The drug does not seem to influence the rate of hepatic regeneration, but it does induce, in both types of liver, a significant increase of GOT and GPT activities, and particularly of the latter.

It is suggested that the hormone may enhance gluconeogenesis by activating the transamination processes; in this connection, regenerating liver does not behave differently from normal liver.

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# AN HYDRAULIC HOMOGENIZER* FOR THE CONTROLLED RELEASE OF CELLULAR COMPONENTS FROM VARIOUS TISSUES**

C. F. EMANUEL AND I. L. CHAIKOFF

*Department of Physiology of the University of California School of Medicine, Berkeley, Calif. (U.S.A.)*

Procedures now available for dispersing tissues yield, as a rule, not only mixtures of cells, nuclei, and mitochondria, but also products of their destruction. An adequate and simple means of controlling the products obtained by tissue homogenization has, so far, not been devised. Ideally, such a procedure should produce only *whole* cells, the *intact* products of whole cells (*i.e.*, nuclei, mitochondria, and microsomes), or the dispersed contents of nuclei plus the other cellular inclusions. In addition, homogenization should be accomplished rapidly, at low temperatures, and should yield metabolically active products. From the standpoint of these requirements, the instrument described here has proved greatly superior to glass homogenizers used in this laboratory.

* Commercially available from Microchemical Specialties Co., 1834 University Avenue, Berkeley 3, California.

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The homogenizer shown in Fig. 1 was designed to function according to Bernoulli's principle (velocity $\propto$ 1/pressure), within the moderate pressures provided by the standard Carver laboratory press. The width of the space through which the tissue brei moves can be varied from 0.00025 to 0.005 inch (6 to 130 microns)* by means of interchangeable rods, thus making possible the controlled release of cellular inclusions. The chief advantages of the instrument are the ease and rapidity with which homogenization can be carried out—even of tissues usually regarded as very difficult to homogenize—and the high yields of intact nuclei that can be obtained. Complete disruption of nuclei can also be accomplished in this homogenizer.

## GENERAL DESCRIPTION OF THE HOMOGENIZER

The apparatus, as shown in Fig. 1, consists essentially of a cylinder (C) through which a piston (B) moves, and a base (O) which is attached to the cylinder by inter-

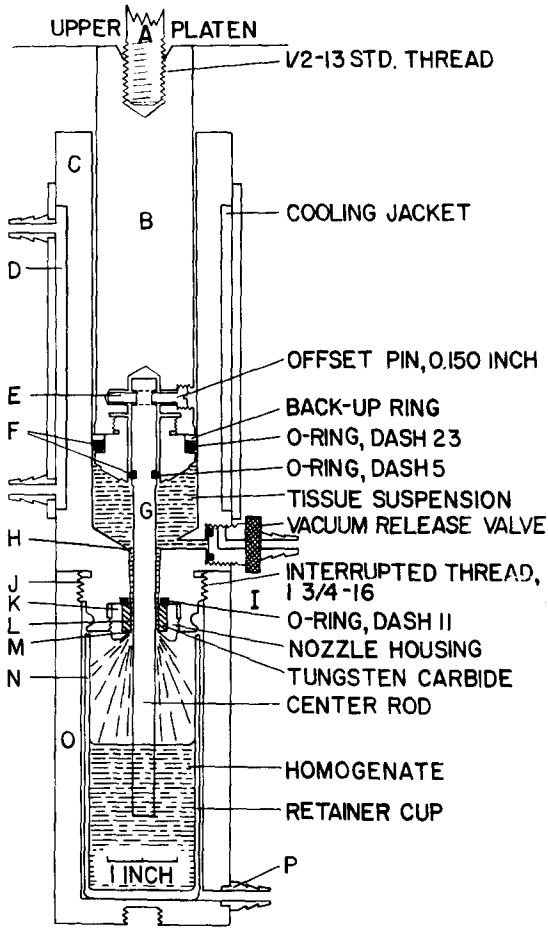


Fig. 1. Cross-section of hydraulic tissue homogenizer.

*These are room-temperature dimensions. The actual orifice dimensions at working temperature (0°) are slightly greater than those recorded.

