ABSTRACTS

(Papers read before the Institute but not published in the Proceedings)

STEROIDS IV.—THE ISOLATION FROM EQUINE PREGNANCY URINE AND CHARACTERIZATION OF A NEW MEMBER OF THE FEMALE SEX HORMONE SERIES. R. D. H. Heard and M. M. Hoffman, Dept. of Biochemistry, Dalhousie University, Halifax, N. S. (Read November 12, 1940). From the non-phenolic extract of equine pregnancy urine there has been isolated a new hydroxy ketone which has been definitely characterized as a Δ-5,7,9-estratrienol-3-one-17, a neutral isomer of estrone in which Ring B instead of Ring A is benzenoid.

Analyses of the hydroxy ketone, its acetate, and oxime established the empirical formula $C_{18}H_{22}O_2$, and the function of the two oxygen atoms. The presence of a benzenoid ring in the nucleus was indicated by a positive xanthoproteic reaction and a yellow colour with tetranitromethane, and the absence of isolated double bonds, by saturation to bromine. Proof of the Δ-5,7,9-estratriene skeleton and of the location of the substituents on C_3 and C_{17} was provided by the identification of the product of hydrogenation. (Published in full in J. Biol. Chem., *138*, (2), 651-665, 1941).

SURVIVAL OF *Eberthella Typhosa* AND *Eschvrichia Coli* ON SALT FISH. M. Frank and E. Hess, Atlantic Fisheries Experimental Station, Halifax, N. S. (Read November 12, 1940). The survival of *Eb. typhosa* and *Es. coli* on salt fish agar and in salt fish broth at 5 to 6°C has been shown to extend over periods of three months and more. On salt fish itself, before drying, survival of these organisms is reduced, particularly for the former organism. Survival is favoured by low storage temperature and heavy contamination. (Published in full in J. Fish. Res. Board Can., *5*, (3), 249-252, 1941).

STEROIDS V.—THE KISHNER-WOLFF REDUCTION OF DEHYDRO-ISOANDROSTERONE. R. D. H. Heard and A. F. McKay, Dept. of Biochemistry, Dalhousie University, Halifax, N. S. (Read December 9, 1940). For the syntheses of certain isomers of the male sex hormone androsterone, a supply of Δ-5-androstenol-3(β) was required. Reduction of the semicarbazone acetate of Δ-5-androstenol-3(β)-one-17 in sodium ethylate at 180° yielded a complex mixture of 17-desoxy steroids, both saturated and unsaturated, from which the desired product was isolated in the form of a molecular co-ordination compound, m.p. 133°, $[\alpha]_D$-65, consisting (bromine titration) of two moles of Δ-5-androstenol-3(β) and one mole of androstanol-3(β). The sparingly soluble digitonide of the latter was separated from the soluble digitonide of the dibromide of the former, to give, after decomposition, the two components in pure state. Also observed in the course of the reaction were epimerization of the 3-hydroxyl group and reduction of the ethenoid linkage to the 5-cis (aetiocholane) as well as the 5-trans (androstane) saturated nucleus. (Submitted for publication in full to J. Biol. Chem.)

THE PHYSIOLOGICAL CONDITION OF CERTAIN BACTERIA IN RELATION TO THEIR "RESTING CELL" ACTIVITY. A. J. Wood, Cornell University, Ithaca, N. Y., now Atlantic Fisheries Experimental Station, Halifax, N. S. (Read December 9, 1940). The influence of growth conditions on subsequent "resting cell" activity of certain Lancefield Group B Streptococci has been investigated. Their dehydrogenase activity was

found to be markedly influenced by the nature of the medium on which the cells were grown. Increasing concentrations of yeast extract in the growth medium, while not materially influencing the total cell crop, produced cells having a much higher dehydrogenase activity per unit of cellular nitrogen. The final hydrogen ion concentration in the growth medium was found to influence activity. If the pH of the medium was permitted to drop to 6.5 or lower the cells recovered from the medium had greatly diminished activity. The significance of these various growth conditions was found to be greater than has been generally recognized. (Submitted for publication in full to J. of Bacteriology).

