Results in 226 Cases

(92 Cases) All Counts in the Same Group			(134 Cases) One or More Counts in a Differ- ent Group from the Remainder, the Initial Count Being:			(50 Cases) Initial Count Under 60 mil./ ml., any Subsequent Count Over 60 mil./ml., the Initial Count Being:		(40 Cases) Initial Count Over 60 mil./ ml., any Subsequent Count Below 60 mil./ml., the Lower Being:		(97 Cases) All 3 Counts Under 60 mil./ml., the Highest Being:	
0-20 mil.	20-60 mil.	Over 60 mil.	0-20 mil.	20-60 mil.	Over 60 mil.	0-20 mil.	20-60 mil.	0-20 mil.	20-60 mil.	0-20 mil.	20-60 mil.
26	27	39	27	67	40	6	44	6	34	26	71

of other infertility factors, of the man proving to be fertile. The second category is the "infertile" group. We have excluded from this investigation all cases of "aspermia" in which no sperms could be found, because in our experience we have very seldom found sperms present in subsequent counts when the initial examination of a centrifuged specimen showed no sperms. It is generally agreed that if the density is below 20 millions per ml. the likelihood of the patient achieving parenthood is remote. This "infertile" group therefore has a range of 0-20 millions per ml. Those with sperm counts between 20 and 60 millions per ml. form a third group of husbands who have a "sporting chance" of achieving parenthood. This we will refer to as the "subfertile" group.

Discussion

In 134 cases in our series (59%) all three counts did not fall into the same group. In 90 cases (40%) at least one count was over 60 millions per ml., but of these 90 cases only 44% gave an initial count over 60 millions per ml. In 50 cases the initial count was under 60 millions per ml. though a subsequent count was over this figure, six cases (12%) showing an initial count of under 20 millions per ml. In 40 cases the initial count was over 60 millions per ml. though a subsequent count was under this figure, six (15%) being under 20 millions per ml.

The question arises whether these variations are the result of a high margin of error due to our technique of counting sperms. It has already been pointed out that the counts have been performed by a number of different individuals, and most of them have been done by technicians. It may well be desirable that the count should be performed by a highly trained seminologist, but this is not likely to be practicable in a busy fertility clinic such as ours, which has dealt with over 1,000 cases in three and a half years. Furthermore, there is no reason to suppose that a sperm count should prove more difficult to perform than a red cell count, and one is in the habit of accepting the result of such a simple haematological investigation even if it is performed in a routine pathological laboratory.

Nevertheless, it should be realized that there is a considerable variability innate in any haemocytometer count, however good the technique employed. Where, as in our work, 150 to 200 cells are counted, a variation of 20% between successive counts might be expected occasionally from that cause alone. For a statistical discussion of this problem Fisher (1948) may be consulted. In addition, it is likely that further errors are caused by the viscous nature of semen and the difficulty of accurate mixing with the diluting fluid. It might also be pointed out that we have not insisted on a constant period of continence, and that the sperm count may vary significantly if this is not done. Nor have we taken steps to see that the interval between the examination of the three specimens is constant. It should be

emphasized, however, that undue insistence on such details may seriously affect the husband's co-operation, which is essential for adequate clinical investigation. Although the variations we have encountered cannot be attributed to one or another of these causes with certainty, we have performed repeated counts on the same specimen of semen on a number of occasions and found that they agreed reasonably well. In one case, for instance, three separate counts on the same specimen of semen gave the following results: (1) 152 millions per ml., (2) 149 millions per ml., and (3) 155 millions per ml.

It would therefore seem unwise to make any attempt to assess male fertility, so far as sperm counts are concerned, unless at least three counts have been considered. Perhaps of more importance still is the contention that one is not justified in reaching any conclusion with regard to the effect of any form of treatment unless at least three counts have been made both before and after treatment.

Summary

A study of 226 cases in which three sperm counts have been performed has led us to conclude that a single count may prove misleading as regards both the fertility of the husband and the effects of any form of treatment of defective spermatogenesis.

