THE EFFECTS OF GONADAL STEROIDS ON CARBOHYDRATE TOLERANCE IN THE RAT.

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A thesis submitted to the University of London for the Degree of Doctor of Philosophy.

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ABSTRACT.

Gonadal steroids and their effects on carbohydrate tolerance have been investigated in the ovariectomized rat.

The study was performed in vivo and was longitudinal in design, with an oral glucose tolerance test (O.G.T.T.) carried out on the same animal after 2 and 4 weeks of steroid administration.

The oestrogens, Oestrdiol benzoate, Mestranol and Ethinyl oestradiol given in pharmacological doses improved glucose tolerance, increased pyruvate levels and generally were without effect or caused a slight decrease in the insulin response during an O.G.T.T.

Progesterone and two classes of progestogen were also investigated. The classes of progestogen were the 19 nor testosterone derivatives, and the 17 alpha hydroxy progesterone derivatives. Norethynodrel and Norethisterone acetate were the steroids investigated in the nor testosterone class and Chlormadinone acetate and Megestrol acetate were the steroids investigated from the progesterone derivative class.

In general, it was found that Progesterone, Norethynodrel, Norethisterone acetate and Chlormadinone acetate did not have any major effects on glucose tolerance. The progestogen, Megestrol acetate was seen to slightly improve glucose tolerance. The O.G.T.T. pyruvate levels were significantly elevated by the 19 nor testosterone derivatives whilst Progesterone and the 17 alpha hydroxy progesterone derivatives gave only marginal elevations.

Elevations in the insulin response during an O.G.T.T. was found with administration of Progesterone and the 17 alpha hydroxy progesterone derivatives but not with the 19 nor testosterone derivatives.

The administration of oestrogens and progestogens in combination with each other were demonstrated to improve glucose tolerance, elevate pyruvate levels and to have little effect or to lower insulin responses during an O.G.T.T.

Mechanisms have been postulated to explain these findings; they are that oestrogens improve glucose tolerance by increasing the activities of enzymes involved with glycogenesis, glycolysis and the Hexose monophosphate shunt in the liver and peripheral tissues and also by the inhibition of gluconeogenesis.
The progestogens alter glucose metabolism by virtue of their oestrogenic properties in the case of the 19 nor testosterone derivatives and their insulinogenic properties in the case of the 17 alpha hydroxy progesterone derivatives.

The importance of the adrenal gland in the mechanism for oestrogen mediated alterations in the metabolism of glucose has also been demonstrated.

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NOTE ON NOMENCLATURE:

Throughout this thesis, steroids have been referred to by their recognized trivial name. The chemical structures and recommended systematic names for these steroids are as set out by the I. U. P. A. C. (Steroids 13, 277 1969) and may be found in Appendix 1.

The names of enzymes discussed in this thesis are as recommended by the I. U. B. (Comprehensive Biochemistry, Vol 13, ed by Florikin and Stotz) and a list with systematic number code may be found in Appendix 11.

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INTRODUCTION

The application of hormonal contraception was introduced by Rock Pincus and Garcia in (1956) and since this time the use of the oral contraceptive has increased in its world wide useage.

It was Waine et al (1963) and Gershberg, Javier and Hulse (1964) who made the first observations that carbohydrate tolerance was impaired in women using combined oestrogen-progestogen therapy for contraceptive purposes. Since this time there has been an accumulation of a mass of data regarding the effects of oestrogens, progesterone and progestogens on carbohydrate tolerance, the pituitary adrenal axis, liver and pancreatic function.

However the exact mechanisms by which these steroid preparations exert their effects still remains unsolved.

OESTROGENS

The influence of the gonads and gonadal steroids on the incidence of diabetes in the rat came under intense study in the late 1940 and early 1950 period. Foglia, Schuster and Rodriguez (1947) showed that ovariectomy greatly increased the frequency of diabetes and castration slightly decreased the frequency of diabetes in the sub totally (95%) pancreatectomized rat. It was also demonstrated by the same investigators that the administration of Oestradiol benzoate to these sub-totally pancreatectomized animals diminished the incidence of diabetes. The alloxan diabetic rat treated with Oestradiol benzoate, also demonstrates on amelioration of the diabetic state, Rodriguez (1954), and Ingle (1941). The workers Ingle, Nezamis and Prestrud (1947) demonstrated in the forced fed normal intact rat, the partially

pancreatectomized rat and the alloxan diabetic rat that there was an increase in glycaemia and glycosuria when these animals were treated with diethylstilboestrol. However Rodriguez (1950), demonstrated that there was a biphasic response involved with such animals and treatment; firstly there was the increase in glycaemia and glycosuria, but after approximately one month of continuous oestrogen treatment, the glycaemia and glycosuria disappeared and there was a permanent protection induced in these animals. This protective action of oestrogens was suggested by Lewis, Foglia and Rodriguez (1950) to be due to the hypertrophy and hyperplasia of the islets of Langerhans in the pancreas. This trophic action of oestrogens on the pancreas does not require the presence of the adrenal or pituitary gland Foglia (1954). Oestrogens have been shown to increase the insulin content of the islets Fraenkal-Conrat et al (1941), Griffiths Marks and Young (1941) and Costrini and Kalkhoff (1971). This insulinogenic effect of oestrogens is reported to require the presence of the pituitary, Fraenkal-Conrat et al (1941). Costrini and Kalkhoff, (1971), demonstrated that the isolated islets of rats that had been treated with Oestradiol benzoate for a period of 3 weeks had an increased capacity to secrete insulin with a glucose stimulus, however Hager et al (1971) demonstrated in a similar study a decrease in the insulin release with a glucose stimulus. Beck (1969) using rhesus monkeys found that Mestranol increased the insulin response during an i. v. glucose tolerance test, whilst Ethinyl-oestradiol and Oestradiol were without effect.

These three steroids did not significantly alter the rate of removal of glucose during the test, although Mestranol was found to increase the resistence to the hypoglycaemic action of exogenously administered insulin.

Experiments in women using the synthetic oestrogens of the oral contraceptive are few in number and with conflicting results. Talaat et al (1965) Di Paola et al (1968) and Yen and Vela (1968) demonstrated that glucose tolerance was unaffected or improved in normal women taking synthetic oestrogens. Recently Spellacy, Buhi and Birk (1972a) have also shown that the carbohydrate tolerance and insulin response was unaffected in women who had been receiving Mestranol or Ethinyl oestradiol for a period of 6 months.

Gershberg et al (1967) found that Mestranol administration to a group of maturity onset diabetics improved their carbohydrate tolerance, without exerting any effect on insulin response. Di Paola et al (1968) showed that increasing the dose of Mestranol was without effect on carbohydrate tolerance and Yen and Vela (1969) demonstrated similar results for studies using Ethinyl-oestradiol.

Di Paola, Robin and Nickolson (1970) using prednisone-glucose tolerance tests demonstrated a biphasic response in women treated with Mestranol, where in the first three months of treatment approximately 55% of the women had abnormal tolerance tests, but after 12 months of continued treatment the percentage of abnormal tests fell to 3%.

Ethinyl-oestradiol in the same study was found not to increase the percentage of abnormal tests.

In conflict with these findings, where oestrogens have been demonstrated to improve carbohydrate tolerance is the work of Goldman and Ovadia (1969), which showed the detioration in the i. v. glucose tolerance with oestrogens. Also Buchler and Warren (1966) demonstrated detioration in the oral and i. v. glucose tolerance tests of pre and post menopausal women on diethylstilboestrol. Pyorala, Pyorala and Lampinen (1967) observed a slower disappearance of glucose after i. v. glucose in women receiving Ethinyl-oestradiol for a short period of time. Insulin data was not available in any of these studies. In general it appears that oestrogen and the synthetic oestrogens exert an effect on carbohydrate metabolism both in animals and the human. The work in animals tends to show that oestrogens ameliorate rather than aggravate experimental diabetes, whilst in man the results are not as clearly delineated. Therefore it suggests that the selection of patients, the type of oestrogen employed, the dosage schedule and duration of treatment are important in defining the effects of oestrogens on carbohydrate tolerance.

PROGESTERONE AND THE PROGESTOGENS

There has been little work done with regard to the effect of Progesterone and the progestogens on carbohydrate metabolism.

The work of Lewis Foglia and Rodriguez (1950) demonstrated that progesterone had no effect on alleviating the incidence of diabetes in the sub-totally pancreatectomized rat.

However Ingle, Beary and Purmalis (1953) showed that Progesterone administration to force-fed sub-totally pancreatectomized rats causes glycosuria with high doses, but was without effect at low doses. Later work by Beck (1969), using rhesus monkeys, showed that Progesterone and the progestogens Norethynodrel, Norethisterone acetate and Chlormadinone acetate cause an increase in the insulin response to an i. v. glucose challenge. Progesterone and the 19 nor testosterone derivatives Norethynodrel and Norethisterone acetate were without effect on fasting glucose or its removal during the i. v test; however Chlormadinone acetate caused a significant increase in the glucose disappearance rate without effecting fasting glucose values.

In similar experiments Costrini and Kalkhoff (1971) and Hager et al (1972) demonstrated that islets isolated from rats treated with Progesterone had an increased insulin response to a glucose stimulus. Goberna et al (1971) demonstrated that Chlormadinone acetate administration to sub-totally pancreatectomized rats decreased the incidence of diabetes as judged by fasting blood glucose and caused an increase in insulin response during an i. v. glucose tolerance test. These same workers found that Chlormadinone acetate given to normal intact rats only marginally increased the 5 minute insulin response to an i. v. glucose tolerance test.

Data in the human with regard to the effects of Progesterone and progestogen steroids is rather scanty and like the oestrogen data, there are many conflicting results.

The work of Goldman, Ovadia and Eckerling (1968) and Yen and Vela (1969) showed that Progesterone given intramuscularly at dose levels of 100-300 mg was without effect on glucose removal after i. v. glucose challenge.

Similar findings were made by Kalkhoff, Jacobson and Lemper (1970) for oral glucose and tolbutamide stimulation tests and it was noted in these experiments that there was an increased insulin response to the stimulus. The progestogen, Medroxyprogesterone acetate given to normal women was shown by Goldman et al (1968) to have no effect on carbohydrate tolerance, however Gershberg et al (1969) demonstrated for this steroid given to pre diabetics and established diabetics that there was impaired glucose tolerance and insulin secretion. Spellacy et al (1970a) also using this progestogen, demonstrated in normal women that insulin and glucose levels were elevated at fasting and during an O.G.T.T.

The 19 nor testosterone progestogens, Norethynodrel and Norethisterone acetate given to normal women have been demonstrated by Di Paola et al (1970), Beck (1969) Laarson-Cohn et al (1969) and Gilfrich et al (1969) to have no effect on glucose tolerance or insulin levels, after a glucose challenge.

The workers Yen and Vela (1969) and Goldman et al (1971) have shown that glucose removal and insulin response after i.v. glucose or argine stimulation were normal in women who had been taking the progestogen Ethynodiol-diacetate. However Spellacy, Buhi and Birk (1972b) showed that there was a significant elevation of blood glucose at fasting and during an O.G.T.T., with parallel increases in the insulin levels. Growth hormone was found not to be altered. Beck (1969) studied the same progestogen in the monkey and found no increase in blood glucose or insulin levels during an i.v. glucose test.

The work of Vermeulen, Daniels and Thiery, (1970) and Larsson-Cohn et al (1969) have shown that Chlormadinone acetate given to normal women is without effect on glucose tolerance, however only the former authors noted an increase in the insulin response to glucose challenge.

Beck (1970) demonstrated a biphasic response in normal and pre-diabetic women treated with Chlormadinone acetate, where there was an early detioration in carbohydrate. tolerance in the first few months, followed by a return to normal, there was no significant change in the insulin response.

Studies on the progestogen, Megestrol acetate by Pyorala et al (1967) and Adams and Wynn (1972) have shown that when this steroid has been used to replace Ethinyl-oestradiol or combined oestrogen-progestogen contraception in women with demonstrated carbohydrate intolerance, there was a marked improvement in glucose tolerance, with a concomittant fall in insulin response.

The effect of 17 alpha-OH-Progesterone caproate on carbohydrate metabolism is conflicting with Benjamin and Casper (1966) reporting an improvement and Schriebman (1968) a detioration in carbohydrate tolerance with use of this steroid.

COMBINED OESTROGEN-PROGESTOGEN PREPARATIONS

The combination of oestrogens and progestogens in oral contraceptive preparations and their effects on carbohydrate tolerance are also very confusing. It may be that there is a potentiation of the adverse effects of one steroid in the presence of another, or it may be that there is an improvement in carbohydrate tolerance, brought about by the favourable effects of one steroid overcoming the adverse effects of the other.

At present, although the literature is voluminous on the effects of the combined oral contraceptive (O.C.), on carbohydrate tolerance, there has not been as yet, a clear definitive assessment of which steroids and in what combination and dose, provide the best contraception with the least perturbation to the normal metabolism of man.

There has been very little work done in animals with regard to the combination of oestrogens and progestogens and their effects on carbohydrate metabolism. Costrini and Kalkhoff (1970) and Hager et al (1972) have shown that isolated islets of rats that had been treated with a combination of Oestradiol benzoate and Progesterone have an increased insulin response to a glucose stimulus. The work of Fenichel et al (1969) showed that there was no effect on glucose levels during an O.G.T.T. in rats treated with Mestranol and Norethisterone or Mestranol and Norethynodrel.

In contrast with the few animals studies done with combined oestrogen-progestogen administration and their effects on carbohydrate tolerance, there is a mass of data for human studies.

There are several excellent reviews that have made an attempt to cover the effects of combined O.C. therapy. Spellacy (1969) Adams and Oakley (1972) and Kalkhoff (1972).

The combination of oestrogen and progestogen may be done in two ways, firstly they may be given together, or secondly in sequence, the latter method being closer to the normal physiology of the female menstrual cycle. The incidence of carbohydrate intolerance seems to be greater for the steroids given together rather than sequential administration, Spellacy (1969), Adams and Oakley (1972). Gershberg, Javier and Hulse (1964) showed that women who had been using combined O.C. from 3 months to 3 years had an O.G.T.T. which became progressively more abnormal.

Wynn and Doar (1966) and (1969) and Spellacy et al (1970b) have shown in longitudinal studies that carbohydrate tolerance was markedly impaired in women using the combined O.C. Both groups of workers agree that approximately 70-80% of the women using combined O.C. had impaired carbohydrate tolerance and Wynn and Doar estimated that 13-15% of this number had developed chemical diabetes. These two groups of workers with Javier, Gershberg and Hulse (1968), all noted hyperinsulinism in the presence of elevated glucose levels. However the latter authors noted that insulin response decreased with increasing duration of treatment. PiSunyer and Oster (1968), in a short term longitudinal study with a Mestranol-Norethynodrel preparation, observed no alteration in glucose tolerance or insulin response. In studies of longer duration, Taylor and Kass (1968) and Danowski et al (1968) also showed that there was no change in carbohydrate tolerance. Unchanged glucose tolerance, but with hyperinsulinism was found by Yen and Vela (1968) in a small number of women on combined O.C. for 3 months. Posner and co. workers (1967a 1967b) found a significant decrease in the glucose disappearance rate during i.v. glucose tolerance tests in women treated with Mestranol and Norethynodrel for 2-3 months, but that this effect was reversed after 4-6 months of continued treatment with these steroids.

Whilst Javier et al (1968) demonstrated a progressive increase in abnormal carbohydrate tolerance with duration of O.C. treatment, Wynn and Doar (1966+1969), and Peterson Steel and Coyne (1966) observed no increase in the deterioration of carbohydrate tolerance with duration of O.C. treatment.

POSTULATED MECHANISMS OF ACTION OF THE GONADAL STEROIDS ON CARBOHYDRATE TOLERANCE

Despite the large amount of data that has accumulated on the effects of oestrogens and progestogens on carbohydrate metabolism and insulin response, very little has been achieved in the way of elucidating the mechanisms by which these steroids induce changes in metabolism.

It may be that in the case where oestrogens improve carbohydrate metabolism there is an increase in the peripheral utilization of glucose brought about by the direct action of oestrogens on liver, peripheral muscle and adipose tissue, or, that there may be a state of induced hyperinsulinism. In the case where oestrogens may cause a detioration in carbohydrate tolerance it may be due to insulin antagonism at the peripheral tissues caused by excess cortisol or growth hormone secretion.

It is well established that glucocorticoids cause impairment of carbohydrate tolerance, and the effects of oestrogens on adrenal function have been well documented. In animal studies Kitay (1963a+1963b) demonstrated that oestrogen treatment of ovariectomized female and male rats but not in the intact female, had a biphasic response in corticosterone production. Corticosterone synthesis was stimulated by low doses and inhibited by high doses of oestrogen,. Kitay (1963) and Kitay, Coyne and Newsom (1965), suggested that these effects were due to increased A.C.T.H. secretion and increased corticosteroidogenesis due to enhanced activity of the adrenal hydroxylating enzymes by oestrogen. McKerns (1961) demonstrated that high doses of oestrogen inhibit the enzyme glucose-6-phosphate dehydrogenase in the rat adrenal, there by decreasing the availability of $NADPH_2$ which is required in the synthesis of corticoids.

In the human, Sandberg, Rosenthal and Slaunwhite (1969) and Plager Schmidt and Staubitz (1964) along with many other workers have shown that plasma cortisol levels, both the transcortin bound and unbound fractions, are greatly increased by oestrogen administration and pregnancy. Burke (1970) has shown that the increase in cortisol levels with oestrogen administration is dose related, with low doses having no effect and high doses increasing cortisol secretion. Oestrogen administration has also been demonstrated to decrease the metabolism of cortisol, Wallace and Carter (1960), Doe et al (1960) and Peterson et al (1960).

Progesterone apparently does not greatly affect plasma cortisol levels, Kalkhoff (1970), although in high doses it may cause displacement of cortisol from transcortin, Sandberg et al (1969). The progestogens Chlormadinone acetate and Megestrol acetate have been reported not to increase plasma cortisol levels in the human, Daly, Elstein and Murray (1968).

Glucocorticoids are known to have potent affects on intermediary metabolism in animals and man. Wynn and Doar (1969) have shown that blood pyruvate levels are increased with glucocorticoid therapy and in women receiving combined O.C. It was suggested by these workers that the alteration in metabolism seen in women using O.C. was due to increased glucocorticoid activity induced by oestrogens.

The involvement of growth hormone (G.H.) in carbohydrate metabolism is also a possibility for it has been described by Frantz and Rabkin (1965) and recently by Thompson et al (1972) and Davidson and Holzman (1973) that oestrogens and O.C. increase G.H. levels and production rates in the human.

The work of Maw and Wynn (1972) showed elevations in fasting G.H. levels in women on O.C. and a normal suppression of G.H. during an O.G.T.T. in the presence of impaired glucose tolerance. They concluded that the increased G.H. secretion was not responsible for impaired glucose tolerance.

Wynn Doar and Cramp (1969) showed that the administration of G.H. had no effect on fasting or O.G.T.T. pyruvate levels.

Progesterone administration has been reported by Kalkhoff (1970) and Beck (1969) to be without effect on G.H. levels. Progestogen administration also has no effect, or in some reports to supress G.H. levels, Spellacy et al (1970), Beck (1970), Yen and Vela (1969) and Simon et al (1967).

In general it is regarded that despite the elevation in G.H. levels seen with oestrogen and combined O.C. administration G.H. does not constitute a major contributing factor toward carbohydrate intolerance.

It is evident that the gonadal and oral contraceptive steroids have marked effects on glucose metabolism and insulin response in the human and experimental animal. The data that exists indicates that oestrogens have a protective action in animals and mildly diabetic patient, but may cause detioration of carbohydrate tolerance in the normal human. The actions of progestogens are less clearly defined, but appear to be without any major untoward effects on carbohydrate tolerance.

Beck (1969) working with monkeys, attempted to separate the actions of the various steroids on carbohydrate metabolism and their insulinogenic properties on a structural basis. He proposed that a partial positive charge at position C5 of the steroid nucleus imparted insulinogenic properties on the steroid, for example Mestranol, Progesterone, Norethynodrel, Norethisterone and Chlormadinone acetate. Unsaturation at the C6 position seen in Chlormadinone acetate and Megestrol acetate he also proposed may decrease the gluconeogenic effects of these two steroids and thereby improve carbohydrate tolerance.

The problems involved with the study of the O.C. in the human are numerous, with type of steroid employed, dosage, route of administration, duration of treatment, type of test used and the lack of adequate control data. It is for these reasons that the animal experiments presented in this thesis were undertaken. The oral glucose tolerance test O.G.T.T., regarded as a more physiological stimulus Kipnis (1969), McIntyre (1969) and a better indicator of carbohydrate intolerance Wynn and Doar (1966) was employed throughout this work.

Therefore it is hoped that some of the results presented in this thesis will increase a little more, the knowledge and understanding of the effects of the gonadal and oral contraceptive steroids on carbohydrate metabolism, and direct further work on this difficult, but very important problem.

CHAPTER 1

EXPERIMENTAL PROCEDURES

1 ANIMALS AND THEIR MAINTENANCE.

11 SURGICAL PROCEDURES.

111 INJECTION REGIME. THE ORAL GLUCOSE TOLERANCE TEST. COLLECTION AND STORAGE OF SAMPLES.

1V CHEMICAL METHODS AND ASSAY PROCEDURE.

V CALCULATIONS AND STATISTICAL ANALYSIS.

V1 EVALUATION OF METHODS.

SECTION 1

ANIMALS:

Female Sprague Dawley rats were maintained in the animal house at St. Mary's Hospital Medical School. These animals were purchased from two commercial suppliers, A. Tuck and Son Ltd., L.A.B.S., Rayleigh, Essex., and O.L.A.C. Southern Ltd., OLAC House, Blackthorn, Bicester, Oxfordshire.

CONDITIONS OF ANIMAL MAINTENANCE:

Care was taken to standardize the conditions of animal maintenance during experiments.

(a) Light and Temperature: The animals were maintained under conditions of standardized temps 21°C and a 12 hour light/dark cycle; light 0700 - 1900 hrs.

(b) Caging: Groups of 5 rats were kept in a large cage, 18"x12"x7"

(c) Food and Water: Animals were fed on 41B pellet food purchased from Christopher Hills, the composition of which was:

Crude Protein	15.9%
Roughage Crude Fibre	5.1%
Crude Oil	3.2%
Carbohydrate	51%

plus essential vitamins and mineral supplement. Drinking water was from the tap, or in the case of Adrenalectomized rats, 0.9% saline was used.

SECTION 11

SURGICAL PROCEDURES:

All surgical procedures required for the experimental work reported in this thesis were performed by the author.

ANAESTHETIC: Ether was used as the anaesthetic throughout the operational procedures.

BILATERAL OVARIECTOMY:

The rat was anaesthetized, placed prone and the fur removed from a small area on the back of the animal. A small vertical mid-line incision was made through the skin over the first lumbar vertebra, the skin retracted on either side ofthe spine so that the peri-ovarian fat deposits could be identified. After incising the fascia over the fat deposits with the tip of scissors, the ovary was exposed th e fallopian tubes and ovarian blood supply ligated and severed before removal of the ovary. The same procedure was used to remove the remaining ovary. The incisions in the fascia and skin were sutured and bathed in antiseptic solution.

Completeness of removal of the ovaries was ascertained by examination of vaginal smears for red blood cells.

BILATERAL ADRENALECTOMY:

The rat was anaesthetized, placed prone and the fur removed from a small area on the back of the animal. A small vertical mid-line incision was made through the skin over the neural spine of the last thoracic vertebra. The skin was retracted on both sides of the spine and the angle between the last rib and the vertebra on one side was carefully explored. With the tip of scissors the fascia was incised and the adrenal gland exposed. The adrenal vein and blood supply were exposed, ligated and severed before the removal of the adrenal gland. The same procedure was used to remove the other gland. The fascia and skin were sutured and bathed with antiseptic solution

Rats were kept on 0.9% saline in place of tap water following the operation.

Surgical removal of the adrenal gland was confirmed by a post mortem examination of the peritoneal cavity for the adrenal tissue.

SECTION III

INJECTION SCHEDULE.

Animals were allowed to recuperate from surgical manipulation for a period of 5 to 7 days before injection schedules were commenced. Steroids for investigation were purchased from Sigma Chemicals, except for Megestrol acetate given by B.D.H. and Chlormadinone acetate a gift from Dr Christie of Syntex Pharmaceuticals. The steroids were solubilised or suspended in corn vegetable oil and given subcutaneously in a volume of 0.10 ml or 0.20 ml at alternate sites daily. The last injection was given the night before the oral glucose tolerance test.

THE ORAL GLUCOSE TOLERANCE TEST. (O.G.T.T.)

Animals were fasted for 16 hours prior to the start of the glucose tolerance test with free access to drinking water or 0.90% saline. All glucose tolerance tests were performed between 10.00 am and 2.00 pm in order to minimize the possibility of diurnal variation and its effects on the glucose tolerance test as described by Ben-Dyke (1971) The animals were brought from the animal house and anaesthetized at 10.00 am with 6.0 mg sodium pentobarbitone (Abbott Labs. Kent. U.K.) given intraperitoneal, subsequent injections were given throughout the glucose tolerance test to maintain the animals in a lightly anaesthetized state.

Sodium pentobarbitone has been reported to have no effect on carbohydrate metabolism (Biebuyck&Alberti (1971) or on the ability of the pancreas to secrete insulin, Kizer, et al (1970)

The animals were kept warm, and glucose solution supplied by St. Marys Hospital Pharmacy at a concetration of 50 g/100ml was given by gastric tube , 1 hour after anaesthesia. The glucose load given to each rat was 2g/kg body weight.
Blood samples were collected at 30 minutes and 45 minutes after anaesthesia, these were fasting 1 and fasting 2 respectively, then at 15. 30, 60, 90 and 120 minutes after the glucose load was given.

COLLECTION OF SAMPLES AND STORAGE. :

Approximately 0. 20-0. 30 ml of mixed arterial-venous blood was obtained from a tail snip by gentle stroking over a period of one minute. The blood was collected into heparinized tubes and after gentle mixing 0. 10 ml of blood was collected with a Hamilton micropipette and squirted into 0. 40 ml of 0. 6 M ice cold perchloric acid, mixed vigorously in order to precipitate blood proteins and inhibit enzyme activity. This precipitated blood sample was immediately centrifuged at 4°C and 2000 rpm, the supernatant removed with a pasteur pipette, placed into Technicon autoanalyzer cups, capped , and stored frozen at -20°C until assayed for pyruvate.

The remainder of the blood sample was immediately centrifuged at 4°C and 2000 rpm . The plasma removed, placed into Beckmann microfuge tubes, capped and stored frozen at -20°C until assayed for glucose and insulin.

SECTION IV

CHEMICAL METHODS AND ASSAY PROCEDURES.

Glucose Estimation. Plasma samples were thawed and well mixed 33μl of plasma was then drawn into a 100 μl Hamilton syringe and diluted to the 100 μl mark using distilled water (1/3 dilution.). The contents of the syringe was then squirted into a Technicon autoanalyzer cup and mixed thoroughly.

Glucose was assayed on the Technicon autoanalyzer using the method of Cramp, D.G. (1967)[..].

In this method glucose is oxidised by glucose oxidase to gluconic acid. Hydrogen peroxide which is also formed in this reaction is broken down to water and oxygen by the enzyme peroxidase in the presence of an oxygen acceptor 0-tolidine hydrochloride which is converted to a coloured compound, the amount of which is read colorimetrically.

Glucose standards were made up fresh before each assay in the range 20 mg/100 ml. to 100 mg/100ml. Quality control serum from Hyland (Travenol Labs.) was also run with each assay in order to minimize between assay variability. Samples from control groups and steroid treated groups were always run in the same assay in order to minimize the variability of assay between these two groups. Glucose values were read from the standard curve and multiplied by the dilution factor 3 in order to give original plasma glucose concentration in mg/100ml.