THE EFFECT OF FLUORIDE ON THE PERMEABILITY OF THE RED BLOOD CELL. Hugh Davson, Dept. of Physiology, Dalhousie University, Halifax, N. S. (Read December 9, 1940). The permeability of the rabbit erythrocyte to potassium induced by treatment with a NaCl-NaF mixture has been investigated. It has been shown that the effect of fluoride is intimately connected with its poisoning action on glycolysis and the evidence suggests that, as a result of this inhibition, an intermediate product in the chain of chemical reactions which constitute the glycolytic process accumulates in the red cell membrane, and so causes it to become permeable to potassium. (Published in full in J. Cellular and Comparative Physiology, *18*, (2), 1941).

PHYTIN AS AN ORGANIC CONSTITUENT OF SOIL. W. J. Dyer, Macdonald College, Que.; now Atlantic Fisheries Experimental Station, Halifax, N. S. (Read January 13, 1941). In the separation of organic phosphorus compounds from soil, it was shown that a material was present which was much more stable than ordinary nucleic acid compounds. A nitrogen free ferric salt was precipitated from a N/6 solution of a soil extract and identified as a phytate, by analysis, reactions, titration with ferric chloride, and behaviour to enzymes. The very insoluble ferric and aluminum phytates were stable to phytase enzyme in acid solution. This is probably the form in which 25 per cent or more of the soil organic phosphorus accumulates in a stable form. (Published in full in Soil Science, *51*, (2), 159-170; (3), 235-248; (4) 323-329, 1941).

THE SYNTHESIS OF SUBSTITUTED PHENANTHRENES RELATED TO THE STEROIDS. W. S. Bauld and C. W. Small, Dept. of Chemistry, Acadia University, Wolfville, N. S. (Read January 13, 1941). The synthesis of 1-methoxy-2, 13-dimethyl-5,6,7,8,9,10,13,14-octa-hydro-phenanthrene and its conversion to 1-methoxy-2-methyl-phenanthrene by selenium dehydrogenation is described. The starting point of the synthesis was o-cresol, which was nitrated, the hydroxy group methylated, nitro group reduced to amine, converted to iodo derivative by diazotization and treatment with potassium iodide, Grignard formed, treated with ethylene oxide to form β (2-methoxy-3-methyl-phenyl) ethyl alcohol. Primary alcoholic group was then chlorinated, Grignard reagent formed, conjugated with ortho-methyl cyclohexane to form 1-[β-(2-methoxy-3-methyl-phenyl) ethyl]-cyclohexan-1-ol. The ring was then closed by 85% H_2SO_4 to form desired compound, analysis of which was in good agreement with the theoretical. The subsequent dehydrogenation of this compound to 1-methoxy-2-methyl-phenanthrene which was identified from melting point in literature, was in accord with the splitting of the C-10 angular methan group in the selenium dehydrogenation of

cholesterol to chrysene, thus confirming steroidal structure as well as providing a route to compounds containing the angular methan group. (Submitted for publication in full to Can. J. Research).

SOMATIC CHROMOSOMES IN *Vicia Faba* L. William H. Feindel, Department of Biology, Acadia University, Wolfville, N. S. (Read January 10, 1941). For a study of chromosome structure, root-tips of *Vicia Faba* L. were fixed in modified Benda's (osmic-chromic-acetic), embedded in celloidin and stained in Haidenhain's haematoxylin. Chromosomes exhibited eight-parted structure at metaphase and quadripartite structure during anaphase, prophase and telophase. Chromomeric appearances as reported by earlier workers are interpreted as tightly coiled or crossed chromonemata.

WINTER GROWTH IN THE VEGETATIVE BUDS OF THE WAGENER APPLE. Hugh P. Bell, Dept. of Biology, Dalhousie University, Halifax, N. S. (Read February 10, 1941). Vegetative buds of the apple were collected from September 26, 1938, to March 18, 1939. The median longitudinal section of each bud was measured. The data collected suggested that a slow but continuous growth in length within the bud proceeds throughout the winter months. The figures were subjected to statistical analysis and found to be significant. (Published in full in Can. J. Research, C, *18*, 585-590, 1940).