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A CASE OF MASCLINOVBLASTOMA

BY

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Rottino and McGrath (1939) suggested the name "masculinovblastoma" for a group of masculinizing ovarian tumours, consisting of large cells which often contain lipid material and which resemble those of adrenal cortex or corpus luteum; such tumours are histologically distinct from arrhenoblastomas, which are also associated with clinical masculinization. Novak (1947) states that masculinovblastomas include adrenal adenomas of the ovary and masculinizing luteomas, the relationship between the two being by no means clear, and adds that such tumours are extremely rare. Curtis (1946) was able to find in the literature only 16 cases in which he considered the diagnosis could be upheld. Iverson (1947) quoted 21 cases of masculinovblastoma, six of which for various reasons he regarded as doubtful members of the group. Pedersen (1947) described another case

and quoted the two cases of Luft, which were not included in Iverson's review. We ourselves found records of six more cases of masculinovoblastoma (Curtis, 1946; Twombly, 1946; Douglass, 1947; Williams and Mendenhall, 1947; Searle, Haines, and Baker, 1948; Winsauer and Manning, 1949).

The case of masculinovoblastoma described below was shown as a case of "virilism? ovarian tumour" at a meeting of the Section of Dermatology of the Royal Society of Medicine on November 17, 1949, before the diagnosis had been confirmed by operation (Forman and Merivale, 1950). We present the complete clinical history of this case before and for eight months after operation, together with laboratory studies of the excretion of certain hormones and the histological report on the tumour.

Case History

The patient, aged 62, an unmarried manageress of a laundry, was first seen at St. John's Hospital for Diseases of the Skin in August, 1949, where she presented with a diagnosis of rosacea. She was noticed to be hirsute over the beard area and to be wearing a wig.

The menarche occurred at the age of 16, after which her periods were irregular, with heavy losses and the passage of blood clots until the age of 30, when they ceased abruptly; they have never recurred. From that time she gradually lost the hair on her head, and hair grew strongly on her chest, abdomen, and back. She volunteered that she became stronger physically, and the veins on her arms became more prominent. Her face became erythrocyanotic, and her breasts decreased in size. She did not suffer menopausal

symptoms at any time, nor did homosexual tendencies develop. Five years previously her abdomen began to swell, but she did not think it had increased much in size in the past four years. She thought that her head was getting bigger. For the past three years she suffered from keratitis intermittently in both eyes.

On examination she was jovial, intelligent, and very cooperative. Her pulse rate and temperature were normal; the blood pressure in the arms and legs was 140/90. Her general appearance is shown in Figs. 1, 2, 3, and 4. She had slight exophthalmos and baldness of the vertex, with a fringe of hair at the sides and back of the scalp, giving the appearance of severe masculine alopecia. Her face was erythrocyanotic, with a stiff growth of reddish hair over the beard area. There was thick hair growth over the front and back of the chest and abdomen. The pubic hair had a masculine distribution. Her breasts were flattened and small; the hymen was intact, and the clitoris enlarged. Her voice was deep and husky.

A smooth hard tumour was felt, filling the abdomen but neither extending into the loins nor entering the pelvis. From its position and size it seemed unlikely that this could be either a masculinizing ovarian tumour or a suprarenal tumour, which were the two diagnostic possibilities under consideration. There was no clinical evidence of ascites.

Rectal examination revealed haemorrhoids. Definite information about the condition of the uterine adnexa could not be obtained by bimanual palpation because of the size of the abdominal tumour.

Laboratory Investigations.—The results of these are summarized in Table I. The patient had mild polycythaemia. There was a flat but not grossly abnormal response to the

FIGS. 1, 2, 3, and 4.—Photographs taken on October 6, 1949.

