and a value of 1 to each day upon which the smears contained nucleated epithelium (non-cornified) but no leucocytes. The total value for estrus days for the nicotine group was 659 and for the controls, 635. The total rat days for the nicotine group was 1242, and for the controls, 1118. By dividing the value of the estrus days by the total rat days, the "estrus index" may be obtained. For the nicotine rats, this index was $659 \div 1242 = 0.53$; for the controls, $635 \div 1118 = 0.57$.

Method II. The second method of evaluation considered only the days of cornification of vaginal epithelium, each day of cornification being given a value of 1. There were 250 rat days of cornification among the nicotine group, and 228 among the controls. The "cornification index" for the nicotine group was $250 \div 1242 = 0.202$; for the control group, $228 \div 1118 = 0.204$.

Thus by each method of comparison it was shown that nicotine had no effect on the estrus cycle of the white female rat.

5510

Fragility of Human Erythrocytes to Certain Salts.*

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Hober[1] determined that the potassium ion had a greater hemolytic effect than the sodium ion by placing red cells in tubes of hypotonic solutions of these salts and noting the time of appearance of hemolysis in the supernatant fluid. Hamburger[2] proved that the sodium and potassium cations could pass in and out of red cells. Ashby[3] incubated mammalian bloods of different species and determined that blood in which the red cells contained excess of potassium showed greater susceptibility to hemolysis by potassium than by sodium salts, and blood in which the red cells contained excess of sodium showed greater susceptibility to hemolysis by sodium than by potassium salts, and attributed these effects to action on the cell

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† I wish to thank the Touro Infirmary for the blood specimens which made this work possible.

[1] Hober, R., *Biochem. Z.*, 1908, **14**, 209.

[2] Hamburger, H. J., *Wiener med. Wochschr.*, 1916, **66**, 521, 575.

[3] Ashby, W., *Am. J. Physiol.*, 1924, **68**, 239, 285.

membrane and colloids of the cell rather than to osmosis. He also showed that the resistance of red cells was increased by standing, by injury of the cells, by increase of the fluid whether isotonic saline or serum, etc., and by carbon dioxide.

Hober and Ashby allowed the solutions to stand until hemolysis occurred. Just how valuable such a procedure might be with reference to the red cell *in vivo* is questioned by the author. There are surely physico-chemical changes in the cell on standing and this changed cell can hardly be compared with the red cell in the living body from a physiological point of view. The author has, therefore, added the blood cells collected by syringe from the patient directly to tubes containing varying concentrations of the salts; sodium chloride, potassium chloride, calcium chloride and magnesium chloride, and has observed the changes. Since the change 5 to 10 minutes after the addition closely corresponded to that of the same solutions 2 hours afterwards when the readings were accomplished with greater ease, the 2 hour readings were accepted as the results. The readings were made at room temperature. The salts were dissolved in distilled water which had a pH of 5.2. The pH of the sodium and potassium chloride solutions remained the same as that of the distilled water but the pH of calcium chloride solutions in the case of the results quoted was 6.2 and that of the magnesium chloride solutions 6.6 to 6.5. A drop of blood was added to the solutions and the reading was made of the concentration at which hemolysis first occurred, approximately 50 specimens being examined.

Hemolysis began in the case of sodium chloride in the 0.40 to 0.45% concentration (44 to 50% isotonic); in the case of potassium chloride in the 0.60 to 0.65% concentration (50 to 54% isotonic); in the case of magnesium chloride in the 1.00 to 1.05% concentration (52 to 55% isotonic) and in the case of calcium chloride in the 0.80 to 0.90% concentration (54 to 62% isotonic). The differences in the tonicity shown in these results would seem to substantiate the conclusions of Ashby and others[4] since greater resistance to hemolysis is shown by hypotonic salt solutions of sodium chloride than potassium chloride, greater resistance by potassium chloride than magnesium chloride and greater resistance by magnesium chloride than calcium chloride. However, the differences are not great and it would be extremely difficult to conclude that the phenomenon was anything but one of osmosis.

As Ashby has shown, increased resistance of the cells was demon-

[4] Simon, A., *Biochem. Z.*, 1929, **170**, 244.

strated in washed and unwashed cells allowed to stand, whether in the clot, in Ringers, in citrate or in normal saline. The author noted, however, that the range from beginning to complete hemolysis was much less in the case of potassium than the other salts. The results with the calcium and magnesium salts were less constant and less reliable than those with the potassium and sodium salts and their readings were less definite in every case in which they were used.

It was attempted to note any change in fragility of red cells when they were incubated in the salt solutions containing 0.0006 molarity of methylene blue under the assumption that the methylene blue would catalyse the absorption of oxygen by the red cells. No change was noted in the methylene blue solutions from that occurring in solutions of the same concentration of salt but containing no methylene blue. The methylene blue solutions were easily read despite the color change.

5511

Influence of Dextrose and of Low Temperatures on Preservation, Transportation and Viability of Malaria Parasites.

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In a study of the factors underlying continued growth and reproduction of malaria parasites *(Plasmodium vivax)* in blood cells removed from the human host, Bass and Johns[1] found that the addition of 0.5% of dextrose to human blood before defibrination apparently prevented an endosmosis into the parasitized corpuscles of toxic substances present in normal serum that quickly destroyed the parasites at body or other temperatures. That by the addition of dextrose the parasites continued their asexual reproduction at a rate directly dependent upon the temperature, up to a maximum of 40°C.

Confronted with the necessity of preserving and transporting blood containing tertian malaria parasites, I have extended these observations on dextrose content and temperature most conducive to prolonging the viability and consequent infectivity of such parasites.

Blood collected from patients presenting the symptoms of thera-

[1] Bass, C. C., and Johns, F. M., *J. Exp. Med.*, 1912, **16**, 567.