8°C. and 360 mm. would fall in the cool temperate steppe formation. If the same values were obtained for a station 2,500 m. above sea level, the formation would be warm temperate montane steppe. Formations with common precipitation and temperature boundaries, but occurring at different elevations in adjacent regions, are termed linked formations because they show closer affinities than other adjacent formations separated by temperature or precipitation boundaries.

The evaporation lines are not essential to the use of the chart. These are inserted to show the other balancing factor in the chart, and the values indicated are thought to represent the number of times the actual precipitation could be evaporated in one year at sea-level atmospheric pressure. No data are available to support these suggestions regarding evaporation.

The chart is designed to make broad divisions and to show actual relations between climatic vegetation formations. Local edaphic conditions, such as salinity or a high water table as well as alteration by man, can obviously change the appearance of the vegetation to a great extent. Two or more names were found necessary at times, partly because formations from both low and high elevations are represented on a two-dimensional chart and partly because certain easily determined factors, such as topography in grass or tree formations and the difference between continental- and mediterranean-type climates, make possible vegetations of a distinct appearance under similar climatic conditions. Further details and examples will be given in a paper now in preparation.

Detection of Hypoglycemic Reactions in the Mouse Assay for Insulin

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In 1946 Thompson (*4*) described a sloping-screen technique for use in the mouse assay of insulin. This technique avoids the subjective selection of convulsive animals necessary in the more conventional methods (*2*, *5*) and reduces personnel requirements.

One disadvantage of Thompson's method, as observed in these Laboratories, lies in the fact that the mice do not fall away from the screen until signs of insulin shock are far advanced, and, even though the mice are immediately injected with glucose, the mortality is high.

Fig. 1 shows a unit which was developed in these Laboratories in an attempt to overcome the difficulty noted.[1] The unit consists of 6 hollow cylinders, 8 inches in diameter and 27 inches long, mounted at an angle of 60° on wooden rollers. Five inches of each end of each cylinder is constructed of 22-gauge galvanized iron, and the central section, 17 inches in length, is constructed from ¼-inch wire mesh. The wooden rollers, motor driven at a constant speed, make contact with the cylinders through plywood bands which encircle the cylinders 2½ inches from top and base. Each cylinder rotates once every 40 seconds. The cylinders may be removed from the rollers for cleaning and emptying. The unit is housed in a room with a temperature range of 23–25° C.

In performing an insulin assay, mice are injected subcutaneously with suitable amounts of the insulin preparations to be compared and are loaded into the rotating cylinders, each of which will readily accommodate 40 animals. The rotation assures that the foothold of the mice is never secure for long. At the first sign of insulin shock they fall away into trays placed beneath the individual cylinders. These contain rabbit chow pellets, and in almost all cases the mice falling into the

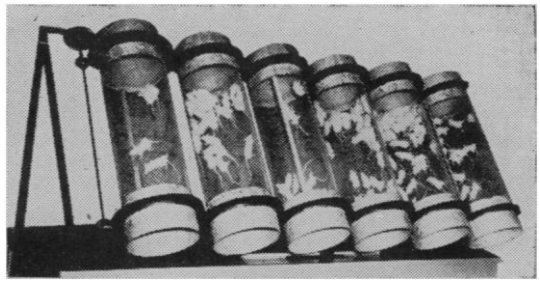

Fig. 1

trays are able to eat sufficient food to relieve their hypoglycemic signs, so that injection with glucose is unnecessary. When a specified interval has elapsed after injection, the mice which have fallen away from the cylinders are counted, the numbers so obtained being used in computing the required estimates of potency and precision.

Results obtained from 35 consecutive assays were utilized in estimating the slope of the logarithm-dosage response relationship. A two-dose technique similar to that outlined by Miller, Bliss, and Braun (*3*) was employed in each assay. The ratio of the higher dose of standard to the lower dose of standard was 2:1 in all cases. The higher dose in the different assays varied from 0.025 to 0.010 I. U. of insulin/mouse. Thirty-six mice were used at each level. In each assay the numbers of reactions which had occurred at 45 and 60 minutes after injection of insulin were noted.

The percentages of animals responding to the two doses of standard were transformed to probits, and the slopes of the logarithm-dosage response curves were computed. The average slopes at 45 and 60 minutes were found to be 3.2 and 3.3, respectively. There was no indication from the χ^2 tests that the slopes varied significantly from one assay to another. The values obtained are of the same order of magnitude as the value 3.5 obtained by Marks and Pak (*2*) and the value 3.0 obtained by Trevan as calculated by Irwin (*1*).

The mortality among the first 18,000 mice placed on test was found to be 1.7 per cent, an appreciable decrease from mortalities of 7–10 per cent experienced in these Laboratories with more conventional procedures. It was found that two operators, using the unit described, could perform all the work necessary for a 1,000-mouse assay in one working day, whereas with the procedure previously used four operators were employed to perform assays of this size. Efficiency was increased somewhat by assaying two unknown samples of insulin (two cylinders per unknown) against one standard (two cylinders) on each run.

