EFFECT OF HUMAN PITUITARY-FOLLICLE-STIMULATING HORMONE AND CHORIONIC GONADOTROPHIN IN STEIN-LEVENTHAL SYNDROME

BY

A. C. CROOKE, M.D., M.R.C.P. W. R. BUTT, Ph.D., A.R.I.C. R. PALMER, M.Sc.
R. MORRIS, F.I.M.L.T.

United Birmingham Hospitals' Department of Clinical Endocrinology, Birmingham and Midland Hospital for Women, and the Metabolic Unit, Little Bromwich General Hospital

R. LOGAN EDWARDS, M.B., B.S., M.R.C.O.G.

University of Birmingham, Department of Obstetrics and Gynaecology, Birmingham and Midland Hospital for Women

C. W. TAYLOR, M.B., Ch.B., F.R.C.O.G.

Department of Pathology, Birmingham and Midland Hospital for Women

R. V. SHORT, B.V.Sc., M.Sc., Ph.D., M.R.C.V.S.

University of Cambridge, Department of Veterinary Clinical Studies

In the normal ovary two distinct types of endocrine cells seem to exist. These are the cells of the theca interna, which contain all the enzyme systems necessary for conversion of progesterone to oestradiol-17 β ; and the granulosa cells, which lack many of these enzymes and therefore produce mainly progesterone (Short, 1962a; Huang and Pearlman, 1962). A change in cell type normally takes place as a result of ovulation.

Characteristically ovulation fails to occur in patients with the Stein-Leventhal syndrome, and the multiple small follicular cysts which persist in the ovaries of these patients contain a fluid which is rich in androstenedione but has little or no oestradiol-17 β or oestrone. It has been postulated that this results from a defect in the enzyme systems responsible for converting androstenedione to oestrone (Short and London, 1961; Short, 1962b). Similar conclusions have been reached by Axelrod and Goldzieher (1962) as a result of *in vitro* incubation studies, and by Mahesh and Greenblatt (1961) from steroid determinations in ovarian tissue.

It is possible that the recovery which often results from surgical trauma of the ovaries in patients with the Stein-Leventhal syndrome is mediated by a change in the type of endocrine cells in the pathological follicles comparable with the changes that occur at ovulation. It is known that such changes occur when large follicles are ruptured mechanically (Harrison, 1946). It seemed to be of interest, therefore, to study the effects of highly potent preparations of human pituitary-follicle-stimulating hormone and human chorionic gonadotrophin (H.C.G.) on the ovarian steroids and pathology in patients with Stein-Leventhal syndrome.

Methods

Patients.—Five patients were investigated, and their histories are given briefly in the Appendix. The nuclear sex, based on the examination of buccal smears, was normal in them all and the urinary excretion of gonadotrophins was in the low normal range. Three were examined by culdoscopy, another had had a laparotomy previously, and one did not have any surgical examination of her ovaries before treatment. These patients were considered to be typical of the Stein-Leventhal syndrome and were waiting for laparotomy to be performed. Dexamethasone 1 mg. thrice daily was given to the first three patients throughout the period of treatment with gonadotrophins and of operation, but the last two had none. The dosage of gonadotrophins was the same in all, but the time interval between doses and the time of operation in relation to doses varied from patient to

patient. The details are shown in Figs. 1 to 5. Specimens of urine were collected 48-hourly throughout the trials and fluid was aspirated from the ovarian cysts at the time of laparotomy.

Gonadotrophins.—Follicle-stimulating hormone was prepared by the method described previously (Butt *et al.*, 1961). The fraction used in these studies was called CP 1. It was about 3,000 times as potent as the international reference preparation for human menopausal gonadotrophin by the augmentation assay for follicle-stimulating hormone and about 50 times as potent by the hypophysectomized-rat-prostate assay for luteinizing hormone (Butt *et al.*, 1962). Two doses, each equivalent to 2,000 mg. of the international reference preparation by the assay for follicle-stimulating hormone were given to each patient. The doses were separated by an interval of 12 days in the first three patients and four days in the last two. The chorionic gonadotrophin was a commercial preparation which was given as a single dose of 12,000 i.u. to each patient two days after the second injection of follicle-stimulating hormone.

Steroid Determinations.—Oestriol was measured in urine by the method of Brown (1955) and pregnanediol by the method of Klopper *et al.* (1955). They were estimated on the 48-hourly collections of urine and expressed as excretion per 24 hours.

Steroids in the cyst fluid were measured by the method of Short (1962b) and in luteal tissue by a slight variation of this procedure (Short, 1962a).

