Some physiological aspects of the zona pellucida. Z. Dickmann and R. W. Noyes. Department of Obstetrics and Gynecology, Stanford University School of Medicine, Palo Alto, California, U.S.A.

To be published in full elsewhere.

Capacitation and the release of hyaluronidase from spermatozoa. C. R. Austin. Division of Experimental Biology, National Institute for Medical Research, London.

The granulosa-cell mass or cumulus, containing the eggs, was recovered from rabbits, rats and hamsters and gently compressed under a coverslip on a slide. When a sperm suspension was drawn under the coverslip so as to surround the cumulus, the gelatinous matrix was dissolved and spermatozoa passed among the follicle cells, but when the suspension was drawn through in a narrow stream and care taken to keep this stream away from the cumulus, spermatozoa swam through the medium, became attached to the surface of the cumulus and quite failed to penetrate into it. On the other hand, spermatozoa found within cumulus masses from mated animals were observed to move steadily through the matrix; these spermatozoa showed the acrosome changes characteristic of capacitation (Austin & Bishop, 1958b). Epididymal or ejaculated spermatozoa failed to penetrate these cumuli also. Evidently, spermatozoa must undergo capacitation before they can make their way into and through the cumulus.

Capacitation is accompanied by a change in the optical property of the acrosome and by its eventual detachment; the sequence of events is also seen upon the death of the cell (Austin & Bishop, 1958a, b).

Bishop & Austin (1957) suggested that red fluorescence of the acrosome after treatment with acridine orange denoted the presence of hyaluronidase. It has now been found that the red fluorescence is abolished by formalin in concentrations between 0.5 and 10%, the effect being shown by progressively more spermatozoa with increasing concentrations. The activity of hyaluronidase in solution was also inhibited by formalin, though at a lower concentration (0.5%).

In ejaculated semen, hyaluronidase release is believed to be a property of
moribund spermatozoa (Mann, 1954). The possibility that release of hyaluronidase on death of the cell is dependent upon detachment of the acrosome has now been tested by killing spermatozoa with two agents: digitonin, which efficiently removes the acrosome, and formalin, which preserves its integrity. In an experiment just completed, the supernatant from formalin-treated rabbit semen had the same hyaluronidase content (2.4 units/ml) as that from untreated semen, whereas the supernatant from digitonin-treated semen contained much more (22 units/ml). Both agents, in the concentrations used, were without effect on hyaluronidase activity.

It is inferred that capacitation causes release of hyaluronidase, through change in the properties of the acrosome. The idea is consistent with the view that hyaluronidase makes possible the passage of the individual spermatozoon through the cumulus (Austin, 1948). A second action of capacitation has already been described (Austin & Bishop, 1958b), namely the removal of the acrosome which must evidently precede penetration of the spermatozoon through the zona pellucida.

Grateful acknowledgment is made to Dr H. Rogers for the hyaluronidase assays.

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An autoradiographic technique for the study of egg transport in the Fallopian tube. J. P. BENNETT and J. C. BOURSNELL. A.R.C. Unit of Reproductive Physiology and Biochemistry, University of Cambridge.

Full report appears in this issue.

A study of egg transport in the Fallopian tube of the rabbit, with the autoradiographic technique. M. J. K. HARPER and L. E. ROWSON. A.R.C. Unit of Reproductive Physiology and Biochemistry, University of Cambridge.

Full report appears in this issue.

Further observations on pregnancy block in mice caused by the proximity of strange males. H. M. BRUCE. Division of Experimental Biology, National Institute for Medical Research, London.

An exteroceptive block to pregnancy in the newly mated female mouse, caused by the proximity of strange males, especially males of a different strain, has previously been described (Bruce, 1959, 1960). The effect has now been produced in the absence of the male, by putting the female in a container in which males had previously been housed on nesting material highly retentive of animal smells. Such material is provided by the white cotton drill of the ordinary laboratory coat. Five males were housed in a glass jar of 2 l capacity for approximately 12 hr, and then transferred to another jar for 12 hr and so on. The mated female followed the males through a succession of six soiled
jars during the 3 days in the test situation. Under these conditions pregnancy was blocked in 28/33 newly mated females.

