PLACENTAL HORMONES.*

BY

J. B. COLLIP,

PROFESSOR OF BIOCHEMISTRY, MCGILL UNIVERSITY, MONTREAL.

As a review of the literature and a progress report of our experiments on this subject has now been published elsewhere,² a summary only of the work to date will now be given.

The sulphosalicylic acid extract of human placenta made by the technique of Wiesner³ has produced, when injected into immature female rats, increased rate of growth of ovary and the phenomena of oestrus. Corpora lutea were present as a rule in the ovaries at the time oestrus was first observed. The increased size of the ovaries was due for the most part to the newly formed luteal tissue. This same extract, when injected into immature male rats, has produced an increased rate of growth of the prostate and seminal vesicles.

Extracts of human placenta made by the use of acetone (equal volume) or alcohol (two volumes) were found to have similar physiological properties.

Acetone or alcoholic extracts of human placenta which have been concentrated and fractionated with alcohol, have been divided into three parts, each of which appears to contain an active principle peculiar to itself and differing in certain properties from each of the other two. The first fraction to be described contains the sex hormone oestrin, now to be known as theelin. It is obtained by repeated extraction with ether of the acetic-acid acidified aqueous concentrate of the original extract, or of alcohol-soluble fractions obtained from the latter. The second fraction represents the 85 per cent. alcohol-soluble material present in the original extract, but from which oestrin has been removed by repeated extraction with ether. Absolute removal of oestrin has been a matter of considerable difficulty, and it has of late been our practice to extract ten times with ether, reacidifying the aqueous phase with acetic acid after each extraction. The name emmenin has been suggested for the oestrogenic active principle of this fraction. The third fraction consists of the material which has been precipitated from the original extracts by alcohol up to 85 per cent. concentration. This precipitate has been repeatedly extracted with water and reprecipitated by alcohol. It has also been extracted with ether to remove oestrin. For convenience this may be termed the anterior pituitary-like substance of the placenta. The physiological properties of the second and third fractions only will be described.

The second fraction referred to above has produced oestrus in immature rats in from three to seven days' time following either oral or subcutaneous administration. In terms of the equivalent of original placenta tissue the smallest amount required to produce this result has been 0.5 gram per day. Animals killed at the time of the induced premature oestrus have shown in gross and microscopic examination the typical oestrous changes in both uterus and vagina. A slight increase in the size of the ovaries has been associated in many instances with increased development of the follicles. Corpora lutea have not been observed in the ovaries at the time of the induced oestrus. Similar results have been obtained with extracts of this fraction, which have been treated with pepsin or trypsin. No demonstrable effects have ever been obtained in male rats, either immature or adult, by the injection or feeding of this fraction. This fraction has been administered to normal adult female rats in doses varying from 1 to 10 grams of placenta daily or bi-daily over long periods. The results of this study demonstrated conclusively that in the normal adult rat no interference with the oestrous cycle, impregnation, pregnancy, or lactation had occurred. It has also been without effect on senile female rats. A certain number of immature rats treated with the 85 per cent. alcohol-soluble fraction appear to be immune to its effects, and for this, as

well as for other reasons, it is not possible at present to define the rat unit with any degree of certainty. Another observation, the significance of which is not clear, is that certain immature rats, which have been injected daily, although giving the usual oestrous response within a few days, nevertheless have failed to show further oestrous cycles during the period of observation, which in some cases has been as long as nineteen days. This is being further investigated. The immature female rat may accept the male at the induced oestrous period, but in our experience implantation has not taken place until the second or third cycle. Apparently corpora lutea must be present in the rat's ovaries if coitus is to be followed by pregnancy. This is in agreement with observations of Crew³ and others that puberty does not necessarily represent maturity.

The physiological effects of the third fraction (85 per cent. alcohol precipitate) have been studied along the same lines as the second fraction above described. Injection of this fraction into immature female rats has been followed, with remarkable uniformity, by the occurrence of oestrus usually on the fourth or fifth day. Animals killed at this time have shown the usual phenomena of oestrus in both uterus and vagina, but, in addition, the ovaries have been found to be considerably enlarged and to contain numerous corpora lutea; in fact, the picture was practically identical with that obtained by the use of Wiesner's sulphosalicylic acid extract. This alcohol-insoluble fraction was found to be ineffective when administered orally, and it was destroyed by boiling. Immature male rats injected with this fraction have manifested a definite increased rate of growth of the seminal vesicle and prostate gland in the course of ten days. When the injections were continued for long periods the controls tended to equal the treated animals in regard to the size of the accessory sexual apparatus. Adult males injected with this hormone also manifested hypertrophy of the accessory glands, but not of the gonads. Adult female rats have continued to be cyclic with daily injections, but manifested slight irregularities at times. Normal pregnancy and lactation have also been observed during such continuous treatment. Post-mortem findings have not as yet been of any significance.