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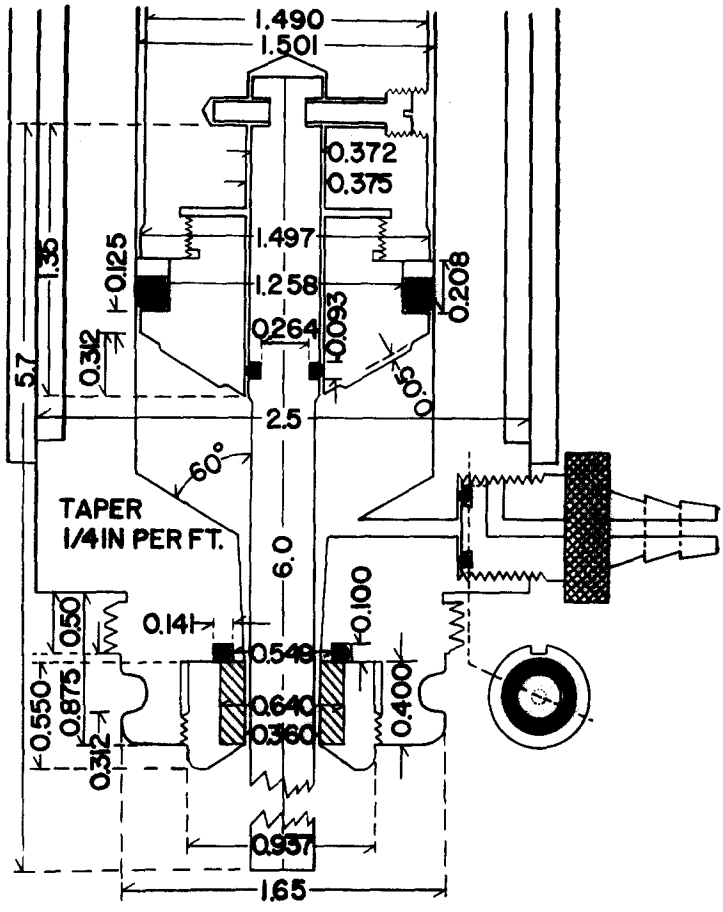


Fig. 2. Diagram showing detailed dimensions of nozzle and piston of homogenizer.

rupted threads (J). Interchangeable rods (G) can be inserted into the head of the piston. The tissue fragments, suspended in buffer, are brought into the cylinder, and the downward movement of the piston ejects the tissue products, at high velocity, through the orifice (M). The products of the homogenization are collected in the base (O). The width of the orifice between the center rod (G) and the nozzle (L) is one of the important factors determining the extent of homogenization and the size of the cellular particles finally obtained. The selection of the orifice size should be based either on an approximate measurement of the cell diameter or on the size of the desired cellular inclusion.

It is important that the rod (retained by an off-set pin (E)) fit loosely into the piston, since the rod's downward—and very slight lateral—movement results in a smooth and efficient ejection of homogenate. The tapered area (H) at the entrance of the region where the tissue fluid moves with highest velocity has been exaggerated in both Figs. 1 and 2 for ease of viewing. The "O" rings (F) enabled us to use moderate pressures with no leakage and, because of their wiping action, increased the recovery of the homogenized tissue.

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Because heat may form in the cylinder (C) as a result of compression, the outside of the cylinder is surrounded by a cooling jacket (D) through which brine cooled at —5° is circulated. When the orifice size is larger than 15 $\mu$, no noticeable heating occurs, and sufficient cooling may be achieved by storing the apparatus in a refrigerator before use.

The pertinent measurements for construction are given in Fig. 2. The rods and nozzle were made of dissimilar materials to prevent cohesion when the width of the orifice is minimum. All parts of the apparatus except the rubber "O" rings (F), the vacuum-release valve (I), the nozzle (L) and its housing (K), and the center rods (G) were constructed from 18-8, Type 303 chrome-nickel steel. The valve, nozzle housing, and rods were machined from 17-4PH stainless steel hardened by heat treatment to over 40 Rockwell C units. (It is imperative that the center rods, especially those of the largest diameter, be machined to exact roundness and constant diameter—$\pm$ 0.0001 inch.) The tungsten carbide nozzle (L) (nickel bonded) was ground with diamond dust to give a close fit into the nozzle housing. Its internal surface was then ground to a uniform diameter and polished. Variations in diameter of the nozzle opening should not exceed $\pm$ 0.0001 inch.