STUDIES ON BROWN HALOPHILIC MOLDS OF THE GENUS *Sporendonema* emend. CIFERRI ET REDAELLI. M. Frank and E. Hess, Atlantic Fisheries Experimental Station, Halifax, N. S. (Read March 10, 1941). Twenty-six cultures of brown halophilic molds, isolated from undried and dried salt fish, air, salt, floor dust and date fruits, were studied. Halophilism, temperature and pH limits for growth, and ability to liquify gelatine and utilize various sugars were compared. Nineteen cultures, including all salt fish isolations, belong to the species *Sporendonema epizoum* (Corda) Ciferri et Redaelli. They are true halophiles, do not grow at 37°C, and do not liquify gelatine; spores are large, and medium to dark brown over the whole temperature range at medium salt concentrations. The other strains are facultative halophilic, grow at 37°C, liquify gelatine, have small spores of light brown colour, and resemble *Torula minuta* Hφye i.e. *Sporendonema minutum* (Hφye) n.comb. (Published in full in J. Fish. Res. Board Can., *5*, (3),287-292, 1941).

STUDIES ON AQUEOUS HUMOUR. H. Davson and C. B. Weld, Dept. of Physiology, Dalhousie University, Halifax, N. S. (Read March 10, 1941). Hodgson (J. Physiol. 1938, 94, 118) has shown that the serum/aqueous chloride in dogs is 0.92. The theoretical ratio on the basis on Donnan equilibrium calculations is 0.96. This excess of chloride is used as evidence that aqueous humour is not a simple dialysate of blood plasma.

We have confirmed Hodgson's finding, obtaining a ratio of 0.922 (standard error of mean .005). Reformed aqueous humour, taken shortly after the original paracentesis, gives a lower chloride and a serum/aqueous ratio approximately unity. Within 5-6 hours the ratio is back to the 0.92 level however. If the lens is removed, the ratio is slightly lowered for a time but after 2-3 months, when the inflammatory process is over and the eye completely healed, the chloride in the aphakic aqueous is at its original level and the serum/aqueous ratio again 0.92.

The serum/aqueous ratio with respect to CO_2 has also been determined and found to be 0.84. The standard deviation is 0.05.

The serum/aqueous ratio of the cation sodium is found to be 1.042 (SD .001). This corresponds closely to the theoretical figure of 1.04. In view of this finding it cannot yet be claimed that the apparent excess of chloride in the aqueous humour has any osmotic significance. (Published in full in Am. J. Physiol., *134*, (1), 1-7, 1941).

STEROIDS VI.—OBSERVATIONS ON THE CONSTITUTION OF ANDROSTANOL-3(β)-ONE FROM EQUINE PREGNANCY URINE. R. D. H. Heard and A. F. McKay, Dept. of Biochemistry, Dalhousie University, Halifax, N. S. (Read March 10, 1941). Previously the authors reported the isolation of an isomer of androsterone which differed from the latter in the orientation of the C_3-OH and the position of the ketonic oxygen atom. Present investigations, concerned with the location of the carbonyl group, surprisingly indicate a 15-keto derivative. Positions 1, 2, 4, and 17 were eliminated by reason of the non-identity of the corresponding diketone with androstanedione-3,17 and its failure to show reactions characteristic of an α or β diketone. The ease of oximation of the urinary androstanol-3(β)-one, and its high levorotation ($[\alpha]_D$-160°), argue against C_{11} or C_{12}, and the negative response in the Liebermann-Burchard test contrasts with the behavior of saturated 6-ketosteroids. The 7-keto compound seemed likely, but, on synthesis, it proved non-identical with the natural. Remaining are positions 15 and 16; a 16-keto derivative, containing the—CH_2-CO-CH_2—linkage, is improbable because of the feeble color developed by the urinary hydroxy ketone with m-dinitrobenzene. (Submitted for publication in full to J. Biol. Chem.).

STEROIDS VII.—THE SYNTHESIS OF ANDROSTANOL-3(β)-ONE-7. R. D. H. Heard and A. F. McKay, Dept. of Biochemistry, Dalhousie University, Halifax, N. S. (Read March 10, 1941). The preparation of androstanol-3(β)-one-7 (m.p. 131°, $[\alpha]_D$-69°) proceeded from the semicarbazone acetate of dehydroiso-androsterone. Reduction (Wolff-Kishner) yielded mainly Δ-5-androstenol-3(β) (m.p. 137°) and androstanol-3(β) m.p. 148°. Oxidation of Δ-5-androstenol-3(β) acetate (m.p. 94°) with warm chromic anhydride gave the acetate of Δ-5-androstenol-3(β)-one-7 (m.p. 174°; λ_{MAX}234mμ; ϵ_{MAX} 11,200) which, on hydrogenation and oxidation, was converted to androstanol-3(β)-one-7 acetate (m.p. 110-113°). (Submitted for publication in full to J. Biol. Chem.).