TABLE I.—Results of Laboratory Examinations Made Before Operation and Not Including Those of Hormone Assays

Test	Result		
Fractional test meal	Achlorhydria		
Histamine test and gastric analysis	Low acid response		
Glucose-tolerance test (50 g. glucose) (true sugar estimated)	Time	Blood Sugar	Urine ₂ Sugar
		mg. per 100 ml.	
	Fasting	77	Nil
	30 minutes	95	"
	60 "	84	"
	120 "	79	"
	150 "	75	"
Urinary creatinine	1.26 g. in 24 hours		
Urinary creatine	0.06 g. in 24 hours		
Plasma chlorides	593 mg. per 100 ml.		
Serum sodium	315 mg. per 100 ml.		
Serum potassium	18 mg. per 100 ml.		
Blood alkali reserve	51 volumes CO ₂ per 100 ml.		
Plasma inorganic phosphorus	4.4 mg. per 100 ml.		
Serum calcium	11.2 mg. per 100 ml.		
Serum alkaline phosphatase	5.8 units per 100 ml.		
Urea clearance	46% average normal		
Serum cholinesterase	105 μ l. CO ₂ per 100 ml.		
Serum Wassermann and Kahn tests	Both negative		
Routine examination of urine	Nothing abnormal detected		
24-hour sample of urine	No Bence-Jones protein detected		

glucose-tolerance test; the serum-albumin level was slightly reduced; and mild renal impairment was revealed by the urea-clearance test. The serum cholinesterase level was high normal. Otherwise no abnormality was detected. The results of hormone estimations made before operation are given in Table II, together with those done six weeks and eight months after operation. Pre-operatively there was a slight increase in 17-ketosteroid excretion, but the amount of 3-(β)-hydroxy-17-ketosteroids was not increased either absolutely or relatively to the α -fraction. Excretion of "11-oxycorticosteroids" was normal, but she was excreting a small but definite amount of pregnanediol, which was more than would be expected from a normal woman of her age. Excretion of oestrogens was minimal. The possible significance of these results is discussed below.

TABLE II.—Results of Urinary Hormone Assays Made Before and After Operation

Hormone	Urinary Excretion in 24 Hours		
	Before Operation	6 Weeks After Operation	8 Months After Operation
Total neutral 17-ketosteroids (ketonic fraction)	31.18 mg.	2.4 mg.	5.6 mg.
3-(α)-hydroxy-17-ketosteroids	30.8 "	—	—
3-(β)-hydroxy-17-ketosteroids	0.38 "	—	—
"11-oxy corticosteroids"	0.12 "	0.099 mg.	0.23 mg.
Pregnenediol-like glucuronide	0.29 "	0.078 "	—
Pregnenediol	5.12 "	1.025 "	—
Oestrone	2.0 "	0.137 "	0.82 mg.
Oestriol	0.7 μ g.	—	—
Oestradiol	None detected	—	—
	0.7 μ g.	—	—

Radiological Examination.—This revealed a pituitary fossa of normal size; no evidence of porosis or exostoses in the skull; and osteoarthritis of the cervical and thoracic spine; the chest was within normal limits; and there were no bony abnormalities of the hands, feet, and pelvis. The heart size was within normal limits, and there was no radiological evidence of a tumour of the thymus.

Masculinization due to ovarian tumour, possibly of the adrenal type, was diagnosed.

Operation.—This was carried out by Mr. Grant Massie; the anaesthetic was given by Dr. T. A. B. Harris. A fibromyoma uteri the size of a football was found occupying the whole of the abdomen. The left ovary, which was atrophic, was attached to it. The right ovary was enlarged to the size of a small orange, and the blood vessels of the right ovarian ligament were also much enlarged. The suprarenals were normal to palpation. No other intra-abdominal abnormality was found. Subtotal hysterectomy and bilateral oophorectomy were performed.

Appearance of the Right Ovary.—Mr. R. E. Horton reported: "The specimen consists of an enlarged ovary 3.8 cm. in diameter, with its Fallopian tube attached. The cut surface of the ovary shows that the normal ovarian tissue has been completely replaced by a tumour which is confined within the ovarian capsule. The tumour is largely of a brown colour on its cut surface, with scattered areas of yellow, and is of moderate vascularity." The report by Dr. S. De Navasquez on the microscopical appearance stated: "The tumour consists of cells which resemble adrenal cortical tissue (Fig. 5), and therefore may be designated a masculinoblastoma."

