showed an increase in acetone body excretion. It is apparent from the data presented that the alcohol of niacin is the most ketogenic, niacin next and niacinamide the least ketogenic.

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Effect of Variations in Osmotic Pressure on Macrophages in Tissue Culture.* (18161)

I. N. DUBIN.† (Introduced by H. L. Stewart.)

From the Department of Pathology and Bacteriology, University of Tennessee College of Medicine, Memphis, Tenn.

In experiments dealing with the cultivation of the exoerythrocytic forms of *Plasmodium gallinaceum* it became necessary to study the effects of variations of the culture medium. This in turn required a knowledge of the range of osmotic pressure tolerated by the malarial parasite and the host cell, the macrophage. This paper presents the results of experiments on the effect of variations in osmotic pressure on macrophages and parasites.

Methods. The tissue culture methods used in the present experiments have been described in detail(1). The macrophages were derived from chick embryo spleens infected with the exoerythrocytic stages of *Plasmodium gallinaceum*. Macrophages were grown *in vitro* at various constant levels of osmotic pressure for 4 days. These levels varied from 3 to 16 atmospheres of pressure at 37°C. The osmotic pressure determinations were made by means of the cryoscopic method. In previous experiments(1) it was determined that the optimal pH range for malarial parasites was pH 7.2 to 8.0, and for macrophages pH 6.8 to 8.2. For this reason the pH of the medium was kept between pH 7.4 and 7.8 in the present experiments. This was done by means of a continuous flow of CO_2 gas mixtures. The nutrient medium consisted of chicken serum diluted with a balanced salt solution.

The osmotic pressure was varied in two ways: (1) by varying the water content only, keeping the relative proportion of ingredients constant, and (2) by varying the content of NaCl. In the first set of experiments hypotonic solutions were prepared by starting off with a mixture of chicken serum and Tyrode's solution and by the addition of varying amounts of water to produce the desired hypotonic solutions. To achieve similar results with hypertonic solutions, a starting hypertonic solution was made from a mixture of 50% chicken serum and 50% triple concentrated Earle's solution(2).‡ Such a mixture has an osmotic pressure of 16 atmospheres at 37°C. Starting with this solution various hypertonic solutions varying from 16 to 8 atmospheres were made by the addition of water. In this set of experiments, however, the percentage of serum varied from one experimental group to another. This necessitated setting up a parallel series of control groups to determine the effect of diluting the serum at constant osmotic pressure. This was done by mixing various concentrations of serum in Tyrode's to match the experimental

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† Present address—Pathology Section, National Cancer Institute, Bethesda, Md.

1. Dubin, I. N., and Yen, C. K., *Arch. Path.*, in press.

2. Earle, W. R., *J. Nat. Cancer Inst.*, 1943, v4, 165.

‡ Triple concentrated Earle's solution was highly alkaline, giving a precipitate of salts. To avoid the precipitate the solution was sterilized by passing through a sintered glass filter under 10 lb pressure of 100% CO_2. It was stored in stoppered bottles under equilibration with the same gas.

groups in which osmotic pressure was varied. This led to the observation that a high concentration of serum was inhibitory to the macrophages and the malarial parasites. This was confirmed in later experiments designed to test the effect of concentration of serum on the cells and parasites.

Consequently, a second set of experiments was set up in which the concentration of serum was kept constant at the optimal level, namely 20%. In this group of experiments osmotic pressure was varied by varying the concentration of NaCl. The chicken serum was mixed with Tyrode's solution which was split into 3 components, (a) a tenfold concentration of NaCl, namely 8.0% NaCl in H_2O, (b) H_2O, and (c) double strength Tyrode's solution from which NaCl was omitted.

Results. The results of the 2 groups of experiments were similar. This is not surprising since NaCl accounts for most of the osmotic pressure in serum and in a serum-Tyrode's mixture. Indeed there was not much difference in the final concentrations of sodium and chloride at any level of osmotic pressure achieved by either of the two methods.§

The optimal growth of macrophages occurred between 7 to 11 atmospheres of pressure. Fifty percent of growth occurred at 5 and 13 atmospheres. Growth ceased at 4 and 15 atmospheres. The parasites similarly exhibited optimal growth between 7 and 11 atmospheres. Fifty percent growth occurred at 6 and 12 atmospheres. There was no growth of parasites at 5 and 14 atmospheres.

Some morphological differences in the macrophages were noted as between the hypertonic and hypotonic solutions. When compared with normal cells, the macrophages in the hypertonic solutions were somewhat smaller and contained slightly less cytoplasm and fewer vacuoles; these cells tended to stain more sharply and brilliantly than the cells in the control group at 8.4 atmospheres. In the hypotonic solutions the cells presented a foamy appearance and the cytoplasm seemed edematous. In addition the cells appeared to stick together, suggesting an increased cohesiveness. The nuclei of such cells were irregular and presented a ragged nuclear membrane.