Recent evidence suggests that the age of the male may be an important factor in determining pregnancy blocking capacity. Young vigorous males 2 to 3 months old proved less efficient than males of 7 or 11 months of age under similar test conditions.

Observations on the time relations of the reaction reveal that the female is most vulnerable to the influence of strange males up to about 48 hr after the stud mating (80% of pregnancies blocked) and that when implantation has started the response rapidly disappears. By Day 7 after coitus the female is not affected by the presence of strange males. During the period of maximum vulnerability even 12 hr of exposure to the presence of strange males is sufficient to cause a block to pregnancy in nearly one-half of the females compared with about 80% if the exposure is maintained for at least 2 days.

Typically, the block to pregnancy following exposure to strange males at 24 hr after finding the vaginal plug is characterized by the return of oestrus within 4 to 5 days (Bruce, 1960), i.e. 3 to 4 days after the introduction of the strange male. The latter relationship is not disturbed by the time post coitum at which exposure took place, so that the interval between the stud mating and exposure to the strange male affects the proportion of females having blocked pregnancies but it does not influence the rate of the reaction.

Submitting the female to a succession of blocked pregnancies does not interfere with her subsequent fertility. Strain differences have not been fully investigated; present indications are that pregnancy block occurs if the strange males are of a different strain from the stud male, irrespective of the strain of the female. The question of imprinting by exposure to males of a different strain before the stud mating, is being investigated.

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The role of odorous substances in mammalian reproduction. A. S. Parkes. Division of Experimental Biology, National Institute for Medical Research, London.

The preceding paper (Bruce, 1960) indicates the conditions under which pregnancy block occurs in newly mated mice in the proximity of strange males, and shows that the operative factor is probably the smell of the male. The source and nature of the odorous substance involved, however, are not known and the work raises the general problem of the occurrence and role of odorous substances, particularly those produced by specific glands, in the reproductive processes of mammals.

Glands producing odorous substances in mammals are of very diverse origin. The preputial glands have this function in the musk deer, the musk rat, and the beaver. The preputial glands of the mouse and rat on the other hand produce little odour detectable by the human nose. Anal and perineal glands
are particularly common in the Mustelidae, and are especially well known in the skunk, the civet, stoat and ferret. Less noxious perineal glands are to be found in the guinea-pig, rabbit and many other animals. Sebaceous glands, specially developed towards the production of odorous substances, are to be found on the flanks in the hamster, shrew, mouse and billy-goat. A complex of sebaceous glands occurs supracaudally in the guinea-pig and produces a pleasant-smelling waxy secretion. Apocrine glands occur on the forearm in the monkey and in the axilla in man. Other well-known superficial glands are the suborbital glands of deer, and the interdigital glands of sheep (Pocock, 1910). Specialized glands, however, are not the only source of odorous substances. Human urine contains a musky steroid Δ^4-androstenol, originally isolated by Broosbank & Haslewood (1950). The faeces, too, cannot be excluded as a source of specific odorous substances of hepatic or other origin.

The extent to which the odoriferous glands are gonad-controlled has not been adequately investigated, but according to Champy (1930) the anal glands of the male guinea-pig atrophy after castration, and the billy-goat loses his mal-odoruous character after castration.

The characteristic smells of animals of different species and of different individuals of the same species undoubtedly play a large part in territory marking and in identification. There is little doubt that they are also concerned in the recognition of oestrous females by the male. Little is known, however, about the effect upon the recipient animal, though preparations of musk and civet are traditionally used in perfumes, because of a supposed aphrodisiac effect. The extensive use of expensive natural products in the perfumery trade has led to a great deal of work on the identification and substitution of the active principles. Thus a macrocyclic compound, muskone, was isolated from the ‘musk pod’ of the musk deer, and has recently been identified in the perineal gland of the boar (Butt, Simpson, Christian & Barnhart, 1960). Civetone was obtained from the perineal gland of the civet, and this compound is of especial interest because its structural formula can be represented as somewhat similar to that of the musk-like urinary steroid mentioned above. It is not to be supposed, of course, that the odorous substances so far isolated from the secretions of the glands provide the whole of the odour. A mixture of substances is involved.

