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

silk tubes results in variation in the amount of material delivered. This is especially undesirable when small amounts of relatively concentrated solutions are being fed. Lehr has discussed the disadvantages of using the wooden, central-hole mouth gag.

The apparatus described here is simple to assemble from parts available in most any animal laboratory. Experience has shown it to be efficient and useful in feeding certain types of solutions, such as oil solutions of carotene and vitamin A. It is adapted especially to feeding small doses when relatively accurate measurement is required.

The apparatus (Fig. 1) consists of a ring stand on which is mounted an ordinary test tube holder in which is fastened a "tuberculin" syringe. Below this a blunt dissecting forceps is clamped in position so that the end of the syringe needle is directly above (about ¼ inch). Obviously, a "hairpin" type of

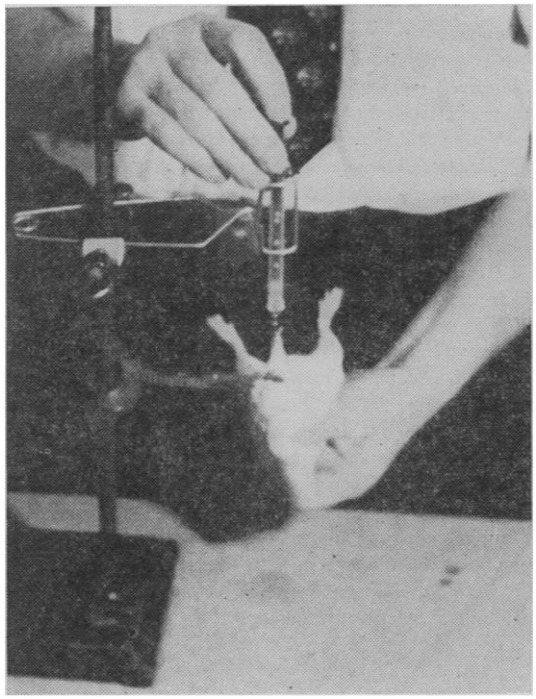

FIG. 1. Apparatus for drop feeding rats without assistance.

gag made from wire or small-diameter glass rod may be substituted for the forceps. However, the forceps has proved to be more satisfactory. Tension can be varied so that it can be adjusted to fit into the mouth of any size rat by adjusting the screw of the ordinary clamp holder, fastening it in position (Fig. 1).

In feeding animals the syringe is filled with the desired quantity of material and placed in the test-tube holder. The animal is grasped with the left hand; ample amounts of skin from the neck and back are held as shown in Fig. 1. With aid of the right hand the forceps is inserted in the animal's mouth. The right hand is then moved to operate the syringe and the feeding is dropped into the open mouth. Fig. 2 illustrates how the tongue should be held with forceps in order to produce a proper opening to receive the liquid. The animal can be immobilized by wrapping in a small towel (*1*), but this is not necessary with the strain of rats used in this laboratory. If the neck skin is held with a firm grasp and the forceps inserted carefully to avoid hurting, there is no inclination on the part of the animal to struggle. The rat invariably swallows the solutions without ejecting them from its mouth. Many substances which the animal will not consume voluntarily can be fed in this manner.

Solutions may be fed as measured by calibration marks on the syringe, or drop weights may be calibrated. Although drop weight is affected by many variables, such as temperature of solution, time required for formation of drops and angle of needle, these factors can be standardized closely enough to insure uniform results. It will be found that counting a given number of standardized drops offers certain advantages in speed and accuracy in some feeding problems. Incidentally, clamping of the syringe in a vertical position, as shown in the figure, eliminates the variable due to holding the needle at different angles while feeding drops.

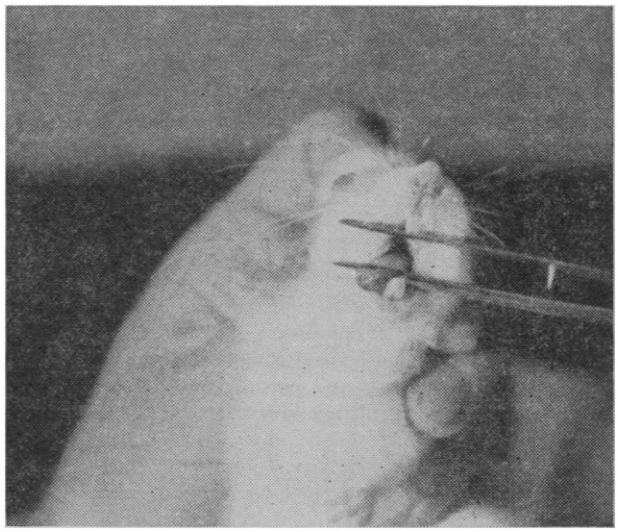

FIG. 2. Position of forceps for drop feeding.

Variation in size of drops can be controlled by using different size needles. The use of a timer with a long sweeping second hand is desirable to standardize timing of drops.

Concentrated solutions make smaller feedings possible and eliminate continual filling of the syringe. Very rapid feeding is possible, especially if the solutions fed are relatively concentrated. For instance, it has been found that an operator can remove an animal from its cage, feed 1–3 drops of solution, and return the animal to its cage in 10 seconds. This is probably faster than would be the case in actual practice, but it gives some idea of what can be expected when using the procedure.

The most desirable feature of the apparatus is that the necessity of an assistant for holding aminals is entirely eliminated. One operator can work practically as fast as two, without sacrifice of accuracy or damage to the animals.

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