

effect of x-rays on bacterial cells was reduced significantly by sodium formate, ethanol, and glycols. On the basis of the present discussion, these compounds would be expected to accelerate the decomposition of  $H_2O_2$  by catalase and thus prevent the accumulation of deleterious concentrations of this oxidant.

The possibility that the radiation protection afforded by sodium nitrite may be mediated through methemoglobin formation is not excluded. This explanation is open to question, however, since the degree of methemoglobinemia induced by the doses of sodium nitrite used by Rust *et al.* (10) (100 mg/kg), which failed to protect, was comparable to that produced by doses of *p*-aminopropiophenone which afforded definite protection (7). Studies directed toward the elucidation of this question are in progress.

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## A Simple Stage-mounted Micromanipulator

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Transplanting inclusions of a few cubic microns volume between living cells is one of the primary objectives of this laboratory. In planning procedures for making such transfers it became apparent that a micromanipulator having the following characteristics was essential:

- 1) Control in three dimensions to a tolerance of about  $1 \mu$ .
- 2) A range of 0.4 mm in each dimension, movement being essentially rectilinear.
- 3) Syringe intake and output with a volume control tolerance of  $1 \times 10^{-12}$  ml.
- 4) Operation under oil immersion with phase contrast objectives.

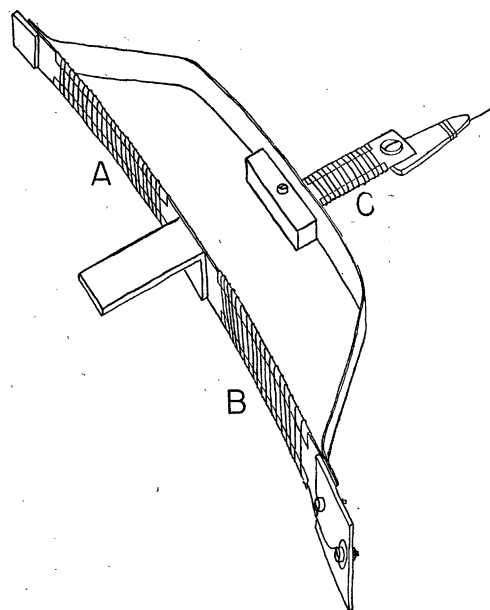


FIG. 1.

The principle utilized in the design of this instrument is that of differential thermal expansion using electrically heated bimetallic elements as motion sources. The foregoing principle has not previously been employed in micromanipulative equipment according to the literature available to us. For its designed function this model has certain advantages over the pneumatic or strictly mechanical manipulators. The primary advantage enjoyed by this manipulator is its small dimensions, which permit it to be mounted on the stage of the microscope. Remote control is effected electrically.

Hypodermic action is obtained by electrically heating a hollow glass needle whose effective volume change depends upon the internal volume of the needle used, the coefficient of expansion of the filling liquid, the operating temperature range of the heater, and the portion of the total volume heated.

Construction of an appropriate needle requires some practice but becomes a simple procedure. The needles used in our study are made from Pyrex glass tubing, and are 2-3 cm in length, with one end closed and the other end drawn out to an internal diameter of approximately  $1 \mu$ . The walls are relatively thin, giving a total external diameter of approximately  $2 \mu$ . The needle is completely filled with freshly distilled water, which has very little dissolved gas in it. The low dissolved gas component prevents separation of gas bubbles from the liquid with which the needle is filled. Such gas bubbles give a "mushy" effect in volume control. When heat is applied to the needle, the liquid expands and ejects a proportional part of its volume; the reverse occurs upon cooling. On occasion, volume control has been stable down to approximately  $1 \mu^3$  ( $1 \times 10^{-12}$  ml).

The organisms being used in our experiments are extremely thermosensitive, yet the heat produced by this

apparatus has never been a source of difficulty. If unshielded, the entire apparatus operates best in a relatively constant air-flow.

In principle, the operation of the "bow" portion of the micromanipulator (Fig. 1)<sup>1</sup> is as follows: There are two heaters on sides *A* and *B*, respectively. These heating coils are wrapped around a continuous bimetallic element. When sides *A* and *B* are heated simultaneously from a reference temperature, they go forward equally and have the effect of ramming the needle forward. If power be decreased on both sides, the cooling which follows permits the bimetallic element to bend back and withdraw the needle from its forward position. If side *A* be heated while side *B* is cooling, the sides will bend forward and backward, respectively, thus producing lateral motion in the direction of side *B*. The converse produces lateral motion in the opposite direction. Attached to the bow is a second bimetallic element (*C*) placed so that its plane is parallel to the stage of the microscope. When this element is heated it bends downward and when permitted to cool it bends upward. This direction of bending is a safety factor to prevent breaking the needle in case power should accidentally be cut off.