There is, therefore, a good deal known about certain odoriferous glands, but discounting the traditions of the perfumery trade, the first demonstration that odorous substances might act as an exteroceptive factor in mammalian reproduction was provided by Whitten (1955), who showed that the introduction of a male into a group of female mice evoked new oestrous cycles so that a peak number of matings took place on the third night; the operative factor was thought to be smell. The Bruce effect, however, appears to be unique in that an exteroceptive factor can prevent the establishment of pregnancy, and it is evidently important to identify the substances concerned. The high discrimination shown by the females in Bruce’s experiments does not necessarily imply that each male mouse produces a different odorous substance. Almost certainly a spectrum of smells is involved, varying slightly in different individuals of the same strain, and substantially between strains. This idea is borne out by the fact that expert perfumers were able to distinguish between jars with cloth
bedding which had contained TO or P male mice on the one hand and G males on the other. It is well known that visual stimulation plays a large part in the reproductive processes of birds and that light and dark act as exteroceptive factors in certain mammals. Bruce’s work will probably lead to the recognition of smell as another and even more important factor in mammalian reproduction.

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Human chromosomes and the testicular squash technique in the study of chromosomal anomalies. J. Hamerton. Guy's Hospital, London.

It is now universally accepted that the human chromosome number is forty-six. Reported cases of variation between populations and the presence of supernumary chromosomes are of doubtful significance.

The development of new techniques and increased clinical interest has resulted in the discovery of a number of chromosomal abnormalities, initially of sex chromosomes, notably Turner's and Klinefelter's syndromes, XO and XXY, respectively. More recently a number of somatic chromosomal aberrations related to various clinical stigmata and referred to generally as Trisomic syndromes, as they are all characterized by the presence of an additional chromosome, have been described.

In a few cases reciprocal translocations are also inferred. All this work has been carried out on somatic material and hence the assignment of the additional chromosome to a particular autosome or group, or the presence of a translocation can only be inferred by matching and measuring. Analysis of chromosomal pairing during the first division of meiosis would provide confirmatory evidence.

Studies of chromosomes in cases of otherwise inexplicable infertility or repeated abortion might show the presence of causatory chromosomal abnormalities.

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Full report to be submitted for publication in J. Reprod. Fertil.

Alkyl sulphonic esters and male rat fertility. H. Jackson, B. W. Fox and A. W. Craig. Christie Hospital and Holt Radium Institute, Manchester.
Full report to be submitted for publication in J. Reprod. Fertil.
Radiation and fertility in the male rat and mouse. A. W. Craig, B. W. Fox and H. Jackson. Christie Hospital and Holt Radium Institute, Manchester.

Full report to be submitted for publication in J. Reprod. Fertil.


Full report appears in this issue.

Early embryonic mortality in the rabbit. C. E. Adams. A.R.C. Unit of Reproductive Physiology and Biochemistry, University of Cambridge.

Earlier experiments on rabbits (Adams, 1960 a, b) provided information on the amount of embryonic mortality occurring before implantation, but their design precluded any analysis of the relationship between mortality and particular stages of pregnancy. Therefore, further experiments were performed with both control and superovulated animals and, in addition, the effect of egg numbers was investigated by means of the egg-transfer technique. Morulae were recovered in homologous plasma from the tubes of superovulated donors 60 hr post coitum (p.c.) and transferred to the uteri of 128 recipients given an ovulating injection (25 i.u. LH) 72 hr previously. The number of implantations in recipients was recorded by laparotomy on the 10th day of pregnancy. The other does were killed at intervals varying from 2 to 6 days p.c. to permit examination and classification of their embryos.

RESULTS

In 24 control and 122 superovulated does, it was confirmed that approx. 5% of pregnancies failed completely before implantation and the cause was found to be lack of fertilization. Furthermore, none of the eggs contained spermatozoa, either in the perivitelline space, zona pellucida, or mucin layer. In twenty-two control does, the mean proportion of eggs fertilized was 98.6% (221 eggs) and the corresponding figure for 114 superovulated does was 96.1% (3659 eggs). In the superovulated does, seventy eggs (1.9%) showed retarded cleavage at recovery, which was performed 60 hr p.c. Regarding the position of the eggs, whether in the tubes or uteri at 60 hr p.c., it was found that they were all still in the tubes of 111 does (91%), both in the tubes and uteri of nine does (7.4%) and solely in the uteri of two does (1.6%). It may be concluded that premature entry of the eggs into the uterus is uncommon even in superovulated animals and consequently is not an important factor in embryonic mortality.