STEROIDS VIII.—THE CONVERSION OF ESTRADIOL TO ESTRONE IN MAN. R. D. H. Heard and M. M. Hoffman, Dept. of Biochemistry, Dalhousie University, Halifax, N. S. (Read April 7, 1941). To substantiate the generally accepted hypothesis that the urinary estrogens, estrone and estriol, arise from the follicular hormone, α-estradiol, the fate in man of injected α-estradiol was investigated. 300 mg. were administered intramuscularly in oil throughout 8 days. The urine (18.7 liters) collected during the administration period and the succeeding fortnight was subjected to hydrolytic treatment (2 hours in the autoclave at 15 pounds, with 40 ml. of concentrated hydrochloric acid per liter) and extracted with benzene. The benzene-soluble material was washed free of acids and divided into a phenolic (890 mg.) and a neutral (957 mg.) fraction. Separation of the ketonic phenols with Girard's Reagent P yielded 40.4 mg. of a mixture of crystals and oil, which, on crystallization from ether, gave estrone melting at 248-253° (Kofler's micromethod; corrected) and at 252-256° on admisture with an authentic specimen (m.p. 256-259°). From the non-ketonic phenols (113 mg.), 9.8 mg. of

unchanged α-estradiol were recovered. The neutral fraction was carefully examined for products of complete saturation of the estrane nucleus, i.e. the estranediols; only dehydro-iso-androsterone, androsterone aetiocholanol-3(β)-one-17, pregnanediol-3(α), 20(α), and cholesterol were encountered. (Published in full in J. Biol. Chem., *140*, 1941, in press).

THE ESTIMATION OF ALLANTOIN IN BLOOD. Helen Wentworth, Dept. of Biochemistry, Dalhousie University, Halifax, N. S. (Read April 7, 1941). A method for the estimation of allantoin in blood based on the Rimini-Schryver reaction is described. 5cc. of mammalian blood are required for an estimation. This method permits of an accuracy of about ±10 per cent using the ordinary Duboscq colorimeter.

THE HATCHING MECHANISM OF SALMON EGGS. F. R. Hayes, Dept. of Biology, Dalhousie University, Halifax, N. S. (Read April 7, 1941). The changes in the force required to break an egg as development proceeds are described and the time of appearance of the hatching enzyme in the perivitelline fluid determined. It is found that the egg shell softens before the hatching enzyme appears. The enzyme acts best in a slightly alkaline medium. (Submitted for publication in full to J. Cellular and Comparative Physiology).

THE GROWTH RATE OF SALMON EMBRYOS. Florence H. Armstrong, Dept. of Biology, Dalhousie University, Halifax, N. S. (Read May 5, 1941). Embryos were weighed periodically during the first winter of development. When the logarithm of the weight is plotted against age, a series of straight lines is obtained, the slopes of which decrease with advancing age. In other words the growth rate is constant for a certain period and then changes abruptly to another rate which in its turn remains constant for a time. In the total time under consideration there are three such periods. During the latter part of development the growth rate of grilse eggs, which are smaller than salmon, was measured. The embryos were found to grow more rapidly than those of salmon and thereby tended to catch up to them in size. (Submitted for publication in full to Can. J. Research).

A DIETARY SURVEY IN HALIFAX. E. Gordon Young, Department of Biochemistry, Dalhousie University, Halifax, N. S. (Read May 5, 1941). A dietary survey comprising 82 families and 385 individuals has been carried out in Halifax by both inventory and individual methods in the income range of $450-$1500 per annum. The results in terms of nutritional essentials have been compared with the Canadian Dietary Standard. The most important findings were a deplorable deficiency in the intake of calcium by the children and of the vitamins, especially B_1 by the whole group. (Published in full in Can. J. Publ. Health, *32*, 236-240, 1941).