against nystatin either by adding ca 0.04 *M* K^+ and NH_4^+ salts (stationary phase cells) or by 0.01 *M* K^+ and NH_4^+ salts and the cofactors essential for a cell-free glycolytic system (log phase cells or protoplasts). Under these conditions there was leakage of K^+, NH_4^+, nucleotides and some protein, although glycolytic activity remained with the treated cells or protoplasts even after repeated washing. Pyruvate decarboxylase in NY-treated cells became accessible to pyruvate ions, but glucose-6-phosphate and fructose-1,6-diphosphate were still not fermented. The rate of CO_2 production by NY-treated cells or osmotically-lysed protoplasts did not exceed 25 to 30% of that obtained with the original preparations. The nature of the membrane alterations is discussed.

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Apparatus for Change of Medium in Extracellular Maintenance *in vitro* of an Intracellular Parasite (Malaria). (26883)

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Methods have been described whereby the avian malarial parasite, *Plasmodium lophurae,* can be removed from its host erythrocytes and maintained extracellularly *in vitro* for up to 4 days at 40°C(1,2). These methods have made possible a study of some of the nutritional requirements of an intracellular parasite itself, as distinguished from the host cell-parasite complex.

To get the best survival and development it was necessary to change the culture fluid after the first 18 hours and at 12 hour intervals thereafter. This was done as follows: Each 50 ml Erlenmeyer culture flask received 0.25 ml of duckling plasma (obtained without use of an anticoagulant). To this was added 0.02 ml of a chick embryo extract (1 10-day embryo ground in 4 ml of glucose-salt solution, mixture centrifuged, supernatant used). The flask was then rotated rapidly in such a way that the plasma clotted as a thin layer along the lower part of the wall of the flask, with the excess sometimes forming a larger clot at the bottom. Each flask then received the culture medium and finally the inoculum of hemolyzed red cells and free parasites prepared by the treatment of blood from an infected duckling with guinea pig

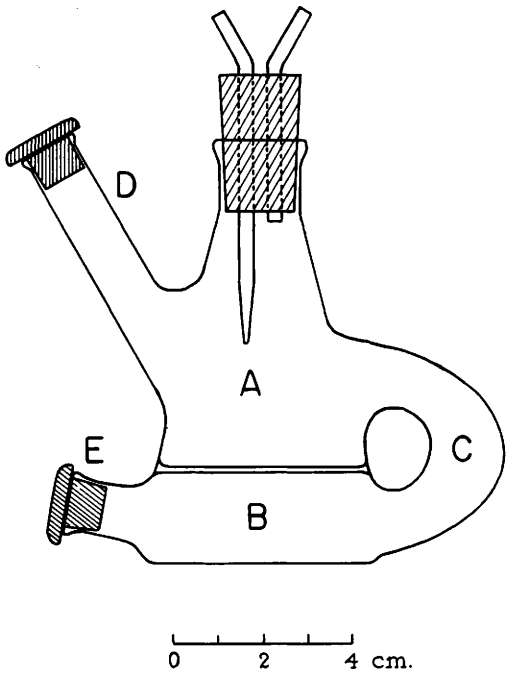

FIG. 1. Diagram of special culture flask with two connected compartments.

serum (as complement) and an anti-duck erythrocyte serum prepared in rabbits. The flasks were placed at 40°C on a rocking device giving 16 complete cycles per minute and a tilt of 20° from the horizontal. The cultures were usually completed at about 4 p.m. (of day 0). By the morning of day 1 approximately half of the free parasite-hemolyzed red cell material had stuck to the layer of plasma clot to form a scum which was washed over by the culture medium as the flask rocked to and fro.

The scum formation made it a relatively simple matter to draw off the fluid in the flask with a pipette and replace it quickly with an appropriate volume of fresh, previously warmed medium. All attempts to separate parasites from old culture fluid by centrifugation and resuspension in fresh medium resulted in damage to the organisms. The change of medium was effected after the first 18 hours (at 9-10 a.m. of day 1) and thereafter at approximately 12 hour intervals.

When survival to the 4th day was being regularly obtained, it became necessary to take care of the cultures in any one experiment on 3 successive nights, a procedure which made difficult any still more prolonged experiment. The following apparatus was accordingly devised.