Pyruvate Estimation: The perchloric acid extract was thawed and well mixed. Pyruvic acid was assayed on the Technicon autoanalyzer using the fluorimetric method of Cramp, D.G., (1968)[...], with only the slight modification of using 0.40M, pH 7.4 potassium phosphate buffer in place of the Triethanolamine hydrochloride buffer used in the original method.

This method, measures the decrease in native fluorescence of the reduced form of nicotinamide adenine dinucleotide, NADH 2 as it is oxidized to NAD^+ when coupled with the enzyme lactate dehydrogenase in the conversion of pyruvic acid to lactic acid.

Pyruvate standards were made up fresh before each assay in the range 0.25 mg/100 ml. to 2.0 mg/100ml. Quality controls were made up fresh before each assay using buffered sodium pyruvate salt from B.D.H. Chemicals and run with each assay in order to minimize between assay variability. Samples from the control groups and steroid treated groups were always run in the same assay in order to minimize variability of assay between these two groups.

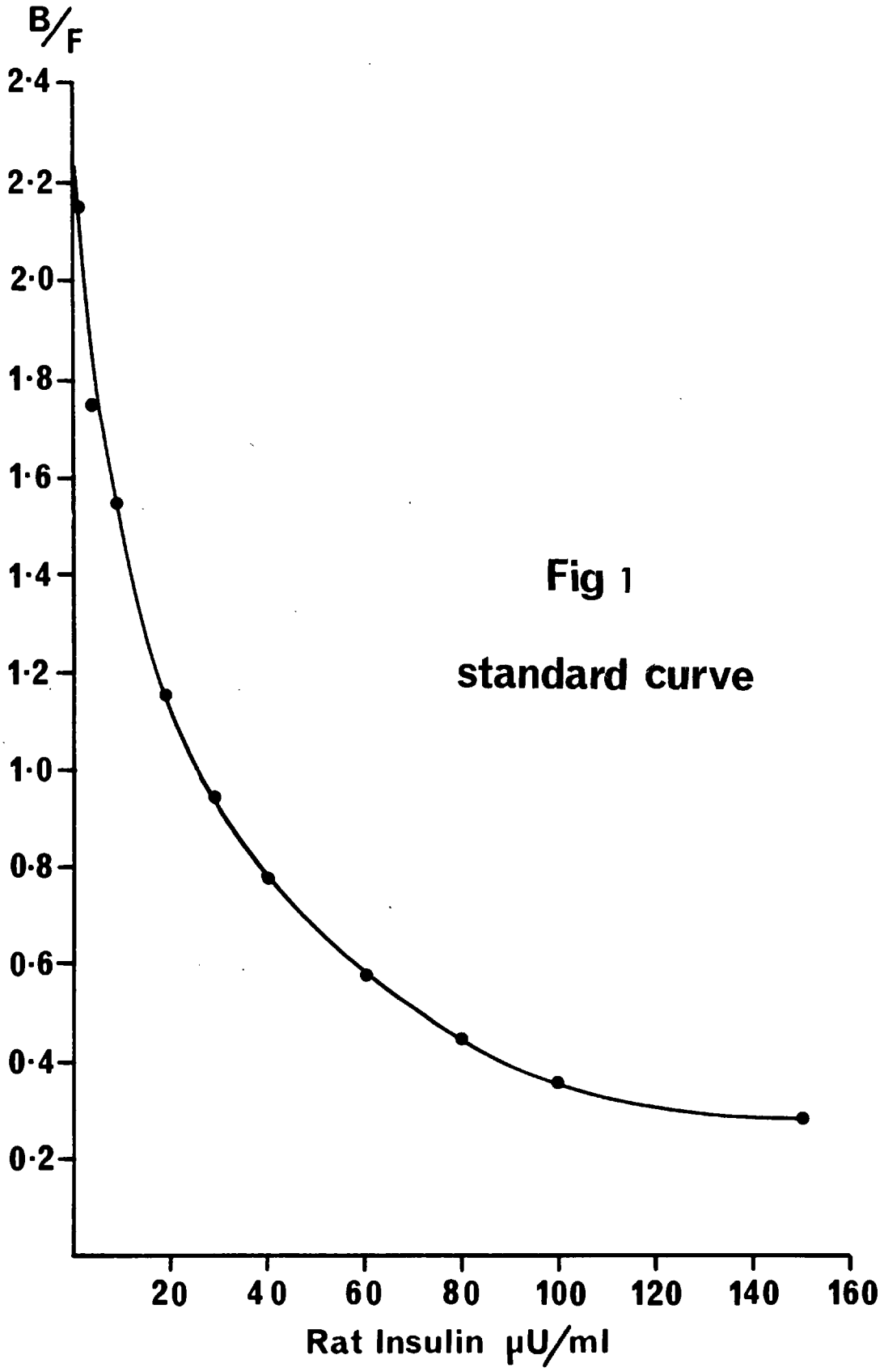


Fig 1

standard curve

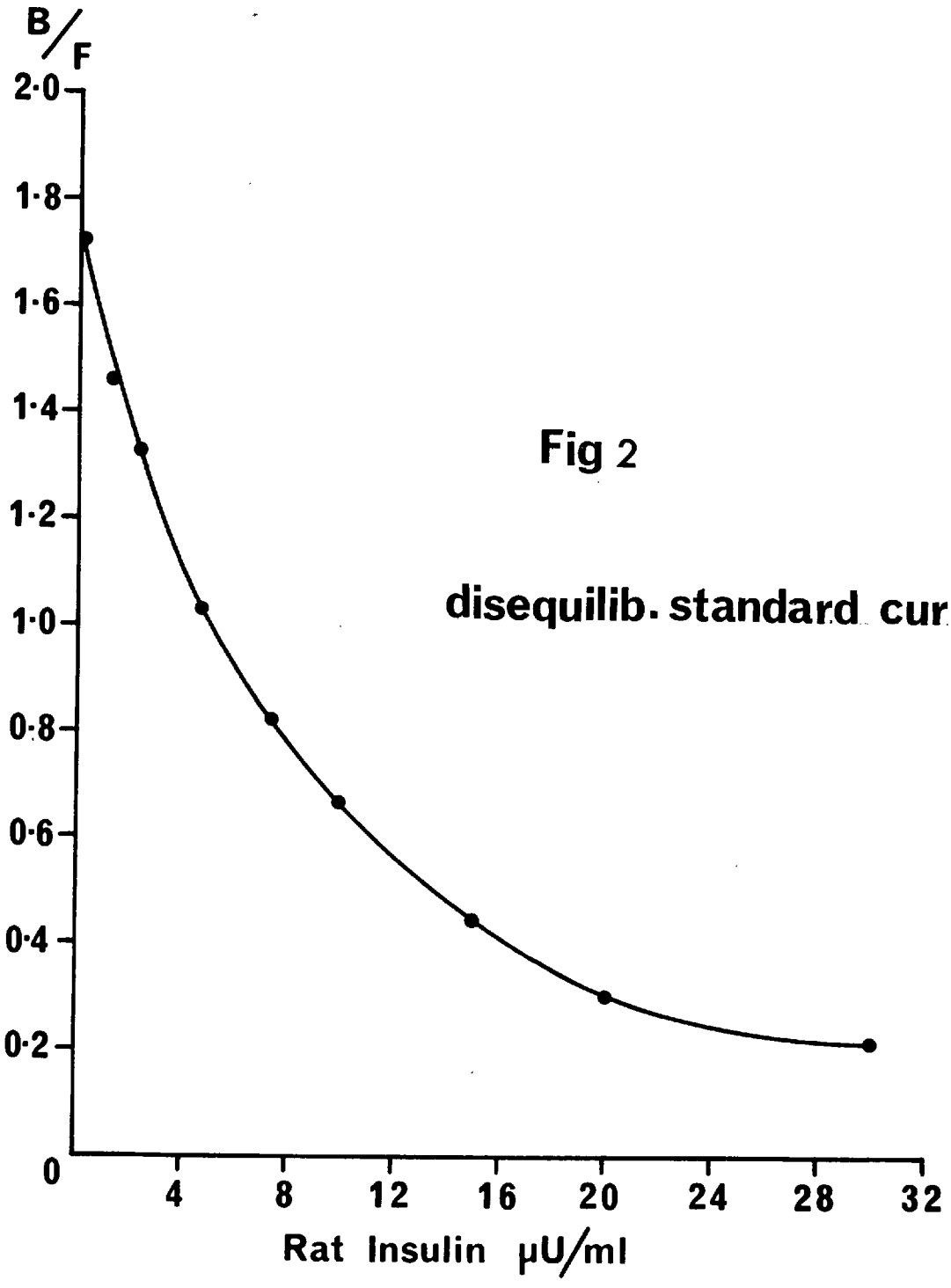


Fig 2

disequilib. standard curve

Pyruvate values were read from the standard curve and corrected for dilution by multiplying the reading by 1.66.

Insulin Immunoassay:

Insulin was assayed using the method of Albano-Ekins, Martiz and Turner (1972) which uses albumin coated charcoal to separate the bound and free hormone. Because of the restricted availability of plasma, the normal procedure was modified in order to handle the smaller sample sizes, 10μl., whilst maintaining precision and reliability of the assay. The plasmas from the control group of animals were always run in the same assay with steroid treated test groups so as to minimize variability between these two groups.

Materials: The assay was carried out in phosphate buffer pH 7.4. A concentrated stock solution of 7.8 g. $NaH_2PO4.\ 2H_2O$ plus 101.2g. Na_2PHO4 in litre was made which is subsequently diluted 1:4 with distilled water. Crystallized bovine plasma albumin (Armour Pharmaceuticals) at a concentration of 0.3g/100ml. was dissolved in the phosphate buffer; this is the working phosphate plasma albumin buffer, PO4/BPA, in which the assay was carried out and all subsequent dilutions of standards, radioactive tracer and antiserum are made.

Standards for the assay were made from rat insulin (Novo Industries, Copenhagen)which was kept as a stock solution of 10 μU/μl. in 0.5g% human serum albumin, veronal acetate buffer pH 8.6 and stored frozen at -20^{o}C. Dilutions of this were made to give the working range of standards.

Insulin ^{125}I Code No. IM 38 with a specific activity of 100 to 200 μCi/μg. was obtained from Amersham Radiochemical Centre, Amersham, England.

The antiserum used in the assay was Wellcome anti-insulin serum Code MR 09/10. This antiserum produced in guinea pig demonstrated very good cross reaction with rat insulin standard (See Fig. 1 and Fig 2.)

The standard curve for the assay was performed in the presence of insulin free plasma so that approximately equivalent protein concentration was maintained in the standards and test plasmas. This insulin free plasma was prepared by mixing fasting plasma with 1g. Norit OL charcoal per 10 ml of plasma for a period of 2 hours followed by filtering and ultracentrifugation for 30 minutes.

The clear plasma was stored in 2 ml. aliquots at 20°C.

METHOD:

The incubation tubes (glass 53 x 10 mm.) were set up in duplicate with the following constituents;

Blank or Control Tube

(i) PO4/B. P. A. Buffer 640μl.

(ii) Insulin ^{125}I 100 μl.

(iii) Plasma 10 μl.

Standard Curve Tube

(i) PO4/B. P. A. Buffer 540 μl.

(ii) Insulin ^{125}I 100 μl.

(iii) Plasma (insulin free) 10 μl.

(iv) Antiserum 50 μl.

(v) Standard 50 μl.

Sample Assay Tube

(i) PO4/B. P. A. Buffer 590 μl.

(ii) Insulin ^{125}I 100 μl.

(iii) Plasma 10μl.

(iv) Antiserum 50 μl.

The plasma samples and quality controls for assay were thawed and mixed thoroughly on a Rotomix, 10 μl. aliquots were taken using a Hamilton syringe. The standards were also added using a Hamilton syringe, insulin ^{125}I tracer, antiserum and PO4/BPA. buffer was added using Hamilton repette syringes.

A pair of blanks containing insulin free plasma or test plasma was sufficient to allow for individual "damage" correction for the standard curve and test plasmas respectively. The "damage" fraction in this instance being defined as that fraction of insulin which is immunologically inactive, or material which will not bind to charcoal. The concentration of insulin ^{125}I tracer and the antiserum used in the assay were diluted appropriately according to the sensitivity required.

The disequilibrium technique, used for maximum sensitivity in the range 0 - 25μU/ml. insulin, had a three day incubation period with antiserum at a concentration of $\frac{1}{750,000}$ final dilution, prior to the addition of 27 pg. insulin tracer; followed by a further three day incubation period after the tracer addition. In the normal assay range 0 - 150 μU/ml., 80 pg. insulin ^{125}I tracer and the antiserum at a final dilution of $\frac{1}{450,000}$ were added on the same day and incubated for a period of 65 hours at 4^{o}C.

The incubation was stopped by the addition of 100 μl. coated Norit OL Charcoal. The charcoal was coated by suspending 1g. of charcoal in 20 mls. of o.3 g/100 ml PO4/B.P.A. buffer for one hour. The charcoal incubation mixture was mixed thoroughly and allowed to stand for 30 minutes at 4^{o}C before centrifugation at 4^{o}C and 2500 rpm. for 30 minutes. The supernatant containing the antibody bound hormone was removed and placed into a plastic vial, capped and counted with its respective counterpart charcoal "button".

Counting was done in either the Wallac 80,000 or Packard 3002 auto gamma spectrometer and count rates of 1 0,000 to 15,000 counts per 5 minutes were regarded as statistically valid for calculation of the B/F ratio.

This B/F ratio = $\frac{\text{(counts in supernatant)}}{\text{counts on charcoal}}$ was calculated and a standard curve plotted, using B/F on the ordinate axis and insulin concentration in μU/ml. on the abscissa. (See figs. 1 and 2). The value read from the standard curve was corrected for dilution to give absolute plasma insulin concentration in μU/ml.

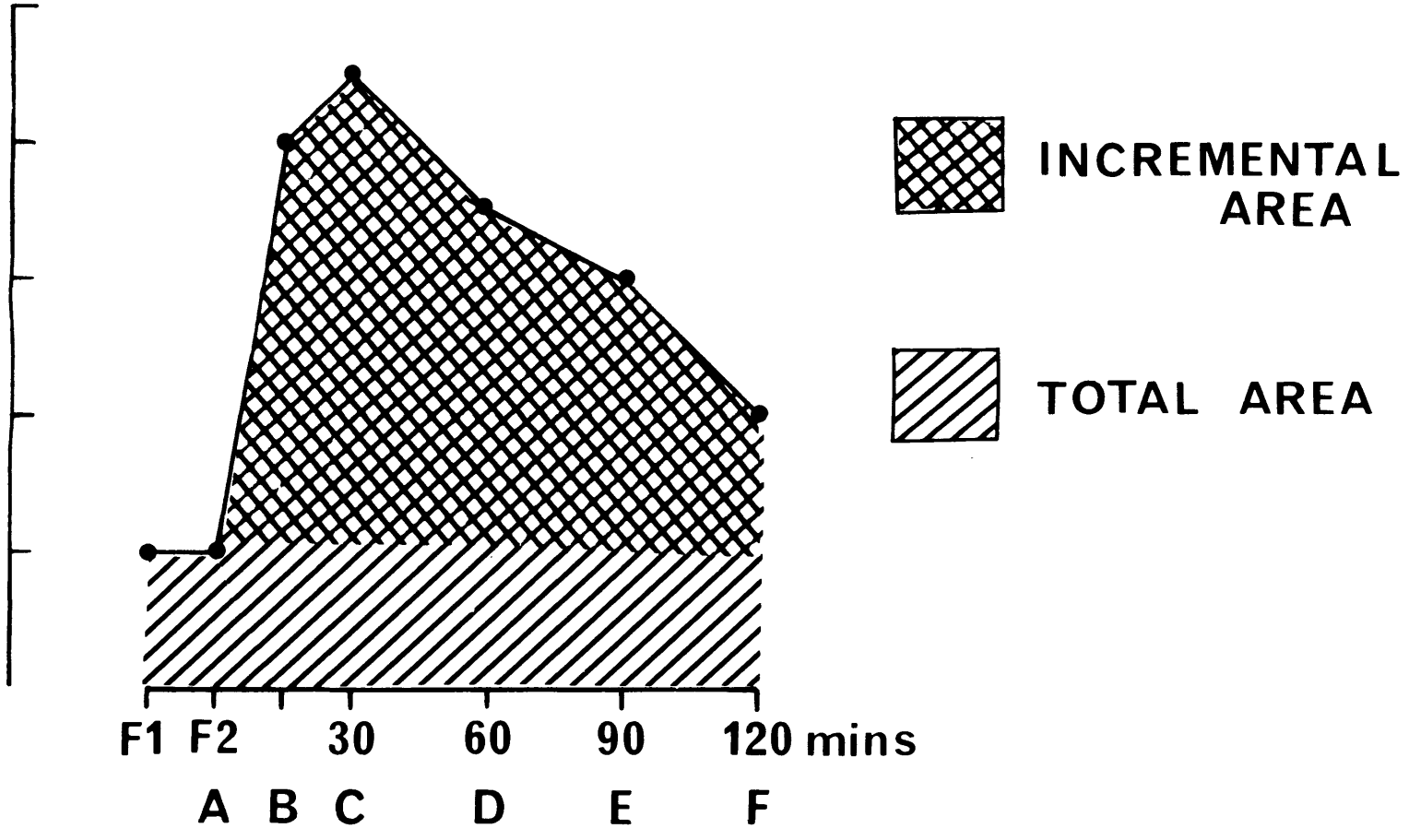
SECTION V

CALCULATIONS:

All calculations and statistical analysis in this work were carried out on the Olivetti Programma 101 computer. The Oral Glucose Tolerance Test (O.G.T.T.) was investigated in two ways.

See Fig. 2.A.

Fig. 2A



i. The mean and standard error of mean was calculated at each time interval for the parameters glucose, pyruvate and insulin. These values plotted against time permitted the indentification of the overall shape and pattern of response for these parameters during the O.G.T.T.

ii. Using the method of triangulation, the 120 minute and 60 minute total and incremental areas were calculated for each of the three parameters. These measurements allowed a quantitative estimate to be made for the metabolism of glucose, the formation of pyruvate and the secretion of insulin during the O.G.T.T.

The area calculations were made using the second fasting sample, since it was found that plasma glucose, insulin and blood pyruvate values were steady at this time after anaesthesia.

Formulae for area calculations, area units are Units-min.

Glucose and Insulin

$$120 \text{ minute total area} = \frac{\frac{1}{2}A + B + 1\frac{1}{2}C + 2D + 2E + F}{2}$$

120 minute incremental area = 120' total area - 4A

$$60 \text{ minute total area} = \frac{\frac{1}{2}A + B + 1\frac{1}{2}C + D}{2}$$

60 minute incremental area = 60' total area - 2A

Pyruvate

$$120 \text{ minute total area} = \frac{1}{2}A + C + D + E + \frac{1}{2}F$$

120 minute incremental area = 120' total area - 4A

$$60 \text{ minute total area} = \frac{1}{2}A + C + \frac{1}{2}D$$

60 minute incremental area = 60' total area - 2A

STATISTICAL ANALYSIS

The Student's t test for unpaired data incorporating Bessel's correction for small numbers in the populations under study was used for testing the difference between means. Because of the large variability sometimes occurring in the data, the variance ratio F was calculated before the t test was applied.

$$F = \frac{\text{Greater estimate of variance of population}}{\text{Lesser estimate of variance of population}}$$

If F with (N-1) degrees of freedom was less than the corresponding value in the 5% table, the variances were regarded as not being significantly different and the Student's t test was applied.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left[\frac{(N_1-1)S_1^2 + (N_2-1)S_2^2}{N_1 + N_2 - 2}\right]\left[\frac{1}{N_1} + \frac{1}{N_2}\right]}}$$

The probability level (p) was determined from the (t) value with $(N_1 + N_2 - 2)$ degrees of freedom. A (p) value < 0.05 was regarded as being significant in the analysis of data.

However if the F. ratio was greater than the corresponding value in the 5% table, the variances were regarded as being significantly different and Cochran and Cox's modification of the t test was applied as follows:-

The variance of each mean was calculated separately.

$$\text{Variance of mean} = \left.\frac{\sum(x - \bar{x})^2}{n-1}\right/ n$$

The variance of the mean difference was the sum of these two variances and the standard error of the difference = sum of variance for each mean.

The ratio $\frac{\bar{x} - \bar{x}_2}{\sqrt{\text{sum of variance for each mean}}} = t$, was calculated.

An approximation to the 5% probability level of this ratio was computed as the weighted means of two values of t. These values were taken from the t-table and were the values corresponding to the t (5%) for (n_1-1) and (n_2-1) degrees of freedom.

The weights were the two variances $\left(\left.\frac{\sum(x - \bar{x})^2}{n-1}\right/ n \right)$ for each mean.

$$5\% \text{ level} = \frac{t_1 \times \text{variances}_1 + t_2 \times \text{variance 2.}}{\text{variance 1} + \text{variance 2}}$$

If t was greater than the Cochran 5% level, the difference between means was regarded as significantly different.

SECTION V1

EVALUATION OF METHODS:

The methods used in the investigation were evaluated by replicate samples. The following table gives the mean and standard deviation for low and high range values for within assay and between assay variability for the three parameters measured.

	WITHIN ASSAY		BETWEEN ASSAY	
	Low range	High range	Low range	High range
Glucosemg/ 100ml	39 ± 1	76 ± 2	41 ± 1	76 ± 2
Pyruvate mg/ 100ml	0.68 ± 0.06	1.35 ± 0.04	0.63 ± 0.08	1.33 ±0.06
Insulin μU/ ml	6 ± 1	46 ± 3	9 ± 2	49 ± 4

CHAPTER 2

SECTION 1

THE EFFECT OF OESTRADIOL BENZOATE AT DIFFERENT DOSES ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE OVARIECTOMIZED RAT.

SECTION 11

THE EFFECT OF THE SYNTHETIC OESTROGEN, MESTRANOL, AT DIFFERENT DOSES ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVEL DURING AN O.G.T.T IN THE OVARIECTOMIZED RAT.

SECTION 111

THE EFFECT OF THE SYNTHETIC OESTROGEN, ETHINYLOESTRADIOL ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE OVARIECTOMIZED RAT.

INTRODUCTION.

In this section of work an attempt is made to define the effects of Oestradiol benzoate, Mestranol and Ethinyl oestradiol on glucose metabolism, pyruvate formation and the insulin response during an O.G.T.T.

The experiments were performed in vivo and are longitudinal in nature, i.e. the O.G.T.T. was done at 2 and 4 weeks, this is roughly equivalent to 5 and 10 cycles respectively in the human, as the rat ovulatory cycle is approximately 3 to 4 days in length.

The doses of the different steroids used were estimated on a weight basis from the work of Beck(1969), carried out in the monkey and are pharmacological rather than physiological doses. However the anovulatory dose of these steroids in the rat is of the order 10-50 μg administered sub-cutaneous, daily, Dorfman(1968)

A wide range of dose was employed to determine if there was a dose related effect of these steroids on the three parameters under investigation, glucose, pyruvate and insulin.