Results

Urinary Steroids.—The results are shown in Figs. 1 to 5. The excretion of oestriol increased nearly threefold in the first three patients when they were given dexamethasone alone. The first injection of follicle-stimulating hormone caused about a threefold increase in the excretion of oestriol in four cases; Case 2 failed to respond. Excretion of pregnanediol increased only in Case 5. The second dose of follicle-stimulating hormone followed by chorionic gonadotrophin caused an increase in the excretion of oestriol and of pregnanediol in all patients. In Cases 2, 4, and 5 oestriol and pregnanediol rose almost simultaneously, but in Cases 1 and 3 oestriol fell as pregnanediol began to rise. In Case 1 there was a secondary peak in the excretion of oestriol which occurred after the rise in excretion of pregnanediol.

Ovarian Steroids.—The concentrations of the various steroids are given in the Table. The haemorrhagic fluid from the ovarian cysts in Case 1 contained no detectable

steroids and progesterone was found in very low concentration in the corpus luteum (Zander *et al.*, 1958). The fluid from the small cysts in Cases 2 and 3 showed a high concentration of androstenedione and no measurable oestradiol-17 β or oestrone; this is characteristic of the Stein-Leventhal syndrome. The fluid from the large follicular cysts in Cases 4 and 5 showed a high concentration of progesterone, a moderate amount of 17 α -hydroxy-progesterone, and some oestradiol-17 β . The only Δ^5 3 β -hydroxy steroid that could be detected was pregnenolone, and this was present only in traces.

FIG. 1.—Excretion of oestriol and pregnanediol in Case 1.

FIG. 2.—Excretion of oestriol and pregnanediol in Case 2.

Pathology

The ovaries in Case 1 were slightly enlarged and contained numerous small cysts and a single large corpus luteum. Histological examination showed condensation of the cortical stroma over the cysts, which were derived from follicles; some of the cysts were lined by a layer of granulosa cells, but this was absent in other cysts which appeared to be undergoing atresia. No excessive theca-interna-cell proliferation was noted and there was no luteinization of the granulosa cells apart from the single recent corpus luteum. There was haemorrhage into the lumen of some of the cysts and areas of haemorrhage in the ovarian stroma.

The ovaries in Case 2 were normal in size and contained numerous tiny cysts (operation record). No tissue was taken for histology on this occasion, portions of ovary having previously been submitted for microscopy. Examination of these sections revealed some condensation of cortical stroma overlying many small cysts derived from follicles. No theca-interna-cell proliferation was noted; a recent corpus luteum was present in one section.

The ovaries in Case 3 were unchanged in size. Histological examination showed a thin rind of condensed cortical stroma beneath which there were numerous small cysts lined by granulosa cells—around this layer there was a thick zone of plump theca interna cells. In some of the cysts the granulosa layer was thinned, fragmented, or absent, but the theca interna layer remained. No recent corpus luteum was found, but remnants of apparent old corpora lutea were present.

The swollen ovarian tissue from Case 4 showed extensive haemorrhage both into the cysts and into the ovarian tissue. Histology showed the large and small cysts to be lined for the most part by a well-formed layer of granulosa cells beneath which there was a thin zone of theca interna cells; some of the cysts were filled with blood. In one or two of the cysts the granulosa cells were luteinized. The ovarian stroma was oedematous and congested, and distended thin-walled blood-vessels were present, particularly in the region of the theca layers around the cysts.

The ovarian tissue from Case 5 showed changes similar to those noted in Case 4—in addition, a recent corpus luteum was found in the right ovary.

Discussion

Several reports have appeared on the treatment of patients with polycystic ovaries using human-pituitary-follicle-stimulating hormone (Gemzell *et al.*, 1958, 1960; Mahesh and Greenblatt, 1961, 1962). Although rather crude preparations were used, follicular development and the formation of corpora lutea were demonstrated. However, none of these workers carried out simultaneous determinations of the steroids in the urine and fluid from the cysts.

In the present investigation dexamethasone was given to the first three patients in order to inhibit the production of steroids by the adrenal cortex. Thus any steroids still present in the urine were presumably derived mainly from the ovaries. It was surprising to observe that dexamethasone apparently caused nearly a threefold increase in the excretion of oestriol; a similar effect is also apparent in the data reported by Netter *et al.* (1960). It seems unlikely that this oestriol was derived from the adrenal cortex, which had been adequately suppressed

as judged by the excretion of other adrenocortical steroids (Butt, Crooke, Morris, and Palmer, to be published). Moreover, it is unlikely to have been derived from the pathological follicles, since the fluid from the cysts of these patients contained no oestrogens. It may have resulted from an increased secretion of endogenous follicle-stimulating hormone acting on normal theca interna cells elsewhere, since evidence from this department has shown that corticoids stimulate the pituitary to secrete this hormone (Brown, 1956; Crooke, 1956; Butt, Crooke, Cunningham, and Palmer (1963).