Culture flask. This was a 50 ml Erlenmeyer flask provided with a compartment (B) below it connected to the main body of the flask (A) by a U-shaped arm (C) (Fig. 1). Opposite this U-shaped arm a long straight side-arm (D) extended from the body of the flask and a short side arm (E) from compartment B. The side arms C and D left the flask at such a height that when 4 ml of medium was rocked to an angle of 20° it did not overflow into C. Arm C purposely had an expanded diameter at the base of the U. If the flask was tipped in the direction of arm C through an arc of over 100° the fluid in A entered arm C and then entered compartment B when the flask was righted. Arms D and E were made to fit red rubber vaccine stoppers. Compartment A was stopped with a silicone rubber stopper bearing delivery tubes for the passage of a slow current of air with 5% CO_2 through the flask.

The changing apparatus. Fig. 2 gives an overall view of this with the doors open. It consisted of 2 main parts—an incubator(1) for the culture flasks and a refrigerator(2) for storage of the fresh medium. Part 1 contained a heating unit, thermostat and a rocking platform equipped to hold 8 of the culture flasks. The rocking was effected by a motor driving an eccenter (No. 1, Fig. 3) which transmitted the motion by a steel band. A switch could be set so that at a desired time a time-clock activated a solenoid which engaged eccenter No. 2. This moved the platform through an arc of 115° from the horizontal (Fig. 3), thereby tipping the flasks in the direction of arm C (Fig. 1) and emptying the culture compartment A. Eccenter No. 2 went through only one revolution before being disengaged by the solenoid, the motion of the platform then returning to eccenter No. 1. At the same time solenoids in the upper unit 2 (Fig. 2) were activated by a relay to open clamps which had been shut on silicone rubber tubing connecting a reservoir tube (Fig. 2(3)) to each culture flask. The opening of the clamps permitted fresh

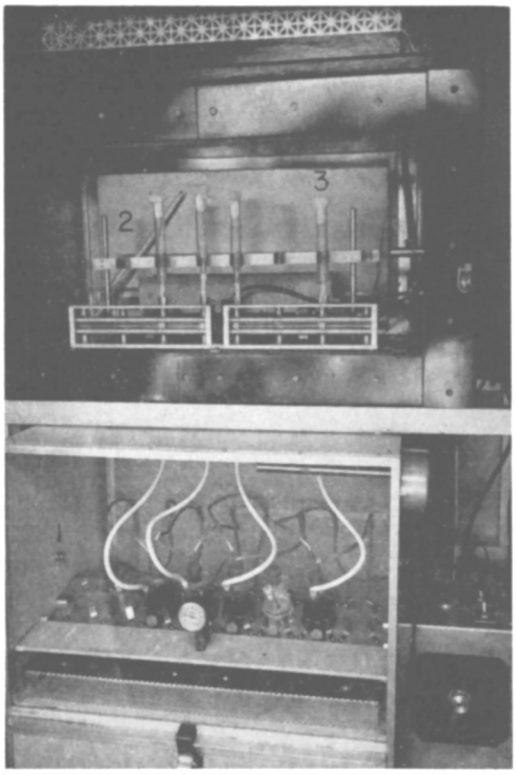

FIG. 2. General view of apparatus (with doors open) just after a change of medium. Unit 2 is supported on a frame over unit 1.

medium to flow into compartment A through side-arm D, after the former had been emptied of the old medium. The solenoids in unit 2 could also be opened and shut by a manually operated switch. Unit 2 was a well insulated refrigerator with a compressor placed above it. It could maintain a temperature of 1-2°C. It contained the 2 clamps activated by the solenoids, each controlling 4 reservoir tubes held in a rack. It was

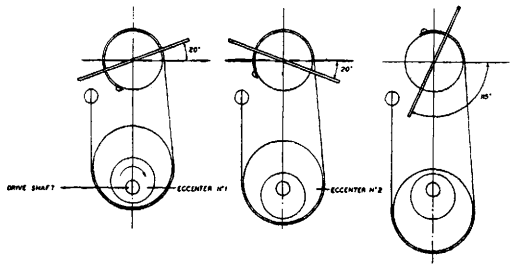

FIG. 3. Diagram illustrating principle whereby rocking motion is changed to a single tipping movement which removes the fluid from the culture chamber.

equipped with a time clock and a small heating unit controlled for a temperature of about 40°C. In this way fresh medium in the reservoir tubes was kept cold until 45 minutes before the time of changing of the medium, when the refrigerator was automatically switched off and the heater on. By the time the change occurred the fresh medium had been warmed.