Fig. 3

OESTRADIOL BENZOATE SERIES AT 2 WEEKS

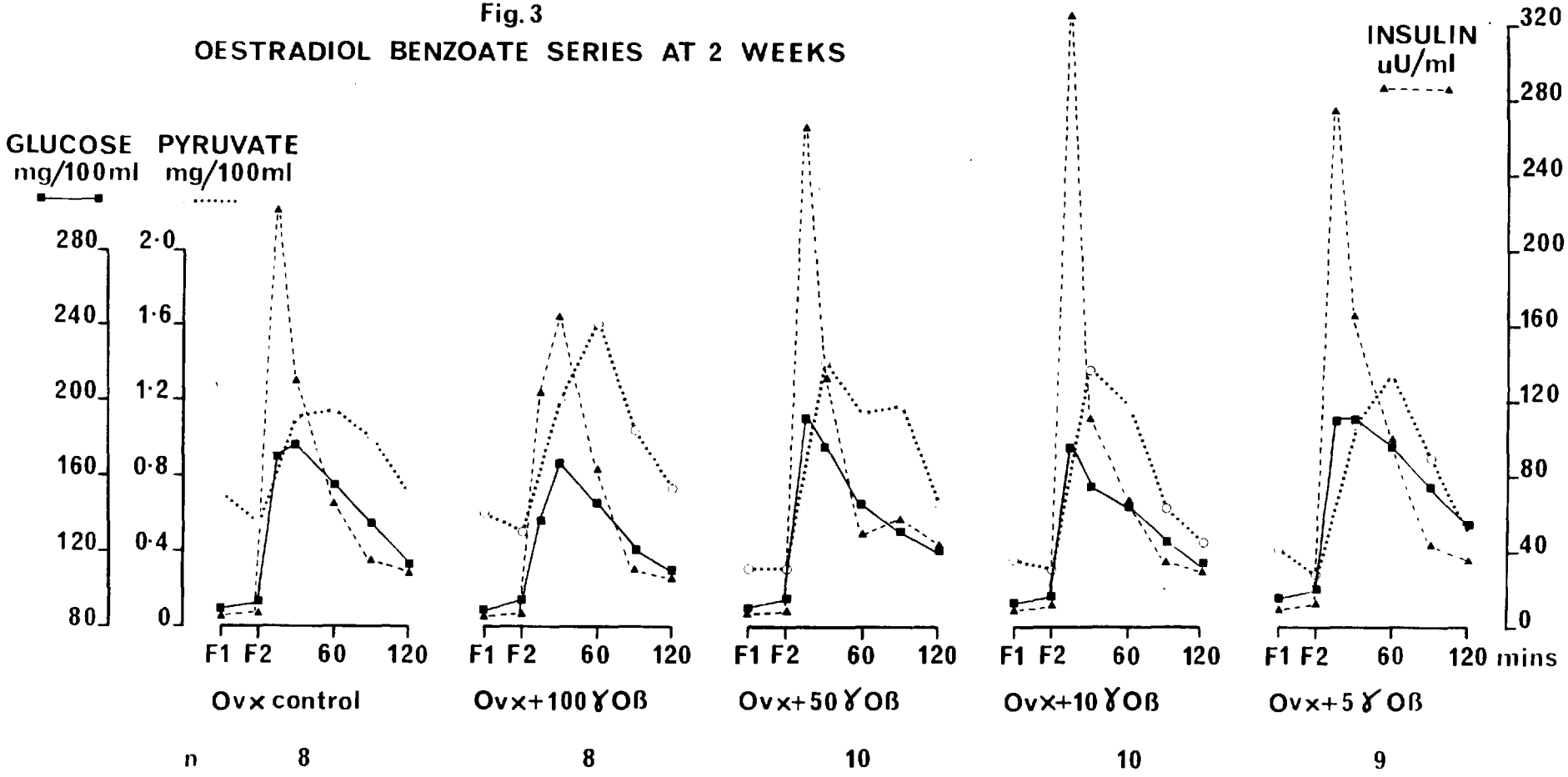


Fig.3A

OESTRADIOL BENZOATE SERIES AT 4 WEEKS

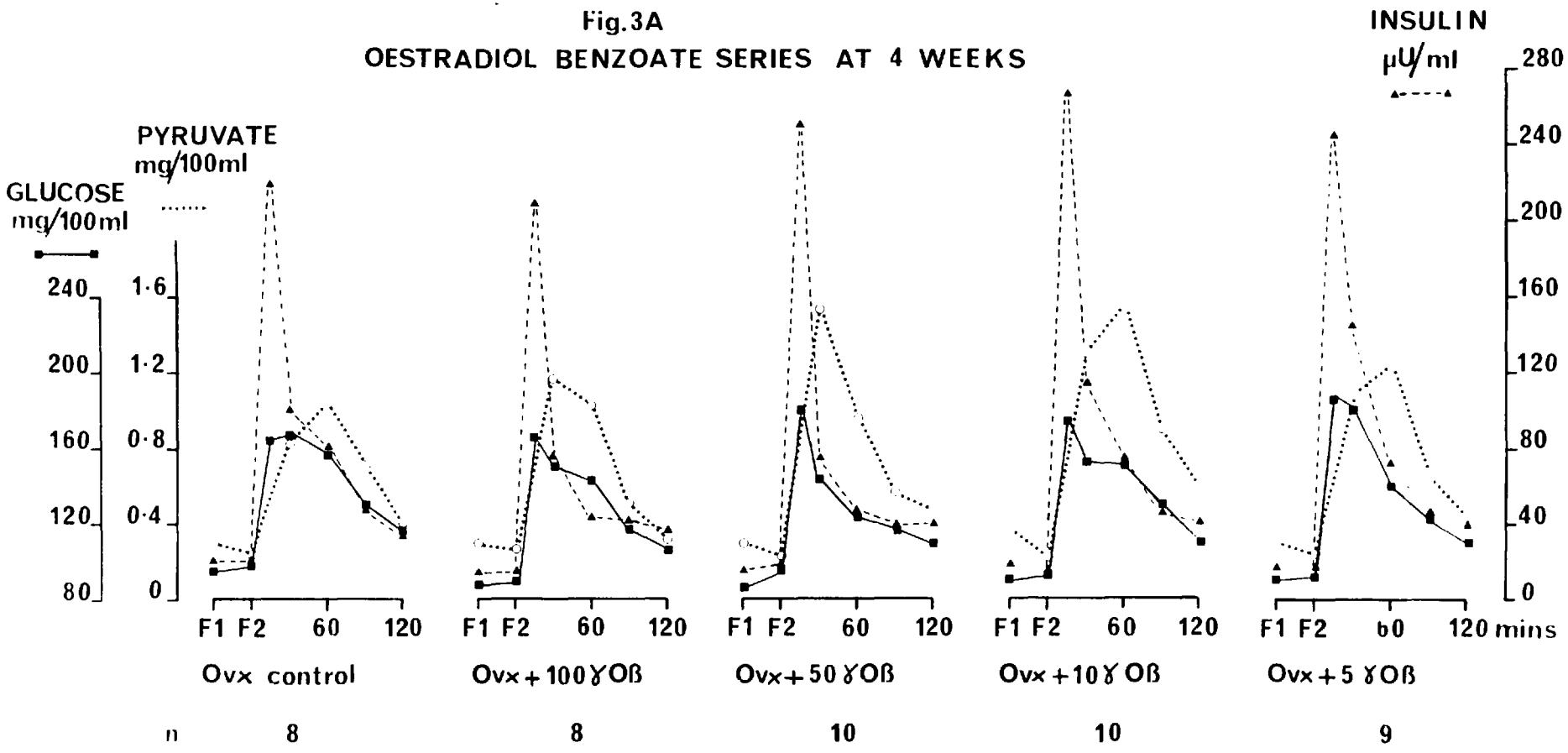


TABLE I

		F1	F2	15'	30'	60'	90'	120'
OVX 0.1 0ml. OIL Control N = 8	Glucose mg/100ml.	89 ± 1	92 ± 2	171 ± 10	177 ± 6	155 ± 4	132 ± 7	112 ± 4
	Pyruvate mg/100ml.	0.71 ± 0.02	0.55 ± 0.07	-	1.09 ± 0.10	1.15 ± 0.08	1.02 ± 0.13	0.68 ± 0.05
	Insulin μU/ml.	9 ± 1	9 ± 2	219 ± 37	131 ± 18	66 ± 10	34 ± 4	28 ± 5
OVX + 5μgE$_2$B N = 9	Glucose	97 ± 2	99 ± 2	190 ± 9	191 ± 7	176 ± 7	154 ± 6	135 ± 4
		< .01	< .025	N.S.	N.S.	< .05	< .05	< .005
	Pyruvate	0.53 ± 0.04	0.29 ± 0.03	-	1.12 ± 0.19	1.33 ± 0.21	0.89 ± 0.11	0.47 ± 0.09
		N.S.	< .05		N.S.	N.S.	N.S.	N.S.
	Insulin	7 ± 1	11 ± 2	273 ± 29	166 ± 31	97 ± 21	43 ± 11	37 ± 7
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX + 10μgE$_2$B N = 10	Glucose	93 ± 1	97 ± 1	178 ± 5	153 ± 5	143 ± 6	126 ± 5	115 ± 3
		< .01	< .05	N.S.	< .01	N.S.	N.S.	N.S.
	Pyruvate	0.38 ± 0.04	0.29 ± 0.03	-	1.38 ± 0.12	1.15 ± 0.13	0.61 ± 0.12	0.45 ± 0.11
		< .05	< .05		N.S.	N.S.	< .05	N.S.
	Insulin	10 ± 1	12 ± 2	334 ± 29	111 ± 21	64 ± 14	35 ± 7	29 ± 5
		N.S.	N.S.	< .05	N.S.	N.S.	N.S.	N.S.
OVX + 50μgE$_2$B N = 10	Glucose	90 ± 1	96 ± 1	190 ± 7	175 ± 5	145 ± 5	131 ± 7	121 ± 7
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Pyruvate	0.34 ± 0.08	0.33 ± 0.03	-	1.41 ± 0.18	1.13 ± 0.16	1.17 ± 0.15	0.64 ± 0.11
		< .05	< .05		N.S.	N.S.	N.S.	N.S.
	Insulin	11 ± 1	11 ± 2	265 ± 18	130 ± 27	50 ± 14	56 ± 14	39 ± 10
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX + 100μg E$_2$B N = 8	Glucose	90 ± 2	94 ± 2	138 ± 7	165 ± 8	144 ± 7	121 ± 5	110 ± 4
		N.S.	N.S.	< .02	N.S.	N.S.	N.S.	N.S.
	Pyruvate	0.60 ± 0.08	0.51 ± 0.05	-	1.24 ± 0.12	1.59 ± 0.20	1.04 ± 0.12	0.73 ± 0.09
		N.S.	N.S.		N.S.	N.S.	N.S.	N.S.
	Insulin	9 ± 1	8 ± 1	124 ± 18	164 ± 44	83 ± 21	31 ± 4	24 ± 3
		N.S.	N.S.	< .05	N.S.	N.S.	N.S.	N.S.

TABLE I: Mean ± SEM O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for oestradiol benzoate series at 2 weeks. (N.S. = not significant.)

(E_2B = Oestradiol benzoate)

TABLE I A

		F1	F2	15'	30'	60'	90'	120'
OVX 0.10ml. Oil Control N = 8	Glucose mg/100ml.	92 ± 2	97 ± 2	166 ± 5	167 ± 4	157 ± 3	128 ± 3	117 ± 4
	Pyruvate mg/100ml.	0.30 ± 0.05	0.22 ± 0.02	-	0.84 ± 0.07	1.05 ± 0.07	0.71 ± 0.13	0.37 ± 0.05
	Insulin μU/ml.	16 ± 2	15 ± 3	219 ± 34	100 ± 10	79 ± 11	48 ± 9	35 ± 6
OVX+5μgE_2B N = 9	Glucose	87 ± 1	91 ± 1	186 ± 8	182 ± 7	142 ± 7	121 ± 5	110 ± 5
			$<.05$	$<.05$	N.S.	N.S.	N.S.	N.S.
	Pyruvate	0.29 ± 0.03	0.24 ± 0.03	-	1.08 ± 0.13	1.24 ± 0.16	0.66 ± 0.13	0.44 ± 0.10
		N.S.	N.S.		N.S.	N.S.	N.S.	N.S.
	Insulin	16 ± 2	14 ± 1	243 ± 43	147 ± 22	73 ± 21	43 ± 7	41 ± 9
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX+10μgE_2B N = 10	Glucose	90 ± 2	92 ± 1	173 ± 5	153 ± 4	152 ± 5	132 ± 4	110 ± 3
		N.S.	N.S.	N.S.	$<.05$	N.S.	N.S.	N.S.
	Pyruvate	0.38 ± 0.01	0.22 ± 0.02	-	1.31 ± 0.21	1.58 ± 0.12	0.90 ± 0.10	0.61 ± 0.07
		N.S.	N.S.		N.S.	$<.05$	N.S.	$<.05$
	Insulin	18 ± 2	16 ± 2	267 ± 36	117 ± 12	74 ± 11	49 ± 6	40 ± 7
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX+50μgE_2B N = 8	Glucose	87 ± 3	93 ± 2	184 ± 8	145 ± 6	124 ± 7	118 ± 5	109 ± 3
		N.S.	N.S.	N.S.	$<.01$	$<.05$	N.S.	N.S.
	Pyruvate	0.27 ± 0.03	0.21 ± 0.02	-	1.55 ± 0.22	0.97 ± 0.22	0.58 ± 0.12	0.47 ± 0.09
		N.S.	N.S.		$<.05$	N.S.	N.S.	N.S.
	Insulin	15 ± 2	18 ± 2	215 ± 59	75 ± 14	43 ± 7	38 ± 5	40 ± 6
		N.S.	N.S.	N.S.	N.S.	$p<.02$	N.S.	N.S.
OVX+100μgE_2B N = 8	Glucose	87 ± 3	88 ± 3	167 ± 6	149 ± 7	143 ± 7	118 ± 3	107 ± 3
		N.S.	$<.025$	N.S.	$<.05$	N.S.	$<.05$	$<.05$
	Pyruvate	0.30 ± 0.02	0.28 ± 0.02	-	1.19 ± 0.24	1.02 ± 0.24	0.52 ± 0.10	0.33 ± 0.02
		N.S.	N.S.		N.S.	N.S.	N.S.	N.S.
	Insulin	14 ± 2	11 ± 2	208 ± 41	77 ± 12	44 ± 6	39 ± 6	37 ± 6
		N.S.	N.S.	N.S.	N.S.	$<.02$	N.S.	N.S.

TABLE IA: Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for oestradiol benzoate series at 4 weeks. (N.S. = not significant)

(E_2B = Oestradiol benzoate)

120 Minute Areas

	Glucose Total Area Δ	Glucose Incremental Δ	Pyruvate Total Δ	Pyruvate Incremental Δ	Insulin Total Δ	Insulin Incremental Δ
OVX 0.10ml. Oil Control N=8	582 ± 11	214 ± 13	3.88 ± 0.19	1.70 ± 0.20	323 ± 38	289 ± 37
OVX + 5μg E_2B N = 9	661 ± 16 < .005	264 ± 16 < 05	3.55 ± 0.53 N.S.	2.39 ± 0.43 N.S.	423 ± 45 N.S.	381 ± 42 N.S.
OVX + 10 μg E_2B N = 10	555 ± 13 N.S.	168 ± 12 < .02	3.51 ± 0.23 N.S.	2.35 ± 0.25 N.S.	366 ± 22 N.S.	320 ± 22 N.S.
OVX + 50μg E_2B N = 10	589 ± 18 N.S.	201 ± 19 N.S.	4.20 ± 0.47 N.S.	2.89 ± 0.40 < .05	362 ± 42 N.S.	318 ± 42 N.S.
OVX + 100μg E_2B N = 8	534 ± 17 < 05	162 ± 19 < .05	4.49 ± 0.42 N.S.	2.44 ± 0.36 N.S.	313 ± 56 N.S.	282 ± 55 N.S.
60 Minute Areas						
OVX 0.10ml. Oil Control N = 8	318 ± 10	135 ± 10	1.94 ± 0.13	0.85 ± 0.09	243 ± 33	224 ± 32
OVX + 5μg E_2B N=9	351 ± 10 < .05	152 ± 9 N.S.	1.92 ± 0.30 N.S.	1.35 ± 0.27 N.S.	312 ± 33 N.S.	291 ± 31 N.S.
OVX + 10 μg E_2B N=10	300 ± 5 N.S.	106 ± 5 < .02	2.10 ± 0.13 N.S.	1.50 ± 0.13 < .005	285 ± 17 N.S.	261 ± 31 N.S.
OVX + 50 μg E_2B N=10	322 ± 8 N.S.	130 ± 9 N.S.	2.15 ± 0.25 N.S.	1.50 ± 0.21 < .05	258 ± 27 N.S.	236 ± 28 N.S.
OVX+100μg E_2B N=8	288 ± 12 N.S.	101 ± 13 N.S.	2.29 ± 0.22 N.S.	1.27 ± 0.20 N.S.	228 ± 47 N.S.	213 ± 46 N.S.

TABLE II: Mean ± S.E.M.(Units-Min.) O.G.T.T. 120 minute and 60 minute total and incremental areas with significance levels for oestradiol benzoate series at 2 weeks. (N.S. = not significant)

(E_2B = Oestradiol benzoate)

120 Minute Areas:	Glucose Total Area (Δ)	Glucose Incremental Δ	Pyruvate Total Δ	Pyruvate Incremental Δ	Insulin Total Δ	Insulin Incremental Δ
OVX 0.10ml. Oil Control N = 8	577 ± 10	190 ± 11	2.90 ± 0.17	2.02 ± 0.21	332 ± 31	272 ± 24
OVX + 5μg. E_2B N=9	569 ± 13 N.S.	202 ± 14 N.S.	3.33 ± 0.42 N.S.	2.38 ± 0.41 N.S.	369 ± 56 N.S.	313 ± 56 N.S.
OVX + 10μg. E_2B N=10	563 ± 8 N.S.	195 ± 9 N.S.	4.12 ± 0.36 < .01	3.33 ± 0.34 < .01	368 ± 30 N.S.	304 ± 27 N.S.
OVX + 50μg. E_2B N=10	520 ± 15 < .01	150 ± 15 < .05	3.44 ± 0.49 N.S.	2.59 ± 0.44 N.S.	287 ± 35 N.S.	216 ± 31 N.S.
OVX + 100μg. E_2B N=8	531 ± 10 < .01	180 ± 13 N.S.	3.02 ± 0.48 N.S.	1.91 ± 0.50 N.S.	290 ± 44 N.S.	245 ± 39 N.S.
60 Minute Areas:						
OVX 0.10ml. Oil Control N = 8	311 ± 5	117 ± 5	1.48 ± 0.08	1.04 ± 0.10	228 ± 21	198 ± 17
OVX + 5μg. E_2B N = 9	323 ± 9 N.S.	141 ± 8 < .05	1.80 ± 0.19 N.S.	1.35 ± 0.17 N.S.	270 ± 44 N.S.	242 ± 44 N.S.
OVX + 10μg. E_2B N = 10	301 ± 4 N.S.	116 ± 4 N.S.	2.22 ± 0.30 < .05	1.80 ± 0.28 < .05	262 ± 22 N.S.	230 ± 21 N.S.
OVX + 50μg. E_2B N =10	286 ± 10 N.S.	101 ± 8 N.S.	2.14 ± 0.29 < .05	1.72 ± 0.27 < .05	207 ± 33 N.S.	173 ± 31 N.S.
OVX + 100μg. E_2B N = 8	288 ± 6 < .02	113 ± 7 N.S.	1.84 ± 0.32 N.S.	1.28 ± 0.33 N.S.	199 ± 28 N.S.	176 ± 26 N.S.

TABLE IIA: Mean ± S.E.M. (Units-Min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels oestradiol benzoate series at 4 weeks. (N.S. = not significant).

E_2B = Oestradiol benzoate

TABLE II B Animal weights. Oestradiol benzoate. series.
Mean ± S.E.M. (body weight in grams)

	Initial	2 weeks	4 weeks
OVX Oil Control	186 ± 4	220 ± 4	252 ± 6
OVX + 5 μg O.B.	194 ± 4	214 ± 8	234 ± 10
OVX + 10 μg O.B.	186 ± 6	214 ± 4	220 ± 6
OVX + 50 μg O.B.	181 ± 5	188 ± 6	202 ± 9
OVX + 100 μg O.B.	212 ± 6	217 ± 6	231 ± 10

(O.B. = Oestradiol benzoate)

SECTION 1 OESTRADIOL BENZOATE SERIES

PROCEDURE:-

Ovariectomized (OVX) rats weighing approximately 200 - 250 gm were given sub-cutaneous injections of Oestradiol Benzoate (O. B.) dissolved in 0. 10 ml corn oil at the following doses; 5 μg, 10 μg, 50 μg and 100 μg. The animals were injected for 2 weeks after which time the first O. G. T. T. was performed, the rats were then maintained for a further 2 weeks at the same dosage shedules, at the end of which the second (4 week) O. G. T. T. was performed. The control animals received 0. 10 ml corn oil only.

RESULTS:

PLASMA GLUCOSE AT 2 WEEKS FIG. 3 ; TABLE 1

Fasting; The second fasting value was used for evaluation.

Animals receiving 5 μg and 10 μg O. B. had significantly elevated mean fasting plasma glucose values compared with control mean value, ($p < .025$) and ($p < .05$) respectively. The 50 μg and 100 μg doses demonstrated no significant difference in their mean fasting values from that of the control value.

DURING THE O. G. T. T.

At the 15 min interval after glucose loading, the only group that had a mean value that was significantly different from the control group was the glucose value for the 100 μg O. B. group, ($p < .02$). At the 30 min interval the 10 μg group had a mean value that was significantly less than the control value, ($p < .01$); the other groups had mean values that were not significantly different from the control value. At the 60, 90 and 120 min. intervals the mean values for the 10 μg, 50 μg and 100 μg groups were lower though not significantly different from the control value. The 5 μg group demonstrated significantly elevated mean values compared with control value, for the 60 min and 90 min values ($p < .05$), and the 120 min interval, ($p < .005$).

In general the 5 μg O. B. group demonstrated mean values that were significantly greater than the respective control mean values, whilst

the 10 μg, 50 μg and 100 μg demonstrated mean values at most time intervals that were lower than the respective mean control values.

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS TABLE 11

120 Minute Glucose Areas:- The 5 μg group total and incremental areas were significantly greater than the respective control areas, ($p < .005$) and ($p < .05$) respectively. The 10 μg group had total and incremental areas that were lower than the respective control areas, however only the incremental area was significantly lower ($p < .02$). The total and incremental areas for the 50 μg group were not significantly different from the respective control areas. The 100 μg group total and incremental areas were significantly less than the respective control areas, ($p < .05$) for both areas.

60 Minute Glucose Areas:- The 5μg group demonstrated a significantly elevated total area, ($p < .05$) but not incremental area compared with respective control areas. The 10 μg group had total and incremental areas lower than the respective control areas, however only the incremental area was significantly lower ($p < .02$). The 50 μg group total and incremental areas were not significantly different from the control areas. The 100 μg group had lower total and incremental areas compared with the respective control areas, however no area was significantly different from control area.

BLOOD PYRUVATE AT 2 WEEKS FIG 3 TABLE 1

Fasting

The second fasting mean pyruvate values are used in evaluation in this section and subsequent sections later in the series. This was done because it was found that at this time interval, approximately 45 mins. after anaesthesia, that the blood pyruvate remained steady. The groups receiving 5 μg, 10 μg and 50 μg O. B. had mean values that were significantly lower than the control mean value, ($p < .05$) for all groups. The 100 μg group mean value was not significantly different from the control mean value.

DURING THE O.G.T.T.

At the 30 min interval the mean values for all O.B. treated groups were greater than the mean control value, however no value was significantly different from the control value. Except for the 10 μg group 90 min value which was significantly lower than the control mean value ($p < .05$); the mean values for all O.B. treated groups at the 60 min, 90 min and 120 minute intervals were not significantly different from the respective control mean values.

MEAN O.G.T.T. PYRUVATE AREAS AT 2 WEEKS TABLE 11

120 Minute Areas:-

The 5 μg, 10 μg and 100 μg groups had total and incremental areas that were greater than the respective control areas, however no area was significantly different from the control areas. The 50 μg total and incremental areas were also greater than the respective control areas, but only the incremental area was significantly greater than the control, ($p < .05$).

60 Minute Areas:-

The total and incremental areas for all O.B. treated groups were greater than the respective control areas. The 10 μg and 50 μg groups had incremental areas that were significantly greater than the control incremental area, ($p < .005$) and ($p < .05$) respectively; the total areas for these two groups were not significantly different from the control areas. The 5 μg and 100 μg groups had total and incremental areas that were not significantly different from the respective control areas.

PLASMA INSULIN AT 2 WEEKS FIG 3 TABLE 1

Fasting:-

All O.B. groups had mean values that were not significantly different from the control mean value.

DURING THE O.G.T.T.

At the 15min interval, the 5 μg, 10 μg and 50 μg groups all had mean values that were greater than the control mean value, however only the 10 μg group value attained significance, ($p < .05$). The 100 μg group had a significantly lower mean value compared with the control, ($p < .05$).

At the 30, 60, 90 and 120 min intervals no O.B. group had a mean value that was significantly different from the control mean value. The 5 μg group mean values at these time intervals had a tendency to be greater than the respective control mean values.

MEAN O.G.T.T. INSULIN AREAS AT 2 WEEKS TABLE 11

120 Minute Areas:-

The total and incremental areas for all O.B. groups were not significantly different from the respective control areas. However there was a tendency for the 5 μg, 10 μg and 50 μg groups to have areas that were slightly greater, and the 100 μg group to have areas that were slightly less compared with control areas.

60 Minute Areas:-

The total and incremental areas for all O.B. groups were not significantly different from the respective control areas. However as for the 120 minute areas there was a tendency for the 5, 10 and 50 μg groups to have areas slightly greater and the 100 μg group to have areas slightly less than the control areas.

GLUCOSE AT 4 WEEKS FIG 3 A TABLE 1A

Fasting:-

All O.B. groups had mean values that were lower than the control mean value, however only the 100 μg group was significantly lower, ($p < .025$).

DURING THE O.G.T.T.

At the 15 min interval the 5 μg O.B. group had a mean value

that was significantly greater than the control mean value ($p < .05$).

At the 30 min. interval the 5 μg group had a mean value that was greater than the control mean value, but was not significant. The 10 μg, 50 μg and 100 μg groups had mean values that were significantly less than the control mean value, ($p < .05$) for 10 μg and 100 μg groups and ($p < .01$) for the 50 μg group. At the 60 min interval all the O.B. groups had mean values that were less than the mean control value, however only the 50 μg group was significantly less, ($p < .05$).

At the 90 min and 120 min intervals the 5 μg, 10 μg and 50 μg groups had mean values that were not significantly different from the control mean value. The 100 μg group had mean values that were significantly lower than the control mean values, ($p < .05$) for both time intervals.

MEAN O.G.T.T. GLUCOSE AREAS AT 4 WEEKS TABLE 11 A

120 Minute Areas:-

The total and incremental areas for the 5 μg and 10 μg O.B. groups were not significantly different from the respective control areas . The 50 μg and 100 μg groups had total areas that were significantly less than the total area for the control, ($p < .01$) for both group areas. The incremental areas for the 50 μg and 100 μg groups were less than the controls but only the 50 μg group was significantly less ($p < .05$).

60 Minute Areas:-

The total and incremental areas for the 5 μg group were greater than the respective control areas, however only the latter area was significant, ($p < .05$). The total and incremental areas for the 10 μg and 50 μg groups were not significantly different from the respective control areas. The 100 μg total and incremental areas were both less than the control areas but only the total area was significant, ($p < .02$).

BLOOD PYRUVATE AT 4 WEEKS FIG 3A TABLE 1A

Fasting:-

The mean values for all O. B. treated groups were not significantly different from the control mean value.

DURING THE O.G.T.T.

At the 30 min interval all O. B. groups had mean values that were greate r than the control mean value, with only the 50 μg group mean value significantly greater than the control ($p < .05$). At the 60 min interval the 10 μg group had a mean value that was significantly greater than the control mean value, ($p < .05$).

At the 90 min and 120 min intervals, the mean values for all O. B. groups were not significantly different from the respective control mean values.

MEAN O.G.T.T. PYRUVATE AREAS AT 4 WEEKS TABLE 11A

120 Minute Areas:-

The total and incremental areas for the 5 μg, 50 μg and 100 μg groups were not significantly different from the respective control areas. The total and incremental areas for the 10 μg group were significantly greater than the respective control areas, ($p < .01$) for both areas.

60 Minute Areas:-

All groups demonstrated total and incremental areas that were greater than the respective control areas, however only the 10 μg and 50 μg groups were significantly greater, ($p < .05$) for both areas for the two groups.

PLASMA INSULIN AT 4 WEEKS FIG 3 A TABLE 1A

Fasting:- All O. B. groups had mean values that were not significantly different from the control mean value.

DURING THE O.G.T.T.

At the 15 min. interval, the 5 μg, 10 μg and 50 μg groups demonstrated mean values ; that were greater than the control mean value but were not significant. The 100 μg group had a mean value less than the control mean value but was not significant.

At the 30 min. interval the 5 μg and 10 μg groups had mean values that were greater than the control mean value, and the 50 μg and 100 μg groups had mean values that were less than the controls, however no value was significantly different from the control value.

At the 60 min interval all O. B. groups had a mean value less than the control mean value, however only the 50 μg and 100 μg groups were significantly less, ($p<.02$) for both groups.

The 90 min and 120 min interval values for all O. B. treated groups were not significantly different from the control mean values.

MEAN O.G.T.T. INSULIN AREAS AT 4 WEEKS TABLE 11A

120 Minute Areas:-

The total and incremental areas for the 5 μg and 10 μg groups were greater and the 50 μg and 100 μg groups less than the respective control areas, however no O. B. group area was significantly different from the respective control areas.

60 Minute Areas:-

The 5 μg and 10 μg groups had total and incremental areas greater, and the 50 μg and 100 μg groups had areas less than the respective control areas, however no area was significantly different from its control area.