Discussion. In the present experiments the optimal growth of macrophages at 37°C occurred between 7 to 11 atmospheres of pressure. White(3) found that in Locke-Lewis solution chick embryo tissues remained in good condition at osmotic values from 3 to 11 atmospheres when the ratio of ingredients was unchanged. Willmer(4) noted that chick embryo tissues (a mixture of epithelium and fibroblasts from intestines) showed optimal growth in concentrations of sodium chloride from 0.4% to 1% (representing a range of from about 4 to 10 atmospheres of pressure).

An interesting effect was that of the dilution of the serum in the culture medium. A high concentration of serum was found to be inhibitory to the macrophages and the malarial parasites. Ebeling(5) observed that the dilution of the tissue culture medium at constant osmotic pressure produced a more extensive zone of cell proliferation but no increase in actual mass of newly formed tissue. Lambert(6) also noted that dilution of plasma with isotonic solution caused a more extensive migration in cultures of cells of the actively migratory type, such as those of the spleen and bone marrow. Dilution with a limited quantity of distilled water produced the same effect. Less actively motile cells (*e.g.* epithelium) were influenced little or not at all by dilution. The effect on cells of the migratory type he considered to be due probably to the reduction in quantity of fibrin in the clot, producing lessened resistance to cell locomotion. This explanation appears improbable, however, in the light of the present experiments. In the experiments described in the present paper the macrophages were grown directly on the glass coverslips without the use of plasma clot, and here also the dilution of the culture medium resulted in

§ Values for sodium levels in chicken serum were kindly supplied by Dr. Richard Overman, Department of Physiology, University of Tennessee, Memphis.

3. White, P. R., *Growth,* 1946, v10, 231.
4. Willmer, E. N., *Brit. J. Exp. Biol.,* 1927, v4, 280.
5. Ebeling, A. H., *J. Exp. Med.,* 1914, v20, 130.
6. Lambert, R. L., *J. Exp. Med.,* 1914, v19, 398.

an increased growth or migration. It seems more likely that the stimulating effect of the dilution of the serum resulted from a dilution of some inhibitory substance in the chicken serum.

Summary. 1. The optimal growth in tissue culture of macrophages and the exoerythrocytic forms of *Plasmodium gallinaceum* occurred between 7 and 11 atmospheres of pressure. Growth was reduced to 50% at 5 and 13 atmospheres.

2. High concentrations of serum (over 50%) were inhibitory to both the malarial parasites and the host cell, the macrophage.

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Changes in the Reactivity of Purified Prothrombin After Freeze Drying.* (18162)

ROBERT I. MCCLAUGHRY, EDNA B. ANDREWS AND WALTER H. SEEGERS.

From the Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Mich.

In work on the purification of prothrombin it became evident that the protein could not be activated as readily as the prothrombin of native plasma. The question arose whether this might be due to an alteration in the reactivity of the prothrombin or to the removal of essential activators. When evidence for the latter possibility became available in abundance, there followed a general tendency to consider the question closed. However, the reactivity of prothrombin can be changed; for example, under proper conditions, thrombin can produce alterations(1). This altered prothrombin can still be converted to thrombin but not with calcium plus thromboplastin plus Ac-globulin.

We have now found that freeze drying of purified prothrombin preparations produces changes which cause the prothrombin to be refractory to the physiological activators. This makes it desirable to avoid the freeze drying technic when the object is to retain purified prothrombin for long periods of time. The change in reactivity of the purified prothrombin is not noticeable immediately after drying. The loss in activity occurs later, and variable periods of time are required for extreme changes to take place. The sequence of events is approximately as follows: First the prothrombin becomes refractory to the activators such as calcium, thromboplastin, and Ac-globulin. Activation with sodium citrate, thrombin and 3-chloro-4,4′-diaminodiphenyl sulfone is then still possible. Later even this activation procedure is no longer effective and finally the dried prothrombin becomes insoluble in physiological saline solution.

Experimental procedures. Purified prothrombin was obtained from bovine plasma (2). The assay for prothrombin involved the use of the modified 2-stage procedure, which supplies Ac-globulin, calcium, and thromboplastin for the activation of prothrombin(3). Thrombin activity was measured by the method of Seegers and Smith(4). The apparatus used for drying prothrombin from the frozen state was similar to that described by Seegers(5).

Time relationships. The loss in activity of a large number of products has been studied

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3. Ware, A. G., and Seegers, W. H., *Am. J. Clin. Path.,* 1949, v19, 471.

4. Seegers, W. H., and Smith, H. P., *Am. J. Physiol.,* 1942, v137, 348.

5. Seegers, W. H., *Science,* 1945, v101, 284.