In twelve superovulated does examined 144 hr p.c., the mean numbers of corpora lutea and of blastocysts recovered were 36.8 and 26.2 respectively, which means that 20.6% of the eggs failed to develop into blastocysts. Of the 351 blastocysts recovered, it was estimated on a size basis that 6.3% showed retarded development and probably succumbed between 96 and 120 hr p.c. Thus, at 144 hr the mean number of 'normal' blastocysts was 27.4 and their mean diameter was 2.35 mm.
When increasing numbers of morulae, namely, five, ten, fifteen, twenty, or twenty-five were transferred to a single uterine horn, the number of implantations also increased, rising from 4.0 to 13.6, though the proportion of embryos surviving showed a significant decrease, falling from 80.0% to 54.3%. In a further experiment, five morulae were transferred to one uterine horn and twenty morulae were transferred to the other horn of nine recipients, which were killed on the 6th day when the embryos were 132 hr old. On the side receiving five morulae (control), the mean number of blastocysts recovered was 4.6 (loss = 7.5%) compared with 15.0 blastocysts (loss = 26.2%) on the experimental side. However, the percentage of retarded blastocysts (<600 µ) was slightly higher on the control side, 8.1% and 3.7% respectively, and there was no significant difference in the mean diameter of the two sets of surviving blastocysts, which measured 1.69 mm and 1.71 mm, respectively. Comparison of the numbers of surviving blastocysts in this series with implantations in the corresponding 'five to twenty' group in which implantation was allowed, indicates that losses between 132 hr and 168 hr may not exceed 6%.

It is thus apparent that the heavier embryonic mortality associated with increasing numbers of eggs occurs mainly between 60 and 96 hr p.c. when blastocyst formation is taking place. It has already been demonstrated that this stage represents a critical phase in embryogenesis, requiring the assistance of special uterine factors (Adams, 1958). Particularly noteworthy is the observation that once the early blastocyst has developed beyond a certain stage (96 hr) its further development appears to be little affected by the number present in the uterine horn.

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Some properties of early embryonic fluids in the rabbit. Cecilia Lutwak-Mann. A.R.C. Unit of Reproductive Physiology and Biochemistry, University of Cambridge.

Up to the stage of the morula, the cells of the segmenting ovum appear tightly packed. The formation within the segmenting zygote of a confluent cavity heralds the onset of the blastocyst stage, and accumulation of fluid within the cavity begins. Very rapid growth is characteristic of this period: the average fresh weight of the rabbit blastocyst is 2 mg at 5 days, 25 mg at 6 days, and over 100 mg on day 7 (from the time of mating). This increase in weight is almost entirely due to intake of fluid, as the dry weight remains very low (1 to 2%).

The present study is concerned with three constituents of the blastocyst fluid, namely bicarbonate, glucose and lactic acid; the presence of glucose and bicarbonate in rabbit blastocysts was detected earlier (Lutwak-Mann, 1954; Lutwak-Mann & Laser, 1954). The free-lying pre-implantation blastocysts were used entire, without previous rinsing; after implantation the fluid was withdrawn from the blastocoelic cavity by aspiration. Parallel chemical determinations were performed on maternal blood-serum, peritoneal fluid and
also on the endometrial secretion collected 24 hr after mating, a stage when in some rabbits the secretion is fairly abundant.

Bicarbonate content was high in unimplanted blastocysts, and declined steeply after implantation. Values expressed as ml CO$_2$/100 ml were 170 and 130 on Days 5 and 6, respectively; 89 on Day 7; 42 on Days 8 to 9. Maternal values were: plasma 50, peritoneal fluid 42, uterine secretion 70.

The glucose content, expressed as mg/100 ml, was 8 to 17 in 6-day blastocysts, but rose to 45 to 80 on Day 7, and reached maternal blood values, 90 to 110, on Days 8 to 9. The uterine secretion contained 10 to 18 mg/100 ml.

Lactic acid content, expressed as mg/100 ml, ranged from 17 to 30 in 6-day blastocysts, increasing rapidly at implantation, and ranging from 130 to 160 on Day 7, and 100 to 110 on Days 8 to 9. Uterine secretion contained 25 mg/100 ml.