Fig. 4

MESTRANOL / ETHINYL OESTRADIOL SERIES AT 2 WEEKS

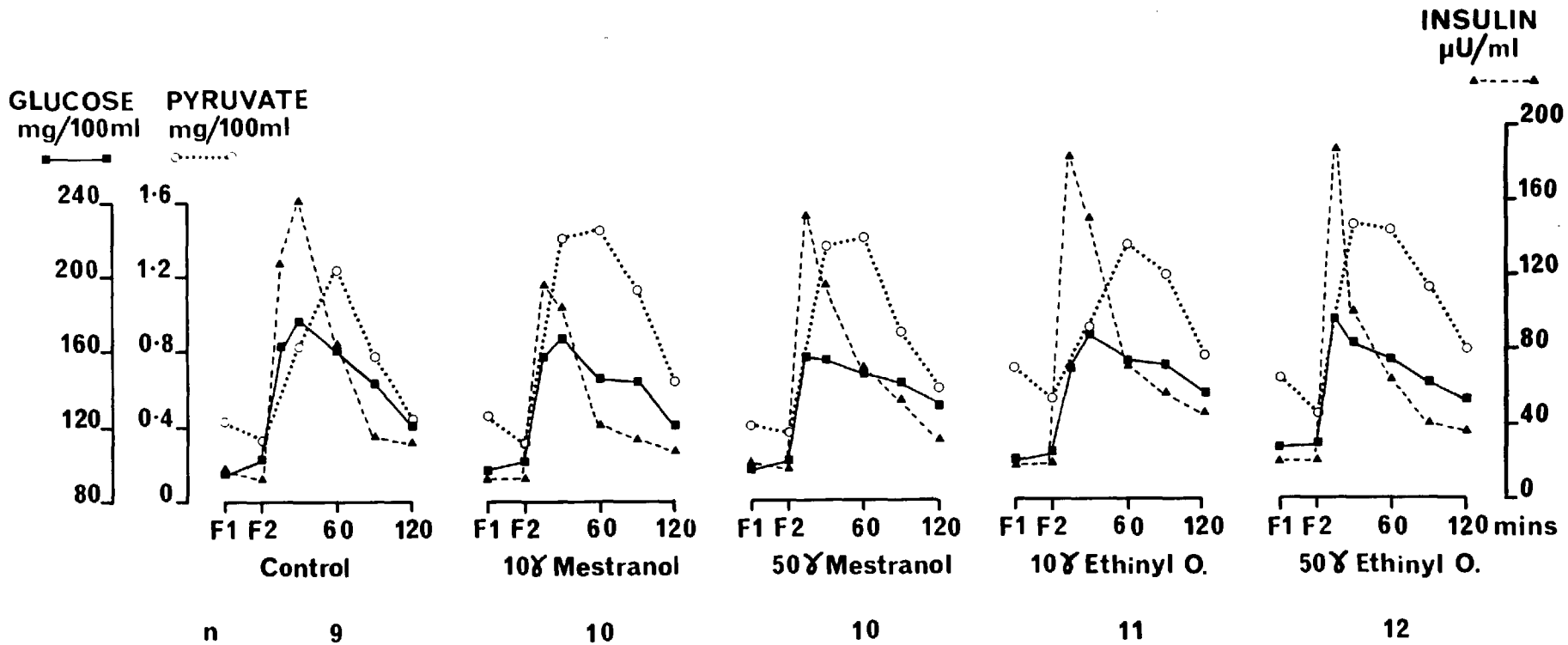


Fig. 4A

MESTRANOL/ETHINYL OESTRADIOL SERIES AT 4 WEEKS

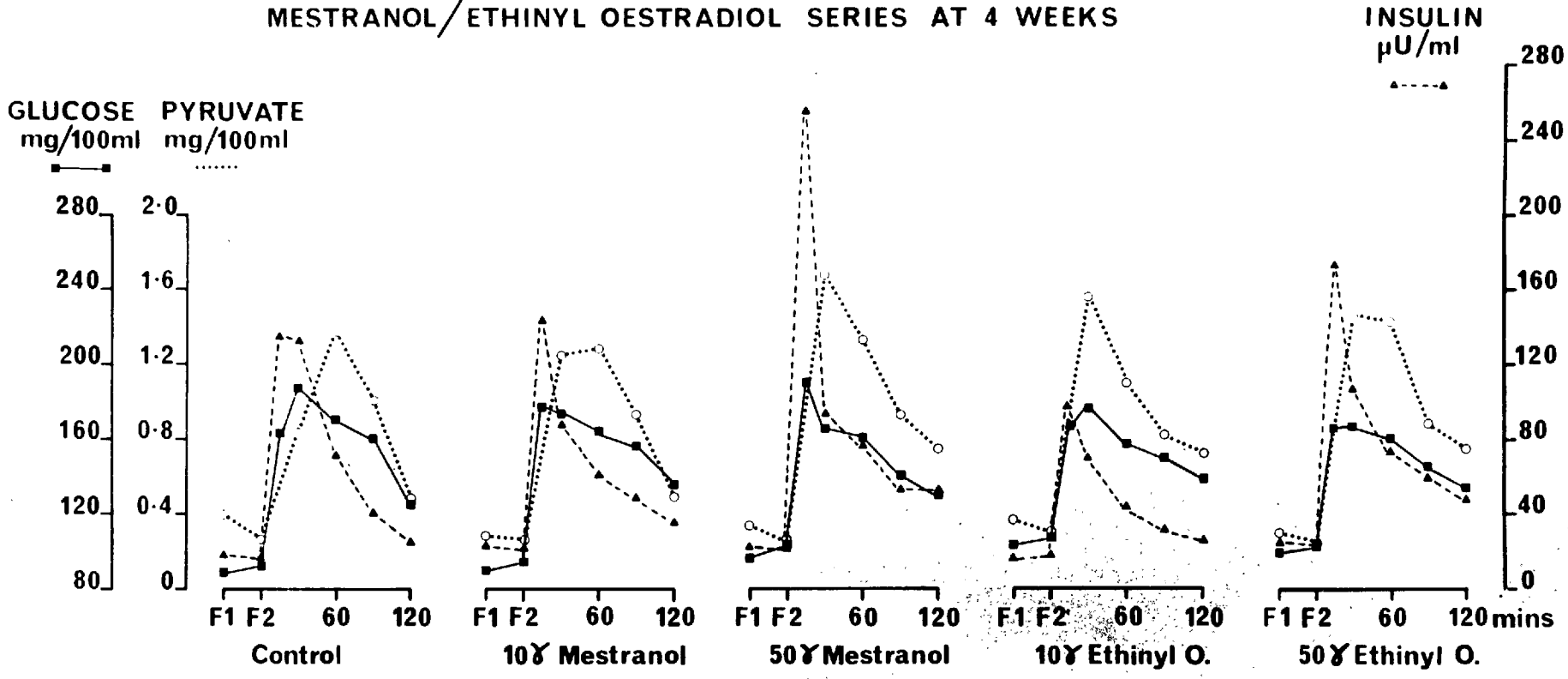


Fig. 5

MESTRANOL - ETHINYL OESTRADIOL SERIES AT 2 WEEKS

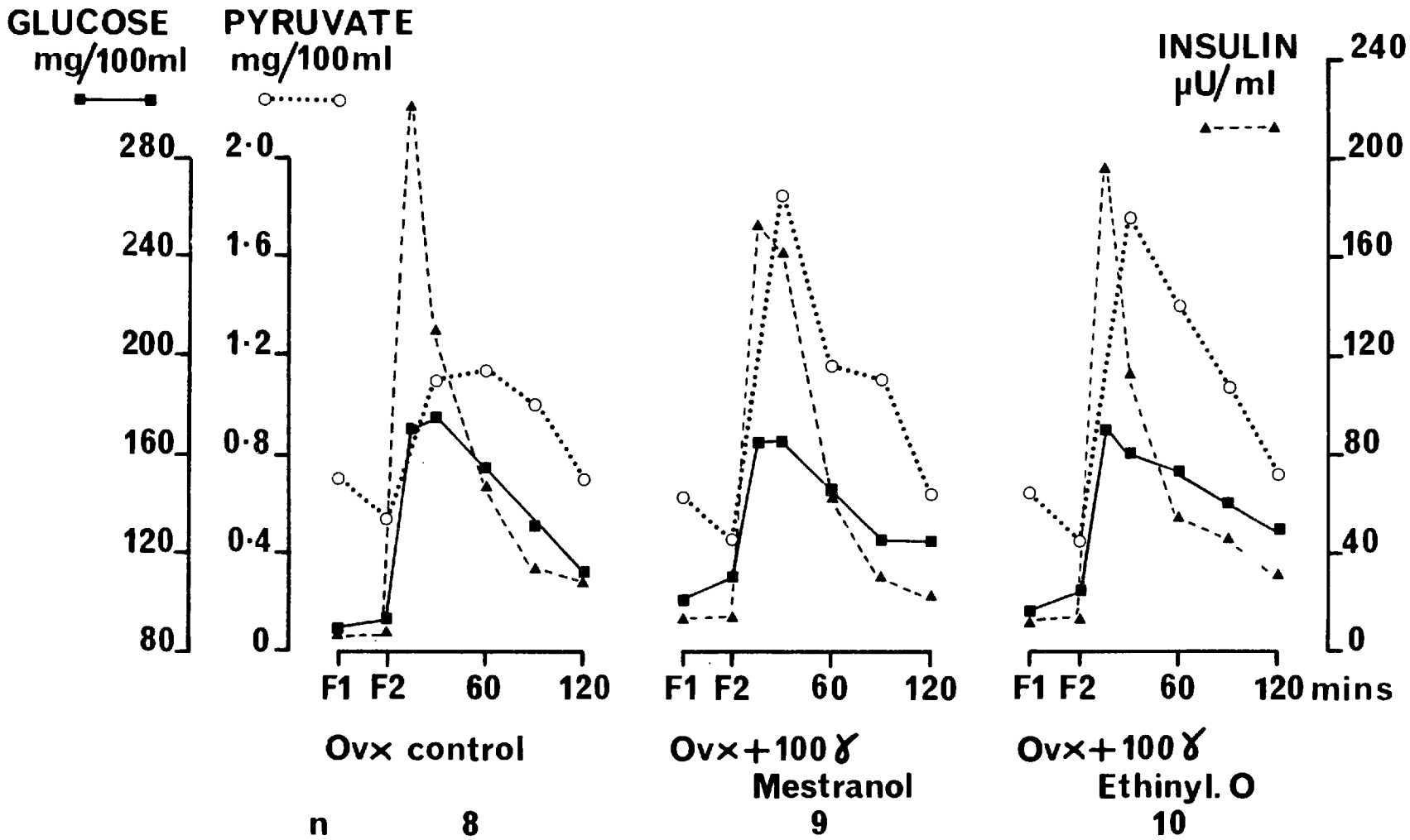


Fig. 5A

MESTRANOL - ETHINYL OESTRADIOL SERIES AT 4 WEEKS

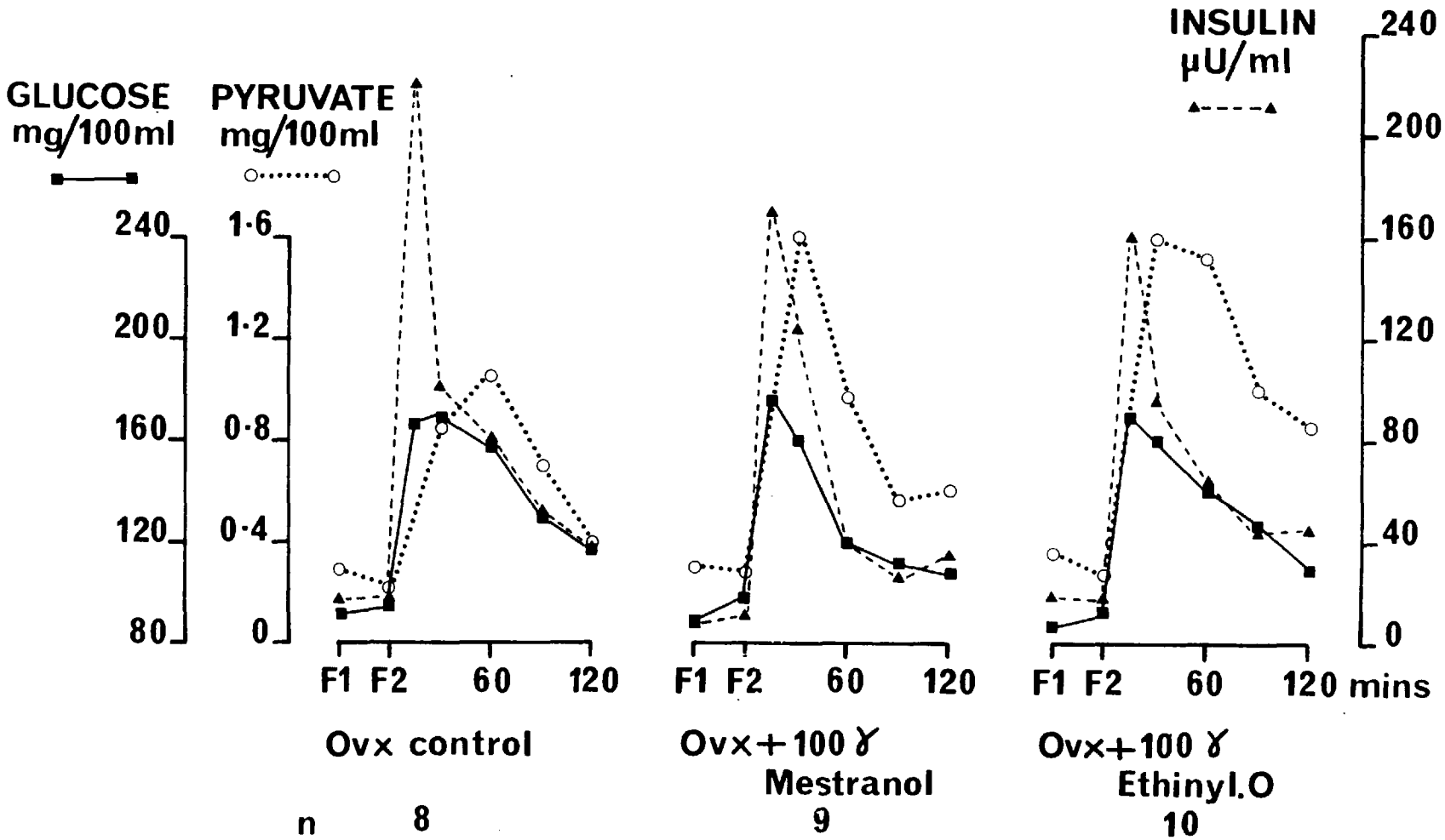


TABLE III

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX 0.10ml Oil Control N =9	Glucose mg/100ml	94 ± 3	101 ± 3	161 ± 8	179 ± 2	161 ± 5	142 ± 6	120 ± 3
	Pyruvate mg/100ml	0.45 ± 0.04	0.32 ± 0.05	---------	0.82 ± 0.05	1.24 ± 0.10	0.77 ± 0.15	0.41 ± 0.04
	Insulin µu/ml	14 ± 1	12 ± 2	128 ± 26	161 ± 20	79 ± 7	36 ± 7	32 ± 5
OVX +10 µg Mestranol N =10	Glucose	97 ± 4 N.S.	101 ± 5 N.S.	159 ± 6 N.S.	167 ± 8 N.S.	145 ± 7 N.S.	143 ± 5 N.S.	120 ± 3 N.S.
	Pyruvate	0.47 ± 0.04 N.S.	0.30 ± 0.03 N.S.	---------	1.39 ± 0.18 <.05	1.44 ± 0.17 N.S.	1.12 ± 0.11 N.S.	0.64 ± 0.09 <.05
	Insulin	12 ± 2 N.S.	13 ± 2 N.S.	117 ± 30 N.S.	103 ± 29 N.S.	43 ± 6 <.001	33 ± 5 N.S.	26 ± 6 N.S.
OVX + 50 µg Mestranol N = 10	Glucose	96 ± 2 N.S.	100 ± 3 N.S.	158 ± 7 N.S.	157 ± 7 <.05	148 ± 4 <.05	142 ± 6 N.S.	129 ± 4 N.S.
	Pyruvate	0.42 ± 0.03 N.S.	0.37 ± 0.02 N.S.	----------	1.38 ± 0.12 <.05	1.41 ± 0.11 N.S.	0.90 ± 0.14 N.S.	0.60 ± 0.08 N.S.
	Insulin	20 ± 2 <.025	18 ± 3 N.S.	154 ± 31 N.S.	117 ± 28 N.S.	69 ± 14 N.S.	54 ± 12 N.S.	31 ± 4 N.S.
OVX + 10 µg Ethinyl - O. N =11	Glucose	102 ± 3 <.05	105 ± 3 N.S.	151 ± 6 N.S.	169 ± 6 N.S.	155 ± 6 N.S.	152 ± 5 N.S.	137 ± 6 <.02
	Pyruvate	0.70 ± 0.09 <.025	0.54 ± 0.05 <.01	----------	0.94 ± 0.10 N.S.	1.36 ± 0.11 N.S.	1.19 ± 0.14 <.05	0.77 ± 0.12 <.025
	Insulin	20 ± 3 N.S.	19 ± 3 N.S.	185 ± 47 N.S.	151 ± 37 N.S.	73 ± 12 N.S.	58 ± 8 <.05	46 ± 9 N.S.
OVX + 50 µg Ethinyl - O. N = 12	Glucose	109 ± 3 <.005	110 ± 3 <.025	177 ± 5 N.S.	175 ± 4 N.S.	156 ± 3 N.S.	141 ± 4 N.S.	135 ± 6 <.05
	Pyruvate	0.64 ± 0.11 N.S.	0.45 ± 0.07 N.S.	----------	1.49 ± 0.20 <.05	1.46 ± 0.10 N.S.	1.14 ± 0.07 <.025	0.79 ± 0.09 <.005
	Insulin	19 ± 2 <.05	20 ± 3 <.05	188 ± 40 N.S.	101 ± 11 <.02	63 ± 9 N.S.	40 ± 4 N.S.	36 ± 3 N.S.

TABLE III

Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin, and blood pyruvate with signifance levels for Mestranol Ethinyl - oestradiol series at 2 weeks.

(N.S. = not significant.)

TABLE III A

		F1	F2	15 min	30 min	60 min	90 min	120 min
VX 0.10 ml	Glucose mg/100ml	89 ± 1	92 ± 1	162 ± 8	188 ± 7	170 ± 5	161 ± 3	127 ± 4
il Control.	Pyruvate mg/100ml	0.40 ± 0.02	0.28 ± 0.03	----------	0.82 ± 0.11	1.39 ± 0.12	1.00 ± 0.68	0.48 ± 0.07
N = 9	Insulin μU/ml	18 ± 3	14 ± 3	136 ± 28	133 ± 17	70 ± 11	41 ± 13	23 ± 4
VX + 10 μg	Glucose	90 ± 3	94 ± 4	179 ± 6	177 ± 7	164 ± 5	158 ± 4	138 ± 5
Mestranol.		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
N =10	Pyruvate	0.30 ± 0.03	0.27 ± 0.03	----------	1.24 ± 0.22	1.27 ± 0.21	0.94 ± 0.12	0.49 ± 0.04
		<.005	N.S.		N.S.	N.S.	N.S.	N.S.
	Insulin	24 ± 4	18 ± 4	145 ± 29	90 ± 16	62 ± 9	49 ± 11	37 ± 5
		N.S.	N.S.	N.S.	<.05	N.S.	N.S.	<.05
VX + 50 μg	Glucose	100 ± 4	103 ± 3	191 ± 6	166 ± 10	161 ± 6	141 ± 4	131 ± 4
Mestranol.		<.02	<.01	<.02	N.S.	N.S.	<.001	N.S.
N =10	Pyruvate	0.33 ± 0.04	0.23 ± 0.03	----------	1.69 ± 0.12	1.32 ± 0.13	0.93 ± 0.10	0.75 ± 0.16
		N.S.	N.S.		<.001	N.S.	N.S.	N.S.
	Insulin	23 ± 3	22 ± 3	256 ± 38	96 ± 20	81 ± 15	54 ± 6	54 ± 7
		N.S.	<.05	<.05	N.S.	N.S.	N.S.	<.005
OVX + 10 μg	Glucose	103 ± 4	107 ± 5	168 ± 9	177 ± 8	157 ± 2	149 ± 7	138 ± 7
Ethinyl - O.		<.05	<.05	N.S.	N.S.	<.05	N.S.	N.S.
N = 7	Pyruvate	0.39 ± 0.02	0.29 ± 0.03	----------	1.58 ± 0.24	1.10 ± 0.27	0.82 ± 0.17	0.74 ± 0.18
		N.S.	N.S.		<.01	N.S.	N.S.	N.S.
	Insulin	16 ± 3	18 ± 4	99 ± 24	70 ± 21	43 ± 6	32 ± 9	26 ± 7
		N.S.	N.S.	N.S.	<.02	N.S.	N.S.	N.S.
OVX + 50 μg	Glucose	100 ± 4	104 ± 3	166 ± 5	167 ± 9	161 ± 6	146 ± 6	135 ± 6
Ethinyl - O.		<.05	<.05	N.S.	N.S.	N.S.	N.S.	N.S.
N = 12	Pyruvate	0.31 ± 0.02	0.22 ± 0.02	----------	1.46 ± 0.11	1.42 ± 0.08	0.88 ± 0.09	0.77 ± 0.09
		<.01	N.S.		<.001	N.S.	N.S.	<.05
	Insulin	27 ± 4	23 ± 2	174 ± 29	109 ± 11	72 ± 5	60 ± 7	51 ± 7
		N.S.	<.025	N.S.	N.S.	N.S.	N.S.	<.005

TABLE IIIA Mean ± S.E.M.

O.G.T.T. Plasma glucose , insulin and blood pyruvate levels with signifance levels for Mestranol - Ethinyl - oestradiol series at 4 weeks

(N.S. = not significant.)

TABLE III B

		F1	F2	15 min	30 min	60 min	90 min	120 min
O.G.T.T at 2	weeks.							
OVX 0.10 ml Oil Control N = 8	Glucose mg/100ml	89 ± 1	92 ± 2	171 ± 10	177 ± 6	155 ± 4	132 ± 7	112 ± 4
	Pyruvate mg/100ml	0.71 ± 0.02	0.55 ± 0.07	----------	1.09 ± 0.10	1.15 ± 0.08	1.02 ± 0.13	0.68 ± 0.05
	Insulin μU/ml	9 ± 1	9 ± 2	219 ± 37	131 ± 18	66 ± 10	34 ± 4	28 ± 5
OVX + 100μg Mestranol. N = 9	Glucose	103 ± 2 <.001	109 ± 2 <.001	165 ± 5 N.S.	165 ± 6 N.S.	144 ± 4 N.S.	126 ± 5 N.S.	124 ± 5 N.S.
	Pyruvate	0.64 ± 0.07 N.S.	0.46 ± 0.03 N.S.	----------	1.83 ± 0.32 <.05	1.18 ± 0.10 N.S.	1.12 ± 0.11 N.S.	0.65 ± 0.1 N.S.
	Insulin	12 ± 2 N.S.	14 ± 2 N.S.	173 ± 36 N.S.	162 ± 26 N.S.	61 ± 9 N.S.	30 ± 3 N.S.	31 ± 6 N.S.
OVX + 100 μg Ethinyl-O. N = 10	Glucose	96 ± 2 <.02	105 ± 2 <.001	172 ± 7 N.S.	161 ± 6 N.S.	152 ± 4 N.S.	141 ± 6 N.S.	130 ± 3 <.005
	Pyruvate	0.62 ± 0.08 N.S.	0.47 ± 0.07 N.S.	----------	1.76 ± 0.18 <.005	1.41 ± 0.13 N.S.	1.06 ± 0.07 N.S.	0.74 ± 0.08 N.S.
	Insulin	12 ± 1 N.S.	12 ± 2 N.S.	195 ± 30 N.S.	113 ± 17 N.S.	56 ± 12 N.S.	48 ± 6 N.S.	31 ± 6 N.S.
O.G.T.T. at	4 weeks.							
OVX 0.10 ml Oil Control N = 8	Glucose	92 ± 2	97 ± 2	166 ± 5	167 ± 4	157 ± 3	128 ± 3	117 ± 4
	Pyruvate	0.30 ± 0.05	0.22 ± 0.02	----------	0.84 ± 0.07	1.05 ± 0.07	0.71 ± 0.13	0.37 ± 0.05
	Insulin	16 ± 2	15 ± 3	219 ± 34	100 ± 10	79 ± 11	48 ± 9	35 ± 6
OVX + 100 μg Mestranol. N = 9	Glucose	93 ± 1 N.S.	97 ± 2 N.S.	177 ± 9 N.S.	161 ± 6 N.S.	120 ± 5 <.001	112 ± 5 <.02	109 ± 5 N.S.
	Pyruvate	0.28 ± 0.05 N.S.	0.27 ± 0.03 N.S.	----------	1.61 ± 0.13 <.001	0.97 ± 0.15 N.S.	0.57 ± 0.08 N.S.	0.60 ± 0.13 N.S.
	Insulin	11 ± 2 N.S.	13 ± 2 N.S.	170 ± 33 N.S.	123 ± 16 N.S.	39 ± 6 <.05	27 ± 4 <.05	35 ± 7 N.S.
OVX +100μg Ethinyl - O. N = 10	Glucose	88 ± 2 N.S.	93 ± 2 N.S.	173 ± 5 N.S.	160 ± 6 N.S.	138 ± 7 <.05	128 ± 5 N.S.	108 ± 2 <.05
	Pyruvate	0.37 ± 0.04 N.S.	0.28 ± 0.03 N.S.	----------	1.59 ± 0.21 <.05	1.53 ± 0.12 <.05	1.02 ± 0.07 <.05	0.86 ± 0.11 <.005
	Insulin	19 ± 3 N.S.	18 ± 2 N.S.	157 ± 20 N.S.	97 ± 13 N.S.	60 ± 11 N.S.	47 ± 5 N.S.	43 ± 6 N.S.

TABLE III B Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with signifance levels for Mestranol - Ethinyl- O estradiol series. at 2 & 4 weeks.
(N.S. =not significant.)

TABLE IV

120 Minute Areas.	Glucose Total Area (Δ)	Glucose Incremental Δ	Pyruvate Total Δ	Pyruvate Incremental Δ	Insulin Total Δ	Insulin Incremental Δ
OVX 0.10ml Oil Control N=9	603±11	199±15	3.19±0.26	1.90±0.19	318±28	270±27
OVX +10μg Mestranol N=10	583±21 N.S.	179±13 <.05	4.41±0.45 <.05	3.23±0.39 <.05	228±38 N.S.	178±34 <.05
OVX +50μg Mestranol N=10	576±14 N.S.	178±12 <.05	4.17±0.26 <.02	2.70±0.25 <.025	306±51 N.S.	235±47 N.S.
OVX+10μg Ethinyl-O. N=11	604±17 N.S.	184±19 N.S.	4.25±0.25 <.01	2.25±0.29 N.S.	365±64 N.S.	291±58 N.S.
OVX+50μg Ethinyl-O. N=12	603±11 N.S.	161±9 <.05	4.85±0.29 <.001	2.96±0.24 <.005	300±34 N.S.	218±27 N.S.
60 Minute AREAS						
OVX 0.10ml Oil Control N=9	321±8	119±8	1.60±0.09	0.95±0.10	227±26	203±29
OVX+10μg Mestranol N=10	303±11 N.S.	101±6 N.S.	2,25±0.26 <.05	1.66±0.23 <.05	160±36 N.S.	135±33 N.S.
OVX+50μg Mestranol N=10	296±8 <.05	97±6 <.05	2,27±0.14 <.005	1.54±0.13 <.005	203±38 N.S.	167±36 N.S.
OVX+10μg Ethinyl-O. N=11	307±9 N.S.	97±9 N.S.	1.93±0.12 <.05	0.93±0.12 N.S.	248±50 N.S.	210±46 N.S.
OVX+50μg Ethinyl-O N=12	317±4 N.S.	96±5 <.02	2.57±0.24 <.005	1.63±0.20 <.02	210±29 N.S.	169±26 N.S.

TABLE IV Mean ± S.E.M. (Units-min) O.G.T.T. 120minute and 60 minute total and incremental areas with sigificance levels for Mestranol Ethinyl-oestadiolseries at 2 weeks.
N,S. =not significant.

TABLE IV A

120 Minute Areas.	Glucose Total Area (Δ)	Glucose Incremental Δ	Pyruvate Total Δ	Pyruvate Incremental Δ	Insulin Total Δ	Insulin Incremental Δ
OVX 0.10ml OIL Control N=9	640 ± 15	274 ± 16	3.62 ± 0.28	2.52 ± 0.24	294 ± 28	239 ± 24
OVX+10μg Mestranol N=10	638 ± 13 N.S.	262 ± 10 N.S.	3.82 ± 0.51 N.S.	2.75 ± 0.44 N.S.	272 ± 30 N.S.	200 ± 23 N.S.
OVX+50μg Mestranol N=10	612 ± 18 N.S.	186 ± 19 < .005	4.44 ± 0.33 N.S.	3.51 ± 0.34 < .05	366 ± 41 N.S.	278 ± 43 N.S.
OVX+10μg Ethinyl-O. N=7	619 ± 19 N.S.	192 ± 27 < .02	4.01 ± 0.71 N.S.	2.57 ± 0.48 N.S.	194 ± 33 < .05	123 ± 22 < .005
OVX+50μg Ethinyl-O. N=12	608 ± 21 N.S.	193 ± 22 < .02	4.27 ± 0.24 N.S.	3.37 ± 0.22 < .02	333 ± 25 N.S.	241 ± 22 N.S.
60 Minute Areas						
OVX 0.10ml Oil Control N=9	330 ± 10	147 ± 11	1.68 ± 0.16	1.13 ± 0.14	207 ± 21	179 ± 22
OVX+10μg Mestranol N=10	328 ± 9 N.S.	141 ± 8 N.S.	2.01 ± 0.32 N.S.	1.48 ± 0.28 N.S.	175 ± 29 N.S.	139 ± 25 N.S.
OVX+50μg Mestranol N=10	327 ± 12 N.S.	114 ± 12 N.S.	2.47 ± 0.15 < .005	2.00 ± 0.17 < .005	246 ± 31 N.S.	202 ± 31 N.S.
OVX+10μg Ethinyl-O. N = 7	322 ± 10 N.S.	109 ± 13 < .05	2.27 ± 0.39 N.S.	1.69 ± 0.34 N.S.	128 ± 28 < .05	92 ± 21 < .02
OVX+50μg Ethinyl-O. N=12	315 ± 11 N.S.	107 ± 12 < .025	2.28 ± 0.13 < .01	1.83 ± 0.13 < .005	211 ± 19 N.S.	165 ± 18 N.S.

