cytes (at least in stained smears) and apparently many injured parasites, but there are others which look quite normal. The periodicity of *Plasmodium relictum* var. *matutinum*, normally very sharp, did not seem to be altered in any way.

Summary. Seven species of avian malaria plasmodia have been successfully preserved for periods up to 90 days by low-temperature freezing. Very rapid freezing and thawing seem of more importance than the occurrence of fairly considerable temperature variations. For maintaining stocks of malaria for research or class use this method of preservation has much potential importance. It is probable that all species of plasmodia can be preserved in this way equally well. And it does not appear that different stages of the parasite are much differently affected.

13753

Apparatus for Rapid, Sterile Removal of Chick Embryos from Eggs.

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Developing chick embryos are now used extensively for the study of viruses and rickettsiae. At present they are used in large numbers for the preparation of vaccines against virus and rickettsial diseases. However, it has always been extremely difficult to sterilize thoroughly the eggshell with ordinary chemical disinfecting agents. In 1939 Penna[1] described a technic in which he employed an oxyacetylene torch for burning the eggshell open while the egg was rotated slowly in an adjustable clamp turned by an electric motor. Penna's technic is satisfactory except that it is too slow. The purpose of this report is to describe a modification of Penna's apparatus which permits a considerably faster removal of embryos under sterile condition.

The apparatus is illustrated in Figs. 1, 2, and 3. The eggs are handled in groups of four carried in shallow metal trays (A). The flame of an oxyacetylene torch (B) is applied at the assumed position of the air sac's margin as each egg is made to rotate through one complete revolution; the optimum rate of turning is dependent on the resistance to burning shown by the shells of a particular lot

[1] Penna, H. A., *Am. J. Trop. Med.*, 1939, **19**, 589.

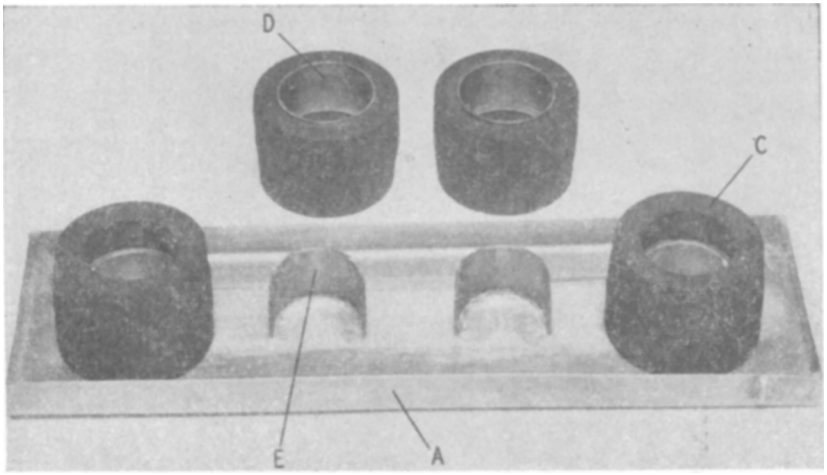

FIG. 1.

Metal tray, showing four guides and the egg holders which successively rotate about them as the holders come into contact with a turning disk. The holders consist of rubber tubing and a short section of thin metal tubing.

of eggs, but is usually of the order of 45 rpm. The metal trays serve to retain the embryonic fluids which occasionally issue forth when a shell cap breaks loose while being flamed. They also perform the same function while the caps are being displaced with a sterile instrument and while the embryos are being removed. The trays and holders can be quickly rinsed in running tap water and then put back into service.

The details of the holders and trays can be seen in Fig. 1. Each holder (C) consists of a 1⅝-in. length of heavy rubber tubing into one end of which is inserted a 1-in. length of brass tubing (D). The outside diameters of the rubber and metal are 2¼ in. and 1⅝ in. respectively; the wall thicknesses, ⅜ and 1/32 in. respectively. Trays (A) are of 0.025-in. monel metal, 12 in. in length, 3½ in. in width, and ⅝ in. in height, soldered on the inside at each corner. Equally spaced along each tray are 4 soldered monel metal guides (E) about which the holders can rotate fairly closely but freely. The guides taper in angular width from their tops to their bases, 2 of them being obtained by sawing through a 1-in. length of 1½-in. tubing at an angle of about 20° to the axis of the tubing. All soldering must be done with a soldering iron rather than a flame to avoid permanently distorting the trays.

Loaded trays are pulled between 2 metal guiding strips (F, Fig. 2), ¼ in. in height, across the top surface of a table-like stand (G) which is covered with sheet metal. Each egg holder in succession

