ment could not be grown in this medium (BV), probably because younger embryos are less capable of synthesizing their own growth factors than older ones. Coconut milk[4] proved to be an excellent source of these additional growth factors necessary for very young embryos. For example, results such as the following were obtained: Pro-embryos, 0.14 mm in diameter (0.00144 mm³) were isolated from ovules of $2n$ plants 14 days after pollination and transferred to media B, BV and BV to which was added non-autoclaved coconut milk, and BV to which was added autoclaved coconut milk. After 4 days in the medium containing non-autoclaved coconut milk 4 of 7 embryos were on the average 1.9 mm long and 0.6 mm in diameter. These embryos grew below the surface of the medium. Two other embryos which were placed at the surface of the medium did not grow and 1 culture was infected. Thus, the 4 embryos that had grown had within 4 days increased their volume over 300 times. After 10 days in culture the two largest of the embryos measured 10×1.3 mm and hence had increased in volume 8,000 times. No growth occurred in the other media.

The following is another example: 7 embryos from ovules of $4n$ plants 11 days after pollination were removed. The embryos were in a slightly more advanced stage of development than the $2n$ embryos mentioned above. They measured 0.3 mm in diameter (0.014 mm³) and showed small cotyledon primordia. After 3 days below the surface of the medium (BV) to which non-autoclaved coconut milk was added all embryos cultured had grown on the average 2.0 mm in length and 0.9 mm in width. This corresponds to a volume increase of 90 times. After 10 days in the above medium the two largest embryos measured 8×1.5 mm, corresponding to a 1,000-time increase in volume. The embryos in the two experiments cited showed a good development of cotyledons and hypocotyl. The primary leaves also developed to a length almost equal to the cotyledons. Roots did not develop, but could be made to develop by transferring the embryos to medium (B) or (BV) without the additional coconut milk. A heat-stable root inhibitor which may be auxin is probably present.

In the case of these $4n$ embryos, growth also occurred in half of the cultures kept on medium (BV) to which autoclaved coconut milk was added. However, no differentiation occurred. After 10 days they had developed into lens-shaped bodies about 2 mm in diameter.

The success of coconut milk in furnishing some accessory substances which stimulate the growth of isolated embryos *in vitro* suggests its applicability to other species and prompts this preliminary report. Ultimately it is hoped to secure information regarding the nature of the substances in coconut milk which give it its peculiar properties.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MINCER ADAPTABLE TO SMALL QUANTITIES OF TISSUE[1]

IN preparing skeletal muscle for oxygen uptake determinations we were confronted with the problem of obtaining a relatively uniform mince of small specimens obtained at biopsy. It became necessary to design and construct the apparatus described here, since we could find no adequate micromincer on the market.

The essential elements of the mincer (Fig. 1) are three telescoping parts: (*1*) The easily removable tubular steel jacket (*A*) which is held in a fixed position during mincing by a pin which fits into a slot in flange *D*. The mince emerges from holes drilled in the side of this tube, and the particle size can be regulated by varying the diameter and number of the holes. (*2*) The steel knife unit (*B*), which is solid at the shank end and slotted, as shown, to engage with pin *E*, and tubular at the opposite end to accommodate the plunger, which forces the contained tissue through

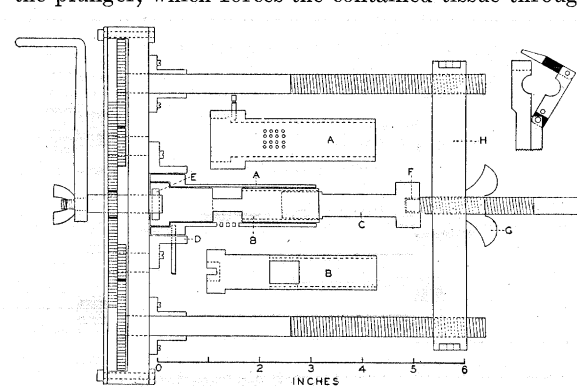

FIG. 1

[4] Coconuts were obtained from the local markets. The activity of the milk from different nuts varied considerably.