Use of the apparatus in a culture experiment. Culture flasks were prepared using compartment A (Fig. 1) in the same way as an ordinary Erlenmeyer flask. At this time arms D and E were stopped with the vaccine stoppers. Care was exercised not to tip the completed flasks more than through a small angle in the direction of arm C. The flasks were placed on the rocking platform and the delivery tubes in the central stopper connected to the source of air with 5% CO_2. The incubator had been previously adjusted to 40-40.5°C, this temperature being read from a small thermometer inserted in the side-arm of an empty flask (Fig. 2). On the following morning, when the scum containing the free parasites had formed, the medium was changed manually in each flask. Each flask was removed individually, the previously mixed medium for the flask was taken up in a 5 ml pipette, the flask was opened at the central stopper and rotated so that the old medium contained in compartment A spilled through C into B, the fresh medium being then immediately added.

For the automatic change, to be effected at night, the fresh medium was mixed in reservoir tubes. These were made of Pyrex glass tubing with an outer diameter of 16 mm and about 12 cm long. The lower end was drawn to an outer diameter of about 7 mm and bore a 25 cm length of silicone rubber tubing (4 mm inside diameter) having at the opposite end a 5-6 cm length of glass tubing of 5 mm outer diameter. The free end of this tube was covered with aluminum foil. The wide end of the reservoir tube bore a gauze and cotton plug. These tubes were sterilized by autoclaving. A small screw clamp was attached and closed at the base of the reservoir tube, and the latter was provided with the fresh medium. The clamps

in upper unit 2 (previously refrigerated) were opened and the foil-capped free end of the tubing was threaded through the clamps and through a small opening which guided it from the bottom of unit 2 into the top of unit 1. With aseptic precautions the aluminum foil cap was removed and a 35 cm long piece of silicone rubber tubing was attached. This bore at its free end a short length of 7 mm glass tubing surrounded at its upper portion by a 2 cm length of silicone tubing. This fitted snugly into arm D of the culture flask, replacing the vaccine stopper, and completed the connection from the reservoir tube to the flask. The clamps in unit 2 were now shut and the screw clamps at the base of the reservoir tubes were opened. The refrigerator was set to change to the heater at 9:15 p.m. and the change of medium was set for 10 p.m. The following morning each connecting tube was removed and replaced with a vaccine stopper in arm D, sterile precautions being observed throughout.

Since the flasks so far used have a lower compartment (B, Fig. 1) able to hold fluid from only 2 changes of medium, this compartment was emptied once each day. A sterile needle was inserted through the vaccine stopper into arm E and the fluid drawn off with a syringe.

Discussion. With the same medium used previously (Trager(2)), extracellular survival and development of *P. lophurae* have been as good in the automatic changer as in experiments in which the medium was changed by pipetting. On day 4 about 80% of the parasites appeared in good connection by morphological criteria. Recently, a modification of the medium has given good survival in the automatic changer to day 5, with infective parasites demonstrated on day 6.

The apparatus here described is not designed for the same purpose as apparatus providing a continuous slow flow of medium. Apparatus of the latter type would not be suitable since the medium used at present for extracellular maintenance of malaria parasites contains labile ingredients, such as adenosinetriphosphate and, in addition, the concentrated red cell extract contains hydrolytic enzymes. Medium held warm in a reservoir providing a slow flow would deteriorate as much as the medium in the culture vessel. With the present method of sudden change, however, the fresh medium can be refrigerated until shortly before it is added to the culture flask. It then provides at the outset high concentrations of labile factors, which presumably are much reduced by the end of the 12-hour period between changes of medium.

Summary. A special flask and mechanical equipment are described which provide for automatic removal at a desired time of old culture fluid and addition of fresh medium to cultures in which extracellular malaria parasites (*P. lophurae*) are attached as a scum to a thin plasma clot lining the wall of the flask.

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Species Dependence of Chlorpromazine Metabolism.* (26884)

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Earlier reports from this laboratory(1,2) have confirmed the importance of sulfoxidation as a pathway for the metabolism of chlorpromazine(3,4). However, the question

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