tually the weight returned to about normal, even though the administration of the same amount of thyroxine was continued. We interpret this as evidence that until the peripheral tissues could acquire a tolerance to the changes induced by thyroxine the adrenal gland was stimulated. After adaptation by the tissues the stimulus to the adrenal was removed.

We agree with Selye that the several changes observed after adrenalectomy are all symptoms of, rather than the primary cause of, the condition of insufficiency, but we do not agree that the primary change is an increase in histamine or similar substance. Rather it is the inability to resist sudden violent changes in the concentration or distribution of electrolytes, and we feel that potassium should be included as at least one of the "toxic metabolites" postulated by Selye.

Brief comment may be made on two points of Selye's note. Although he stressed the emergency action of the adrenal cortex it is now known that the cortex is essential for life with or without a stress on the animal. There is only one known treatment by which a normal condition can be maintained in an adrenalectomized animal without the use of cortin. This treatment is the use of an enormously high intake of sodium chloride with sodium citrate or bicarbonate and a minimal intake of potassium. It has not been shown that such treatment modifies in any way the detoxification of histamine or like substances.

In all the conditions given by Selye as suitable to produce the "alarm reaction" there is a rise in the concentration of potassium in the serum. Adaptation may not prevent a rise in the concentration of potassium, but it does permit the animal organism to withstand the effects of such an increase.

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THE DISAPPEARANCE OF INJECTED EPINEPHRINE IN THE ANIMAL BODY[1]

ALTHOUGH several hypotheses have been advanced by various authors to explain the rapid disappearance of epinephrine from the blood after injection, none of these theories have been proved adequate. The general belief held was that destruction occurred mainly in the liver. From the present work it appears that the liver can not assume the major rôle in the destruction of epinephrine, since ligation of the blood supply to that organ does not alter the pressor effect of the active principle. Similar experiments with the spleen and kidney indicated that these organs also play unimportant rôles. These findings are substantiated by the fact that nephrectomy does not retard the disappearance of the active principle from the blood.[2] Other theories have been advanced that the nerve endings in the walls of the blood vessels lose their sensitiveness under the influence of epinephrine and relax. However, the pressor effect may be maintained for a long time by a continuous flow of dilute epinephrine solution into the jugular vein. The blood can not actively destroy epinephrine during the short duration of the pressor effect, since it was found by the present workers that freshly drawn blood or a phosphate buffer (pH 7.3) containing 0.5 per cent. H_2O_2 oxidized only 25 per cent. of a solution of epinephrine (1:1000) when allowed to remain in contact for a period approximating the normal duration of physiological activity. Oxidized epinephrine has no effect on blood pressure.[3]

The period of existence of ephinephrine in the circulation has been studied by several investigators. It was believed that at least 75 per cent. of intravenously injected epinephrine disappeared within 15 seconds after injection and, when hypertension subsided, no trace of the drug could be further demonstrated.[4] Weiss and Harris[5] believed that epinephrine is still left in the circulation after the blood pressure returns to normal. These investigators showed this by allowing blood, from which epinephrine seemed to have disappeared, to flow into an artery which had been previously ligatured; a constriction was observed. This observation has been confirmed by the present workers in the following way: Several samples of blood were removed from the circulation of an anesthetized cat at various intervals during epinephrine hypertension, and the presence of the hormone tested on a contracting gut preparation. It was observed that epinephrine remained in the circulation in small quantities for three minutes after the blood pressure returned to normal.

A few experiments on the products of destruction of epinephrine in the animal body have been reported by other investigators. The results which were obtained could not be interpreted. Embden and Von Furth[6] fed epinephrine to a rabbit orally after sewing up the rectum, and afterwards isolated from its urine a yellow product. They were unable, however, to determine its structure or suggest any possible relation to the epinephrine molecule. In the present experiments,

[1] From the Departments of Chemistry and Physiology, University of Saskatchewan.

[2] M. A. Goldzieher, "The Adrenals." The Macmillan Company, 1929.

[3] S. S. Weinstein and R. J. Manning, *Proc. Soc. Exp. Biol. and Med.*, 32: 1096, 1935.

[4] M. A. Goldzieher, *loc. cit.*

[5] O. Weiss and J. Harris, *Pfluger's Archives*, 103: 510, 1904.