TABLE IV A Mean ± S.E.M. (Units-min) O.G.T.T. 120 minute and 60 minute total and incremental areas with signifance levels for Mestranol Ethinyl-oestradiol series at 4 weeks (N.S. =not significant)

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas						
OVX 0.10ml Oil Control. N =8	582 ± 11	214 ± 13	3.88 ± 0.19	1.70 ± 0.20	324 ± 38	289 ± 37
OVX + 100μg Mestranol N =9	565 ± 14 N.S.	131 ± 12 <.001	4.64 ± 0.32 N.S.	2.79 ± 0.35 <.05	313 ± 34 N.S.	258 ± 35 N.S.
OVX + 100μg Ethinyl - O. N =10	590 ± 12 N.S.	171 ± 11 <.02	4.83 ± 0.19 <.005	2.95 ± 0.31 <.01	304 ± 33 N.S.	258 ± 29 N.S.
60 Minute Areas.						
OVX + 0.10ml Oil Control N =8	318 ± 10	135 ± 10	1.94 ± 0.13	0.85 ± 0.09	243 ± 33	224 ± 32
OVX + 100μg Mestranol N =9	306 ± 7 N.S.	88 ± 6 <.001	2.65 ± 0.22 <.02	1.73 ± 0.24 <.05	242 ± 31 N.S.	214 ± 32 N.S.
OVX + 100μg Ethinyl -O. N =10	309 ± 7 N.S.	99 ± 5 <.005	2.70 ± 0.16 <.005	1.76 ± 0.19 <.005	213 ± 26 N.S.	190 ± 24 N.S.

TABLE IV B Mean ± S.E.M. (Units - min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Mestranol - Ethinyl oestradiol series at 2 weeks. (N.S.= not significant)

TABLE IV C

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total Area	Pyruvate Incremental △	Insulin Total Area	Insulin Incremental △
120 minute Areas						
OVX 0.10ml Oil Control N =8	577 ± 10	190 ± 11	2.90 ± 0.17	2.02 ± 0.21	332 ± 31	272 ± 24
OVX + 100μg Mestranol. N =9	521 ± 17 <.02	127 ± 15 <.005	3.58 ± 0.30 N.S.	2.49 ± 0.35 N.S.	264 ± 33 N.S.	213 ± 26 N.S.
OVX + 100μg Ethinyl-O. N =10	550 ± 12 N.S.	177 ± 15 N.S.	4.71 ± 0.33 <.001	3.57 ± 0.32 <.005	284 ± 32 N.S.	214 ± 28 N.S.
60 Minute Areas						
OVX 0.10ml Oil Control N =8	311 ± 5	117 ± 5	1.48 ± 0.08	1.04 ± 0.10	228 ± 21	198 ± 17
OVX + 100μg Mestranol N =9	294 ± 10 N.S.	97 ± 9 N.S.	2.23 ± 0.19 <.005	1.68 ± 0.20 <.02	200 ± 28 N.S.	175 ± 24 N.S.
OVX + 100μg Ethinyl - O· N =10	299 ± 7 N.S.	112 ± 9 N.S.	2.49 ± 0.24 <.005	1.92 ± 0.24 <.01	185 ± 22 N.S.	150 ± 20 N.S.

TABLE IV C Mean ± S.E.M. (Units - min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Mestranol- Ethinyl - oestradiol series at 4 weeks.

(N.S. = not significant)

TABLE IV D Animal weights. Mestranol and Ethinyl -oestradiol series.

Mean ± S.E.M. (body weight in grams)

	Initial	2 weeks	4 weeks
OVX Oil Control	238 ± 4	255 ± 6	274 ± 6
OVX + 10 μg Mestranol	226 ± 6	200 ± 6	220 ± 6
OVX + 50 μg Mestranol	234 ± 3	208 ± 3	220 ± 4
OVX + 10 μg Ethinyl- O.	241 ± 5	226 ± 5	222 ± 7
OVX + 50 μg Ethinyl - O.	236 ± 4	200 ± 4	218 ± 4
OVX Oil Control	186 ± 4	220 ± 4	252 ± 6
OVX + 100 μg Mestranol	211 ± 5	190 ± 4	194 ± 8
OVX + 100 μg Ethinyl - O	210 ± 4	202 ± 6	217 ± 9

(Ethinyl - O. = Ethinyl oestradiol)

SECTION 11 MESTRANOL SERIES

PROCEDURES:-

Ovariectomized (OVX) rats weighing 200 - 250 gm were given sub-cutaneous injections of Mestranol dissolved in 0.10 ml corn oil at the following doses, 10 μg, 50 μg and 100 μg. The control animals received 0.10 ml oil only. The animals were injected for 2 weeks, after which time the first O.G.T.T. was performed, the animals were then maintained for a further 2 weeks at the same dosage schedule at the end of which, the second (4 week) O.G.T.T. was performed.

RESULTS:-

PLASMA GLUCOSE AT 2 WEEKS FIGS. 4 + 5 TABLES 111 + 111B

Fasting:-

The 10 μg and 50 μg groups had mean values that were not significantly different from the control mean value. The 100 μg group had a mean value that was significantly greater than the control value ($p < .001$).

DURING THE O.G.T.T.

At the 15 min interval, all groups had mean values that were lower than their respective control mean values , however no group value was significantly different from the control value.

At the 30 min interval, the mean values for all mestranol groups were less than their respective control mean value, however only the 50 μg value was significantly less, ($p < .05$):

At the 60 min interval all mestranol groups had mean values that were lower than their respective control mean values, however only the 50 μg group had a mean value that was significantly less than its control value, ($p < .05$).

At the 90 min and 120 min intervals the mean values for all mestranol groups were not significantly different from the mean values for their respective control groups.

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS TABLES 1V+1VB

120 Minute Areas:-

All mestranol groups had total and incremental areas that were lower than their respective control areas. The incremental areas for the 3 groups being significant, ($p < .05$) for the 10 μg and 50 μg groups and ($p < .001$) for the 100 μg group.

60 Minute Areas:-

All mestranol groups demonstrated total and incremental areas that were less than their respective control areas. The 50 μg group total and incremental areas were both significantly less, ($p < .05$) for both areas; the 100 μg group incremental area was significantly less than its control area, ($p < .02$).

BLOOD PYRUVATE AT 2 WEEKS FIG. 4 + 5; TABLE 111 + 111B

Fasting:-

The mean values for all mestranol groups were not significantl different from their respective control mean value.

DURING THE O.G.T.T.

The 10 μg and 50 μg groups had mean values at 30, 60, 90 and 120 minutes that were elevated in comparison to the respective control mean values, however only the 30 min value for both groups was significantly greater than the control, ($p < .05$) for both the two groups.

The 100 μg group had a 30 min value that was significantly greater than its control mean value ($p < .05$), subsequent time interval values were not significantly different from control values.

MEAN O.G.T.T. PYRUVATE AREAS AT 2 WEEKS TABLES 1V+1VB

120 Minute Areas:-

The 10 μg group had significantly elevated total, ($p < .05$) and incremental, ($p < .05$) areas compared with control areas. The 50 μg group also had significantly elevated total, ($p < .02$) and incremental ($p < .025$) areas compared with respective control areas. The 100 μg group whilst both total and incremental areas were elevated in comparison to the controls only the incremental area was significantly greater, ($p < .05$).

60 Minute Areas:-

The 10 μg group had a significantly elevated total, ($p < .05$) and incremental ($p < .05$) area compared with respective control areas. The 50 μg group total and incremental areas were significantly greater than the respective control areas, ($p < .005$) for both areas. The 100 μg group had significantly elevated total, ($p < .02$) and incremental, ($p < .05$) areas compared with respective control areas.

PLASMA INSULIN AT 2 WEEKS FIGS. 4 + 5; TABLES 111 + 111B

Fasting:-

There was a tendency for the 50 μg and 100 μg groups to have mean values greater than their respective control mean values, however no mestranol group had mean values significantly different from their control value.

DURING THE O.G.T.T.

With the one exception, the 10 μg group 60 min. interval value there was no significant difference between the respective control values and mean values of the mestranol treated groups at all time intervals. The level of significance for the 10 μg group 60 min mean value, which was less than its control value was ($p < .001$).

MEAN O.G.T.T. INSULIN AREAS AT 2 WEEKS TABLES 1V + 1VB

120 Minute Areas:-

The total and incremental areas for all mestranol treated groups were less than their respective control areas however they were not significantly different. The 10 μg group incremental area was one exception, which was significantly less than its control area, ($p < .05$).

60 Minute Areas:-

The total and incremental areas for all mestranol groups were not significantly different from their respective control areas.

PLASMA GLUCOSE AT 4 WEEKS FIGS. 4A + 5A; TABLES 111A+ 111B

Fasting:-

All mestranol mean fasting values had a tendency to be higher than their respective control values, however only the 50 μg group had a mean value that was significantly greater than the control, ($p < .01$).

DURING THE O.G.T.T.

At the 15 min interval all mestranol groups had mean values that were greater than their respective control mean value, however no group had a value that was significantly greater than the control value. At the 30, 60 and 90 minute intervals, the mean values for the mestranol treated groups were lower than the mean values for their respective control values. The 90 min value for the 50 μg group was significant at ($p < .001$) and the 60 min and 90 min values for the 100 μg group significant at ($p < .001$) and ($p < .02$) respectively.

The 120 min interval mean values for the 10 μg and 50 μg groups demonstrated higher values and the 100 μg group lower values than their respective control mean values, but no group value was significantly different from the control value.

MEAN O.G.T.T. GLUCOSE AREAS AT 4 WEEKS TABLES 1VA+1VC

120 Minute Areas:-

The total and incremental areas for all mestranol treated groups were lower than their respective control areas. The 50 μg and 100 μg group incremental areas were significantly less than their respective control areas ($p < .005$) for both groups, the 100 μg group total area was also significantly less ($p < .02$) than its control area.

60 Minute Areas:-

The total and incremental areas for all mestranol groups were less than their respective control areas, however no group area was significantly different from its control.

BLOOD PYRUVATE AT 4 WEEKS FIGS. 4A + 5A; TABLES 111A + 111B

Fasting:-

The mean values for all mestranol groups were not significantly different from their respective mean control value.

DURING THE O.G.T.T.

At the 30 min interval the mean values for all mestranol groups were greater than the respective control mean value. The 50 μg and 100 μg groups had values significantly greater than their control value ($p < .001$) for both groups.

At the 60, 90 and 120 min intervals, the mean values for all mestranol groups whilst showing a tendency to be less than their respective control values no group value was significantly less than its control.

MEAN O.G.T.T. BLOOD PYRUVATE AREAS AT 4 WEEKS TABLES 1VA + 1VC

120 Minute Areas:-

The total and incremental areas for all mestranol treated groups were greater than their respective control areas.

However only the 50 μg incremental area was significantly greater than its control, ($p < .05$).

60 Minute Areas:-

The total and incremental areas for all mestranol groups were greater than their respective control areas. Significant elevations over the control areas were found in the 50 μg total and incremental areas, ($p < .005$) for both areas, and the 100 μg group total and incremental areas ($p < .005$) and ($p < .02$) respectively.

PLASMA INSULIN AT 4 WEEKS FIGS. 4A + 5A; TABLES 111A+ 111B

Fasting:-

The 10 μg and 50 μg group mean values had a tendency to be greater than the control mean value, with the 50 μg group mean value significantly greater than its control ($p < .05$). The 100 μg group had a mean value that was not significantly different from its control value.

DURING THE O.G.T.T.

The 10 μg group had mean values that were little different from the respective control mean values, except the 30 min mean value for the 10 μg mestranol group was significantly less than the control mean value ($p < .05$).

The 50 μg group mean values were generally greater than the respective control mean values, with the mean values at 15 min and 120 min for the mestranol group significantly greater than the control mean value, ($p < .05$) and ($p < .005$) respectively.

The 100 μg group generally had mean values that were less than the respective control mean values, with the 60 and 90 min values significantly less than the control value ($p < .05$) for both; the 120 min value was not significantly different from the control value.

MEAN O.G.T.T. INSULIN AREAS AT 4 WEEKS TABLES 1VA+1VC

120 Minute Areas:-

The 10μg and 100 μg groups had lower and the 50 μg group greater, total and incremental areas in comparison with respective control areas; however no area for the mestranol group was significantly different from its respective control area.

60 Minute Areas:-

The 10 μg and 100 μg groups had lower and the 50 μg group greater, total and incremental areas in comparison with respective control areas, however no area for the mestranol groups was significantly different from its respective control area.

SECTION 111 ETHINYL-OESTRADIOL SERIES

PROCEDURE:-

Ovariectomized (OVX) rats weighing 200-250 gm were given sub-cutaneous injections of Ethinyl-Oestradiol dissolved in 0.10 ml corn oil at the following doses 10 μg, 50 μg and 100 μg. The control animals received 0.10 ml corn oil only. The animals were injected for 2 weeks at the end of which time the first O.G.T.T. was performed, the animals were then maintained for a further 2 weeks at the same dosage schedule at the end of which time, the second (4week) O.G.T.T. was performed.

RESULTS:-

PLASMA GLUCOSE AT 2 WEEKS FIG. 4+5; TABLE 111+111B

Fasting:-

The mean value for all Ethinyl-oestradiol groups were greater than their respective control values. The 50 μg and 100 μg groups had mean values significantly greater than their respective control mean values ($p < .025$) and ($p < .001$) respectively.

DURING THE O.G.T.T.

There was no apparent trend for the Ethinyl-oestradiol groups to have mean values that were either greater or less than their respective control mean values. The only values that were significantly different from the controls occurred at 120 min interval where all three Ethinyl-oestradiol groups had mean values, greater than their respective control mean values; 10 μg ($p < .02$), 50 μg ($p < .05$) and 100 μg ($p < .005$).

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS TABLES 1V+1VB

120 Minute Areas:-

The total areas for the three Ethinyl-oestradiol groups were lower though not significantly different from their respective control areas. The incremental areas for the three groups were also lower than their respective control areas with the 50 μg and 100 μg groups significantly less ($p < .05$) and ($p < .02$) respectively. The 10 μg group was not significantly different from its control.

60 Minute Areas:-

The total areas for the three Ethinyl-oestradiol groups were lower though not significantly different from their respective control areas; the incremental areas were all . . lower than their respective control areas. The 50 μg and 100 μg groups were significantly lower ($p < .02$) and $p < .005$) respectively. The 10 μg group was not significantly different from its control.

BLOOD PYRUVATE AT 2 WEEKS FIGS. 4+5 TABLES 111 + 111B

Fasting:-

The only group that was significantly different from its control was the 10 μg mean value which was greater, ($p < .01$).

DURING THE O.G.T.T.

The mean values for all Ethinyl-oestradiol groups at all time intervals were greater than their respective control mean values. At the 30 min interval, the 50 μg and 100 μg groups had mean values that were significantly greater than their respective control values, ($p < .05$) and ($p < .005$) respectively. The 10 μg group mean value was not significantly different from its control mean value.

At the 60 min interval all Ethinyl-oestradiol group had a mean value greater though not significantly different from respective control mean value.

At the 90 min interval, the 10 μg and 50 μg Ethinyl-oestradiol groups had mean values that were significantly greater than the

control mean value ($p < .05$) and ($p < .025$) respectively. The 100 μg group value was not significantly different from its control value.

At the 120 min interval, the 10 μg and 50 μg group mean values were significantly greater than the control mean value, ($p < .025$) and ($p < .005$) respectively. The 100 μg group mean value was not significantly different from its control value.

MEAN O.G.T.T. BLOOD PYRUVATE AREAS AT 2 WEEKS

TABLES IV + IV B

120 Minute Areas.

The 10 μg total and incremental areas were greater than their respective control areas, however only the total area was significantly greater than the control, ($p < .01$). The 50 μg group had total and incremental areas that were significantly greater than the respective control areas , ($p < .001$) for total area and ($p < .005$) for the incremental area.

The 100 μg group had significantly elevated total and incremental areas compared with its respective control areas, ($p < .005$) for total area and ($p < .01$) for incremental area.

60 Minute Areas:

The 10 μg group had total and incremental areas that were greater than the respective control areas , however only the total area was significantly greater than the control, ($p < .05$).

The 50 μg group had significantly elevated total and incremental areas compared to control areas ,($p < .005$) and ($p < .02$) respectively. The 100μg group had significantly elevated total and incremental areas compared to respective control areas, ($p < .005$) for both total and incremental areas.

PLASMA INSULIN AT 2 WEEKS FIGS. 4 + 5 TABLES 111 + 111B

Fasting:-

The 10 μg and 50 μg groups had mean values greater than the control mean value, with the 50 μg group value significantly greater ($p < .05$) than the control. The 100 μg group had a mean value that was not significantly different from its control value.

DURING THE O.G.T.T.

At the 15 min interval the mean values for the 10 μg and 50 μg groups were greater and the 100 μg group mean value less than their respective control mean value, however no Ethinyl-oestradiol group had a mean value that was significantly different from its control value. At the 30 min interval the mean values for all Ethinyl-oestradiol groups were less than their respective control mean values, with the 50 μg group having a significantly less mean value ($p < .02$) compared with its control.

At the 60 min interval all the Ethinyl-oestradiol groups had mean values that were less than their respective control mean values, however no group value was significantly different from its control.

At the 90 min and 120 min intervals all values for the Ethinyl-oestradiol groups were greater than the respective control mean values, with the 10 μg group 90 min value significantly greater ($p < .05$) compared with the control.

MEAN O.G.T.T INSULIN AREAS AT 2 WEEKS TABLE 1V + 1VB

120 Minute Areas:-

The 10 μg group had total and incremental areas that were greater and the 50 μg and 100 μg groups' areas less than the respective control areas. However no area for the Ethinyl-oestradiol groups was significantly different from that of its control areas.

60 Minute Areas:-

The 10 μg group had total and incremental areas that were greater than the respective control areas, whilst the 50 μg and 100 μg groups had areas that were less than their respective control areas.

However no Ethinyl-oestradiol group had an area that was significantly different from its respective control area.

PLASMA GLUCOSE AT 4 WEEKS FIGS. 4A + 5A; TABLES 111A+111B

Fasting:-

The 10 μg and 50 μg groups had mean values that were significantly greater than the control mean value, ($p < .05$) for both groups. The 100 μg group had a mean value that was approximately the same and not significantly different from the control mean value.

DURING THE O.G.T.T.

At the 15 min interval the Ethinyl-oestradiol groups had mean values that were approximately the same as the respective control mean values, with no value significantly different from its control value.

At the 30, 60, 90 and 120 min intervals, the mean values for the Ethinyl-oestradiol groups had a tendency to be less than their respective control mean values, with the exceptions 10 μg and 50 μg 120 min values which were greater. The only mean values that were significantly less than the respective control mean values was the 10 μg group 60 min value and the 100 μg group 60 and 120 min values; ($p < .05$) for all values.

MEAN O.G.T.T. AREAS AT 4 WEEKS TABLES 1VA + 1VC

120 Minute Areas:-

All Ethinyl-oestradiol groups had total and incremental areas that were less than their respective control areas, however only the 10 μg and 50 μg groups incremental areas were significantly less than the control incremental area ($p < .02$) for both groups.

60 Minute Areas:-

All Ethinyl-oestradiol groups had total and incremental

areas that were less than their respective control areas with the 10 μg and 50 μg incremental areas significantly less than the control incremental area; ($p < .05$) and ($p < .025$) respectively.

BLOOD PYRUVATE AT 4 WEEKS FIG. 4A + 5A; TABLES 111A+111

Fasting:-

The mean values for all Ethinyl-oestradiol groups were not significantly different from their respective control mean values.

DURING THE O.G.T.T.

At the 30 min interval, all Ethinyl-oestradiol groups had mean values that were significantly greater than the respective control mean value; 10 μg group ($p < .01$), 50 μg group ($p < .001$) and 100 μg group ($p < .05$).

At the 60 min interval, the 10 μg and 50 μg groups had mean values that were not significantly different from the control mean value, however the 100 μg group had a mean value significantly greater than its control ($p < .05$).

At the 90 min interval, the 100 μg group had a mean value that was significantly greater than the control value ($p < .05$), the 10 μg and 50 μg groups had values that were not significantly different from the control value.

At the 120 min interval, all Ethinyl-oestradiol groups had mean values that were greater than the respective control mean value. The 50 μg and 100 μg group values were significantly greater than their respective control values, ($p < .05$) and ($p < .005$) respectively; the 10 μg group value was not significantly different from the control value.

MEAN O.G.T.T. BLOOD PYRUVATE AREAS AT 4 WEEKS
TABLES 1VA + 1VC

120 Minute Areas:-

All Ethinyl-oestradiol groups had total and incremental areas greater than their respective control areas.

The 10 μg group had total and incremental areas that were not significantly different from the control areas. The 50 μg group had a significantly elevated incremental area ($p < .02$), however total area was not significant in comparison with control areas. The 100 μg group had significantly elevated total and incremental areas compared with control areas, ($p < .001$) and ($p < .005$) respectively.

60 Minute Areas:-

All Ethinyl-oestradiol groups had total and incremental areas greater than their respective control areas. The areas for the 10 μg group were not significantly different from the control areas. The 50 μg group total and incremental areas were significantly greater than the control areas, ($p < .01$) and ($p < .005$) respectively. The 100 μg group also had significantly elevated total ($p < .005$) and incremental ($p < .01$) areas in comparison with control areas.

PLASMA INSULIN AT 4 WEEKS FIGS. 4A + 5 A TABLES 111A + 111B

Fasting:-

The 50 μg group had a significantly greater mean value than the control mean value ($p < .025$). The 10 μg and 100 μg groups had mean values that were greater though not significantly different from respective control values.

DURING THE O.G.T.T.

At the 15 min interval, the 10 μg and 100 μg groups had mean values lower and the 50 μg group a mean value greater than the respective control mean values, however no value was significantl different from the controls.

At the 30 min interval all groups had mean values less than the respective control mean values with the 10 μg group value significantly less than the control value, ($p < .02$)

At the 60, 90 and 120 min intervals, the mean values for the three groups had a tendency to be neither greater or less than the respective control mean value. However the 50 μg group 120 min value was significantly greater than its control value ($p < .005$).

MEAN O.G.T.T. INSULIN AREAS AT 4 WEEKS TABLES 1VA + 1VC

120 Minute Areas:-

The 10 μg group had total and incremental areas that were significantly less than the control areas, ($p < .05$) and ($p < .005$) respectively for the two areas. The 50 μg group had total and incremental areas that were greater than the respective control areas, however the areas were not significantly different. The 100 μg group total and incremental areas were less than their respective control areas, but were not significant.

60 Minute Areas:-

The 10 μg group, total and incremental areas were significantly less than their respective control areas, ($p < .05$) and ($p < .02$) respectively. The 50 μg group had total and incremental areas greater and the 100 μg group had total and incremental areas less than their respective control areas, however no area was significantly different from its control area for these two groups.

SUMMARY OF RESULTS FOR OESTROGEN SERIES.

GLUCOSE.

Oestradiol benzoate administered in a low dose range 5 μg and 10 μg for 2 weeks had a tendency to increase the mean fasting glucose level, however with 4 weeks of continued administration the mean fasting levels for all doses of Oestradiol benzoate were less than the mean fasting levels for the controls. The oestrogenic steroids Mestranol and Ethinyl oestradiol at all dose levels, 10 μg 50 μg and 100μg had a tendency to increase the mean fasting glucose levels after 2 and 4 weeks of administration.

In general the administration of Oestradiol benzoate at 50 μg and 100μg dose levels and the two oestrogenic steroids Mestranol and Ethinyl oestradiol at all dose levels, 10μg , 50 μg and 100μg for periods of 2 and 4 weeks increased the rate of glucose removal during an oral glucose test.

The administration of low doses of Oestradiol benzoate , 5 μg and 10 μg for 2 weeks were without effect or had a slight tendency to decrease the rate of glucose removal during an O. G. T. T.

PYRUVATE.

After 2 weeks of Oestradiol benzoate administration the mean fasting pyruvate levels for the 5 μg, 10 μg and 50 μg groups were lower than the control mean value , however after 4 weeks of continued administration the mean fasting levels for alldose levels of Oestradiol benzoate were not significantly different from the control mean value.

The oestrogenic steroids Mestranol and Ethinyl oestradiol administered for 2 weeks and 4 weeks were without any significant effects on mean fasting pyruvate levels.

The administration of these three steroids for 2 and 4 weeks significantly increased the pyruvate levels during an O. G. T. T.

INSULIN.

In general, the insulin levels closely followed those of glucose.

The Ethinyl oestradiol group had fasting values that had a tendency to be greater than the respective control values , whilst the Mestranol and Oestradiol benzoate groups had fasting values that were not greatly different from those of the control.

The insulin response during the O.G.T.T. was brisk for all three steroids at both the 2 and 4 week intervals. The Oestradiol benzoate group demonstrated a biphasic insulin response at the 2 and 4 week intervals with increased insulin response for low dose levels and decreased insulin response at high dose levels.

The insulin responses for Mestranol and Ethinyl oestradiol groups were generally similar to or less than their respective control insulin response.

DISCUSSION.

The results obtained in this section indicate that Mestranol , Ethinyl oestradiol and high doses of Oestradiol benzoate(O. B.) administered for periods of 2 and 4 weeks improve carbohydrate tolerance, suggesting that these steroids increase the metabolism of glucose in ovariectomized rat. This observation would be in agreement with the work of Taalat et al (1965b) who demonstrated improved carbohydrate tolerance in the rabbit treated with Oestradiol dipropionate and also for women treated with the same oestrogen Taalat et al (1965a).

The low dose levels of (O. B.) 5 μg and 10 μg studied in the experiments of this section were observed to be without effect or to slightly impair carbohydrate tolerance. This finding may be related to the increased circulating levels of corticoids the effects of which are known to increase insulin resistance and decrease glucose uptake by the tissues , Ingle (1945) and Franckson et al (1953) for the rat and Basteine et al (1953), Burns et al (1953) and West (1957) for the human. Evidence for the increased circulating levels of corticoids with oestrogen administration for the human is well documented, Sandberg, Rosenthal and Slaunwhite (1969) and Burke (1969a + b) and for the rat Kitay (1963) and Braidman (1972) These two latter authors demonstrated that rats given 5 μg to 10 μg O. B. had increased corticosterone levels , but with increased dose of O. B. , the levels of corticosterone returned to normal.

The fasting data in oestrogen treated animals is difficult to interpret because of the many complex factors involved in glucose homeostasis. Oestrogen administration is known to effect important hormones involved with glucose regulation, for example growth hormone G. H. , thyroxine and the glucocorticoids.

Matute and Kalkhoff (1973) have shown that oestrogen administration to rats, increased glycogenesis and also increased the rate of glycogenolysis during fasting over that of the normal rat. This latter effect could contribute to elevated fasting glucose levels, seen with Mestranol and Ethinyl-oestradiol treated animals.

The improvement in the rate of glucose metabolism in the absence of hyperinsulinism seen in these exerpiments for oestrogen treated animals, is possibly due to a direct action of these steroids on liver and peripheral tissues. There is evidence to suggest that this may in fact be the case, since rats receiving oestrogen have increased liver glycogen content, Griffiths et al (1944)., Waalas (1952a), Ingle (1959) and Matute and Kalkhoff (1973) and also increased glycogen stores in uterine and diaphragm muscle, Waalas (1952b) and McKerns et al (1958).

The adipose tissue of rats treated with low doses of oestrogen has been shown to increase glucose uptake and its conversion to triglyceride, Gilmour and McKerns (1966). The workers Kissebah, Harrigan and Wynn (1972) and Kekki and Nikkila (1971) have demonstrated in women receiving O.C. therapy that there is an increase in the synthesis of triglyceride. Therefore it may be expected that animals treated with oestrogens would show an increase in body weight, however such is not the case for body weight was found to be decreased in these experiments and others, Grosvenor and Turner (1959) and Keller, Richardson and Yates (1969).