The entire apparatus is mounted on a Bakelite block, which is in turn provided with a vertical motion screw, and is hinged at the front end. Counter-sunk into this piece of Bakelite are two Alnico magnets, which hold the entire apparatus steady on a small steel plate mounted on the microscope stage (Fig. 2).<sup>2</sup> In operation, the apparatus is moved by

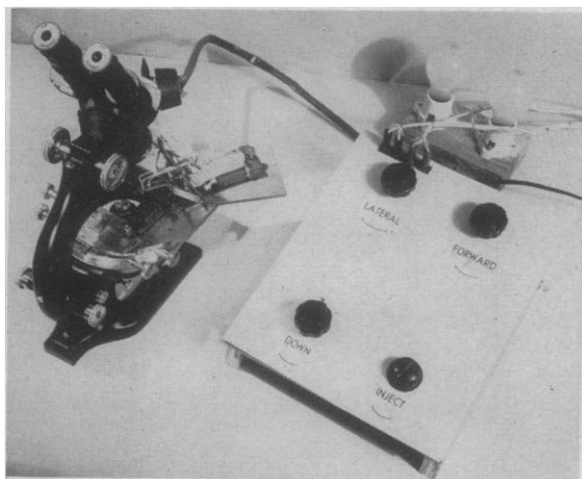


FIG. 2.

hand so that the needle tip comes into view in the microscopic field, then control is taken over electrically for micromanipulative procedures. Such manual control of gross motion has been found entirely adequate for our purposes. The apparatus has two main features in its favor: (1) Since the small steel plate

<sup>1</sup> The magnet-attached bow-base was designed and fabricated by Bailey Moore of this institution.

<sup>2</sup> Manufacture of this very simple, cheap micromanipulator is planned by the American Optical Company.

is attached directly to the microscope stage, no heavy additional stand is needed. (2) The entire apparatus is very close to its work and is remarkably free from vibration.

The period of movement from one side of the field to the other (under 1.25 N.A. oil immersion) is approximately 12 sec. The control, being electrical, is subject to additional refinement, such as a "joystick," or any number of modifications that the individual might wish to insert.

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## Comparative Histological Studies of Endocrine Glands of Yellow (*A<sup>y</sup>a*) and Non-agouti (*aa*) Mice in Relation to the Problem of Hereditary Obesity<sup>1</sup>

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Yellow mice (*A<sup>y</sup>a*) show a hereditary tendency to become obese. The yellow gene in mice (*A<sup>y</sup>*) causes obesity irrespective of color combinations carried with it, even when the yellow coat color is suppressed, as in albino combinations. The increased weight is due to excess fat, which is deposited particularly around the viscera and in the subcutaneous region (1). Obesity in these mice is due to increased food intake (2-4), as well as to less energy expended in body work (4). The latter fact agrees with the observation that these mice have a lower basal metabolism than normal mice (5).

Comparative growth curves have shown that the adiposity is more marked in yellow females than in yellow males. Also, ovariectomized nonyellow females become as obese as normal yellow females (1, 6). It was further pointed out (6) that both male and female yellow mice show a striking decline in obesity after 18 months of age. Obese yellow females show decreased fertility compared to other mice (1, 5); this has been denied (7), but the evidence in support of the latter view is not strong. Weitze (7) performed parabiotic experiments between various combinations of yellow and nonyellow mice. Her results indicate that the endocrine system of yellow mice is definitely related to the development of obesity in these animals. She studied the histology of the pituitary gland in obese mice and reported it to be normal.

The picture is further complicated by the observation that inbred yellow mice fail to become obese when fed normal laboratory diets (6), although they attain a body weight slightly greater than that of their nonyellow littermates (8). The tendency for inbred yellows to remain slightly heavier than nonyellows was also observed in the present study (Fig. 1), and, if

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<sup>2</sup> Rosalie B. Hite predoctoral fellow, Feb. 1951-Sept. 1951.