To obtain information on osmotic conditions within the early embryos and their environment, freezing-point depression was determined by the micro-method of Ramsey & Brown (1955).* The values were as follows, blastocyst fluid: 5 days, 0.547; 6 days, 0.564; 7 days, 0.532; 8 days, 0.548; endometrial secretion, 0.536; serum 0.625; peritoneal fluid, 0.545. The values at 7 days were slightly but distinctly lower than at 6 or 8 days; this may be partly due to the previously established decrease at 7 days in the sodium content of the blastocyst fluid (Lewis & Lutwak-Mann, 1954).

Bicarbonate, glucose and lactic acid concentrations were next investigated in 6- and 7-day blastocysts removed from rabbits which had been pretreated with various agents shown in an earlier study adversely to affect the rabbit blastocyst (Hay, Adams & Lutwak-Mann, 1960). The interference by these agents with embryonic development was reflected in the chemical composition of the blastocyst fluid, in that the level of bicarbonate, glucose, and lactic acid was distinctly ‘retarded’ in relation to the chronological age of the embryos.

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The Third Oliver Bird Lecture. Sterilization of the male by cadmium salts. J. Pařízek. Laboratory of Physiology and Pathophysiology of Metabolism, Prague, Czechoslovakia.

Full report appears in this issue.

Fertility in cryptorchidism. L. Stuart Scott. Male Infertility Clinic, Western Infirmary, Glasgow.

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Recent observations on bacterial invasion of the cervix. Mary Barton. Royal Free Hospital, London.

Cervical dysfunction is one of the commonest infertility factors. Non-traumatic

* These measurements were carried out by Lord Rothschild’s assistant, Mr Nichol, working on behalf of the A.R.C.
dysfunction exhibits two common types. In both, the mucus contains numerous polymorphs in any stage of the cycle, and both tend to repel sperm invasion and to impair survival of spermatozoa within the cervical mucus. Type one is not inflammatory and is due to failure of oestrogenic stimulation. Hence none of the characteristic changes of the follicular phase are present. Type two is essentially an inflammatory condition and pathognomically it closely follows the pattern of infections of the nasopharyngeal tract, falling as it does into two stages: (1) an acute catarrhal phase with voluminous mucous flow; (2) the stage of invasion by pathogenic culturable organisms when the erosive action of the discharge becomes marked. The non-invadible mucus of Stage 1 may yield a negative culture from routine inocula. It may well reflect a virus infection. But the diagnosis requires repeated bacteriological examination of specimens, preferably to be collected during the postovulatory phase of the cycle. If and when pathogenic organisms are demonstrable by culture, sensitivity tests will determine the choice of antibiotic. Failing a positive culture, broad spectrum antibiotics may often be effective. A combination of local and systemic therapy yields the best results. But cervical infection frequently reflects a fairly general condition of the pelvic organs and the relapse rate is high, especially where extragenital areas of infection exist concomitantly (cf. Sandler’s study (1958) of the relationship between nasopharyngeal sepsis and infertility).

Prolonged supervision is required. Eradication of infection is recommended before routine fertility tests are proceeded with. In cases reported in 1948 (Barton & Wiesner, 1948) the great majority of presumptive pathogens recovered from the cervix were gram negative organisms with a prevalence of *Escherichia coli* and *Bacillus proteus*. In the last two years or so the frequency of such infections has greatly diminished and chromogenic staphylococci and various streptococci are more commonly encountered.

In the last series of cervical inocula cultured between December 1959 and May 1960, twenty-six cases yielded profuse growths on routine media; eleven (42%) consisted of *Staphylococcus aureus* coagulase positive; eight (34%) consisted of *Streptococcus faecalis*; *B. coli* was recovered in two cases only (8%). This contrasts with findings in a comparable series examined in 1948 in which there were nineteen cases of *B. coli* infection (76%). Both series of cultures were carried out by Dr C. J. C. Britton.

The presence of pathogenic bacteria is of more than local importance since they may yield endotoxins that are toxic or even lethal to the foetus. Dr Thiervers of Washington University has shown that the ovicidal action of micro-organisms resides in their lipopolysaccharides. The ovicidal toxins known to date have been obtained from gram negative organisms but Thiervers has recently obtained ovicidal lipopolysaccharides from a gram positive streptococcus. Unfortunately these toxins are not antigenic and it does not seem possible to immunize potential mother animals against the ovicidal toxins (J. B. Thiervers, personal communication). Hence he thinks it is essential to try to eradicate the infection by other means such as suitable antibiotics.