## PREPARATION OF THE HOMOGENATE

Eight to 10 g of tissue are finely minced with scissors and suspended in 40 ml of buffer. We found that a buffer-to-tissue ratio (v/w) of 5 or more results in the most complete cell breakage when the glass–pestle type of homogenizer described by WILBUR AND ANDERSON[1] is used. This ratio may be reduced to 2.5 in our homogenizer without greatly impairing the efficiency of the homogenization. Further reduction of the buffer-to-tissue ratio, however, does interfere with the completeness of homogenization. There is practically no lower limit to the amount of tissue which can be treated, provided the buffer volume is maintained at 20 ml or more; the greatest efficiency is, in fact, obtained under conditions of high dilution. It should be remembered that operation of this instrument depends upon the velocity of the tissue suspension passing the orifice (M), and that high velocities can be achieved only if the viscosity is low, *i.e.*, when the buffer-to-tissue ratio is high. If the volume of the tissue suspension is less than 10 ml, little homogenization will occur.

A tissue suspension can be introduced into the cylinder in three ways: (1) The base is separated from the cylinder, and the piston is pulled back so as to withdraw the center rod from the orifice. The mixture is then poured through the nozzle directly into the inverted cylinder; the piston is then pushed up (with the cylinder still inverted) until the center rod obstructs the nozzle opening. (2) The piston with its center rod is separated from the cylinder, the opening at the bottom of the nozzle is closed with a stopper, and the tissue mixture is poured into the open top end of the cylinder. The piston is then replaced and pushed past the "O" ring, the cylinder is inverted, the stopper is cautiously removed (or the vacuum-release valve is opened), and the piston is pushed so that the center rod closes the nozzle opening. (3) The tissue mixture containing fragments no larger than 2 to 3 mm can also be introduced through the vacuum-release valve (shown at (I)). In this case, the homogenizer is assembled and placed in the Carver press with the head of the piston resting at the bottom of the cylinder. The vacuum-release valve (I) is opened by one turn, counter

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clockwise, of its knurled knob. A tygon tube is then attached to the valve, the open end of the tube being immersed in the tissue suspension. The suction created by pulling the cylinder downward draws the tissue mixture into the bottom of the cylinder. The vacuum-release valve is then closed.

The assembled apparatus containing the tissue mixture is placed in a Carver press with its platens 15 inches apart. The lower platen is raised slightly until the homogenizer piston almost touches the upper platen. A ½-inch bolt (A, Fig. 1) is screwed into the piston manually to insure that the homogenizer is centered. The piston is now rapidly pushed into the cylinder. One excursion of the piston—2 to 3 minutes—is sufficient to homogenize the tissue to the extent determined by the size of the annular orifice. The piston can now be readily withdrawn from the cylinder by opening the vacuum-release valve (I).

For most purposes, the homogenate is collected in a plastic cup (N) made to fit the inside of the base. Some foaming occurs, which may result in a slight loss when the volume of the recovered homogenate exceeds 50 ml. In that case, it is desirable to collect the homogenate in a collapsed rubber condom. In this way, 20 g of tissue can be homogenized with 50 ml of buffer, and the products can be completely recovered. The comminuted connective tissue quickly floats to the top of the homogenate.

To homogenize large volumes of a tissue or cell suspension, the plastic collecting cup is removed, and a delivery tube is attached to the air-vent hose connection (P, Fig. 1). The homogenizer is filled by method 3, described above, and the homogenization of the tissue is carried out in the usual manner. The filling and homogenization can now be done repeatedly without dismantling the apparatus.

TABLE I

SHOWING THE EFFECTS UPON DNA YIELDS OF PRETREATMENT[2]OF LIVER AND OF THE SIZE OF OF THE HYDRAULIC HOMOGENIZER

In all experiments, 8 g of rat liver were homogenized with 40 ml of buffer

| *Expt.* | *Pretreatment of liver* | *Hydraulic homogenization* | | | |
|---|---|---|---|---|---|
| | | *Times* | *Orifice thickness inches** | *Maximum pressures attained p.s.i.* | *Stumpf test mg per 100 g fresh tissue* |
| 1 | Minced with scissors | Twice (1) | 0.0015 | 2000 | |
| | | (2) | 0.0015 | 200 | 185 |
| 2 | Minced with scissors | Twice (1) | 0.0015 | 2500 | |
| | | (2) | 0.0010 | 3250 | 152 |
| 3 | Minced with scissors | Twice (1) | 0.0015 | 3000 | |
| | | (2) | 0.0005 | 11000 | 117 |
| 4 | Minced with scissors | Once only | 0.0015 | 2000 | 145 |
| 5 | Minced with scissors | Once only | 0.0010 | 16,000 | 167 |
| 6 | 2 strokes of glass homogenizer | Once only | 0.0015 | 1200 | 224 |
| 7 | 2 strokes of glass homogenizer | Once only | 0.0010 | 8000 | 153 |
| 8 | 2 strokes of glass homogenizer | Once only | 0.0005 | 13000 | 124 |
| 9 | 2 strokes of glass homogenizer | Once only | 0.0004 | 15000 | None |
| 10–21 | 200 strokes of glass homogenizer | | | | |

