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

for 30 minutes, rapidly filtered with suction on a Buchner funnel, washed with a little cold acetone, and again extracted for 30 minutes with acetone, using in this and in all the following extractions a weight of acetone equal to the weight of the original batch of hearts. In all, four extractions were made After the final extraction the material was ground in a mortar, spread out on an evaporating dish, and freed of the last traces of acetone and water by keeping it from 24 to 48 hours in a large, evacuated desiccator, over $CaCl_2$ or H_2SO_4.

The yield of dry substance is about 18 per cent of the weight of the fresh hearts used. The material is a very light, tawny-colored powder containing many fibers that remain unpowdered.

For the purpose of sterilization the powder was transferred, in amounts of 1–2 grams, to sterile, large, cork-stoppered centrifuge tubes, the tubes filled with acetone for 1–2 hours, and centrifuged. The excess acetone was pipetted off under sterile conditions and the tubes left overnight in a desiccator *in vacuo* to get rid of the acetone. The powder was then transferred with sterile precautions to ampoules which were sealed *in vacuo* and kept in the icebox.

Whenever heart extract is required, a sample of the powder is extracted for 24 hours, with 22 volumes of Tyrode's solution diluted with enough distilled water to obtain a final isotonic extract. In this way an extract is obtained which in its water content corresponds to that from an equivalent weight of fresh hearts made by our standard method (*2*). The extract from the dried powder, just as the fresh extract (*1*), can be diluted with an equal volume of Tyrode's solution without apparently diminishing its growth-promoting effect on cell colonies.

TABLE 1

COMPARISON OF THE SIZES OF CULTURE (MM.²) GROWN FOR SIX DAYS IN THE PRESENCE OF AN EXTRACT OF ACETONE-DESICCATED CHICKEN HEARTS (A) AND IN A PROTECTIVE MEDIUM (TYRODE'S SOLUTION) (B)

No. of culture	A	B
12203	97	8
12204	100	7
12221	96	5
12222	88	4
12223	96	13
12224	134	20
Average.....	101.8	9.5

The tests of growth-promoting power of the extract from acetone-desiccated tissue powder were made on standardized cultures of fibroblasts in Carrel flasks. The solid phase of the medium consisted of 0.5 ml. of chicken plasma and 1 ml. of Tyrode's solution coagulated with 1 drop of dilute embryo extract, while the liquid phase consisted of 1 ml. of the solution to be tested. The tests were always performed by comparing the growth of the two sister halves of a single standard culture.

It was found that the extract from the acetone dried heart powder has intense cell growth-promoting power, producing cultures with areas about 10 times as large as those of cultures growing in a protective medium (plasma + Tyrode's solution) (Table 1). A comparison of the growth-promoting effect of this extract with that of fresh adult heart extract, using both in standard concentrations, shows that the former is somewhat more potent (Table 2).

TABLE 2

COMPARISON OF THE SIZES OF CULTURE (MM.²) GROWN FOR SIX DAYS IN THE PRESENCE OF AN EXTRACT OF ACETONE-DESICCATED CHICKEN HEARTS (A) AND AN EXTRACT OF FRESH CHICKEN HEARTS (B)

No. of culture	A	B
12205	80	40
12206	92	50
12209	80	96
12210	114	105
12225	94	50
12226	80	68
12337	115	72
12338	116	65
12341	96	88
12342	88	58
12375	63	52
12376	80	72
12379	51	64
12380	45	58
Average.....	85.3	67.0

Samples of acetone-desiccated chicken hearts have now been kept for five months and still fully retain their original activity.

An identical procedure performed on adult chicken brains has yielded a dried brain powder giving an extract with the same cell growth-promoting properties as extracts of the original brain tissue.

Acetone desiccation of pulped chicken embryos of various ages (8–18 days of incubation) has also yielded a dried powder which provides a growth-promoting extract as active as fresh chicken embryo extract made from the corresponding amount of fresh embryos.

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Oral Administration of Small Doses of Liquids to Laboratory Animals

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The problem of feeding small quantities of substances, especially liquids, to laboratory animals is of importance in many experimental procedures. Feeding from dishes has certain undesirable features, such as failure of animals to consume the dose completely and exposure of the material to destructive and contaminating effects.

Stomach-tube feeding as described by Marks (*2*) and recently by Lehr (*1*) is used widely but has some disadvantages. Considerable experience and skill are necessary with metal-type tubes to avoid injury to animals. Rubber tubes are sometimes cut by animals' teeth, and slight pressure on rubber and