In general there was good agreement between the daily excretion of urinary steroids and the concentration of steroids in the ovarian cysts and luteal tissue. In Case 1 laparotomy was performed six days after the last injection of gonadotrophin. By this time the urinary oestriol and pregnanediol had risen and fallen again to control levels and the concentration of progesterone in the large corpus luteum was extremely low, suggesting that it had ceased to function. The numerous small cysts which had persisted were largely filled with recent blood clot and no steroids were detected in the fluid from them. The pattern of steroid excretion which followed the second injection of follicle-stimulating hormone in this patient resembled an exaggerated pattern of the normal menstrual cycle with oestriol rising to a peak at mid-cycle and falling again as the pregnanediol began to increase. This was followed by a secondary rise in the excretion of oestriol coinciding with the peak in excretion of pregnanediol and similar to that which is sometimes seen in the normal cycle (Brown and Matthew, 1962). Laparotomy revealed the development of multiple follicles, and it is suggested elsewhere from a study of patients with secondary amenorrhoea given similar treatment that the large secondary rise in excretion of oestriol is indicative of multiple ovulation (Crooke *et al.*, 1963).

Case 2 failed to respond to the first injection of follicle-stimulating hormone and at operation only five days later the ovaries still contained numerous tiny follicles typical of the Stein-Leventhal syndrome. The fluid from these cysts contained a high concentration of androstenedione and no detectable oestradiol-17 β or oestrone. Subsequently the patient responded well to the second injection of follicle-stimulating hormone followed by chorionic gonadotrophin, the pattern of excretion resembling somewhat that in Case 1.

Case 3 showed a striking increase in the excretion of oestriol after the second injection of follicle-stimulating hormone and chorionic gonadotrophin but no change in pregnanediol at the time of operation only two days after the injection of chorionic gonadotrophin. Subsequently there was slight rise in pregnanediol. The ovaries contained numerous tiny follicles with fluid which had a high concentration of androstenedione and no detectable oestradiol-17 β or oestrone. Perhaps the defect persisted in this patient because luteinization had not yet occurred at the time of operation, leaving the affected cells in the theca interna still functional. Presumably the oestrogen was produced by normal theca interna cells elsewhere in the ovary.

Cases 4 and 5 responded to treatment with a much greater excretion of oestriol and pregnanediol than the others. Both patients had enormous ovaries, but the one (Case 4) who had the greater response also had the larger ovaries. Neither was treated with dexamethasone, but it seems unlikely that its absence was responsible

for the increased excretion of oestriol. It is more likely that this was due to the shorter interval of time between the two injections of follicle-stimulating hormone. The fluid from the cysts of both patients contained oestradiol-17 β , a very high concentration of progesterone, and little androstenedione, in striking contrast to the usual findings

FIG. 3.—Excretion of oestriol and pregnanediol in Case 3.

FIG. 4.—Case 4.

FIG. 5.—Case 5.

in untreated patients with the Stein-Leventhal syndrome. The steroid pattern together with the macroscopic appearance of the ovaries at laparotomy suggested that many follicles had developed in response to treatment with gonadotrophins and were now undergoing luteinization. The evidence is not sufficient to establish whether they were derived from the original pathological cysts or were fresh ones, but histologically there was extensive haemorrhage both into the stroma and into some of the cysts which were becoming luteinized.

The remarkable response of patients with the Stein-Leventhal syndrome to surgical wedge resection of the ovaries is difficult to explain except on a basis of destruction of a sufficient number of cystic follicles to alter the abnormal hormonal secretion. If these follicles can be destroyed by treatment with gonadotrophins a medical cure may be possible.

Summary

Five patients with Stein-Leventhal syndrome were treated with a highly potent preparation of human pituitary follicle-stimulating hormone and chorionic gonadotrophin. The first three were also given dexamethasone simultaneously to inhibit the production of adrenocortical steroids. A laparotomy was performed in each case at a different time interval after the commencement of treatment, and fluid was taken from the cystic follicles for determination of ovarian steroids. The results were generally in good agreement with urinary excretion of oestriol and pregnanediol. All responded to stimulation with gonadotrophins, some showing great enlargement of the ovaries and increases in the excretion of oestriol and pregnanediol and little androstenedione in the fluid from the cysts.

The histological findings in the ovaries are discussed.