It is reasonable to suppose that the apparatus might also

[1] The authors wish to express appreciation to W. Parkinson and D. P. Joel for technical assistance in construction of the apparatus.

be employed to assay compounds counteracting the action of insulin (cortical and pituitary hormones), central nervous system depressants (anesthetics and hypnotics), and compounds affecting neuromuscular transmission (curare), as well as in studies relating to the development of tolerance to certain drugs.

In assaying drug samples of unknown origin by means of the apparatus described, special attention will have to be paid to the qualitative relationship between unknown and standard material. The results of an assay will be misleading if the mice react to some agent in the unknown preparation other than the specific substance under study.

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Apparatus for Mustard Gas Treatment[1]

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The early work which led to the discovery that mustard gas could induce mutations in *Drosophila melanogaster* as efficiently as X-rays was carried out by pumping mustard gas dissolved in cyclohexane at intervals into air flowing past the flies (*1*). This method was rather unsatisfactory, as apparently similar treatments gave widely discordant results, and a few accidents occurred among those handling the mustard gas. The new apparatus to be described has several advantages over the old: (1) Dosage is controlled and repeatable; (2) a single charge of mustard gas lasts for many experiments; (3) contact with mustard gas is avoided except during recharging; (4) another substance in addition to mustard gas is not used; and (5) the apparatus is ready for use at any time.

The following train attached to an aspirator is set up in a fume cupboard: air intake with cotton-wool filter; tap; bubbling device (two potash bulbs) containing pure mustard gas; three-way tap; trap; *Drosophila* vial; trap; wash-bottles containing HNO_3. The aspirator consists of two winchester quarts fitted in series in the usual way. Rubber connections are employed throughout.

The insects are exposed by placing them in a clean, empty vial in the apparatus and turning the three-way tap to admit air. When the rate of flow of air through the wash-bottles has been adjusted (about 3 bubbles/second), the taps are turned to admit the air via the mustard gas. This air is saturated with mustard gas vapor, and the amount depends (but only slightly) on the temperature. Using Florida-4, an inbred stock, at a temperature of approximately 10° C., the flies show some discomfort after about 8 minutes, after 20 minutes do not often travel the entire height of the vial, and after 30 minutes a few remain motionless on the bottom of the vial. At the close of the run, the two-way tap is shut, and the three-way tap adjusted to admit air. A period of about 10 minutes is allowed to wash away all traces of mustard gas. The flies rapidly recover when the mustard gas is no longer admitted. During the run, the operation of the wash-bottles and bubblers causes the air flow to surge; this is regarded as an advantage, as it ensures thorough mixing of the air in the exposure chamber.

If a large number of flies is used, the vial becomes very moist, because the flies excrete much fluid when the mustard gas reaches them. If a piece of coarse filter paper is placed in the vial, most flies rest upon this, and the moisture is absorbed and evaporates more easily in the air stream.

As originally designed, the concentration of mustard gas in the air stream could be raised by heating the bulbs and thus increasing the vapor pressure of the mustard gas. To prevent subsequent condensation, the first trap and fly vial must then be raised to the same or a greater temperature. No need was found for this arrangement, however, and all dosage work has been performed by varying the time of exposure.

Of course, this arrangement controls only the external concentration of mustard gas. The amount which reaches the nucleus of any organ will vary, due to differences of chemical composition of the organ and consequent solubility of mustard gas. Though constant for similar organisms, the apparatus cannot, like X-ray machines, deliver an equal dose to the nucleus of all nuclei of any species exposed in the same way.

With this apparatus, Florida-4 males exposed for 15 minutes show about 5 per cent sex-linked mutations, using the ClB test.

[1] All expenses in connection with this work were borne by the British Empire Cancer Campaign.

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Acetone-desiccated Adult Tissues as a Source of Cell Growth-promoting Extracts

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During the course of our studies on the growth-promoting effect of adult tissue extracts on cell colonies *in vitro*, it became obvious that a starting material combining the characteristics of stability, uniformity, and easy sterilizability would facilitate the continuation of the work and obviate the tedious necessity of preparing extract under sterile conditions, from freshly removed tissue, each time a new experiment was contemplated. Acetone-desiccated adult chicken hearts were found to constitute such a material.

The procedure used was as follows: Hearts of adult chickens were packed in ice and sent to the laboratory as soon as convenient. Following gross dissection in order to remove the large vessels and fat tissue, the hearts were "homogenized" in a blender with as little physiological saline solution as possible to allow for smooth running of the machine, and the resulting mash was mixed with five times its weight of acetone, previously cooled in the icebox. The mixture was left in the icebox