Dr Kass of Boston has shown that untreated bacilluria may result in abortion (J. B. Thiervers, personal communication), thus confirming independently my own experience.
The similarity between cervicitis and the common cold has suggested a clinical experiment in which subjects received 1 g Redoxon (Roche) or a placebo daily for a period of 6 months observation. Fifty cases have been fully recorded but the results have not yet been evaluated. Recent observations have suggested that in cases where neither administration of oestrogens nor clearance of infection restore the cervical flow to normal character or volume, administration of pilocarpine hydrobromide in the ovulatory phase (in a daily dose of 10 to 12 mg) may be effective. This procedure may be of particular value in post-operative stenosis of the cervical canal and external os.

Rapid distension of the os with increased flow of mucus similar to the pilocarpine effect is seen when fertile semen is placed in contact with the cervix in the ovulatory phase. This response may occur within a few minutes or up to 3 hr and may last for 24 hr. Possibly semen contains an activating compound which in normal conditions in part contributes to the receptivity of the cervix.

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Results of artificial insemination (husband). G. I. M. Swyer. University College Hospital, London.

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Amenorrhoea and oligomenorrhoea in relation to subfertility. D. Maxwell. 24 Park Street, Windsor.

Oligomenorrhoea was defined as the 'unphysiological absence of menstruation of short or long duration', and it was considered impossible to separate amenorrhoea from oligomenorrhoea.

A comprehensive analysis of the causes was shown and the functional causes of amenorrhoea were discussed in some detail with allusions to the literature. Consideration was then given to the purely clinical aspect of oligomenorrhoea cases. These were classified as follows:

1. Environmental — example well seen in the hospital nurse.
2. Fear of pregnancy — exemplified by extramarital intercourse, and in unmarried girls who have had intercourse.
3. The amenorrhoea of mental disease.
4. Cases complaining essentially of subfertility, who have oligomenorrhoea.
5. Hyperinvolution.

The meeting was asked to discuss, in particular, the treatment of the subfertile case presenting oligomenorrhoea. It was stressed that there was no certain
drug on the market, nor method of treatment, that would certainly make a case ovulate.

Investigation of these cases was alluded to — also the fact that substitution therapy, if given clinically, might make the patients bleed — thus making them, and the husbands, happier but, on the other hand, this treatment might inhibit the pituitary.

The Stein-Leventhal Syndrome symptoms were carefully enumerated and discussed by Mr Maxwell and Dr Swyer.

The work of Bailey of Manchester was referred to and it was agreed that a typical Stein-Leventhal Syndrome might be widespread where all the classical symptoms were not present but which responded — in many cases — to wedge resection of the ovaries.


The first essential in the endocrine management of amenorrhoea and oligomenorrhoea is to exclude both physiological amenorrhoea and that requiring surgical treatment and to select for hormone treatment those cases likely to respond. Various forms of primary amenorrhoea due to developmental failure were described, and endocrine therapy recommended in genital hypoplasia, hypothyroidism and ovarian agenesis. Secondary amenorrhoea may occur in the Stein-Leventhal syndrome, where hormone treatment has been found useless. It may be associated with the Simmonds-Sheehan syndrome, lactation or other conditions associated with pregnancy. Psychogenic amenorrhoea may result from stress, both of minor and of major degree, and here, the induction of bleeding by endocrine methods may have considerable psychotherapeutic value. Oestrogens alone, combined preparations of oestrogen and progesterone and progestational 19-norsteroids have been used with satisfactory results.

Low-dosage pituitary irradiation. L. H. Walter. Postgraduate Medical School, Hammersmith Hospital, London.

The author gave a brief review of the history of the use of ovarian and pituitary irradiation in the treatment of amenorrhoea and infertility. The fact that irradiation is always destructive and not stimulating in its action and the possible genetic hazards of ovarian irradiation were considered. A technique for treating the pituitary was described for the treatment of cases of infertility and oligomenorrhoea in which no organic disease had been found and who have not responded to other types of therapy.