[1] Supported by the Wisconsin Alumni Research Foundation.

a square window cut into this tube immediately adjacent to the solid portion. This window is of such a size as to correspond with the area in which the holes are drilled in the outer jacket (*A*). The edge of the square window (or windows) is filed to form a cutting knife or knives if more than one window is made as in the larger units. This tubular knife fits snugly within the outer jacket and rotates freely when activated by the pin *E*. (*3*) The brass plunger (*C*) which telescopes snugly into the open end of the hollow knife is able to rotate with it since it operates freely on the bearing at *F*, thus preventing the maceration of tissues which would occur if the plunger were fixed. The outer jacket and the knife can be made more economically of seamless steel tubing fitted into brass parts to form the shank, thus reducing the amount of machine work necessary. These three mincing parts are readily interchangeable and may be removed quickly from the activating mechanism for loading and cleaning either by retracting the bearing *F* after loosening the thumbscrew *G* or by removing the supporting bar *H* from the threaded rods.

The activating mechanism is mounted on a heavy board by supporting arms attached to the gear box at such a height that the crank may be turned readily. Rotation of the knife and the advancement of the plunger occur simultaneously when the crank is turned. The two threaded rods are geared to the crank and engage the crossbar *H*. By means of this mechanism, the plunger is advanced one sixteenth of an inch for each ten turns of the knife, thus assuring the same uniform rate of tissue advancement and cutting irrespective of the speed at which the crank is turned. This is an absolute essential if a uniform particle size of tissue is to be obtained. Since the plunger can not be advanced without simultaneous operation of the knives it is impossible to squeeze tissues through the openings in the outer jacket without this material being cut, an occurrence common to the Latapie type mincer. The activating mechanism could be improved mechanically by making it possible to alter as desired the ratio of tissue advancement to knife speed. Other modifications, such as a mechanical drive and a lathe-bed type of arrangement for supporting the mincing unit, would add to the convenience but also increase the cost.

By constructing several sizes of the three essential mincing parts, all having uniform dimensions at the shank end, and by using interchangeable casings and knives, we have found it convenient to mince quantities of tissue from 0.25 to 30 grams. Dr. A. E. Axelrod, of the Department of Biochemistry, is using a small mincing unit of 4 mm plunger diameter which will deliver 200 milligrams of tissue from a 250-milligram rat heart. The efficiency of delivery is much greater with larger units, although a small waste of tissue is inevitable because of the small dead space between the knives.

Values for Q_{O_2} obtained on tissues minced with this apparatus compare very favorably with those obtained from the larger Latapie mincer. This mincer will cut soft tissues like brain or liver into discrete particles. Dr. V. R. Potter[2] has found that this apparatus yields a liver mince of "the critical particle size needed to permit adequate inward diffusion of oxygen with minimum loss of cytochrome due to outward diffusion." Fibrous mammary tumors, cartilage and even soft bone, which are refractory to mincing with the Latapie or simple pressure mincers, are reduced readily in the apparatus as described.

The mechanical features were designed by J. S. Hipple, Medical School mechanician, who also constructed the apparatus.

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A COMBINED FIXATIVE AND STAIN FOR THE CILIA AND TRICHOCYSTS OF PARAMECIUM

THE combined fixative and stain described here offers numerous advantages over the methods now used for the demonstration of trichocysts and cilia. The structures are stained instantaneously and the normal contour of the animals is faithfully preserved. The trichocyst stain is prepared as follows: Copper sulphate, 5 per cent., 50 cc; hydrochloric acid, 0.1N, 12 drops; blue ink, 5 drops.

If it is desired to stain the cilia only, the hydrochloric acid is omitted from the formula. To use the stain, add two drops to the culture on the slide, place cover glass and examine. The best preparations are usually found around the edges.

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