* The orifice thickness in microns is obtained by multiplying these values by $2.54 \cdot 10^4$.

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PREPARATION OF AN HOMOGENATE CONTAINING INTACT NUCLEI

As already pointed out, the orifice between the central rod and the nozzle determines the size of the cellular components in the homogenate. To provide intact nuclei, the correct orifice size (38 $\mu$) is created by use of the appropriate center rod. Hence, as the piston and center rod move downward, the tissue pieces are fragmented until, at the orifice exit (M), the size of the largest component in the homogenate can hardly exceed that of a whole cell. However, efficient bursting of the cells actually occurs as a result of the velocity-pressure changes and other physical forces acting at the orifice. With the proper choice of orifice size and a favorable tissue-to-buffer ratio, a high yield of intact nuclei can be obtained.

To obtain maximum yields of nuclei it is important to mince or disperse the tissue before placing it in the hydraulic homogenizer. Relatively soft tissues can be minced with scissors or, preferably, subjected to brief treatment (two or three strokes) in a large glass homogenizer* modeled after that described by WILBUR AND ANDERSON[1].

The hydraulic homogenizer described was originally designed to facilitate the isolation of deoxyribonucleic acid (DNA). In particular, it was our desire to subject tissues with unfavorable nuclear–cytoplasmic ratios (*i.e.*, low in DNA) to a homogenization process which would be so complete that almost all nuclei would be left free and intact. If the nuclei of such an homogenate could be quantitatively separated from the remaining cellular debris, a ready source of DNA would become available from almost any tissue. The details of separation of nuclei by adsorption, as well as the subsequent isolation of their DNA, are treated elsewhere[2].

The number of intact nuclei released is, of course, a measure of homogenization effectiveness. But instead of counting free nuclei, we measured the total DNA content of the isolated nucleus preparation. Two measures of DNA recovery were employed: the Dische colorimetric test, as modified by STUMPF[3]; and the actual weight of isolated fibrous material. This colorimetric method was carried out on the initial DNA filtrates obtained by extraction of the concentrated nucleus preparation with saturated salt solution. Some interference from foreign materials was to be expected at this point, and for this reason we compared the values obtained by this colorimetric procedure with the actual weights of the isolated, fibrous NaDNA dried *in vacuo* over $Mg(ClO_4)_2$ and NaOH. Quantities of DNA lower than about 140 mg % could not be precipitated quantitatively with alcohol (see Expts. 3, 8, and 9, Table I).

The effects of homogenization conditions upon DNA yields are shown in Table I. While mincing

THE ANNULAR ORIFICE

| *DNA recoveries* |
|---|
| *Isolated*<br>*mg per 100 g fresh tissue* |
| 225 |
| 155 |
| (Too low for quantitative precipitation) |
| 125 |
| 177 |
| 228 |
| 143 |
| (Too low for quantitative precipitation) |
| (Too low for quantitative precipitation) |
| Isolated: range 133–189; mean 154 |

* Our glass homogenizer, which had a capacity of 120 ml, was provided with a jacket through which cold brine (—5°) circulated. The annular clearance between the glass walls and the lucite pestle was 0.0045 inch proximally and 0.006 inch distally.

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by scissors is, of course, convenient, it should be noted that brief pretreatment of the liver in the glass homogenizer, before hydraulic homogenization, improved the DNA yield somewhat. This improvement results from the fact that such a suspension has greater mobility when moving through the hydraulic homogenizer. The orifice dimension used in experiment 6 (0.0015 inch) is slightly larger (38 $\mu$) than the average liver cell (20 $\mu$)[4], and when this orifice was used in hydraulic homogenization, the DNA yield amounted to about 225 mg per 100 g of fresh liver. This value is in good agreement with the 200 to 260 mg per 100 g of liver reported by a number of investigators[5]. The use of an orifice smaller than 0.0015 inch results in some degree of nuclear destruction and loss of DNA. In fact, when orifices of 0.0005 and 0.0004 inch were used for hydraulic homogenization, nuclear destruction was so extensive that little or no DNA was recovered (Expts. 8 and 9, Table I).

