

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.

References

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Apparatus for Mustard Gas Treatment¹

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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 HNO₃. 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.

Reference

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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

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