SPECIAL ARTICLES

SYNTHESIS OF SUBSTANCES OF POSSIBLE PHYSIOLOGICAL ACTIVITY

THE methods developed by Fieser and Hershberg[1] for the synthesis of phenanthrene and hydrophenanthrene dicarboxylic anhydrides have been applied to the preparation (E. B. H.) of hydroxy and methoxy derivatives which are of interest for their possible oestrogenic activity. The condensation of succinic anhydride with 1-methoxynaphthalene afforded a suitable starting material for the synthesis of 9-methoxyphenanthrene-1, 2-dicarboxylic anhydride (m.p. 249–250°, corr.) by reduction, condensation of the ester with oxalic ester, cyclization and dehydrogenation. Plans to obtain the 7-substituted isomer by a similar process from the known γ-(6-methoxy-1-naphthyl)-butyric acid[2] were abandoned with the appearance of a paper by Cohen, Cook and Hewett[3] anticipating this part of our program. The 6-methoxy and the 6, 7-dimethoxy derivatives of octahydrophenanthrene-11, 12-dicarboxylic anhydride were prepared by the addition of butadiene to the unsaturated anhydrides obtained from anisol and from veratrol by condensation with succinic anhydride, reduction, ester condensation and cyclization. The ethers were demethylated after hydrogenation of the active ethylenic linkage. 6-methyl-7-hydroxyoctahydrophenanthrene-11, 12-dicarboxylic anhydride (m.p. 134.5–135.5°, corr.) was prepared similarly from γ-(3-methyl-4-methoxyphenyl)-butyric acid.[4]

As a further approach to the oestrone type of structure, the anhydride of phenanthrene-1, 2-dicarboxylic acid was converted into 1′, 3′-diketo-1, 2-cyclopentenophenanthrene (M. F. and E. B. H.) by condensation of the dimethyl ester with ethyl acetate. 1′, 3′-diketo-3, 4-cyclopentenophenanthrene (m.p. 201.4–202°, corr.) was obtained similarly.

We may report also the synthesis (M.F. and E.B.H.) of chrysene, 2, 3-dimethyl-6, 7-acechrysene (m.p. 222.6–223.1°, corr.), and 6, 7-dimethyl-3, 4-benzphenanthrene (m.p. 94.5–95°, corr.) from starting materials already described, by obvious extensions of the hydrocarbon synthesis developed by Fieser and Hershberg. The last two substances are being tested for carcinogenic activity. In connection with the latter problem 5, 10-dimethyl-1, 2-benzanthracene (m.p. 147–147.5°, corr.; picrate, m.p. 173.7–174.2°, corr.) has been synthesized for comparison with cholanthrene (M. S. N.). The method consisted in the reaction of *o*-tolylmagnesium bromide with naphthalene-1, 2-dicarboxylic anhydride to give a keto acid which yielded β-(*o*-toluyl)-naphthalene on decarboxylation, addition of the methyl Grignard reagent to the ketonic group of the keto ester, reduction of the resulting lactone by Martin's modification[4] of the Clemmensen method, cyclization to an anthrone and reduction with zinc and alkali.

By a reaction analogous to the reported Diels-Alder addition to cyclic unsaturated anhydrides, a new type of hydrophenanthrene derivative of interest in connection with the morphine problem has been made available (H. L. H.). Butadiene and 2, 3-dimethylbutadiene were successfully added to 3, 4-dihydro-1-naphthoic ester affording, after hydrolysis, 5, 8, 9, 10, 13, 14-hexahydrophenanthrene-13-carboxylic acid (morphine numbering), m.p. 137–137.5°, corr., and its 6, 7-dimethyl derivative, m.p. 162–162.5°, corr. These yielded phenanthrene and 2, 3-dimethylphenanthrene on dehydrogenation. The compounds are of significance because of the presence of a carbon-substituent in the position (C_{13}) assumed in the Gulland-Robinson formula for morphine to be occupied by the ethanamine chain. To provide a closer approach to the morphine structure a general synthesis of the required starting materials has been developed: α-keto-δ-phenylvaleric acid (m.p. 61–62°) is obtained by the acid hydrolysis of ethyl α-oxalyl-γ-phenylbutyrate and the ester is cyclized to 3, 4-dihydro-1-naphthoic acid. The 3-methoxy derivative of the hydrophenanthroic acid has been obtained and other syntheses are in progress.

The publication of the results of these and other experiments has been deferred for a time, pending the preparation for the press by one of us (L. F. F.) of the posthumous papers of the late Samuel C. Hooker.

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1 L. F. Fieser and E. B. Hershberg, *Jour. Am. Chem. Soc.*, 57: 1508, 1851, 2192 (1935).
2 A. Butenandt and G. Schramm, *Ber.*, 68: 2083, 1935.
3 A. Cohen, J. W. Cook and C. L. Hewett, *Jour. Chem. Soc.*, 52, 1936.
4 E. L. Martin, *Jour. Am. Chem. Soc.*, in press.