The human pituitary glands were part of a collection organized by the Clinical Endocrinology Committee of the Medical Research Council. They were supplied by the Department of Biochemistry, Cambridge University, through the courtesy of Professor F. G. Young. The chorionic gonadotrophin was a gift from Messrs Paines and Byrne Ltd. We are grateful to Organon Laboratories Ltd. for a grant for technical assistance.

APPENDIX

Case 1

A married Indian woman aged 27 had had normal menstruation since the age of 11 except for two spells of amenorrhoea, each lasting for about three months, which occurred during her teens. Since the age of 20 she had had longer spells of amenorrhoea, lasting for about six months and separated by spells of regular menstruation of similar duration. Latterly she had had vaginal bleeding induced by oestrogens. She first noticed the growth of

downy hair on her lips at 18, which increased in coarseness and spread to the sides of her face and chin until she had to shave on alternate days. It also increased on her abdominal wall and legs.

She was admitted to hospital for investigation and treatment with gonadotrophins. Subsequently at operation she was found to have slightly enlarged ovaries which contained numerous follicular cysts up to about 0.5 cm. diameter and a single large corpus luteum. The cysts were punctured and the fluid was collected for examination. Both ovaries were then wedge resected. Macroscopically most of the cysts were seen to be filled with recent blood-clot. The corpus luteum was divided and part taken for biochemical investigation.

Case 2

A single girl aged 19 had had regular menstruation from the age of 12 to 17, when she had bled for six weeks continuously. This was followed by a spell of amenorrhoea. At the age of 16 there had been an abrupt growth of hair on her face, completely changing her appearance within a period of six months. Eighteen months later, at 17, she was admitted to another hospital where a laparotomy was performed. Both ovaries were enlarged and contained numerous small follicular cysts. Bilateral wedge resections were carried out, leaving about a normal amount of ovarian tissue on each side. Histological examination showed the cortical stroma to be somewhat condensed over the cysts, but there was no great proliferation of cells in the theca interna. Remnants of previous luteinized follicles and a single large corpus luteum were present. After the operation menstruation returned to normal but the growth of hair increased; she became depressed and threatened to commit suicide.

She was admitted for further investigation and treated with gonadotrophins. Laparotomy was performed five days after the first injection. She was found to have numerous adhesions binding the ovaries, Fallopian tubes, and bowel together. These were freed and a blocked left tube was repaired. Both ovarian remnants were about the size of normal ovaries, but they contained numerous small cysts. These were punctured and the fluid was collected, but no tissue was taken for histological examination.

Case 3

A married woman aged 24 started to menstruate at the age of 13 and had regular periods for the first six months only. Subsequently they recurred at about six-monthly intervals, and at 21 she was given chorionic gonadotrophin, 500 I.U. twice weekly, for six months. Previously she had had a little downy hair on her lips and chin, but during treatment it had increased rapidly, became coarse, and spread to the sides of her face. After the stopping of injections the growth diminished somewhat and the hair became finer. She had married at 20 but failed to conceive, and subsequently gained about 12 lb. (5.4 kg.) in weight. She was admitted to hospital and examined under anaesthesia. The uterus was normal in size, but culdoscopy revealed polycystic ovaries about 4 by 3 cm. She was treated with gonado-

Concentration of Steroids in Fluid from Cysts and in Luteal Tissue

Case No.	Vol. (ml.)	Preg-nenolone	17 α -OH-Preg-nenolone	D.H.A.	Pro-gesterone	17-OH-Pro-gesterone	Andro-stenedione	Oestrone	Oestradiol-17 β	Oestriol	19-OH-Androstenedione	20 α -OH-Pregn-4-en-3-one
<i>Cyst Fluid—Results as μg./100 ml.</i>												
1	1.06	—	—	—	<10	<10	<10	<5	<5	—	—	—
2	0.8	—	—	—	12	12	63.0	<6	<6	—	—	—
3	3.0	<1.5	—	<1.5	5	88.5	130	<2	<2	—	—	—
4	10.0	<0.5	<0.5	<0.5	348	102	12	2	34	<0.5	<10	<10
5	20.0	0.5	<0.3	<0.3	550	124	22	<1	17	<0.3	<5	5.5
Lt. ovary												
5	12.0	0.8	<0.4	<0.4	592	148	18.4	3.8	15.4	—	<8	<8
Rt. ovary												
<i>Luteal Tissue—Results as μg./100 g.</i>												
1	1.49	—	—	—	23.5	<7	<7	<7	<7	—	—	<7

trophins, and two days after the last injection a laparotomy was performed. The ovaries were unchanged in size; the cysts were punctured and the fluid was collected for examination, and bilateral wedge resections were then performed.