[6] E. Embden and O. Von Furth, *Beitr. Zeits. Chem. Physiol. Path.*, 421, 1904.

rabbits were kept on a strictly controlled diet for 48 hours prior to the injection of epinephrine, and the phenolic content of the urine was determined in a 24-hour specimen. An increase in phenolic substances was obtained after epinephrine injection equivalent to 80 per cent. of the injected drug. A portion of the urine was acidified with acetic acid and allowed to stand for 48 hours, filtered, hydrolyzed by NaOH and concentrated under reduced pressure. The residue was extracted several times with 90 per cent. alcohol, and the extract reduced to dryness. The dark brown mass was extracted with ether. Upon evaporating the ether extract, a small quantity of crystalline material was obtained which gave characteristic tests for protocatechuic acid. The amount of material isolated was too small for combustion analysis. It would appear highly probable that protocatechuic acid may be an end product of epinephrine, since injected protocatechuic acid is excreted partly unchanged and partly as an ethereal sulfate.[7] Furthermore, Dakin[8] showed that phenyl serine and phenyl-glyceric acid are oxidized to benzoic acid and that p-hydroxy proprionic acid is oxidized to p-hydroxy benzoic acid. Comparing these structures with that of epinephrine, it is conceivable that a similar oxidation process might take place in the animal body yielding protocatechuic acid.

As an alternative hypothesis for the rapid disappearance of epinephrine from the blood, the following may be suggested from a critical consideration of the present findings; epinephrine is not destroyed by the blood nor to any significant extent by specific organs, but passes rapidly through the capillaries into the tissues, where it is oxidized to a physiologically inactive substance, possibly protocatechuic acid.

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MENINGOCOCCUS INFECTION OF THE CHICK EMBRYO[1]

GOODPASTURE and Anderson[2] have recently reported on the use of the chick embryo in the study of infections by various types of bacteria. They followed the technique previously described by Goodpasture and Buddingh[3] for the cultivation of vaccinia virus. This method offers a distinct advantage in that the behavior of infectious agents can be studied *in vivo* in a uniform sterile living culture medium. Observations on some of the phenomena of invasion and the earlier stages in the pathogenesis of various infections can be made with comparative ease and simplicity. Furthermore, the possibility of cultivating microorganisms which heretofore have been maintained with difficulty on artificial media or in laboratory animals presents itself.

In this communication we wish to report our findings on the cultivation of the *Micrococcus meningitidis* by this method.

A pure culture possessing all the staining and morphological characteristics of *Micrococcus meningitidis* was obtained directly from the spinal fluid of a patient with the typical clinical picture of cerebrospinal meningitis. Fermentation reactions were typical; it was agglutinated by a polyvalent antimeningococcus serum and the Type I monovalent serum (Gordon's classification) in dilutions of 1–100. No agglutination took place in normal horse serum.

The microorganism from the first culture obtained directly from the spinal fluid was inoculated onto the chorio-allantoic membrane of chick embryos twelve days old. A platinum loopful of the 18-hour blood agar slant culture was used as inoculum. At the end of 24 hours the majority of the embryos had died from the infection. In the remaining ones, Gram-negative diplococci in large numbers, both intra- and extracellular, could be demonstrated by smears made from the membranal exudate. Transfers by a platinum loopful of membranal exudate to fresh 11- or 12-day-old embryos have been made every 24 hours. The purity of the culture has been controlled by stained smears and inoculation of the membranal exudate on blood agar slants. Agglutination and fermentation reactions have been set up at frequent intervals. In this manner the strain has been passed through 100 serial transfers in the chick embryo without loss of its type specificity.

Throughout this period of investigation the infection has been uniformly lethal for the chick embryo. Death usually occurred 24 to 48 hours after inoculation. Cultures from the heart's blood of the embryo were usually positive for the meningococcus, indicating that, besides infection of the membrane, the embryo itself is also invaded.

A histological study of the lesion in the chorio-allantoic membrane and the chick embryo was undertaken. Twenty-four- and 48-hour membranal lesions and embryos were fixed in Zenker's (10 per cent. acetic acid) and embedded in paraffin. Sections were stained with hematoxylin and eosin and by Giemsa's method to demonstrate microorganisms.

Grossly the membranal lesion is not very striking. Twenty-four hours following inoculation there is slight cloudiness and swelling flecked with small streaks and patches of hemorrhage. Microscopically,

[7] E. Baumann, *Zeits. physiol. Chem.*, 1: 263, 1877.
[8] H. D. Dakin, "Oxidation and Reduction in the Animal Body," Longmans, Green and Company, 1922.
[1] Aided by a grant from the Division of Medical Sciences, Rockefeller Foundation.
[2] E. W. Goodpasture and K. Anderson, *Am. Jour. Path.*, 13: 149, 1937.
[3] E. W. Goodpasture and G. J. Buddingh, *Am. Jour. Hyg.*, 21: 319, 1935.