FIG. 5.—Photomicrograph showing cells containing lipid material. ($\times 500$.)

Convalescence was stormy. Pulmonary embolus occurred on the third day after operation, and was treated with heparin by intravenous drip. Haemorrhage occurred from the operation site for 48 hours, so the heparin was stopped and a blood transfusion was given. A second pulmonary embolus occurred 12 days after operation and the patient developed auricular fibrillation. She then developed a right-sided pleural effusion, from which a large amount of sterile blood-stained fluid was repeatedly aspirated. A pulmonary infection caused by *Bact. coli* and *Pseudomonas pyocyanea* was controlled by streptomycin and "aureomycin." Auricular fibrillation continued for several weeks, and the patient was discharged two months after operation.

In March, 1950 (four months after operation), the haemoglobin was 9.5 g. per 100 ml. of blood. The facies was less plethoric, and a few fine hairs were seen growing on the scalp. The patient believed that the beard hairs were not growing so stiffly and that the hair on her chest was less thick.

In July (eight months after operation) she was in good health and had returned to work. Hirsutism was far less pronounced over the chest and abdomen, the latter being free of hair and the upper limit of pubic hair being horizontal. The beard was not so thick, and a few fine hairs could be seen over the scalp. She had put on weight, with the deposition of fat over the lower trunk and buttocks.

Discussion

The clinical features of this case were both striking and distinctive. The syndrome of defeminization and masculinization described by Novak (1947) was present in advanced degree and left little doubt about the diagnosis, though it was necessary to exclude Cushing's syndrome and tumour of the suprarenal cortex. On clinical grounds a diagnosis of Cushing's syndrome was unlikely in view of the absence of striae, obesity, and hypertension, although the erythrocyanotic facies, hirsutism, and slight polycythaemia were suggestive. Radiologically there was no osteoporosis.

The excretion of "11-oxy corticosteroids," which is often increased in Cushing's syndrome and tumours of the suprarenal cortex (Talbot, Albright, Saltzman, Zygmuntowicz, and Wixom, 1947), was normal. The excretion of 17-ketosteroids, which was slightly increased, would have done equally well for a case of Cushing's syndrome or masculinizing ovarian tumour, but was against a diagnosis of suprarenal cortical tumour, in which very high levels of excretion are usually encountered. Furthermore, the excretion of 3-(β)-hydroxy-17-ketosteroids was normal, whereas in tumours of the suprarenal cortex the excretion of this fraction is usually markedly increased, owing to the large amounts of dehydroisoandrosterone excreted (Mason and Engstrom, 1950). It is worth recalling that Cushing's syndrome can occur in cases of masculinoblastoma (Kepler, Dockerty, and Priestley, 1944; Greene and Lapp, 1944), and it is interesting that it has not yet been reported in any case of arrhenoblastoma.

Hyperostosis of the skull was a feature of five recorded cases of masculinoblastoma (Novak and Wallis, 1937; Rottino and McGrath, 1939; Mayer, 1942; Luft, 1944; Pedersen, 1947), but was absent in our case. So far this abnormality has not been reported in a case of arrhenoblastoma.

Pedersen (1947) has drawn attention to the frequency with which fibromyoma uteri and masculinoblastoma are both present in the same patient (7 of 20 cases reported by 1947), and Büttner (1933) made the same observation for arrhenoblastoma. Shaw (1948) estimated

that 15% of all women over the age of 35 suffer from fibromyomata, and it is conceivable that, in view of the rarity of masculinizing ovarian tumours, the association is fortuitous.

The levels of excretion of 17-ketosteroids reported in cases of masculinovoblastoma vary from within the normal range, as in the case described by Pedersen (1947), to 158 mg. in 24 hours in the pregnant patient of Searle, Haines, and Baker (1948). The case of Kepler, Dockerty, and Priestley (1944) excreted 54 mg. in 24 hours, that of Winsauer and Manning (1949) 43 mg., and one of the cases reported by Luft (1944) 23 mg. It follows that estimation of these metabolites is not of much help diagnostically except to exclude adrenocortical tumour.