The liver is probably the main organ involved in the metabolism of an oral glucose load since it has been estimated that 60-70% of an oral glucose load is metabolized by the liver, Kipnis (1968). In the liver there is an important basic fact and that is, that there is no permeabililty barrier to glucose. The liver is able to take up glucose when the peripheral glucose levels exceed 150 mg per 100 ml or release glucose when the levels fall below this threshold value, Cahill et al (1959).

Therefore the metabolism of glucose will be chiefly determined by the balance between uptake and release of glucose by the liver cells which in turn is governed by the activity of the key gluconeogenic and glycolytic enzymes, Weber et al (1967). It is possible therefore, that elevations seen in the pyruvate levels during an O.G.T.T. are due to increased metabolism of glucose via the glycolytic and/or the Hexose mono phosphate shunt pathways (see Appendix 11), brought about by the direct action of the oestrogenic steroids on liver enzymes.

There is little to be found in the literature regarding the effects of oestrogen administration on liver enzymes concerned with carbohydrate metabolism. Wynn and Doar (1969), and (Doar 1969) demonstrated that the elevated pyruvate levels observed in women using O.C. were due to an increased production rate and not a decrease in the rate of its removal. However McKerns and Bell (1960) and McKerns and Clynes (1961) have shown that glycolytic activity of adipose tissue and liver slices from rats is unaffected with oestrogen treatment, whilst Hagerman (1962) working with liver slices from pregnant rats has shown an increased metabolism of glucose via the glycolytic pathway with elevations in pyruvate levels and lipid synthesis.

The workers Hori and Matsui (1970) have shown in the livers of rats treated with oestrogen the activity and de-novo synthesis of the enzyme, glucose-6-phosphate dehydrogenase is markedly increased. This enzyme is the first in the enzyme series involved in the Hexose mono-phosphate shunt pathway. The enzyme has also been shown to have increased activity in the mammary tissue and uterine muscle of female rats given oestrogen, Richards and Hilf (1972) and Hilf et al (1972). Oestrogen administration to rats is known to increase prolactin release, Meyer and Clifton (1956) and corticotrophin Gemzell (1952). Both of these trophic hormones have been shown in vitro, to increase the activity of the Hexose mono-phosphate shunt pathways in adipose tissue Winegrad et al (1959).

The role of insulin in improving carbohydrate tolerance in oestrogen treated rats is probably only secondary in respect to the effects already discussed. It is known from earlier work of Lewis et al (1954) that oestrogens cause hyperplasia and hypertrophy of the islets of Langerhans and that the insulin content of the islet is increased, Fraenkal Conrat et al (1941). However despite this, the insulin response during an O.G.T.T. performed on rats receiving high doses of oestrogen; 50 μg and 100 μg is either equal to, or lower than the response for a rat not receiving oestrogen. The only explanation available for such findings, in the absence of evidence to suggest that the oestrogens cause marked increases in the metabolism of insulin, is that the low insulin response is in accord with the lower plasma glucose levels, or that there may be a fault in the glucose trigger release mechanism for insulin secretion from the islet, Hager et al (1972).

Insulin has well recognized actions on the activity of key glycolytic enzymes in the liver Weber et al (1966) and peripheral tissues, by increasing their de-novo synthesis and release from inhibition by metabolites such as free fatty acids and reduced NAD. Insulin also increased the de-novo synthesis of enzymes involved in lipogenesis and the Hexose mono-phosphate shunt, Ashmore and Weber (1968).

It is probable therefore, that insulin has a function in maintaining the levels of key glycolytic enzymes and their activation during an oral glucose challenge in the oestrogen treated rat.

Oestrogen administration may also effect carbohydrate metabolism in a manner secondary to those already discussed. It has been shown by Ray Foster and Lardy (1966) and Veneziale et al (1967) that the tryptophan metabolite, quinolinic acid, inhibits the key gluconeogenic enzyme phospho-enol-pyruvate carboxykinase. Rose (1966), has shown that oestrogens influence the metabolism of tryptophan in man by effecting certain key vit B6 dependent enzymes and has recently demonstrated, Rose (1973) that women on O.C. have elevated urinary levels of quinolinic acid after a tryptophan load in comparison with levels for normal female subjects. Braidman and Rose (1971) demonstrated that there was increased activity of the liver enzyme tryptophan oxygenase in oestrogen treated rats. Therefore it may be possible, and Krebs (1970) has postulated, that quinolinic acid may be a metabolite involved in the control of gluconeogenesis.

Matute and Kalkhoff (1973) have shown that oestrogen administration decreased the conversion of pyruvate to glucose. This latter finding may suggest that elevations in O.G.T.T. pyruvate levels seen in the experiments presented in this section and in women on O.C., Wynn and Doar (1969), may be caused by a decrease in the rate of removal of pyruvate as well as an increase in the rate of formation of pyruvate.

In summary it seems possible to postulate, that oestrogens improve carbohydrate tolerance in the rat by acting directly and/or indirectly on the liver and peripheral tissue enzymes concerned with the metabolism of glucose and gluconeogenesis.

CHAPTER 3

SECTION 1 — THE EFFECT OF PROGESTERONE AT DIFFERENT DOSES ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE OVARIECTOMIZED RAT.

SECTION 11 — THE EFFECT OF THE PROGESTOGENS NORETHYNODREL AND NORETHISTERONE ACETATE AT DIFFERENT DOSES ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE OVARIECTOMIZED RAT.

SECTION 111 — THE EFFECT OF THE PROGESTOGENS CHLORMADINONE ACETATE AND MEGESTROL ACETATE AT DIFFERENT DOSES ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE OVARIECTOMIZED RAT.

INTRODUCTION.

In this section of the work the natural hormone Progesterone and the synthetic Progestogens of the O.C., Norethynodrel , Norethisterone Acetate, Chlormadinone Acetate and Megestrol Acetate are studied.

The experiments were performed in vivo and are longitudinal in nature, with an O.G.T.T. performed at 2 and 4 weeks which is roughly equivalent to 5 and 10 cycles in the human. This procedure was used in order to obtain evidence for alterations in glucose, pyruvate and insulin responses with duration of treatment.

The steroids were administered in the pharmacological dose range taken from the work of Beck(1969) in monkeys. However the anovulatory dose of these steroids in the rat is in the 0.50-2.00 mg range sub-cutaneous, daily, Dorfman(1968)

The employment of a dose range of these steroids provides a means of estimating if there is a dose related effect on the three parameters under investigation, glucose, pyruvate and insulin.

Fig. 6

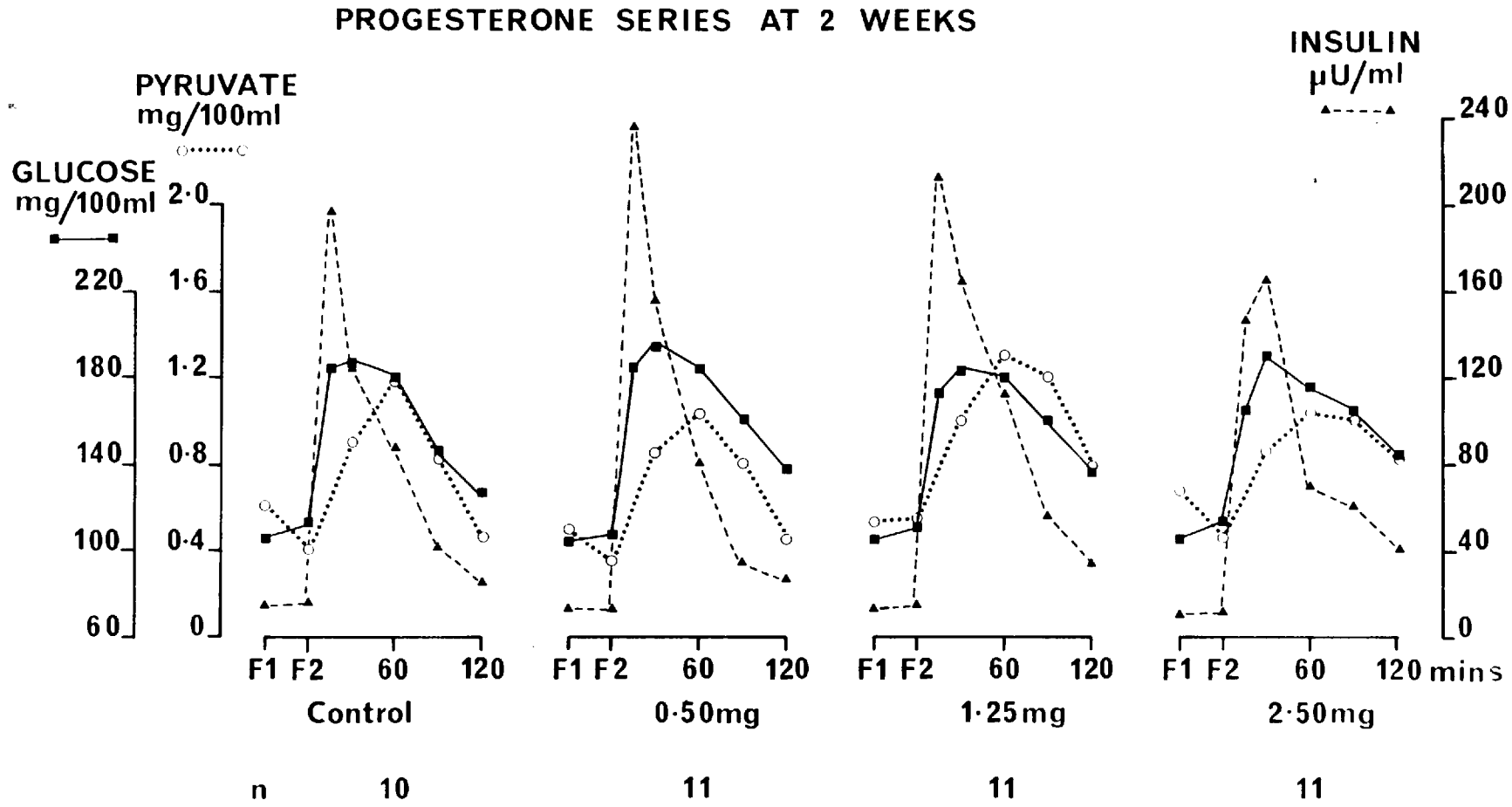

PROGESTERONE SERIES AT 2 WEEKS
PYRUVATE
mg/100ml
GLUCOSE
mg/100ml
INSULIN
μU/ml
220
180
140
100
60
2·0
1·6
1·2
0·8
0·4
0
240
200
160
120
80
40
0
F1 F2 60 120
Control
0·50mg
1·25mg
2·50mg
mins
n 10 11 11 11

Fig. 6A

PROGESTERONE SERIES AT 4 WEEKS

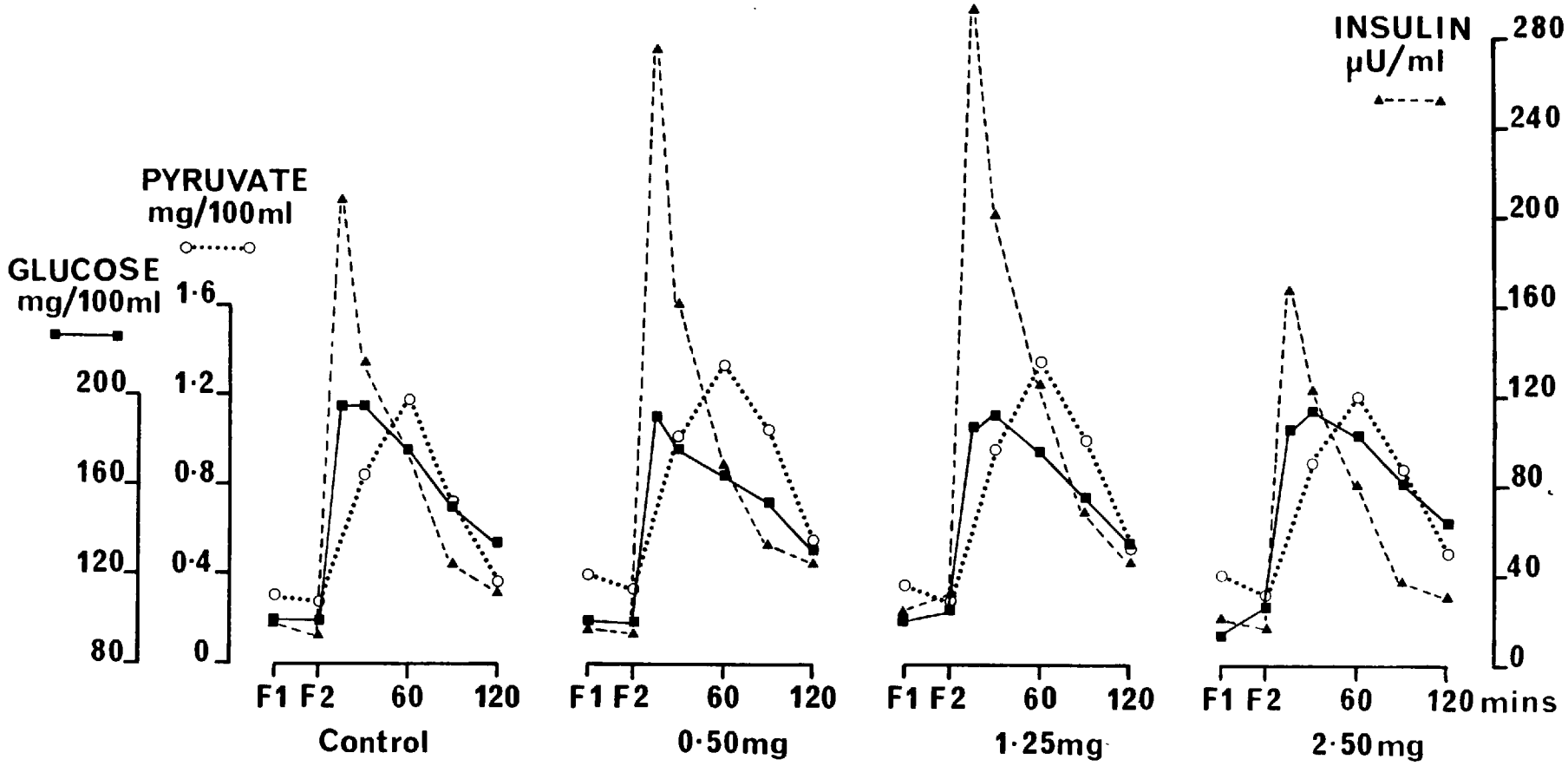


Fig. 7

PROGESTERONE SERIES

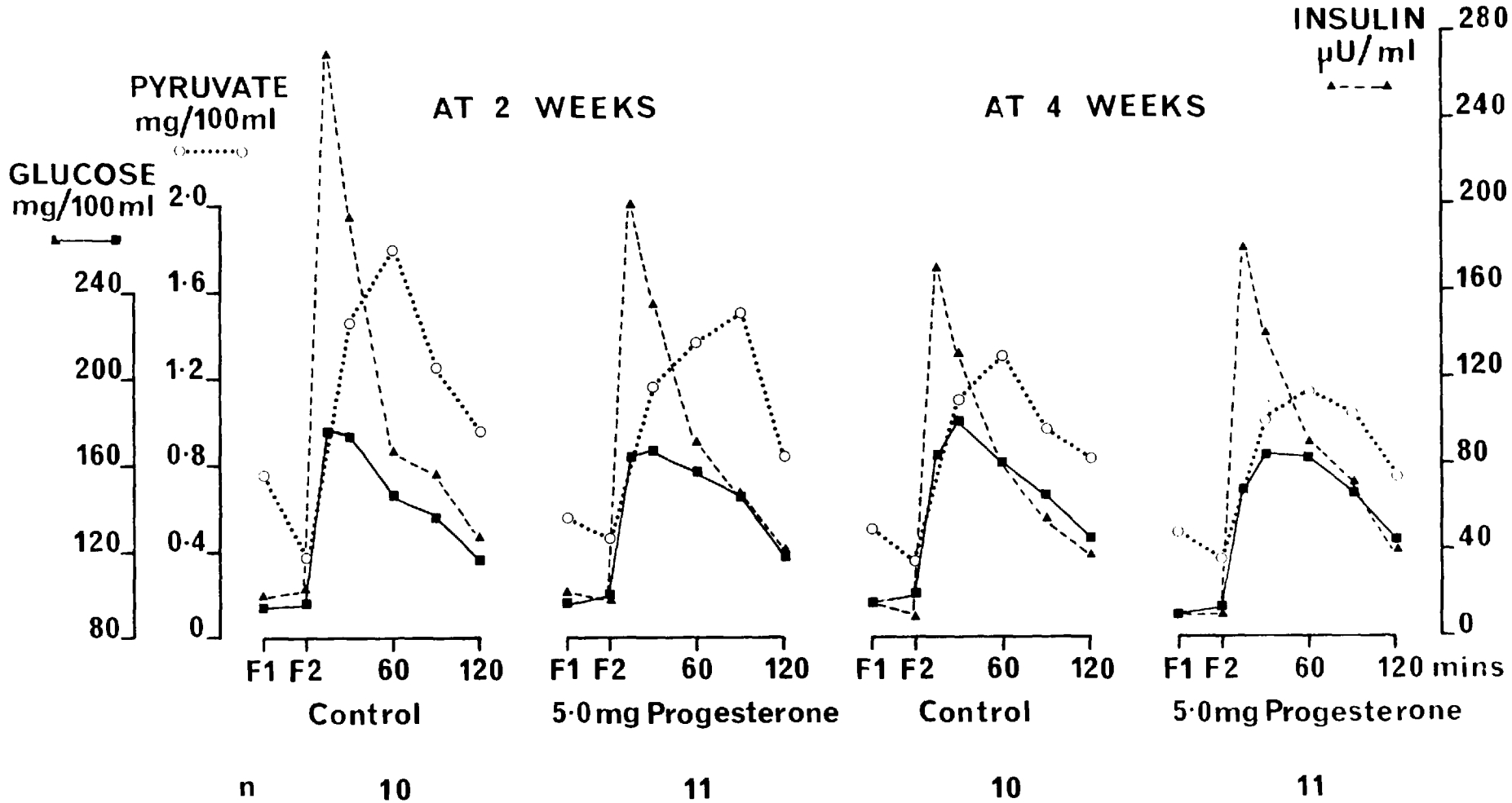


TABLE V

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX 0.20 ml	Glucose mg/100 ml	106 ± 3	112 ± 4	183 ± 8	186 ± 8	179 ± 4	148 ± 66	127 ± 6
Oil Controls	Pyruvate mg/100 ml	0.63 ± 0.04	0.41 ± 0.03	----------	0.90 ± 0.06	1.19 ± 0.08	0.86 ± 0.14	0.47 ± 0.05
N = 10	Insulin μU/ml	15 ± 2	16 ± 3	229 ± 42	152 ± 36	88 ± 9	42 ± 4	24 ± 3
OVX + 0.50 mg	Glucose	105 ± 2	110 ± 2	184 ± 5	194 ± 8	184 ± 5	160 ± 4	138 ± 4
Progesterone.		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
N = 11	Pyruvate	0.50 ± 0.05	0.35 ± 0.03	----------	0.86 ± 0.25	1.03 ± 0.10	0.80 ± 0.07	0.45 ± 0.02
		N.S.	N.S.		N.S.	N.S.	N.S.	N.S.
	Insulin	13 ± 2	11 ± 1	239 ± 20	158 ± 22	80 ± 10	36 ± 5	28 ± 3
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX +1.25 mg	Glucose	106 ± 2	111 ± 2	172 ± 4	184 ± 10	182 ± 8	161 ± 7	137 ± 5
Progesterone		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
N = 12	Pyruvate	0.51 ± 0.06	0.52 ± 0.04	----------	1.01 ± 0.08	1.32 ± 0.12	1.21 ± 0.11	0.73 ± 0.04
		N.S.	< .025		N.S.	N.S.	N.S.	< .001
	Insulin	13 ± 2	15 ± 2	212 ± 20	166 ± 30	116 ± 13	58 ± 6	33 ± 3
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	< .05
OVX +2,50 mg	Glucose	107 ± 3	113 ± 2	165 ± 6	191 ± 9	176 ± 7	144 ± 4	144 ± 4
Progesterone		N.S.	N.S.	< .025	N.S.	N.S.	N.S.	< .025
N = 12	Pyruvate	0.67 ± 0.03	0.44 ± 0.04	---------	0.87 ± 0.04	1.03 ± 0.08	1.00 ± 0.10	0.81 ± 0.10
		N.S.	N.S.		N.S.	N.S.	N.S.	< .005
	Insulin	10 ± 1	12 ± 1	147 ± 17	166 ± 30	71 ± 8	60 ± 11	41 ± 7
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	< .05

TABLE V Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for Progesterone series at 2 weeks.

(N.S. = not significant.)

TABLE V A

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX 0.20 ml	Glucose mg/100ml	100 ± 2	101 ± 2	198 ± 7	197 ± 6	176 ± 2	151 ± 5	132 ± 6
Oil Control	Pyruvate mg/100ml	0.30 ± 0.04	0.28 ± 0.03	----------	0.83 ± 0.12	1.19 ± 0.09	0.72 ± 0.12	0.36 ± 0.04
N = 10	Insulin μU/ml	19 ± 2	14 ± 3	209 ± 26	136 ± 18	96 ± 15	48 ± 5	33 ± 4
OVX + 0.50 mg	Glucose	100 ± 2	102 ± 2	190 ± 9	177 ± 5	162 ± 5	152 ± 5	129 ± 5
Progesterone.		N.S.	N.S.	N.S.	< .02	< .02	N.S.	N.S.
N = 11	Pyruvate	0.41 ± 0.05	0.33 ± 0.04	----------	1.04 ± 0.07	1.34 ± 0.13	1.05 ± 0.06	0.51 ± 0.0
		N.S.	N.S.		N.S.	N.S.	< .02	< .02
	Insulin	18 ± 2	16 ± 3	275 ± 35	152 ± 20	85 ± 12	53 ± 6	43 ± 8
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX + 1.25 mg	Glucose	101 ± 3	104 ± 4	185 ± 7	191 ± 9	175 ± 5	156 ± 8	135 ± 7
Progesterone		N.S.	N.S.	N.S.	N.S.	N.S.	N.S	N.S.
N = 11	Pyruvate	0.35 ± 0.04	0.28 ± 0.04	----------	0.98 ± 0.11	1.35 ± 0.12	1.00 ± 0.09	0.55 ± 0.(
		N.S.	N.S.		N.S.	N.S.	N.S.	< .05
	Insulin	24 ± 3	28 ± 3	292 ± 43	200 ± 24	128 ± 18	66 ± 7	46 ± 6
		< .001	< .001	N.S.	< .05	N.S.	< .05	N.S.
OVX + 2.50 mg	Glucose	93 ± 2	103 ± 3	187 ± 6	196 ± 10	182 ± 4	160 ± 3	142 ± 3
Progesterone		< .05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
N = 11	Pyruvate	0.41 ± 0.04	0.31 ± 0.03	----------	0.89 ± 0.07	1.20 ± 0.10	0.88 ± 0.10	0.49 ± 0.05
		< .05	N.S.		N.S.	N.S.	N.S.	N.S.
	Insulin	20 ± 2	16 ± 2	169 ± 25	123 ± 18	79 ± 9	48 ± 8	31 ± 4
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

TABLE VA Mean ± S.E.M. Plasma glucose, insulin and blood pyruvate with significance levels for Progesterone series at 4 weeks.
O.G.T.T. (N.S. =not significant)

		F1	F2	15 min	30 min	60 min	90 min	120 min
O.G.T.T. at	2 weeks							
VX 0.20 ml	Glucose mg/100ml	92 ± 2	95 ± 2	175 ± 6	173 ± 8	143 ± 6	134 ± 5	117 ± 5
Oil Control.	Pyruvate mg/100ml	0.74 ± 0.09	0.38 ± 0.03	----------	1.47 ± 0.18	1.80 ± 0.21	1.25 ± 0.17	0.97 ± 0.17
N=10	Insulin μU/ ml	19 ± 3	22 ± 2	270 ± 21	194 ± 37	87 ± 11	77 ± 11	46 ± 6
OVX +5.0mg	Glucose	95 ± 2 N.S.	99 ± 3 N.S.	162 ± 5 N.S.	163 ± 7 N.S.	159 ± 4 N.S.	146 ± 7 N.S.	117 ± 3 N.S.
Progesterone.	Pyruvate	0.56 ± 0.03 < .05	0.47 ± 0.04 N.S.	----------	1.18 ± 0.12 N.S.	1.38 ± 0.17 N.S.	1.50 ± 0.12 N.S.	0.83 ± 0.09 N.S.
N =11	Insulin	21 ± 3 N.S.	23 ± 2 N.S.	201 ± 14 < .05	153 ± 37 N.S.	88 ± 19 N.S.	63 ± 9 N.S.	36 ± 6 N.S.
O.G.T.T. at	4 weeks.							
OVX 0.20 ml	Glucose	94 ± 2	99 ± 2	163 ± 6	181 ± 7	161 ± 7	148 ± 5	126 ± 4
Oil Control.	Pyruvate	0.49 ± 0.05	0.34 ± 0.04	----------	1.07 ± 0.12	1.29 ± 0.19	0.97 ± 0.14	0.81 ± 0.11
N =10	Insulin	13 ± 2	16 ± 2	170 ± 31	132 ± 19	82 ± 11	56 ± 6	36 ± 4
OVX +5.0 mg	Glucose	90 ± 2 N.S.	93 ± 2 N.S.	150 ± 3 N.S.	164 ± 6 N.S.	162 ± 5 N.S.	147 ± 6 N.S.	127 ± 4 N.S.
Progesterone.	Pyruvate	0.48 ± 0.05 N.S.	0.36 ± 0.03 N.S.	----------	1.00 ± 0.17 N.S.	1.17 ± 0.14 N.S.	1.03 ± 0.18 N.S.	0.75 ± 0.16 N.S.
N =11	Insulin	17 ± 4 N.S.	11 ± 1 N.S.	181 ± 18 N.S.	140 ± 28 N.S.	87 ± 9 N.S.	61 ± 6 N.S.	42 ± 4 N.S.

TABLE V B Mean ± S.E.M. O.G.T.T. Plasma glucose , insulin and blood pyruvate with significance levels for Progesterone series at 2 & 4 weeks (N.S. = not significant.)

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX 0.20 ml Oil Control N = 10	649 ± 15	201 ± 26	3.88 ± 0.24	1.79 ± 0.23	340 ± 39	278 ± 35
OVX + 0.50 mg Progesterone N = 11	677 ± 8 N.S.	238 ± 9 N.S.	3.08 ± 0.15 N.S.	1.69 ± 0.17 N.S.	371 ± 25 N.S.	327 ± 24 N.S.
OVX + 1.25 mg Progesterone N = 11	663 ± 23 N.S.	219 ± 26 N.S.	4.16 ± 0.26 < .05	2.14 ± 0.24 N.S.	426 ± 34 N.S.	367 ± 37 N.S.
OVX + 2.50 mg Progesterone N = 12	667 ± 19 N.S.	216 ± 14 N.S.	3.51 ± 0.18 N.S.	1.75 ± 0.19 N.S.	352 ± 40 N.S.	306 ± 40 N.S.
60 Minute Areas.						
OVX 0.20 ml Oil Control. N = 10	348 ± 9	124 ± 13	1.69 ± 0.10	0.89 ± 0.08	241 ± 32	210 ± 30
OVX + 0.50 mg Progesterone N = 11	357 ± 6 N.S.	137 ± 8 N.S.	1.55 ± 0.10 N.S.	0.85 ± 0.09 N.S.	280 ± 23 N.S.	258 ± 22 N.S.
OVX + 1.25 mg Progesterone N = 11	343 ± 13 N.S.	121 ± 15 N.S.	1.92 ± 0.14 N.S.	0.94 ± 0.12 N.S.	293 ± 26 N.S.	262 ± 27 N.S.
OVX + 2.50 mg Progesterone N = 12	342 ± 11 N.S.	117 ± 8 N.S.	1.59 ± 0.07 N.S.	0.72 ± 0.08 N.S.	244 ± 32 N.S.	213 ± 29 N.S.

