about the same response suggests that the visual changes, like the basal metabolic rate, are functions of general metabolism. Similarly, it is known that the rate (although not the extent) of regeneration of visual purple *in vitro* is increased by raising the temperature of the system.[7] It may be stated, however, that maximal visual responses occurred in experiments 4 and 6 before changes in the basal metabolic rate took place. Moreover, no general correlation was found between the level of the basal metabolic rate and the speed or extent of dark adaptation in other subjects. In one patient with classical myxedema (B.M.R. −26) the dark adaptation was normal. In 4 control subjects, whose basal metabolic rates were −17, −19, −16, and −15, respectively, the dark adaptation curves were normal.

An increase in the circulating available Vitamin A does not account for the visual response, since in all experiments but one the serum Vitamin A fell to lower levels during therapy.

The improvement in speed and extent of rod adaptation was greater than that of cone adaptation. In this respect the response resembles that to Vitamin A therapy,[7] and is in contrast to the slight and equal lowering of the rod and cone thresholds observed to accompany recovery from anoxemia.[8] The changes appear, therefore, to represent an alteration in the speed and extent of visual purple regeneration rather than a local effect upon the nervous mechanism. The decrease in serum Vitamin A level accompanying the improvement in dark adaptation suggests that the feeding of thyroid extract or of α-dinitrophenol to these patients facilitated the utilization of Vitamin A by the visual mechanism.

11930

Hemolytic Effect of Gramicidin.

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Gramicidin, a bactericidal agent isolated by Dubos[1] from cultures

[7] Zewi, M., *Act. Soc. Sc. Fenn.*, 1939, New Series B, **2**, 1.

[8] McFarlane, R. A., and Evans, J. N., *Am. J. Physiol.*, 1939, **127**, 37; McDonald, R., and Adler, F. H., *Arch. Ophth.*, 1939, **22**, 1.

[1] Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1, 11; *Ann. Int. Med.*, 1940, **13**, 2025.

of a sporulating soil bacillus, has a marked bactericidal effect against gram-positive bacteria, both *in vitro* and *in vivo*. However, this substance is very toxic for mice, rabbits and dogs when administered by the intravenous route. Early in the course of a study on the action of gramicidin by means of tissue culture methods, it was observed that gramicidin exerted a strong hemolytic action on rabbit's erythrocytes suspended in the tissue culture medium. The lots of gramicidin used in this work were furnished respectively by Dr. Rene J. Dubos and by Sharp and Dohme. The preparation of gramicidin used in this study was the alcohol-soluble, water-insoluble fraction which recently has been described and named tyrothricin by Hotchkiss and Dubos.[2] The tissue culture preparation employed in this study was similar to the one used by King, Henschel, and Green[3] to demonstrate hemolysin activity. The culture was planted on a 22 mm round coverslip and consisted of a drop of heparinized rabbit's plasma and 3 drops of tissue extract made by extracting 7-day chick embryos with rabbit's serum. A sufficient amount of rabbit's erythrocytes was added to make a 5% suspension in the final clot. Because gramicidin is insoluble in saline solution, suspensions were prepared by making suitable dilutions of a 2% solution of gramicidin in 95% alcohol with Tyrode's solution. Similar dilutions of 95% alcohol in Tyrode's solution were used as controls. In those cultures that contained 0.005 mg of gramicidin per cubic centimeter of culture medium, hemolysis was visible after incubation for 3 hours at 37.5°C, and the red cells were completely hemolyzed at the end of 16 hours of incubation. This amount, 0.005 mg of gramicidin, has been found to be bactericidal for pneumococci in this preparation.* Cultures containing 0.0025 mg of gramicidin showed complete hemolysis after incubation for 24 hours at 37.5°C. No hemolysis was observed in control cultures containing comparable dilutions of alcohol.

Greater concentrations of gramicidin were necessary to cause complete hemolysis of erythrocytes in blood agar plate preparations containing 0.6 cc of defibrinated rabbit's blood and 12 cc of nutrient agar. Preparations containing 0.05 mg of gramicidin per cubic centimeter of blood agar showed complete hemolysis only after 36 hours of incubation at 37.5°C. Small, circumscribed areas of hemolysis, apparently surrounding small aggregates of gramicidin were observed after 48 hours of incubation in blood agar plates con-

[2] Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, 1940, **136**, 803.

[3] King, J. T., Henschel, A. F., and Green, B. S., *J. A. M. A.*, 1939, **113**, 1704.

* To be reported.

taining 0.005 mg of gramicidin per cubic centimeter of medium.

Suspensions of 1% washed sheep's cells containing 0.01 mg of gramicidin per cubic centimeter were completely hemolyzed after one hour in a water bath at 37.5°C. Hemolysis was complete in 24 hours in tubes containing 0.001 mg of gramicidin per cubic centimeter of red cell suspension. The addition of small amounts of pooled guinea pig serum caused a slight but definite diminution of the hemolytic activity of gramicidin. The destruction of complementary activity of the serum by heating to 56°C for 30 minutes did not alter the inhibition of hemolysis. Attempts to neutralize this hemolytic effect by the addition of varying amounts of cholesterol to solutions of alcohol and suspensions of gramicidin were unsuccessful.

Further studies must be made to determine whether or not there is a relationship between the hemolytic effect of gramicidin and the hemolysin produced by the soil bacillus from which gramicidin is extracted. The description by MacLeod, Mirick and Curnen[4] of the effect of the intravenous administration of gramicidin on animals makes it seem likely that the hemolytic activity of gramicidin may play an important part in the toxicity of this substance. How far the hemolytic effect accounts for the total toxicity must be determined by *in vivo* experiments; however, the hemolytic activity of gramicidin is great enough in the presence of constituents of the blood to render inadvisable the intravenous use of this substance in bactericidal concentrations.

Summary. Gramicidin has a powerful hemolytic action against rabbit's and sheep's erythrocytes *in vitro*. This activity is marked even in the presence of serum, plasma and tissue extract. The presence or absence of complement did not appear to affect the process.

11931

In vitro Maintenance of Mammalian Endocrine Organs.

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The cultivation of endocrine organs was undertaken primarily with the hope that the secretory activity of such organs and their

[4] MacLeod, C. M., Mirick, G. S., and Curnen, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 461.