

than acid. This holds good especially if we compare the stronger (N/100 to N/500) concentrations. Weaker solutions (N/2000 to N/5000) of acid extract more stain than the stronger solutions of acid; and the difference between acid and alkali almost disappears in the case of weaker concentrations. In watery solutions of acid and alkali the extraction is somewhat better than if the acid and alkali are used in the 3 per cent to 10 per cent NaCl solutions. In pure H₂O and in neutral 1 per cent to 10 per cent NaCl solutions only a trace of eosin is extracted.

Summary.—Acid and alkali behave oppositely as far as their extracting power towards an acid and an alkaline dye is concerned. Addition of NaCl inhibits the extracting power of stronger concentrations of acid and in certain cases also that of alkali towards neutral red. Neutral NaCl solutions on the other hand extract neutral red better than water. How far in these effects the dissociation depressing action of NaCl or other actions of NaCl are concerned is uncertain. These experiments demonstrate that there exists a certain similarity between the action of acid and alkali on amoebocytes on the one hand, and on filter paper on the other hand; but this similarity is incomplete. It therefore appears probable that the conditions determining the staining of cell granules by neutral red differ in some essential respects from those determining the staining of filter paper.

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The effect of insulin upon the metabolism of certain bacteria.

ARTHUR ISAAC KENDALL.

*[From the Department of Bacteriology and Public Health,
Washington University School of Medicine, St. Louis, Mo.]*

The striking pharmacological effect of administering insulin to a diabetic person suggests that a similar response might possibly be induced in cultures of bacteria that do not utilize glucose, provided, of course, that insulin alone, and no supplementary factor, is responsible for the phenomenon.

Four organisms that do not utilize glucose (or any other carbo-

hydrate) were tested with this possibility in view. They were *Bacillus alcaligenes*, *Micrococcus catarrhalis*, a vibrio isolated from a diseased alimentary tract, a previously undescribed coccus. None of these microbes induces measurable chemical changes in glucose, so far as available methods indicate.

Plain glucose and glucose-insulin broths were prepared, and inoculated with parallel amounts of the respective organisms. Incubation was practiced for 6, 24, 48 and 72 hours respectively. At these several intervals the various media were examined for evidence of fermentation, that is, for change in hydrogen ion concentration, titratable acidity, and for gas formation. None occurred. The reaction changes were uniformly toward the alkaline side, indicating the utilization of the protein constituents for energy. In other words, insulin failed to induce these non-glucose fermenting organisms to utilize the sugar.

Shortly after these observations were completed, Noyes and Estill¹ reported experiments indicating that insulin increased the rate of fermentation of glucose, when it was added to cultures of *Bacillus bulgaricus* and *Bacillus acidophilus*. Their procedure was to add to a milk-broth-glucose solution some insulin, and, with suitable controls without insulin, observe the titratable acidity at suitable intervals. They reported that a 20 to 25 per cent increase in acidity was detectable in cultures of *Bacillus bulgaricus*, considerably less in corresponding cultures of *Bacillus acidophilus*, where insulin was used.

These experiments were repeated, following the technique of Noyes and Estill, but with negative results. Both the coagulation time, and the rate and intensity of acid production proceeded at a uniform rate in cultures with and without insulin. It is possible that the organisms used in the two laboratories were not the same. All that can be said is that the cultures of *Bacillus bulgaricus* and *Bacillus acidophilus* tried here were typical in all respects.

It is important for several obvious reasons to know whether any bacteria can be influenced in their glucose metabolism by insulin. Therefore a third series of tests were set up, using a variety of microbes of varying fermentation reactions, and of widely different proteolytic capacities. They included: the dysentery bacilli, typhoid, the paratyphoid bacilli, the Morgan

¹ *Proc. Nat. Acad. Sc.*, 1924, x, 415.

bacillus, coli, proteus, cholera and *Streptococcus hemolyticus*, *Straphylococcus aureus*, and types of pneumococci. The cultures were examined under parallel conditions in glucose and glucose insulin broths, at the same intervals as were those in the first series. It should be stated that 20 units of insulin were used for every 50 cc. of broth culture. A Lilly preparation, which gave satisfactory clinical reactions, was employed. In every instance the results were negative.

These observations seem to indicate that insulin added to glucose media has no apparent effect either upon the rate or the intensity with which several types of bacteria utilize glucose for energy, as measured by changes in titratable acidity and by changes in hydrogen ion concentration.

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Identification of two types of mononuclear phagocytes in the
peripheral blood of rabbits.

F. A. McJUNKIN.

[From the Department of Pathology, Washington University
School of Medicine, St. Louis, Mo.]

Mallory¹ in 1898 named the mononuclear phagocyte of typhoid fever lesions endothelial leukocytes. Since that time cumulative evidence has given much support to the view that the mononuclear tissue and blood phagocytes arise from endothelium. Aschoff and Kiyono² by means of vital dyes traced these free cells to the reticulo-endothelium. Sabin³ in the chick embryo saw endothelial cells detach themselves from the walls of veins and become free in the lumen of the vessel; and this author,⁴ after marking the vascular endothelium by the intravenous injection of carbon in suspension, observed the carbon-laden endothelial cells to un-

¹ Mallory, F. B., *J. Exp. Med.*, 1898, iii, 611.

² Aschoff, L., and Kiyono, K., *Folia. Haematol.*, 1913, xv, 383.

³ Sabin, F. R., Contributions to Embryology, 1920, iii, Carnegie Inst. Wash. Pub. No. 272, p. 213.

⁴ McJunkin, F. A., *Am. J. Anat.*, 1919, xxv, 27.