TABLE VI Mean ± S.E.M(Units - min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Progesterone series at 2 weeks. (N.S. = not significant)

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX +0.20 ml Oil Control. N =10	666 ± 9	263 ± 9	3.06 ± 0.25	1.95 ± 0.22	367 ± 40	320 ± 41
OVX + 0.50 mg Progesterone. N =11	631 ± 16 N.S.	226 ± 13 <.05	3.89 ± 0.20 <.025	2.52 ± 0.29 N.S.	421 ± 42 N.S.	360 ± 40 N.S.
OVX + 1.25 mg Progesterone. N =11	660 ± 16 N.S.	245 ± 18 N.S.	3.74 ± 0.26 N.S.	2.63 ± 0.22 <.05	519 ± 46 <.025	409 ± 41 N.S.
OVX + 2.50 mg Progesterone. N = 12	678 ± 16 N.S.	265 ± 11 N.S.	3.07 ± 0.24 N.S.	2.14 ± 0.24 N.S.	322 ± 31 N.S.	258 ± 27 N.S.
60 Minute Areas.						
OVX + 0.20 ml Oil Control . N = 10	360 ± 7	159 ± 7	1.57 ± 0.15	1.01 ± 0.15	258 ± 31	234 ± 31
OVX + 0.50 mg Progesterone. N = 11	334 ± 8 N.S.	131 ± 6 <.01	1.88 ± 0.11 N.S.	1.21 ± 0.14 N.S.	304 ± 32 N.S.	267 ± 34 N.S.
OVX + 1.25 mg Progesterone. N = 11	349 ± 10 N.S.	142 ± 11 N.S.	1.79 ± 0.16 N.S.	1.24 ± 0.16 N.S.	367 ± 35 <.05	312 ± 33 N.S.
OVX + 2.50 mg Progesterone. N = 12	357 ± 11 N.S.	150 ± 9 N.S.	1.64 ± 0.11 N.S.	1.03 ± 0.11 N.S.	220 ± 21 N.S.	187 ± 19 N.S.

TABLE VI A Mean ± S.E.M. (Units - min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Progesterone series at 4 weeks (N.S. = not significant.)

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas at 2 weeks						
OVX + 0.20 ml Oil Control. N =10	576 ± 16	195 ± 16	5.19 ± 0.54	3.66 ± 0.50	469 ± 37	381 ± 39
OVX +5.0 mg Progesterone. N =11	590 ± 15 N.S.	197 ± 14 N.S.	4.79 ± 0.35 N.S.	2.85 ± 0.43 N.S.	390 ± 56 N.S.	297 ± 59 N.S.
60 Minute Areas at 2 weeks						
OVX + 0.20 ml Oil Control. N =10	313 ± 10	122 ± 10	2.56 ± 0.27	1.80 ± 0.26	326 ± 30	282 ± 31
OVX +5.0 mg Progesterone. N = 11	307 ± 7 N.S.	110 ± 6 N.S.	2.15 ± 0.17 N.S.	1.18 ± 0.19 N.S.	265 ± 35 N.S.	219 ± 41 N.S.
120 Minute Areas at 4 weeks						
OVX + 0.20 ml Oil Control N = 10	614 ± 15	222 ± 17	3.90 ± 0.40	2.54 ± 0.36	343 ± 27	281 ± 26
OVX + 5.0 mg Progesterone. N = 11	594 ± 15 N.S.	222 ± 13 N.S.	3.75 ± 0.44 N.S.	2.32 ± 0.45 N.S.	369 ± 24 N.S.	325 ± 24 N.S.
60 Minute Areas at 4 weeks						
OVX + 0.20 ml Oil Control N = 10	220 ± 36	126 ± 8	1.88 ± 0.20	1.20 ± 0.18	228 ± 21	197 ± 21
OVX + 5.0 mg Progesterone N = 11	302 ± 7 N.S.	116 ± 6 N.S.	1.76 ± 0.23 N.S.	1.05 ± 0.22 N.S.	242 ± 22 N.S.	220 ± 22 N.S.

TABLE VI B Mean ± S.E.M. (Units min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Progesterone series at 2&4 weeks (N.S. =not significant)

TABLE VI C

Animal weights. Progesterone series.

Mean ± S.E.M. (body weight in grams)

	Initial	2 week	4 week
OVX Oil Control	200 ± 4	248 ± 6	265 ± 6
OVX + 0.50 mg Progesterone	205 ± 5	255 ± 8	278 ± 10
OVX + 1.25 mg Progesterone	205 ± 3	252 ± 3	268 ± 3
OVX + 2.50 mg Progesterone	205 ± 3	258 ± 6	283 ± 8
OVX Oil Control	224 ± 7	245 ± 7	256 ± 9
OVX + 5.00mg Progesterone	220 ± 4	241 ± 6	252 ± 7

SECTION 1 PROGESTERONE SERIES

PROCEDURE: Ovariectomized (OVX) rats weighing 200-300g were given sub-cutaneous injections of Progesterone at the following dose levels, 0.50 mg, 1.25 mg, 2.50 mg and 5.0 mg dissolved in 0.20ml corn vegetable oil. Control animals received 0.20ml corn oil only.

The animals were treated for 2 weeks, at the end of which time the first O.G.T.T. was performed; the animals were maintained for a further 2 weeks at the end of which the second O.G.T.T. (4 week) was performed.

RESULTS:

PLASMA GLUCOSE AT 2 WEEKS FIG 6 + 7 TABLES V VB

Fasting: There was no significant difference in mean values for any of the progesterone treated groups from the values of their respective controls.

DURING THE O.G.T.T.

There was no significant difference in mean values between the progesterone treated groups and their respective control values at all time intervals, with the one exception, the 2.50mg group 15 min mean value which was significantly lower ($p < 0.025$) and the 120 min. value significantly greater ($p < 0.025$) than the control values. Overall there was a tendency for the mean glucose values for all progesterone groups to be higher than their respective control values during the O.G.T.T.

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS: TABLES V1+V1B

120 Minute Areas: There was a very marked trend for mean areas, for both total and incremental areas for all progesterone treated groups to be greater than their respective control mean areas, however no area reached significance.

60 Minute Areas: Both total and incremental areas for all progesterone groups were not significantly different from the areas for their respective controls.

There was no apparent trend for the steroid group total areas to be either greater , or less than the control areas, however the incremental areas had a tendency to decrease with increasing dose of Progesterone when compared to respective control areas.

BLOOD PYRUVATE AT 2 WEEKS FIGS 6 + 7 TABLES V + V B

Fasting. There was no significant difference in mean values for the 0.50 mg , 2.50 mg and 5.00 mg groups compared to the mean values for their respective controls. The 1.25 mg group had a mean value significantly greater ($p<0.025$) than its control value.

DURING THE O.G.T.T.

At the 30 min , 60 min, and 90 min time intervals there was no significant difference between the mean values for any of the Progesterone groups and their respective mean control mean values At the 120 min interval, the 1.25 mg and 2.50 mg group values were significantly greater than the control mean value , ($p<.001$) and ($p<.005$) respectively. The 0.50 mg and 5.00 mg groups had mean values that were not significantly different from their control mean value.

MEAN O.G.T.T. BLOOD PYRUVATE AREAS AT 2 WEEKS:

TABLES VI + VI B

120 Minute Areas. The total and incremental areas for the 0.50 mg 2.50 mg and 5.00mg groups were not significantly different from their respective control areas . The 1.25 mg group demonstrated a significantly elevated total area ($p<.05$) but not incremental area, when compared to control areas.

60 Minute Areas . The total and incremental areas for all Progesterone treated groups were not significantly different from their respective control areas.

PLASMA INSULIN AT 2 WEEKS FIGS. 6 + 7 TABLES V + VB

Fasting:- There was no significant difference between control mean values and progesterone treated mean values.

DURING THE O.G.T.T.

At the 15 min interval the 0.50 mg, 1.25 mg and 2.50 mg group mean values were not significantly different from the control value. The 5.0 mg group had a significantly lower mean value ($p < 0.05$) than its control value.

At the 30 min, 60 min and 90 min intervals the mean values for all progesterone groups were not significantly different from their respective control values.

At the 120 min time interval the 1.25 mg and 2.50mg groups were significantly greater than the control value, ($p < 0.05$) for both groups. The 0.50 mg and 5.0 mg group values, were not significantly different from their control values.

Overall there was a tendency for the lower doses of progesterone 0.50 mg and 1.25 mg to increase the levels of insulin whilst the higher doses 2.50 and 5.0 mg had a tendency to lower the level of insulin during the O.G.T.T.

MEAN O.G.T.T. INSULIN AREAS AT 2 WEEKS TABLES V1 + V1B

120 Minute Areas:-

The total and incremental areas for all steroid treated groups were not significantly different from the respective control areas. Both the total and incremental areas for the 0.50mg and 1.25 mg groups, were greater than the respective control area, whilst the 2.50 mg and 5.00 mg groups had a tendency for the total and incremental areas to be approximately the same or less than their respective control area.

60 Minute Areas:-

The total and incremental area values for the 0.50 mg and 1.25 mg groups were higher than those of the control but did not attain significance.

The 2.50mg and 5.00mg groups demonstrated mean values that were approximately equal to or less than the respective control area.

PLASMA GLUCOSE AT 4 WEEKS: FIGS. 6A + 7 TABLES VA+VB

Fasting: There was no significant difference between mean values for progesterone treated groups and their control values.

DURING THE O.G.T.T.

The mean values for the 1.25, 2.50 and 5.00 mg progesterone groups at all time intervals were not significantly different from their control values. The 0.50mg group, 30 and 60 min values were significantly less than their control values, ($p < .02$) for both time intervals; the 15, 90 and 120 min values were not significantly different from the controls.

MEAN O.G.T.T. GLUCOSE AREAS AT 4 WEEKS: TABLES V1A V1B

120 Minute Areas:

The 0.50mg group demonstrated total and incremental areas that were lower than the respective control areas, however only the incremental area reached significance ($p < .05$).
The 1.25, 2.50 and 5.00mg groups total and incremental areas were not significantly different from their respective control areas.

60 Minute Areas:

The total and incremental areas for the 0.50mg group were lower than the respective control areas, but only the incremental area was significantly different ($p < .01$)

The 1.25, 2.50 and 5.00mg group total and incremental areas were not significantly different from the respective control areas.

BLOOD PYRUVATE AT 4 WEEKS FIGS 6A + 7 TABLES VA + VB

Fasting:

The mean values for all progesterone treated groups were not significantly different from the mean values for the respective controls.

DURING THE O.G.T.T.

There was no significant difference between mean values for the 1.25, 2.50 and 5.00mg progesterone treated groups and their respective mean control values at the 30, 60 and 90 minute intervals. The 0.50mg group had mean values greater, but not significant for the 30 and 60 minute intervals, however the values at the 90 and 120 minute intervals were significantly greater than the control values, ($p < .02$) for both.

MEAN O.G.T.T. BLOOD PYRUVATE AT 4 WEEKS: TABLES V1A + V1B

120 Minute Areas:

The total and incremental areas for the 250mg and 5.00mg groups were not significantly different from the control areas. The 0.50 and 1.25mg groups, total and incremental areas were greater than the respective control areas, with the 0.50mg total area and the 1.25mg incremental area reading significance at ($p < .025$) and ($p < .05$) respectively.

60 Minute Areas:

The total and incremental areas for all progesterone groups were not significantly different from their respective control areas, however the 0.50mg and 1.25mg groups demonstrated a tendency to be greater than the control areas.

PLASMA INSULIN AT 4 WEEKS FIGS. 6A + 7 TABLE VA + VB

Fasting:

The mean values for 0.50mg, 2.50mg and 5.00mg groups were not significantly different from the control values. The 1.25mg values were both significantly greater than the controls, ($p < .001$) for both values.

DURING THE O.G.T.T.

At the 15 min interval no progesterone treated group mean value was significantly different from the respective control mean value. At the 30 min interval only the 1.25mg mean value which was greater than the control mean value, reached significance, ($p < .05$). All progesterone groups had 60 min values that were not significantly different from the control mean values. At the 90 min interval the 0.50mg, 2.50mg and 5.00mg groups had mean values that were not significantly different from the respective mean control value. The 1.25mg group had a mean value that was significantly greater ($p < .05$) than the control value.

At the 120 min interval, there was no progesterone treated group that had a mean value significantly different from the respective mean control value.

MEAN O.G.T.T. INSULIN AREAS AT 4 WEEKS TABLES V1A+V1B

120 Minute Areas

The total and incremental areas for the 0.50mg and 1.25mg progesterone groups were greater than the respective control areas, however only the 1.25mg total area was significantly greater ($p < 0.025$). The 2.50mg and 5.00mg groups had total and incremental areas that were not significantly different from the control areas.

60 Minute Areas

The 0.50mg and 1.25mg groups had total and incremental areas greater than the control areas, however only the 1.25mg group total area was significantly greater, ($p < .05$). The total and incremental areas for the 2.50mg and 5.00mg groups were not significantly different from their respective control areas.

SECTION 11: NORETHYNODREL AND NORETHISTERONE ACETATE SERIES

PROCEDURE:-

Ovariectomized (OVX) rats weighing 200-300gm were given sub-cutaneous injections of either Norethynodrel or Norethisterone acetate. The doses used, were 250 μg and 500 μg for both progestogens studied. These doses were given in 0.10 ml corn oil; the control animals received oil vehicle only.

The animals were treated for 2 weeks, at the end of which time the first O.G.T.T. was performed; the animals were maintained for a further 2 weeks when the second (4 week) O.G.T.T. was performed.

RESULTS:-

PLASMA GLUCOSE AT 2 WEEKS FIG. 8 TABLES V11 V111

Fasting:-

Considering the second fasting sample there was no significant difference between control mean value and any of the progestogen treated group mean values. However there was a tendency for the mean values of the lower dose range for both progestogens to be lower than the control mean value, with the first fasting sample reaching significance, ($p < .05$) for Norethynodrel and, ($p < .005$) for Norethisterone Acetate.

DURING THE O.G.T.T.

At the 15 min interval both progestogens at the two dosage levels, demonstrated elevated mean values compared to the control mean value, with the 500 μg dosages for both progestogens reaching significance at ($p < .05$) and the 250 μg Norethisterone Acetate group ($p < .01$).

Fig. 8

NORETHYNODREL / NORETHISTERONE ACETATE SERIES AT 2 WEEKS

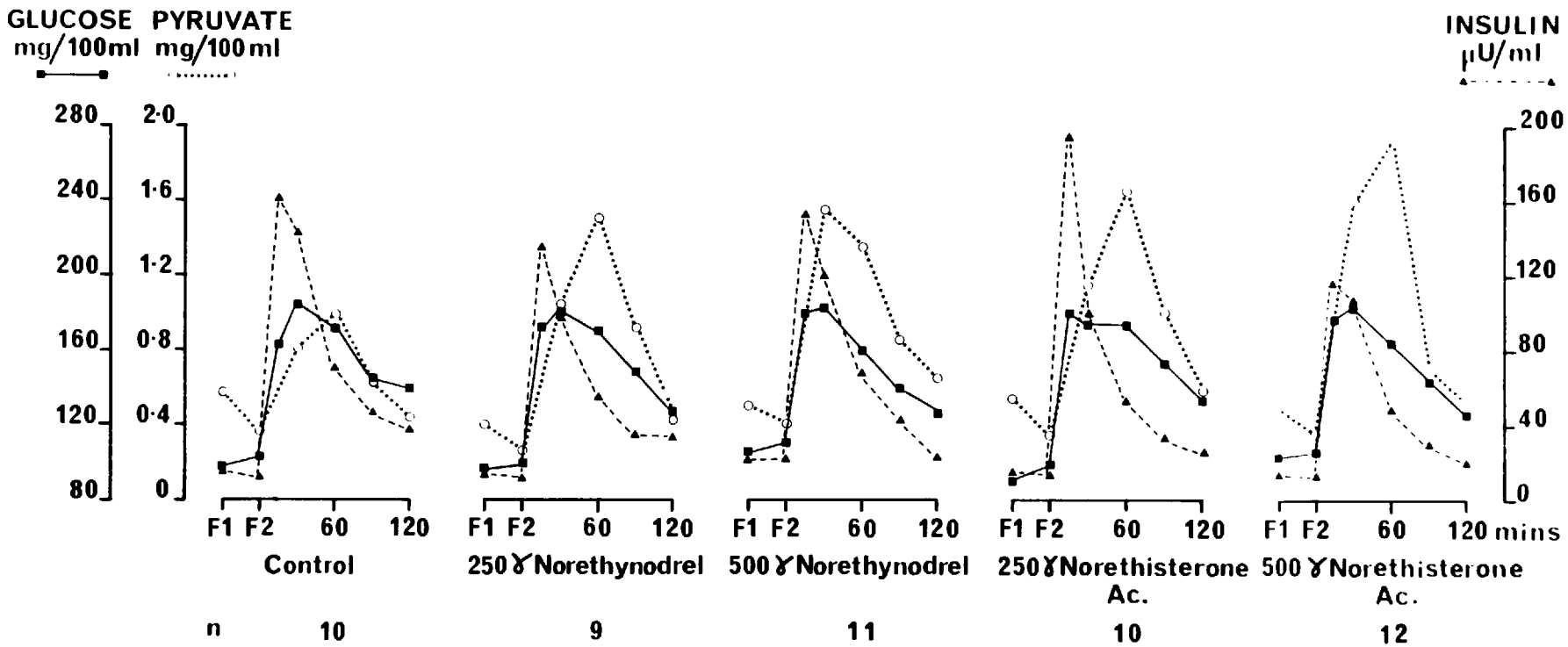


Fig. 8A

NORETHYNODREL / NORETHISTERONE ACETATE SERIES AT 4 WEEKS

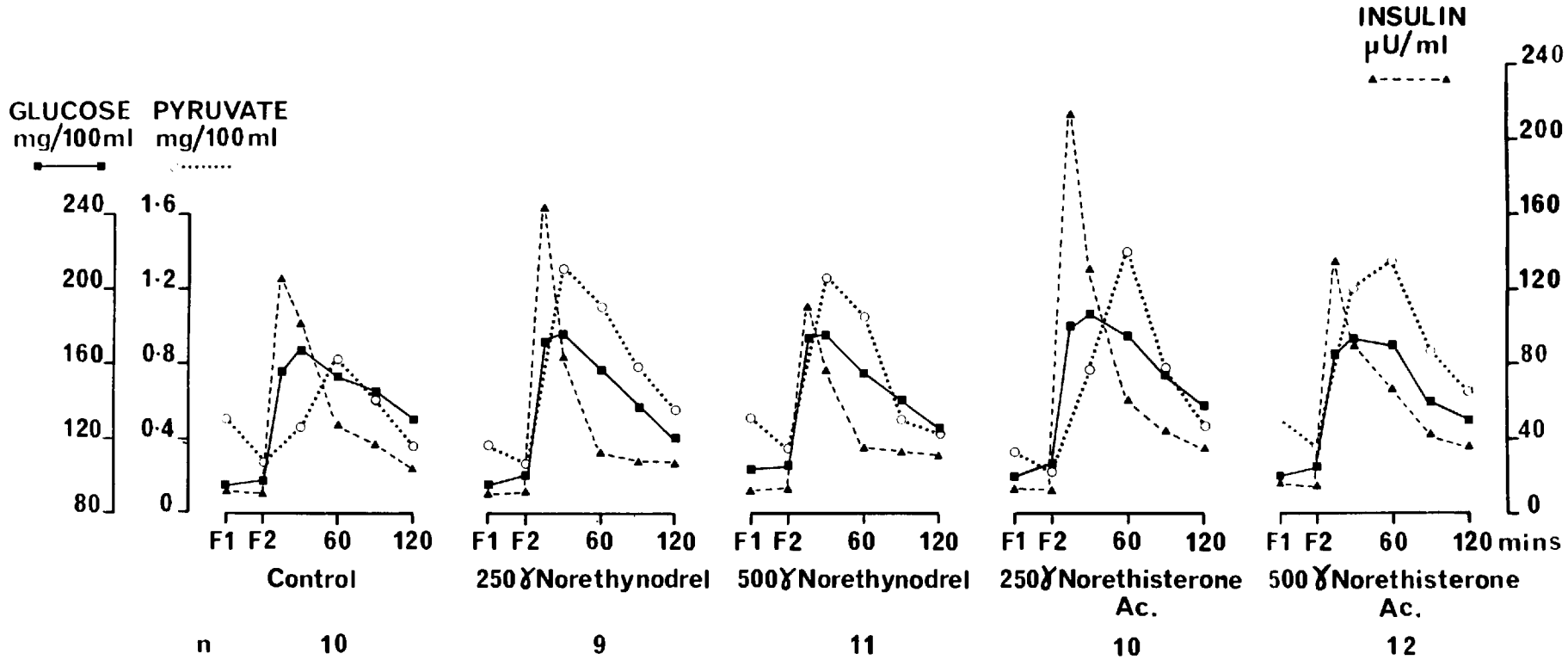


TABLE VII

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX 0.10 ml	Glucose mg/100ml	100 ± 1	104 ± 2	161 ± 5	186 ± 7	172 ± 6	145 ± 6	139 ± 6
Oil Control	Pyruvate mg/100ml	0.58 ± 0.05	0.36 ± 0.03	----------	0.81 ± 0.06	0.99 ± 0.13	0.64 ± 0.12	0.44 ± 0.04
N = 10	Insulin μU/ml	18 ± 4	14 ± 3	160 ± 27	142 ± 14	69 ± 8	47 ± 5	38 ± 4
OVX +250 μg	Glucose	97 ± 2	100 ± 2	172 ± 5	180 ± 7	171 ± 5	148 ± 5	128 ± 4
Norethynodrel		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Pyruvate	0.42 ± 0.01	0.26 ± 0.03	----------	1.03 ± 0.13	1.50 ± 0.08	0.91 ± 0.09	0.41 ± 0.02
N = 9		< .05	< .05		N.S.	< .05	N.S.	N.S.
	Insulin	14 ± 3	16 ± 2	136 ± 19	99 ± 14	55 ± 8	35 ± 6	33 ± 5
		N.S.	N.S.	N.S.	< .05	N.S.	N.S.	N.S.
OVX + 500 μg	Glucose	106 ± 2	109 ± 2	181 ± 7	182 ± 4	160 ± 7	141 ± 6	126 ± 6
Norethynodrel		< .05	N.S.	< .05	N.S.	N.S.	N.S.	N.S.
	Pyruvate	0.50 ± 0.04	0.41 ± 0.03	----------	1.56 ± 0.16	1.35 ± 0.13	0.87 ± 0.09	0.64 ± 0.11
N = 11		N.S.	N.S.		< .001	N.S.	N.S.	N.S.
	Insulin	21 ± 2	21 ± 2	153 ± 22	121 ± 20	68 ± 9	43 ± 7	35 ± 3
		N.S.	< .05	N.S.	N.S.	N.S.	N.S.	N.S.
OVX + 250 μg	Glucose	91 ± 2	99 ± 2	183 ± 6	176 ± 4	173 ± 3	154 ± 6	134 ± 7
		< .005	N.S.	< .01	N.S.	N.S.	N.S.	N.S.
Norethisterone	Pyruvate	0.54 ± 0.05	0.36 ± 0.03	----------	1.17 ± 0.13	1.67 ± 0.16	0.99 ± 0.13	0.58 ± 0.06
Acetate		N.S.	N.S.		< .05	< .005	N.S.	N.S.
N = 10	Insulin	15 ± 2	14 ± 1	193 ± 28	100 ± 16	53 ± 8	33 ± 5	26 ± 5
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVX + 500 μg	Glucose	101 ± 3	105 ± 2	179 ± 7	182 ± 6	164 ± 4	139 ± 4	125 ± 4
		N.S.	N.S.	< .05	N.S.	N.S.	N.S.	< .05
Norethisterone	Pyruvate	0.48 ± 0.05	0.35 ± 0.02	----------	1.59 ± 0.11	1.93 ± 0.11	0.71 ± 0.10	0.53 ± 0.07
Acetate		N.S.	N.S.		< .001	< .001	N.S.	N.S.
N = 12	Insulin	12 ± 1	10 ± 1	118 ± 11	108 ± 25	49 ± 8	29 ± 6	21 ± 4
		N.S.	N.S.	N.S.	N.S.	N.S.	< .05	< .01

TABLE VII Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for Norethynodrel- Norethisterone Acetate series at 2 weeks

(N.S. = not significant.)

TABLE VII A

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX +0.10 ml Oil Control N = 10	Glucose mg/100ml	95 ± 2	98 ± 2	154 ± 5	169 ± 7	152 ± 6	146 ± 5	129 ± 5
	Pyruvate mg/100ml	0.52 ± 0.05	0.27 ± 0.03	----------	0.46 ± 0.06	0.83 ± 0.12	0.60 ± 0.10	0.33 ± 0.04
	Insulin μU/ml	12 ± 1	11 ± 1	128 ± 24	99 ± 21	47 ± 7	38 ± 5	23 ± 2
OVX +250 μg Norethynodrel N = 9	Glucose	97 ± 1 N.S.	100 ± 1 N.S.	171 ± 8 N.S.	175 ± 7 N.S.	158 ± 6 N.S.	138 ± 7 N.S.	119 ± 4 N.S.
	Pyruvate	0.37 ± 0.04 <.02	0.24 ± 0.02 N.S.	----------	1.32 ± 0.23 <.005	1.11 ± 0.12 N.S.	0.79 ± 0.16 N.S.	0.55 ± 0.09 <.05
	Insulin	12 ± 1 N.S.	12 ± 2 N.S.	164 ± 41 N.S.	84 ± 18 N.S.	32 ± 7 N.S.	28 ± 4 N.S.	26 ± 3 N.S.
OVX + 500 μg Norethynodrel N = 11	Glucose	103 ± 2 <.005	105 ± 2 <.02	173 ± 6 <.05	177 ± 4 N.S.	154 ± 5 N.S.	139 ± 5 N.S.	127 ± 4 N.S.
	Pyruvate	0.51 ± 0.04 N.S.	0.32 ± 0.04 N.S.	----------	1.25 ± 0.22 <.02	1.05 ± 0.16 N.S.	0.49 ± 0.07 N.S.	0.46 ± 0.07 N.S.
	Insulin	12 ± 1 N.S.	13 ± 1 N.S.	110 ± 17 N.S.	77 ± 16 N.S.	34 ± 4 N.S.	32 ± 5 N.S.	31 ± 4 N.S.
OVX + 250 μg Norethisterone Acetate N =10	Glucose	101 ± 2 <.05	106 ± 3 <.05	180 ± 8 <.02	187 ± 5 N.S	175 ± 6 <.02	153 ± 6 N.S.	139 ± 5 N.S.
	Pyruvate	0.33 ± 0.01 <.001	0.21 ± 0.02 N.S.	----------	0.78 ± 0.20 N.S.	1.40 ± 0.14 <.01	0.75 ± 0.14 N.S.	0.46 ± 0.13 N.S.
	Insulin	14 ± 2 <.01	13 ± 2 N.S.	213 ± 26 <.05	130 ± 15 N.S.	61 ± 8 N.S.	44 ± 7 N.S.	35 ± 7 N.S.
OVX + 500μg Norethisterone Acetate N = 12	Glucose	99 ± 2 N.S.	103 ± 2 N.S.	165 ± 4 N.S.	174 ± 5 N.S.	170 ± 3 <.01	140 ± 4 N.S.	130 ± 4 N.S.
	Pyruvate	0.35 ± 0.03 <.005	0.51 ± 0.03 <.001	----------	1.20 ± 0.17 <.005	1.37 ± 0.11 <.005	0.87 ± 0.13 N.S.	0.65 ± 0.14 N.S.
	Insulin	19 ± 2 <.005	17 ± 2 <.02	139 ± 16 N.S.	90 ± 11 N.S.	67 ± 9 N.S.	41 ± 4 N.S.	36 ± 3 N.S.

