later in greater detail.

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A BODY PLETHYSMOGRAPH FOR MEASURING RESPIRATORY VOLUMES WITH HIGH RESPIRATORY RATES

WHEN animals which are deficient in sweat glands, *i.e.,* dog, cat, rabbit, etc., are exposed to heat they develop a peculiar type of respiration called "heat polypnea" or "panting." The ventilation and respiratory rate increase, while the tidal volume decreases. With dogs respiratory rates may reach over 300 per minute. In measuring respiratory volumes of panting animals it is customary to use a tracheal canula or mask connected through valves to a recording system such as a respirometer or gas meter. Objections to this method include the following: (1) either anesthetics must be used or the respiratory pattern becomes subject to artificial alterations due to pressure stimuli, pain, etc.; (2) the dead space of the apparatus does not match that of the normal animal; (3) canulae impede air movement; (4) the purpose of panting is to blow air over the moist surface of mouth, tongue and pharynx for purposes of evaporation and this cooling mechanism is lost to the canulated animal; (5) moving mechanical systems have the disadvantages of having appreciable inertia and of giving false records when the respiratory period approaches or becomes less than the natural period of the moving system. In order to

[1] George S. Avery, Jr., Paul R. Burkholder and Harriet B. Creighton, *Am. Jour. Bot.*, 24: 226–232, 1937.

[2] Sam Granick and H. W. Dunham, Papers, Mich. Acad., 22: 69–78, 1936.

eliminate the above sources of error, the recording plethysmograph shown schematically in Fig. 1 was built.

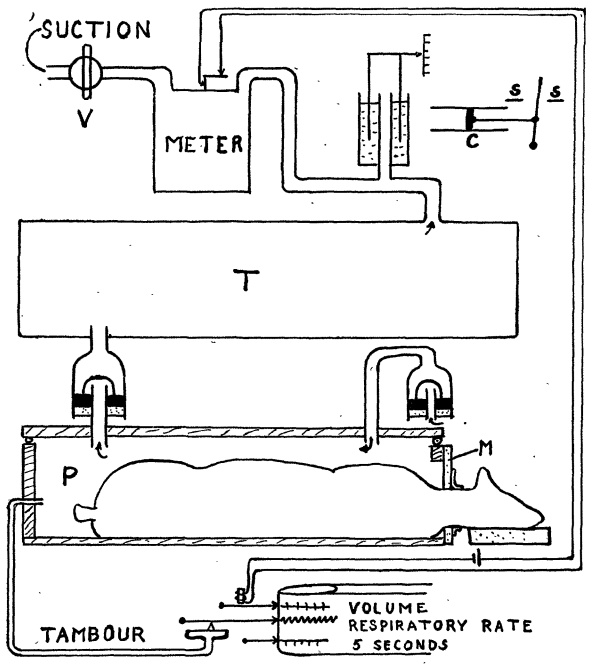

FIG. 1

The recumbent dog lies within a box (P) closed except for valves and tambour outlets, with head protruding through a seal. The seal consists of a spongy rubber mat with a hole just large enough to permit the head of the dog to go through. A circular hole is cut in a piece of dental rubber dam and this is placed around the dog's neck to fit tightly. Adhesive tape seals the rubber dam to the dog's neck and the rubber mat, which is then clamped by a brass frame tightly to the edge of a large hole in the plethysmograph. When carefully placed a dog experiences no discomfort and a trained animal will lie quietly for six hours in the apparatus. The lid of the apparatus is clamped on tightly, soft rubber tubing acting as a washer.

Each of the two box valves consists of a half ping-pong ball seated on mercury. The inlet valve admits room air to the plethysmograph during expiration. During inspiration this air is forced through the outlet valve into a large 150 liter sealed tank (T). From the tank (T) an outlet tube leads to a sensitive spirometer and a dry gas meter. The cylinder of the spirometer is of aluminum, displaces 20 ml per cm and is carefully counterweighted. The meter has electrical contacts which record every one fifth of a revolution representing about 750 ml between contacts. A manually operated valve (V) controls the measured amount of air drawn from (T).

In operation the dog draws air into (P) by expiration and forces the air into (T) during inspiration. Pressure builds up in (T) causing the cylinder of the spirometer to rise. Suction then withdraws air from (T) until the spirometer has returned to its original position. During rapid respirations of 300 per minute, to which the small spirometer can not respond, the large air volume in (T) has added to it by rapid pulsations relatively small quantities of air which build up the pressure. The large air volume acts as a pneumatic cushion to the air pulsations. It is important to have the tank (T) at a slight positive pressure, otherwise air would be drawn through the entire system without breathing. A kymograph record contains a 5 seconds time line, a tambour tracing of rate and a record of the volume of air passing through the plethysmograph, as indicated by the meter contacts.

The apparatus was tested by using a cylinder and piston arrangement (C). The free open end of this cylinder was placed through a hole in the mat (M). Closed containers approximately equal in volume to the dogs were placed in (P). The piston was then moved back and forth between stops (S) at rates simulating respiration. The volume of air drawn into and expelled from (P) could be computed from the stroke and area of the piston.

With this testing procedure the following methods of recording the ventilation rate were tested: (a) a large tambour over a large hole in (P); (b) the small spirometer directly connected to (P); (c) the inlet valve connected to the spirometer and the outlet valve discharging to the room air; (d) the inlet valve opening to the room and the outlet valve discharging air through a wet or dry gas meter; (e) the differential method as described above. As a result of these tests it was found that for respiratory rates exceeding 100 per minute method (e) was the only one giving satisfactory checks. The other methods (a)–(d) depended on some mechanical recorder stopping and starting with each respiration. For rapid respirations the starting inertia was too great to be overcome by the low driving pressures of the plethysmograph.

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