POTASSIUM: A BASAL FACTOR IN THE SYNDROME OF CORTICOADRENAL INSUFFICIENCY

AN increasing number of regulatory functions is now being ascribed to the adrenal cortex. These might be controlled by a number of hormones elaborated by the gland, or possibly the manifold effects of adrenal insufficiency are due to the breakdown of a single regulatory mechanism, to which the other effects are secondary.

The morbid anatomy of animals dying of adrenal insufficiency does not give any definite indications as to the cause of death; more striking changes are found in the blood chemistry during the course of the syndrome. These are, briefly, lowered sodium and

glucose contents, low alkaline reserve, high non-protein nitrogen and high plasma potassium. Of these changes perhaps the least attention has been paid to the last named.

A rise in blood potassium in both experimental adrenal insufficiency[1,2,3,4] and in crises of Addison's disease[5] has, however, been reported in the literature. It has also been found that corticoadrenal extracts lower the high blood potassium of adrenal insufficiency.[4] The increases reported are open to objection for two reasons. In the human the potassium rise might be due to hemolysis of red cells with a release of their high potassium content. In experimental animals it has been found that repeated withdrawal of large amounts of blood will of itself raise blood potassium.[6] Since the cat has been shown to have identical values for cells and plasma the work here reported is not open to the first objection. A new method[7] for determining potassium in 0.2 cc of ear-blood eliminated the second.

The object of the research briefly reported in the present paper was to elucidate the following points:

(1) Is adrenal insufficiency necessarily associated with disturbances in potassium metabolism?

(2) Can the symptoms of adrenal insufficiency be reproduced in normal animals by raising the plasma potassium to the same extent by injection?

(3) What is the mechanism whereby the adrenal cortex regulates potassium metabolism?

The first question was answered by a series of analyses performed on 27 cats, from which it followed that the normal blood potassium level for cats is 19.9 (14.0–25.8) mg per cent. In adrenal insufficiency the average of 51 analyses was 29.9 (15.6–46.1) mg per cent. The values found in the second group varied widely for different animals or for the same animals on different occasions; they tended to rise with the development of the syndrome.

Somewhat greater difficulties were encountered in finding a means of maintaining high blood potassium in normal animals. Studies[8] of the effects of intravenous injections of small amounts of potassium salts showed that 60 mg of potassium (as 2 per cent. KCl) led to an increase of 50 per cent. in plasma K, which was back to normal within 6 minutes, whilst injection of larger amounts caused death. The method finally evolved in the present research was to give intraperitoneal injections of 10 per cent. KCl, in amounts of a quarter of the lethal dose, at appropriately spaced intervals. Cats so treated will exhibit progressively the gross symptoms of adrenal insufficiency when the blood potassium level remains higher than 30 mg per cent. for several hours. If the injections are made at more frequent intervals, plasma potassium will continue to rise, until death ensues shortly after a value of 60 mg per cent. has been attained.

In correlating the observed symptoms of adrenal insufficiency and of experimental potassium poisoning with the blood potassium level we found that anorexia seems to be present at about 30 mg per cent. If this level is maintained for some days asthenia also appears. In acute cases, however, the severe muscular asthenia seems to be correlated with a level greater than 40 mg per cent., and terminal convulsions with blood values of 50 mg per cent. or more.

In the bilaterally adrenalectomized cat, death results within a few hours of a single injection of potassium chloride not exceeding one fifth of the ordinary lethal dose. During the period following injection, the animals will pass rapidly through the stages of corticoadrenal insufficiency to the terminal stage of complete prostration, which would otherwise have been reached many days later. The rise in blood potassium is much greater and more prolonged than that found in the normal animal. As in the normal cat, death takes place when the blood potassium rises to 60 mg per cent. The findings at autopsy conform to those ordinarily found in terminal corticoadrenal insufficiency. The same applies to normal cats in chronic or acute potassium poisoning; in addition, the microscopic anatomy of their adrenal cortex indicates that it has been concerned in the process.

The question remains as to whether the high blood potassium of adrenal insufficiency is a consequence of renal retention alone, whence cortin would be essential for normal potassium excretion, or whether the rise follows from augmented liberation of intracellular potassium due to cortin deficiency. The rise in potassium should be roughly parallel in tissues and blood if the former factor is alone involved. Actually the values found for skeletal muscle are slightly lower than normal, whence it is concluded that both of the above factors are concerned in the rise in blood potassium.

Other symptoms of the syndrome may be considered to be secondary to the rise of potassium. Thus, depletion of sodium may be ascribed to the diuresis due to high potassium.[9] Low blood sugar has been observed

[1] E. Baumann and S. Kurland, *Jour. Biol. Chem.*, 71: 281, 1926.

[2] A. B. Hastings and E. L. Compere, *Proc. Soc. Exp. Biol. and Med.*, 28: 376, 1931.

[3] C. I. Urechia, G. Benetato and M. Retezeanu, *C. R. Soc. Biol.*, 119: 439, 1935.

[4] R. L. Zwemer and R. C. Sullivan, *Endocrinology*, 18: 97, 1934.

