and hematocrit dropped from 6.8 million, 15.3 g and 47 cc to 2.13 million, 4.2 g and 11.5 cc before pyridoxine was administered on the 116th day.

Pyridoxine, 60 μg daily by mouth, was followed by a rise of reticulocytes to 14.4% and a rapid rise in red blood cells and hemoglobin. After 18 days of therapy the red blood cell count was 4.7 million, hemoglobin 11.5 g, and hematocrit 42 cc. The green pigment-producing compound disappeared from the urine within 48 hours.

Pyridoxine-deficient rats whose urine yields large amounts of green pigment-producing substance do not develop such profound anemia as the dogs. Fifteen pyridoxine-deficient rats had hemoglobin values varying from 11.4 to 14.2 g per 100 cc, averaging 13.0 g. The hemoglobin of similar pyridoxine-fed rats varied from 15.2 to 16.5 g, averaging 15.8 g per 100 cc.

Conclusions. 1. Pyridoxine-deficient dogs excrete small amounts of a compound which can be converted to a green pigment by ferric ammonium sulphate. 2. Pyridoxine-deficient rats do not develop as profound anemia as the deficient dogs.

We wish to thank Mrs. D. Parsons for making the hemoglobin determinations and red cell counts on the rats.

13752 P

Preservation of Avian Malaria Parasites by Low-Temperature Freezing.*

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Since malaria plasmodia cannot be readily cultivated it has been necessary to maintain them in laboratory animals wherever they are used for research or classroom material, and hence Coggeshall's[1] demonstration that malaria parasites of the monkey could be preserved for at least 70 days† in the frozen state seemed to have much potential importance. For laboratory use the avian malarias are even more widely employed, and so experiments were undertaken to apply this technic to them.

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1 Coggeshall, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 499.

† Later extended to 151 days. Horsfall, F. L., *J. Bact.*, 1940, **40**, 559.

So far *Plasmodium cathemerium, circumflexum, hexamerium, lophurae, nucleophilum, relictum* var. *matutinum, rouxi,* and *vaughani* have been successfully preserved in this way. A total of 32 cases have been infected with parasites frozen for periods up to 90 days. For *lophurae,* ducks served as hosts; in all other cases female canaries were used.

The technic of freezing and thawing was not different than that used by Coggeshall and others, except that a small electric rotator was used to rapidly revolve the tubes containing the infective mixture during the freezing and thawing process. For convenience the freezing was done in a beaker of alcohol maintained at the correct temperature by being kept in the freezing cabinet, rather than in a specially prepared mixture of alcohol and solid carbon dioxide. Thawing was done in water at 42°C. Small Pyrex test tubes measuring 10 x 1 cm were used for containers, and the amount of blood frozen at a time was generally about 300 cmm. It appeared to make little difference whether this was defibrinated, or diluted with a little physiological citrate solution.

The most important factors in maintaining viability seemed to be speed in the freezing and thawing process, but—contrary to the claims made by some others—fairly considerable variations in temperature did not appear to be of great importance. It was found that the temperature of the alcohol in which the tubes were immersed varied occasionally as much as 25°C, depending on whether dry ice had been freshly added or was getting low. Temperature determinations made under these two conditions showed a range of from about −78°C to −55°C.

No differences were observed in the ability of different species of plasmodia to withstand freezing, nor is there reason to think that they cannot be maintained for periods considerably longer than the maximum so far obtained of 90 days. Coggeshall's success in keeping monkey plasmodia for 70 days has already been cited, and recently Kessler[2] has been able to preserve *Bartonella muris* in this way for 11 weeks.

Nor does the virulence of the plasmodia seem much affected. Incubation periods are in some cases quite long, as, for example, when *Plasmodium nucleophilum* was kept frozen for 60 days. Ordinarily parasites would have been demonstrable within a week or less, but in this case almost two weeks was required. This suggests that a considerable proportion of the parasites are killed. Microscopic examination of frozen material shows many destroyed erythro-

2 Kessler, W. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 238.

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.