Case 4

A single woman aged 27 started to menstruate at the age of 14. She was regular for the first year and then had only one or two periods yearly until she was 21, when she was treated with stilboestrol. Since then she has had scanty irregular periods lasting two to four days at intervals of one to four months. She first noticed the growth of hair on her lips and chin at the age of 16. It became coarse and spread on to her breasts, abdominal wall, and legs. She complained of headache, pain in the limbs, and depression, for which she was treated by a psychiatrist. Her weight was constant. She was admitted and examined under anaesthesia, and her pelvis appeared to be normal. Culdoscopy was unsatisfactory. The left ovary was poorly visualized and its size could not be estimated. The left Fallopian tube was red and deformed. There was a tubovarian mass in the right side, but the size of the ovary could not be established. She was given gonadotrophin, and four days after the last injection a laparotomy was performed. The left ovary was enormous, measuring about 16 by 10 cm. It contained numerous haemorrhagic cysts and enlarged follicles. The Fallopian tube was stretched over its apex and was bound down by numerous adhesions into the pouch of Douglas. The right ovary was not recognized. The right Fallopian tube was enlarged with hydrosalpinx, and ended blind. It was thought that the right ovary might have become bound to the left with adhesions, but no line of demarcation could be established. The follicles were punctured and the fluid was collected and a wedge resection then performed.

Case 5

A married woman aged 25 had vaginal bleeding at the age of 13. It continued for a year, during which time she was given numerous injections. Then, after a few months' amenorrhoea, she had a further spell of bleeding which lasted for six months. This continued, usually with only short intervals, until after she married at the age of 22. Since then the intervals have got longer, usually two to four months, and the bleeding shorter, usually eight to eleven days, but there has been heavy loss of blood, the haemoglobin falling on occasions to 40% and she has had to have blood transfusions. During this time she has had dilatation and curettage on three occasions—at 14, 18, and 21—with very temporary improvement only. She wanted children but failed to conceive, and had gained about 20 lb. (9 kg.) in weight since she married. She developed a slight growth of hair on her lips and chin.

She was admitted to hospital and examined under anaesthesia. The uterus was bulky, the sound passing 8 cm. Culdoscopy revealed the right ovary to be about 3½ by 2 cm. and the left about 4 by 3 cm. They each contained a ruptured follicle and a few translucent cysts. She was given gonadotrophin, and four days after the last injection a laparotomy was performed. The peritoneal cavity contained blood from ruptured ovarian follicles. Both ovaries were greatly enlarged. The right was about 8 by 4.5 cm. and contained a large corpus luteum, and the left was about 6 by 4 cm. Both contained enlarged haemorrhagic follicles and numerous cysts. The follicles were punctured and the fluid was collected and bilateral wedge resections then performed.

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HUMAN ANTITETANUS SERUM IN THE TREATMENT OF TETANUS

BY

MAURICE ELLIS, M.A., M.B., B.Chir., F.R.C.S.

Surgeon in Charge of the Casualty Department and Surgeon to the Tetanus Unit, the General Infirmary at Leeds

Clinical tetanus manifests itself by a complete disorganization of the reflexes in the nervous system, producing the well-known manifestations of trismus and convulsions in the severe case. The neurotoxin reaches the central nervous system, where it acts on the susceptible cells, thereby producing these effects. In spite of this very severe disorganization of the nervous system which is present during the actual disease, when the patient recovers, by whatever means of treatment, the nervous system returns completely to normal. The neurotoxin must therefore have a transient effect on the nervous system, and when cure has taken place it has been eliminated or neutralized in some way. One of the possibilities, it seemed to me, was that the body had manufactured its own antitoxin during the course of the disease and thereby had neutralized the neurotoxin.

Stafford, Turner, and Goldman (1954) suggested the possibility of using human antitoxin for the treatment or prophylaxis of tetanus. The main reasons they gave for this were the elimination of the possibility of anaphylaxis due to the foreign proteins in the horse serum. Rubbo and Suri (1962) gave an account of their experiences in the use of human immune globulin for the prophylaxis of tetanus. When the tetanus unit was formed in this hospital in 1954 it seemed worth while trying human antitoxin in the treatment of clinical tetanus to see if the disease could be cut short and risks of long-continued positive-pressure respiration could be minimized.

This paper gives an account of (a) the method of the collection of human antitoxin; (b) a review of the titres of antitoxin in the serum collected, with the implications on prophylaxis; and (c) the results of the use of this serum in some 26 out of the 57 cases treated in this unit over the past years.

Collection of Serum

The story of the collection of the serum is very gratifying to relate. About 160 subjects volunteered to give blood. The organization for the immunization of