In experiments 10 to 21, we made use of what we consider to be optimum conditions for mechanical homogenization in the glass homogenizer. Even with the laborious application of 200 vertical strokes (the *minimum* effective number), the highest yield was about 190 mg per 100 g of fresh tissue—a figure lower than that obtained in experiment 6 with the hydraulic homogenizer.

## COMMENT

The extent of cellular destruction or dispersal of a tissue by the hydraulic homogenizer will depend upon: (1) the type and preliminary treatment of tissue; (2) the size of the annular orifice; and (3) the buffer-to-tissue ratio. Our experience with cells and tissues that are easily homogenized (*e.g.*, liver, brain, spleen, kidney, fish gonads, ascites cells, myxomycetes, and the protozoan *Tetrahymena*) showed that an orifice size of 0.0015 inch (rod diameter 0.357 inch) resulted in a rapid and essentially complete cell breakage. When the annular orifice is as small as 0.0004 inch, complete destruction of nuclei as well as of cells occurs. Certain tissues, such as thyroid, muscle, mammary gland, salivary gland, and many invertebrate tissues, are difficult to homogenize. It was found necessary to subject these tissues first to one excursion through a large orifice (rod diameter, 0.355 or 0.356 inch) and then through a small orifice (rod diameter, 0.3580 inch). Plant foliage is readily homogenized in one step by the use of a large orifice (*e.g.*, rod diameter 0.355 inch).

Four rods with diameters of 0.355, 0.357, 0.3580, and 0.3590 inch (a greater precision is required in the construction of the two larger rods) were found in the homogenization of most tissues. Pressures in excess of 2,000 or 3,000 pounds per square inch (p.s.i.) are rarely attained when the annular orifice size is larger than 0.001 inch. When the orifice is about 0.0005 inch, or when the tissue being homogenized contains much fibrous material, pressures as high as 12,000 to 14,000 p.s.i. may be developed.

Cooling of the apparatus by as much as 20 degrees (*i.e.*, to 0°) is recommended to counteract the slight heating that develops from the pressures created when the smallest orifice openings are used. This cooling enlarges the orifice by as much as 4 $\mu$; hence cells or cellular inclusions that are about 6 $\mu$ or less in diameter cannot be completely disrupted by the homogenizer.

The use of this apparatus in the preparation of particulate fractions from thyroid, mammary, and salivary tissue has been reported. Such preparations, when in-

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cubated with radioactive iodide, were found to convert the inorganic iodine into protein bound form[6].

SUMMARY

The construction and operation of an hydraulically-driven tissue homogenizer are described.

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# A RAPID METHOD FOR PREPARING POLYMERIZED DEOXYRIBONUCLEIC ACID FROM TISSUES, BASED ON SEPARATION OF NUCLEI*

C. F. EMANUEL AMD I. L. CHAIKOFF

*Department of Physiology of the University of California School of Medicine, Berkeley, Calif. (U.S.A.)*

Present methods for isolating deoxyribonucleic acid (DNA) suffer from one or more of the following disadvantages: (1) they are time-consuming and cumbersome; (2) they can be used only for tissues like thymus and sperm, in which the nuclear/cytoplasmic ratio is quite high; or (3) they require use of either strong acids and bases or high temperatures. Recently we reported a method for the isolation of DNA from fish sperm which, because of the mild conditions employed, yielded a highly polymerized product[1]. But this method, as well as those so far devised for thymus, cannot readily be applied to most other tissues because of their relatively high content of non-nuclear materials. It seemed to us that the isolation of DNA from ordinary tissues could be accomplished provided those tissues could be homogenized in such a way as to release intact nuclei, and provided the nuclei could be separated from the large mass of non-nuclear materials. A rapid method for preparing an homogenate with a high yield of intact nuclei was presented in the preceding paper[2].

The present report describes a method for the quick separation of these nuclei from extraneous cellular elements. This method makes use of the strong affinity of nuclei for a negatively-charged, inert material, and thereby avoids the usual lengthy centrifugation procedures. Since the adsorbing material appears to have no affinity

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