[5] G. Maranon, J. A. Collazo, J. Barbudo and I. Torres, *Arch. med. cir. y especialidad*, 37: 893, 1934.

[6] S. E. Kerr, *Jour. Biol. Chem.*, 67: 694, 1926.

[7] R. Truszkowski and R. L. Zwemer (unpublished).

[8] J. L. D'Silva, *Jour. Physiol.*, 82: 393, 1934.

[9] L. Blum, E. Aubel and R. Levy, *Bull. Mem. Soc. Med. Hop., Paris*, 45: 1504, 1921.

after injection of potassium.[10] Muscular weakness has long been known to be associated with high blood potassium, and since this applies also to cardiac and smooth muscle it accounts for the circulatory disturbances. Dehydration of the blood, often reported in adrenal insufficiency, may be considered a consequence of diuresis and stasis. Finally, the lesions found in the kidney in potassium poisoning are of the same type as those in adrenal insufficiency. The renal lesions, low filtration pressure and diminished circulatory volume would also account for the uremic symptoms of late adrenal insufficiency.

Considerable variations in the survival time may be the result of the application of other mechanisms which may be brought into play, such as selection or avoidance of food and muscular activity. The latter has been shown to lower muscle potassium[11] and increase muscle sodium, thus bringing about the reverse changes in the blood. An adrenal insufficient animal as a rule does not tend to move unless disturbed and any excessive activity thus produced shortens its survival period. This would agree with the observations that muscles from adrenalectomized animals fatigue readily and that normal animals can be protected against fatigue by injections of cortin. It has been found that patients with Addison's disease have a greatly increased capacity for doing work after cortin administration.[12] Preexisting lesions of the kidneys would also lead to a more rapid course of the syndrome. Where the survival time is long, the results of individual sensitivity of different organs will complicate the picture, and death may often result from secondary causes. For these reasons it is not claimed that the terminal stages of the syndrome are in every case identical with those of potassium poisoning, but it is felt that the latter is the basal cause.

Preliminary experiments performed on mice, rats and guinea pigs have afforded some evidence that cortin has a protective action against potassium. Work is now in progress on the application of this finding to the assay of cortin.

Feeding insufficient animals with high potassium diets was found to aggravate the condition in cats, and in rats during the first few days following adrenalectomy. Similar results with dogs have been reported recently.[13]

In the therapy of Addison's disease it might be of greater importance to eliminate potassium from the diet than to administer high doses of sodium chloride, and probably the best results could be expected if both of these measures were used. Finally, cortin administration would appear to be indicated in many other conditions involving a rise in blood potassium, such as nephritis, traumatic shock and certain acute febrile diseases.

[10] E. Kylin and A. Engel, *Klin. Wochnschr.*, 4: 653, 1925.

[11] W. O. Fenn, *Am. Jour. Physiol.*, 116: 47, 1936.

[12] F. A. Hartman, G. W. Thorn, L. M. Lockie, C. W. Greene and B. D. Bowen, *Jour. Am. Med. Assoc.*, 98: 788, 1932.

[13] E. C. Kendall, H. L. Mason, C. S. Myers and W. D. Allers, *Jour. Biol. Chem., Proc.*, XXX: lvii, 1936.

Conclusion

It is believed on the basis of the above findings that an important function of the adrenal cortex is the regulation of potassium metabolism and that the various known symptoms of corticoadrenal insufficiency may be explained in terms of a disturbance of corticoadrenal-potassium interrelations. It is further considered that the beneficial action of cortin in certain other pathologic conditions would suggest that the same mechanism is involved.

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DIETARY PRODUCTION AND PREVENTION OF ANEMIA IN LARVAL AMBLYSTOMA

Amblystoma larvae growing rapidly on a diet of raw beef liver developed long and pointed gill filaments with an abundance of red blood cells flowing through the capillary network. In contrast, larvae fed on raw beef muscle ate less, grew more slowly and had shorter gill filaments with a relatively smaller quantity of blood. This growth rate was raised when the beef muscle was powdered and formed 60 per cent. of a synthetic ration containing carbohydrate, fat, cod-liver oil and yeast; there was also an abundance of blood with deeply colored red cells and its rapid flow through the marginal vessels rounded the ends of the gill filaments. On a diet with the same protein: carbohydrate: fat ratio and the same vitamin supplement, but deriving the protein from a highly purified casein and from powdered milk, larvae grew poorly and had short curling gills with pointed filaments. By use of a low-powered microscope it was seen that the gill circulation of these larvae carried few and pale erythrocytes as compared with that of larvae fed on the synthetic beef muscle diet, and these observations were confirmed by standard blood tests (red cell counts and Newcomer readings of hemoglobin). It was possible to alter the severity of the disease and to vary the length of time before death by quantitative regulation of the diet, but development was rarely carried through the first visible changes preliminary to metamorphosis.

Supplementation of the anemia-producing diet with iron and copper, separately and combined at three different levels, had no effect upon the retarded growth nor upon the anemia. The disease was less severe