We have not been able to find any records of the excretion of 3-(β)-hydroxy-17-ketosteroids in these cases. In our patient this was normal, as was her "11-oxycorticosteroid" excretion, suggesting that there was no increase of metabolic or secretory activity which could be specifically referred to the suprarenal cortex—a point of some interest in view of the histological findings.

After removal of the tumour, the 17-ketosteroid excretion fell well below normal, but had risen again eight months after operation to a low normal figure. The "11-oxycorticosteroid" excretion behaved in the same way.

The excretion of pregnanediol before operation was 2 mg. a day, estimation by two methods agreeing closely. Pregnanediol is a urinary excretory product of progesterone (Heard, Bauld, and Hoffman, 1941; Sommerville and Marrian, 1950) and of deoxycorticosterone (Cuyler, Ashley, and Hamblen, 1940). It is excreted in measurable quantities in the urine during the luteal phase of the menstrual cycle and during pregnancy, and is not excreted after the menopause, or only in very small amounts. In spite of her age, our patient excreted an amount as great as that of many normal women of sexual age during the luteal phase of the menstrual cycle. This finding, coupled with a slightly increased excretion of 3-(α)-hydroxy-17-ketosteroids, led us to make a very tentative diagnosis before operation of adrenal-type ovarian tumour, on the grounds that it indicated the presence of hyperactive adrenal tissue.

The case of masculinovoblastoma of Hamblen and Davies, quoted by Iverson (1947), excreted no pregnanediol, and extracts of the tumour showed no progestational activity. On the other hand, Twombly's (1946) patient excreted 18.5 mg. of pregnanediol a day, and endometrial biopsy revealed a marked decidual reaction; 17-ketosteroid excretion was 21 mg. a day. The histological report showed, as these findings suggest, that this was a case of masculinizing luteoma. Reconsideration of our case in the light of this one suggests the possibility that the tumour cells were derived, if not from luteal cells, at least from cells which had retained some luteal function—a speculation quite compatible with the results of the hormone assays. No endometrial biopsy was done, and in view of her age it is unlikely that it would have shown anything significant.

Oestrogen excretion was minimal, as might be expected of a woman past menopausal age.

Hormone estimations made six weeks after operation all showed a marked fall in excretion, pregnanediol now being virtually absent from the urine. Eight months after operation the excretion of 17-ketosteroids had risen to a low normal level, that of 11-oxycorticosteroids was normal, and pregnanediol excretion was minimal.

We have had to restrict our investigations of hormone excretion to those estimations which are readily available in a routine department, and it is clear that neither we nor other workers have found the answer to the most interesting problem of all in these patients—What is the hormone or group of hormones which produces the really remarkable physical, and in some cases psychological (Pedersen, 1947), changes of sexual characteristics from which they suffer? It is possible that procedures involving large-scale extractions and isolations from pooled urine may provide at least part of the answer.

Appendix on Technical Methods Used

The routine blood and urine estimations were carried out by methods described by King (1946).

Total neutral 17-ketosteroids were estimated by a modification of existing methods described in detail elsewhere (Merivale, 1951).

The 17-ketosteroid fractions were estimated by the method of Frame (1944).

The "11-oxycorticosteroids" were estimated by the method of Talbot, Saltzman, Wixom, and Wolfe (1945).

Pregnanediol-like glucuronide was estimated by the method of Bissett, Brooksbank, and Haslewood (1948).

Pregnanediol was estimated by the method of Sommerville, Gough, and Marrian (1948).

Extraction of the phenolic fraction of the urine and preparation of fractions for oestrogen assay were made by the method of Pincus and Pearlman (1942).

We are grateful to Miss M. Ord for the serum-cholinesterase determination; to Professor J. M. Robson for the assays of the oestrogen fractions; and to Mr. A. C. Engel for the photographs. We should like to express our appreciation of the good humour and cheerful, intelligent co-operation of the patient herself, without which the work we were able to do would have been made very much more difficult.

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