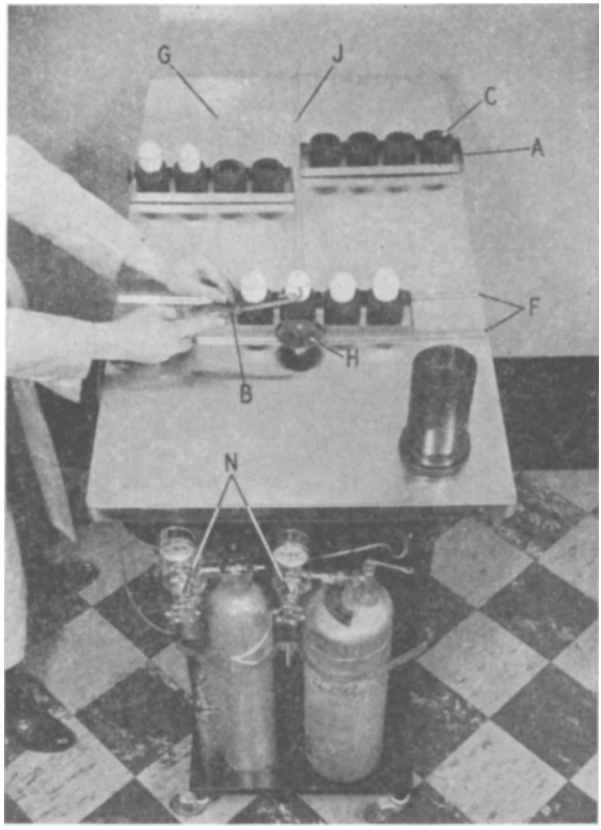

FIG. 2.
Apparatus in operation, showing the oxyacetylene torch being applied to incubated eggs as the first step in the removal of the shell caps and the sterile extraction of the embryos.

comes into contact with a rotating 3⅛-in. disk (H) which causes the holder itself to turn about its own guide. Except at its hub, this disk is about ⅛ in. thick and of slightly conical shape for purposes of drainage. The peripheral edge is rounded and knurled. With ample lateral freedom the shaft passes to a motor (I) beneath the top through a hole of considerably larger diameter, into which is forced a thin protective metal sleeve that extends almost to the disk. A third metal strip (J) serves to separate the incoming and outgoing trays. The left edge of the left guiding strip is provided with a downward slope, and one can with a little practice learn to maneuver the trays quickly with one hand into or out of the guiding channel without disturbing the orientation of the eggs.

The motor (I, Fig. 3) is a universal, 110-volt, variable speed

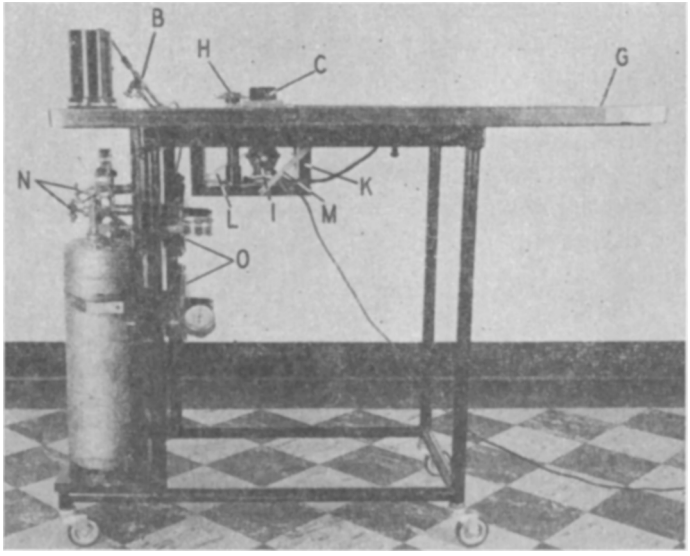

FIG. 3.
Side view of apparatus, showing the system of gas pressure regulation and the method of mounting below the top surface a low-speed motor which furnishes the power for the turning disk that engages the egg holders.

type of 1/30 horsepower, with a built-in gear reduction system yielding a shaft speed of approximately 30 rpm at rated load, or roughly 60 rpm with no applied load. A rheostat regulates the speed. A hinged motor mounting (K) permitting lateral movement, a coil spring (L) for lateral tension, and a limiting stop (M) complete the essential mechanism. However, some form of slip clutch to protect the gears is recommended.

The main stem of the torch is formed from 3/16-in. copper tubing, and the brass burner tip is provided with a jet hole .02 in. in diameter. For the first stage of reduction the oxygen and acetylene regulators (N) are initially adjusted to supply the respective pressures of 20 and 10 lb per sq in. A second stage of reduction is furnished by two low-pressure Hoke Phoenix regulators (O), each with fixed adjustments which insure a steady gas supply at a pressure of 2 lb per sq in. Final regulation is made with needle valves. Adjustments once properly made do not need to be repeated. The regulating system described has given satisfactory service over a long period of time.

Recently Dr. Kenneth Goodner in this laboratory has found that ordinary illuminating gas can be substituted for the acetylene, and in spite of the lower flame temperature it appears to work equally

well with a hole of .028-in. diameter. Furthermore, the gas pressures apparently do not have to be adjusted as critically as with the hotter flame. The illuminating gas is taken directly from the laboratory supply line, and only a single regulator and the needle valve at the torch are used to control the flow of oxygen.

Extensive experience with the apparatus during more than a year of operation has shown that a single operator can take care of from 1000 to 1200 eggs per hour.

13754

Toxicity of 3,3′-Methylenebis (4-hydroxycoumarin).

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Dicumarol,* chemically 3,3′-methylenebis(4-hydroxycoumarin), is the hemorrhagic principle isolated by Link and his coworkers.[1, 2] It can now be prepared synthetically by improved methods.[3] The substance is responsible for the "sweet clover disease" of cattle,[4, 5] and lowers prothrombin level of blood plasma.[6–10] In view of the recent clinical interest in dicumarol,[11–16] a systematic study of its toxicity became highly desirable.

* Dicumarol is the collective trademark of the Wisconsin Alumni Research Foundation, which controls the use thereof.

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