REMARKS.

One must conclude from the results outlined above that the human placenta contains at least three distinct active principles, or hormones. These three substances are alike in that all have been observed to be oestrogenic in the immature female rat. The active principle present in the second fraction (85 per cent. alcohol-soluble) is very similar to oestrin in its ultimate physiological effects. It is ineffective, however, in the castrate, and hence it may be concluded that it functions directly or indirectly by stimulation of the intact ovary. It also differs from oestrin in the degree to which it is effective by oral administration. The third fraction prepared from the alcohol-insoluble material of the original extract is similar to the other two in that it is oestrogenic. It differs from the emmenin fraction on three main points: (1) it is ineffective by the oral route; (2) it produces corpora lutea in the immature rat coincident with induced oestrus; (3) it causes an increase in the rate of growth of the accessory sex glands in the immature male.

As yet we have been unable to obtain with surety from pig and cow placenta any of the above fractions other than oestrin. This observation, which must be given further study, suggests that in the human placenta hormones exist which are absent in lower mammalian forms. This suggestion receives support from the observation that pregnancy urine of certain of the lower forms has failed to give an Aschheim-Zondek pregnancy test when injected into immature mice.

The two oestrogenic placental hormones other than oestrin (theelin) which have been described appear to us to be the counterparts respectively in placenta of Prolan A (oestrogenic) and Prolan B (oestrogenic and luteinizing) of urine described by Zondek and Aschheim.^{1,6,7} We are also

* Read in opening a discussion in the Section of Obstetrics and Gynaecology at the Annual Meeting of the British Medical Association, Winnipeg, 1930.

of the opinion that these active principles are the specific products of placental tissue functioning, and are actively related to the condition of pregnancy. The functional interrelationship which must exist between the pituitary sex hormones and those of the placenta can in the light of our present knowledge only be conjectured. Much more research must be done in this field before the data now available can be satisfactorily correlated.

It is a great pleasure for me to state that through the kindness of the Medical Research Council and the

National Institute for Medical Research I have recently been given facilities to repeat much of the work outlined above in Dr. H. H. Dale's laboratory at Hampstead.

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NOTES ON THE CLINICAL USE OF CERTAIN PLACENTAL EXTRACTS.*

BY

A. D. CAMPBELL AND J. B. COLLIP.

(From the Departments of Obstetrics and Gynaecology and Biochemistry, McGill University, Montreal.

THE process of menstruation in the normal state occurs every twenty-eight days from puberty to the menopause, except as interrupted by pregnancy and lactation. Variations in the length of the interval, the duration of the epoch, or in the amount of blood lost or endometrial tissue shed may not necessarily be considered pathological so long as such variations represent individual peculiarities. The conception that physiological changes in the endometrium leading to menstruation are dependent upon chronological ovarian changes is now generally accepted. Similarly, deviations from normal menstrual cycles, whether amenor-

* Read in the Section of Obstetrics and Gynaecology at the Annual Meeting of the British Medical Association, Winnipeg, 1930.

rhoea, polymenorrhoea, or haemorrhage resulting from non-malignant lesions of the endometrium, may likewise be considered as secondary to deranged ovarian function.

Since Knauer¹ in 1896 first demonstrated the effect on the endometrium of ovarian transplants, scores of workers have used various products of glandular origin with a view to correcting menstrual disorders. The demonstration that alcoholic extracts of the human placenta can be separated into three definite fractions, each having distinctive physiological properties, as described in the previous paper, suggests that a clinical trial of each of these fractions should be made. The observations which are herein reported deal in the main with the possible therapeutic value of the 85 per cent. alcohol-soluble fraction (menmenin). A few clinical experiments with the so-called anterior pituitary-like fraction of the placental extract (85 per cent. alcohol precipitate) are also included. As yet similar experiments involving the oestrin-containing fraction have not been carried out. One hundred and twenty-three cases of deranged ovarian function as judged clinically have been treated and observed for periods

FIG. 1.—Five characteristic cases. Note in Case A decreasing intermenstrual periods, and in Case R slight lengthening of interval and period, cycle being established in both cases at twenty-eight days. Case G stopped her extract two days before the period on one occasion, and only had partial relief. With proper administration complete relief was obtained. The fifth case was a problem; even with varying dosage she received no benefit.