TABLE VII A Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate for Norethynodrel - Norethisterone Acetate series with significance levels at 4 weeks. (N.S. = not significant.)

TABLE VIII

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX + 0.10 ml Oil Control N = 10	640 ± 13	225 ± 18	2.85 ± 0.30	1.41 ± 0.32	318 ± 26	263 ± 23
OVX + 250 μg Norethynodrel N = 9	629 ± 12 N.S.	229 ± 13 N.S.	3,78 ± 0.24 <.05	2.76 ± 0.22 <.005	252 ± 26 N.S.	188 ± 22 <.05
OVX +500 μg Norethynodrel N = 11	618 ± 16 N.S.	183 ± 16 N.S.	4.30 ± 0.21 <.001	2.72 ± 0.25 <.005	300 ± 26 N.S.	216 ± 25 N.S.
OVX +250 μg Norethisterone Acetate N = 10	643 ± 12 N.S.	248 ± 14 N.S.	4.29 ± 0.36 <.01	2.85 ± 0.32 <.005	274 ± 35 N.S.	218 ± 34 N.S.
OVX +500μg Norethisterone Acetate N = 12	618 ± 8 N.S.	197 ± 11 N.S.	4.67 ± 0.30 <.001	3.27 ± 0.27 <.001	231 ± 25 <.05	189 ± 24 <.05
60 Minute Areas.						
OVX +0.10 ml Oil Control N = 10	339 ± 9	132 ± 10	1.48 ± 0.13	0.76 ± 0.13	220 ± 18	193 ± 17
OVX + 250 μg Norethynodrel N = 9	331 ± 8 N.S.	131 ± 7 N.S.	1.91 ± 0.15 <.05	1.39 ± 0.15 <.005	173 ± 19 N.S.	141 ± 17 <.05
OVX + 500 μg Norethynodrel N = 11	335 ± 7 N.S.	117 ± 8 N.S.	2.44 ± 0.19 <.001	1.64 ± 0.18 <.001	206 ± 22 N.S.	164 ± 22 N.S.
OVX +250 μg Norethisterone Acetate N = 10	335 ± 7 N.S.	138 ± 5 N.S.	2.18 ± 0.19 <.01	1.46 ± 0.17 <.01	202 ± 27 N.S.	174 ± 27 N.S.
OVX + 500 μg Norethisterone Acetate N = 12	335 ± 6 N.S.	124 ± 7 N.S.	2.73 ± 0.15 <.001	2.03 ± 0.16 <.001	167 ± 18 N.S.	146 ± 18 N.S.

TABLE VIII Mean ± S.E.M. (Units - min) O.G.T.T. 120 minute and 60 minute total and incremental areas with significance levels at 2 weeks for Norethynodrel-Norethisterone Acetate series.

(N.S. = not significant.)

TABLE VIII A

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX Oil Control N = 10	590 ± 13	197 ± 15	2.27 ± 0.27	1.22 ± 0.28	236 ± 34	194 ± 35
OVX + 250 μg Norethynodrel. N = 9	596 ± 16 N.S.	197 ± 18 N.S.	3.61 ± 0.37 <.02	2.66 ± 0.38 <.01	221 ± 34 N.S.	172 ± 29 N.S.
OVX + 500 μg Norethynodrel. N = 11	602 ± 13 N.S.	183 ± 13 N.S.	2,79 ± 0.30 N.S.	1.56 ± 0.20 N.S.	199 ± 19 N.S.	147 ± 20 N.S.
OVX + 250 μg Norethisterone Acetate N = 10	655 ± 17 <.01	230 ± 14 N.S.	3.26 ± 0.37 <.05	2.41 ± 0.39 <.05	329 ± 30 N.S.	279 ± 26 N.S.
OVX + 500μg Norethisterone Acetate N = 12	612 ± 6 N.S.	198 ± 11 N.S.	3.93 ± 0.42 <.05	2.53 ± 0.40 <.05	268 ± 21 N.S.	201 ± 21 N.S.
60 Minute Areas.						
OVX Oil Control N = 10	304 ± 8	108 ± 8	1.00 ± 0.12	0.51 ± 0.12	164 ± 29	143 ± 29
OVX + 250 μg Norethynodrel. N = 9	320 ± 7 N.S.	121 ± 8 N.S.	1.99 ± 0.24 <.05	1.52 ± 0.24 <.05	164 ± 31 N.S.	139 ± 29 N.S.
OVX + 500 μg Norethynodrel. N = 11	322 ± 8 N.S.	112 ± 7 N.S.	1.73 ± 0.20 <.05	1.10 ± 0.15 <.02	134 ± 14 N.S.	108 ± 14 N.S.
OVX + 250 μg Norethisterone Acetate N = 10	344 ± 8 <.005	132 ± 6 <.05	1.62 ± 0.23 <.05	1.19 ± 0.15 <.05	237 ± 22 N.S	212 ± 21 N.S.
OVX + 500μg Norethisterone Acetate N = 12	324 ± 6 N.S.	118 ± 5 N.S.	2.06 ± 0.22 <.001	1.36 ± 0.21 <.01	175 ± 14 N.S.	141 ± 14 N.S.

TABLE VIII A Mean ± S.E.M. O.G.T.T. 120 minute and 60 minute total and incremental areas with significance levels at 4 weeks for Norethynodrel-Norethisterone Acetate series

(Units-min)

(N.S. = not significant.)

TABLE VIII B Animal weights . Norethynodrel and Norethisterone acetate series.

Mean ± S.E.M. (body weight in grams)

	Initial	2 week	4 week
OVX Oil Control	222 ± 8	249 ± 7	271 ± 9
OVX + 250 μg Norethynodrel	219 ± 7	223 ± 8	241 ± 10
OVX + 500 μg Norethynodrel	224 ± 3	216 ± 6	234 ± 6
OVX + 250 μg Norethisterone acetate.	217 ± 6	240 ± 7	262 ± 8
OVX + 500 μg Norethisterone acetate	215 ± 4	235 ± 7	252 ± 6

At the 30, 60 and 90 minute intervals there was no significant difference between mean values for the controls and progestogen treated groups.

At the 120 minute interval only the 500 μg Norethisterone Acetate group mean value was significantly different and lower than the control value, ($p < .05$).

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS TABLE V111

120 Minute Areas:-

The total and incremental areas for both progestogens at the two dosage levels were not significantly different from the respective area values for the controls. There was a tendency for the 500 μg dosage, for the two progestogens, to have total and incremental areas less than the control areas.

60 Minute Areas:-

The total and incremental areas for both progestogens at the two dosage levels were not significantly different from the respective control areas; as for the 120 minute areas there was a tendency for the 500 μg dosage for both progestogens to have areas less than the control areas.

BLOOD PYRUVATE AT 2 WEEKS FIG 8 TABLES V11 + V111

Fasting:-

Considering the second fasting samples, the only group that was significantly different from the control group was the 250 μg Norethynodrel group which had a mean value less than the control, ($p < .05$).

DURING THE O.G.T.T.

At the 30 min interval the mean values for both progestogens, at both dosage levels, were greater than the control mean value.

The 500 μg Norethynodrel group was significantly greater than the control, ($p < .001$) and the 250 μg and 500 μg Norethisterone Acetate groups were also significantly greater than controls, ($p < .05$) and ($p < .001$) respectively.

At the 60 min interval all mean values for both progestogens at the two dosage levels were greater than the control mean value. The 250 μg Norethynodrel group was significantly greater, ($p < .05$) than the control value. The 250 μg and 500 μg Norethisterone Acetate groups were also significantly greater than the controls, ($p < .005$) and ($p < .001$) respectively.

The 90 and 120 minute interval mean values, for both progestogens at the two dosage levels, were greater than the mean control values, but none attained significance.

MEAN O.G.T.T. BLOOD PYRUVATE AREAS AT 2 WEEKS: TABLE V111

120 Minute Areas:-

There was significant elevations in total and incremental area mean values for both progestogens at both dose levels over the mean values for the total and incremental areas of the control group.

The 250 μg Norethynodrel group attained significance levels for total and incremental areas of ($p < .05$) and ($p < .005$) respectively.

The 500 μg Norethynodrel group had significance at ($p < .001$) and ($p < .005$) for total and incremental areas respectively.

The 250 μg Norethisterone Acetate group attained significance levels for total and incremental areas of ($p < .01$) and ($p < .005$) respectively.

The 500 μg Norethisterone Acetate group was significant at a level of ($p < .001$) for both total and incremental areas.

60 Minute Areas:-

The total and incremental areas for both progestogens at the two dose levels were greater than the respective control areas. The 250 μg Norethynodrel group had significantly elevated total area, ($p < .05$) and incremental area, ($p < .005$) over the respective control areas.

The 500 μg Norethynodrel group had significantly elevated total and incremental areas over the respective control areas, ($p < .001$) for both areas.

The 250 μg Norethisterone Acetate group had total and incremental areas that were significantly greater than the respective control areas, ($p < .01$) for both areas. Also the 500 μg Norethisterone Acetate group had areas significantly greater than the respective control areas, ($p < .001$) for both areas.

PLASMA INSULIN AT 2 WEEKS FIG. 8 TABLES V11 + V111

Fasting:-

There was no significant difference between mean fasting values for 250 μg Norethynodrel and 250 μg and 500 μg Norethisterone Acetate groups, and the control mean value. The 500 μg Norethynodrel had a significantly elevated value over that of the control ($p < .05$).

DURING THE O.G.T.T.

At the 15 min interval there was no significant difference between the mean values for the controls and the progestogen mean values, at both dose levels.

At the 30 min interval all progestogen mean values were less than the control mean value, however only the 250 μg Norethynodrel group was significantly less ($p < .05$).

At the 60 min interval all progestogen mean values were less than the control mean value but none attained significance.

At the 90 min interval all mean values for the progestogen groups were less than the control value with only the 500 μg Norethisterone Acetate being significantly less ($p < .05$).

At the 120 min interval all progestogen mean values were less than the control mean value with the 500 μg Norethisterone Acetate attaining significance ($p < .01$).

MEAN O.G.T.T. INSULIN AREAS AT 2 WEEKS :TABLE V111

120 Minute Areas:-

The progestogen treated groups demonstrated total and incremental areas that were less than the respective control areas. The 250 μg Norethynodrel incremental area , and both the total and incremental areas for the 500 μg Norethisterone Acetate group were significantly less than the respective control areas ($p < .05$), for both progestogens and their areas.

60 Minute Areas:-

The progestogen total and incremental areas were less than the respective control areas, however only the 250 μg Norethynodrel incremental area was significantly less than the control area ($p < .05$).

PLASMA GLUCOSE AT 4 WEEKS FIG 8A TABLE V11A

Fasting:-

The mean fasting value for the 250 μg Norethynodrel group were not significantly different from the control value. The 500 μg Norethynodrel group and 250 μg Norethisterone Acetate group mean values were significantly elevated over the value for the controls, ($p < .02$) and ($p < .05$) respectively. The 500 μg Norethisterone Acetate group mean value was not significantly different from the control value.

DURING THE O.G.T.T.

At the 15 min interval the 250 μg Norethynodrel group and 500 μg Norethisterone Acetate group had mean values that were not significantly different from the control value. The 500 μg Norethynodrel group and 250 μg Norethisterone Acetate group had mean values significantly greater than the control mean value, ($p < .05$) and ($p < .02$) respectively.

At the 30 min interval no progestogen group mean value was significantly different from the control.

At the 60 min interval the mean values for the Norethynodrel progestogen were not significantly different from the control mean value, however the two dose levels for Norethisterone Acetate were significantly greater than the control value, ($p < .02$) for the 250 μg group and ($p < .01$) for the 500 μg group.

At the 90 min and 120 min intervals no progestogen group had a mean value that was significantly different from the control value.

MEAN O.G.T.T. PLASMA GLUCOSE AREAS AT 4 WEEKS
TABLE V111A

120 Minute Areas:-

The Norethynodrel group at both dose levels had mean values for total and incremental areas that were not significantly different from the respective mean control values.

The 250 μg Norethisterone Acetate group had a significantly elevated total area ($p < .01$) compared with control total area, however incremental areas were not significantly different. The 500 μg Norethisterone Acetate group had mean values for total and incremental areas that were not significantly different from respective mean value control areas.

60 Minute Areas:-

All progestogen groups had a tendency to have greater mean value total and incremental areas than the respective mean values for the control areas. The only group that demonstrated significantly elevated areas over the control areas was the 250 μg Norethisterone Acetate group, which had significance at, ($p < .005$) and ($p < .05$) for total and incremental areas respectively.

BLOOD PYRUVATE AT 4 WEEKS FIG. 8A TABLE V11A

Fasting:-

The only group that had a mean value significantly different from the control mean value was the 500 μg Norethisterone Acetate group which was greater ($p < .001$).

DURING THE O.G.T.T.

At the 30 min interval all progestogen groups had a mean value that was greater than the control mean value, with the 250 μg and 500 μg Norethynodrel groups reaching significance at ($p < .005$) and ($p < .02$) respectively and the 500 μg Norethisterone Acetate group at, ($p < .005$).

At the 60 min interval all progestogen groups had mean values greater than the controls with the 250 μg and 500 μg Norethisterone Acetate groups reaching significance at ($p < .01$) and ($p < .005$) respectively.

At the 90 and 120 minute intervals, the mean values for all progestogen groups although greater than the control mean value, no group was significantly different.

MEAN O.G.T.T. PYRUVATE AREAS AT 4 WEEKS TABLE V111A

120 Minute Areas:-

All progestogen groups had total and incremental areas that were greater than the control total and incremental areas. The 250 μg Norethynodrel group had significantly elevated total, ($p < .02$) and incremental ($p < .01$) areas, over that of the respective control areas. The 500 μg Norethynodrel group did not attain significance. The 250 μg and 500 μg Norethisterone Acetate groups had significantly elevated total and incremental areas over the respective control areas, ($p < .05$) for all areas.

60 Minute Areas:-

All progestogen groups had significantly elevated total and incremental area values over the respective values for the control areas.

The levels of significance for the 250 μg Norethynodrel group was ($p < .05$) for both total and incremental areas. The level of significance for the 500 μg Norethynodrel group was ($p < .05$) for the total area and ($p < .02$) for the incremental area. The significance levels for the 250 μg Norethisterone Acetate group were ($p < .0$) for both total and incremental areas.

The 500 μg Norethisterone Acetate group significance levels were ($p < .001$) for the total area and ($p < .01$) for the incremental area.

PLASMA INSULIN AT 4 WEEKS FIG 8A TABLE V11A

Fasting:-

The only group that was significantly different from the control value was the 500 μg Norethisterone Acetate group, which had a mean value greater than the control mean value, ($p < .02$).

DURING THE O.G.T.T.

At the 15 min interval, the only progestogen group that was significantly different from the control value was the 250 μg Norethisterone Acetate group, which had a mean value greater the mean control value, ($p < .05$). At the subsequent time intervals 30, 60, 90 and 120 min none of the progestogen groups had mean values that were significantly different from the controls.

MEAN O.G.T.T INSULIN AREAS AT 4 WEEKS : TABLE V111A

120 Minute Areas:-

The total and incremental areas for all progestogen groups were not significantly different from the respective control areas.

60 Minute Areas:-

The total and incremental areas for all progestogen groups were not significantly different from the respective control areas.

SECTION 111 MEGESTROL ACETATE: CHLORMADINONE ACETATE SERIES:-

PROCEDURE:-

Ovariectomized rats (OVX) weighing 200-300 gm were given sub-cutaneous injections of either Chlormadinone Acetate or Megestrol Acetate dissolved in 0.10 ml vegetable oil at the following doses 100 μg, 250 μg and 500 μg.. The control animals received 0.10 ml oil only.

The animals were treated for 2 weeks, after which time the first O.G.T.T. was performed, the animals were then maintained for a further 2 weeks when the second (4 week) O.G.T.T. was performed.

PLASMA GLUCOSE AT 2 WEEKS: FIGS. 9+10 TABLES 1X+1XA

Fasting:-

The mean values for the progestogen Chlormadinone Acetate were not significantly different from the control mean value. The mean values for the 100 μg and 500 μg Megestrol acetate groups were not significantly different from the control mean value, however the 250 μg group mean value was significantly lower than the control mean value, ($p < .01$).

DURING THE O.G.T.T.

It should be noted at this point, that the control group at this 2 week interval had a rather abnormally shaped glucose tolerance curve in comparison to other control groups observed during these investigations. The Megestrol Acetate groups also had a peculiar tolerance curve, where a "humping" effect was seen at the 60 minute interval after glucose.

Fig. 9

CHLORMADINONE ACETATE—MEGESTROL ACETATE SERIES
AT 2 WEEKS

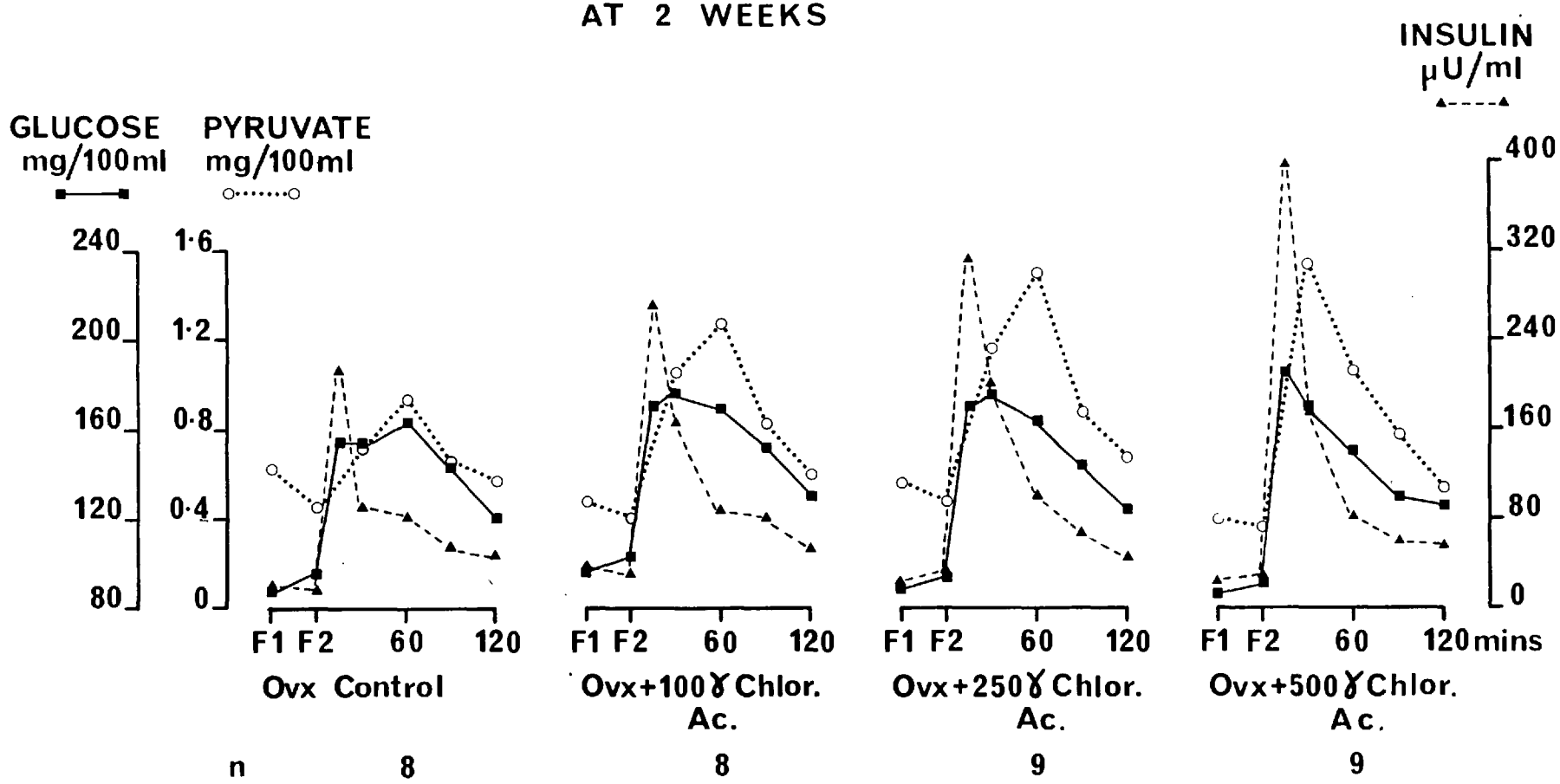


Fig.9A

CHLORMADINONE ACETATE–MEGESTROL ACETATE SERIES
AT 4 WEEKS

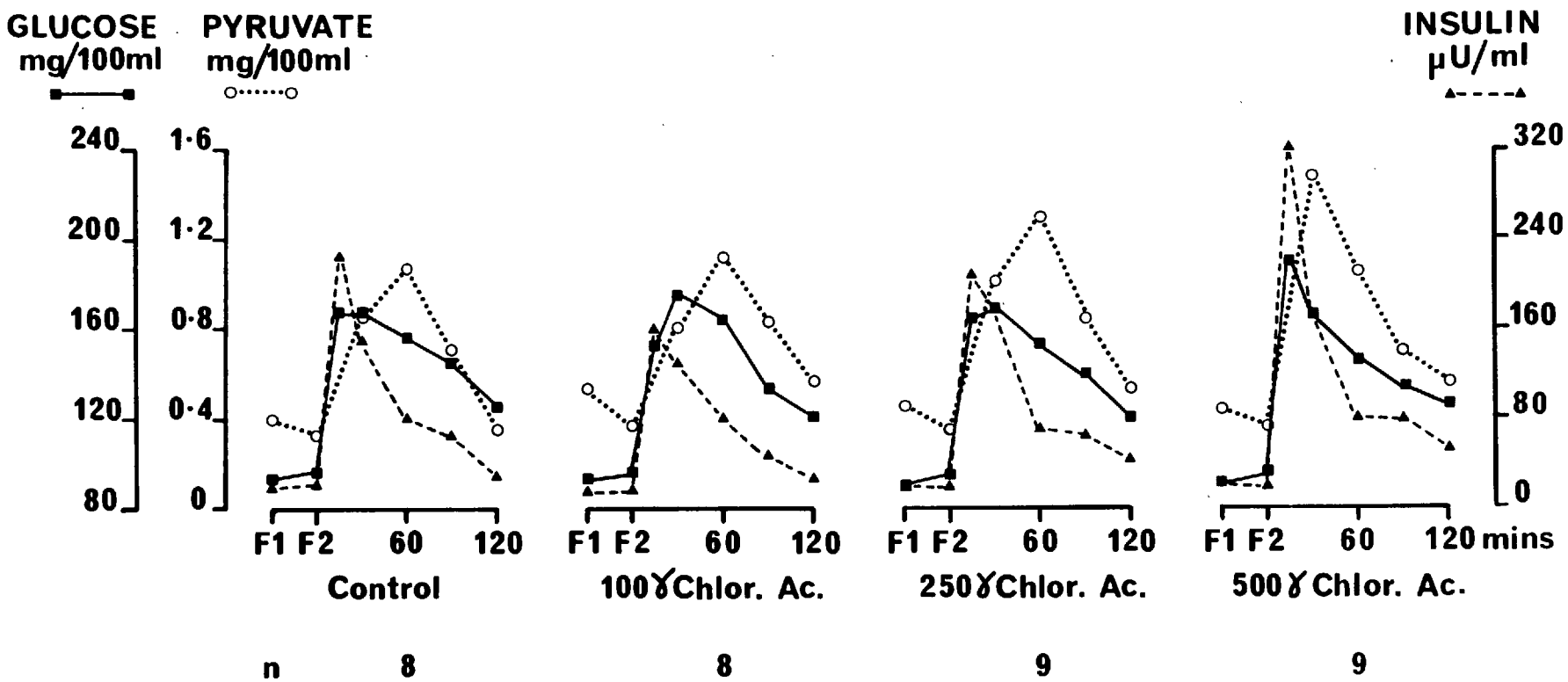


Fig. 10

CHLORMADINONE ACETATE—MEGESTROL ACETATE SERIES AT 2 WEEKS

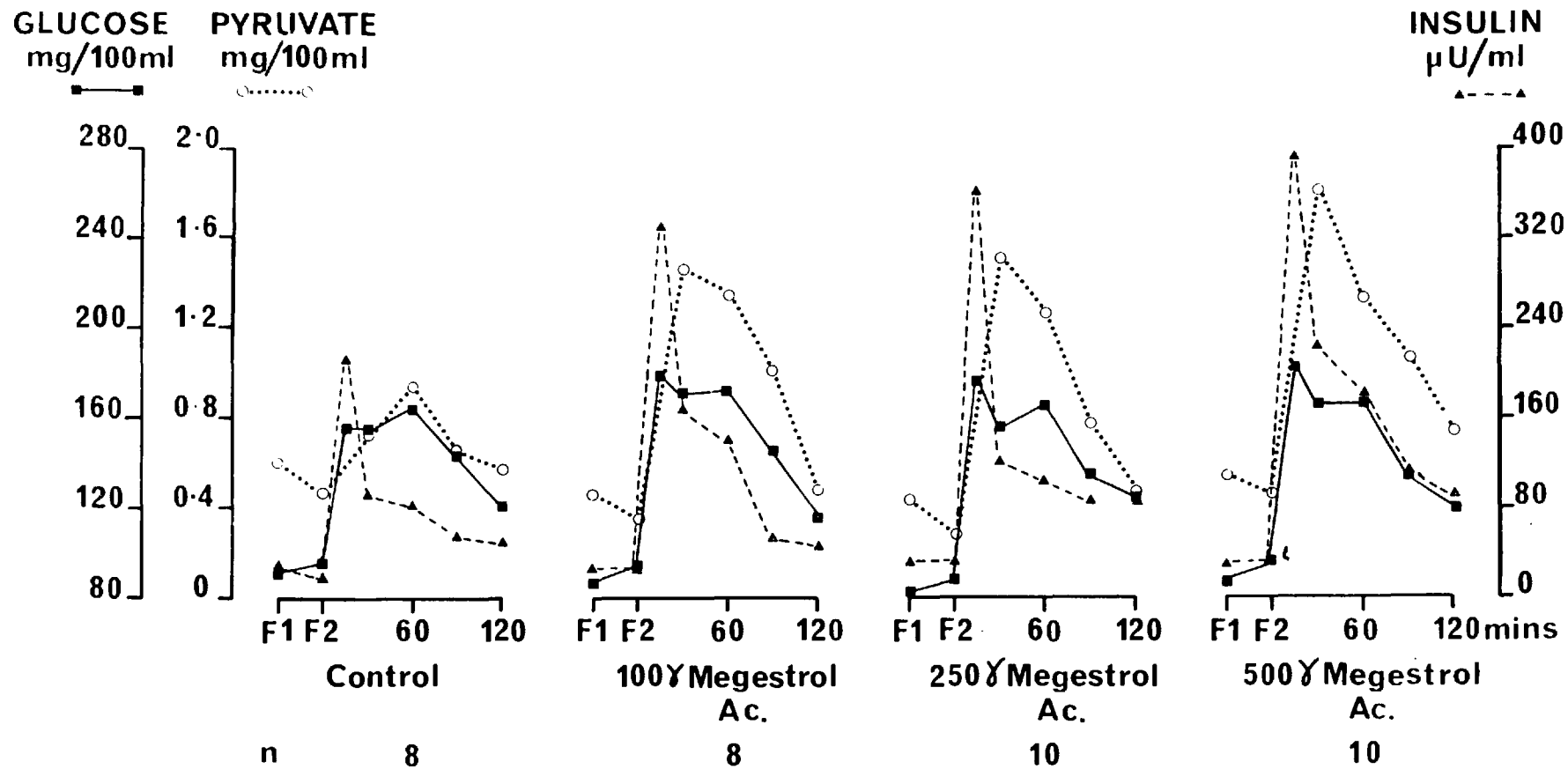
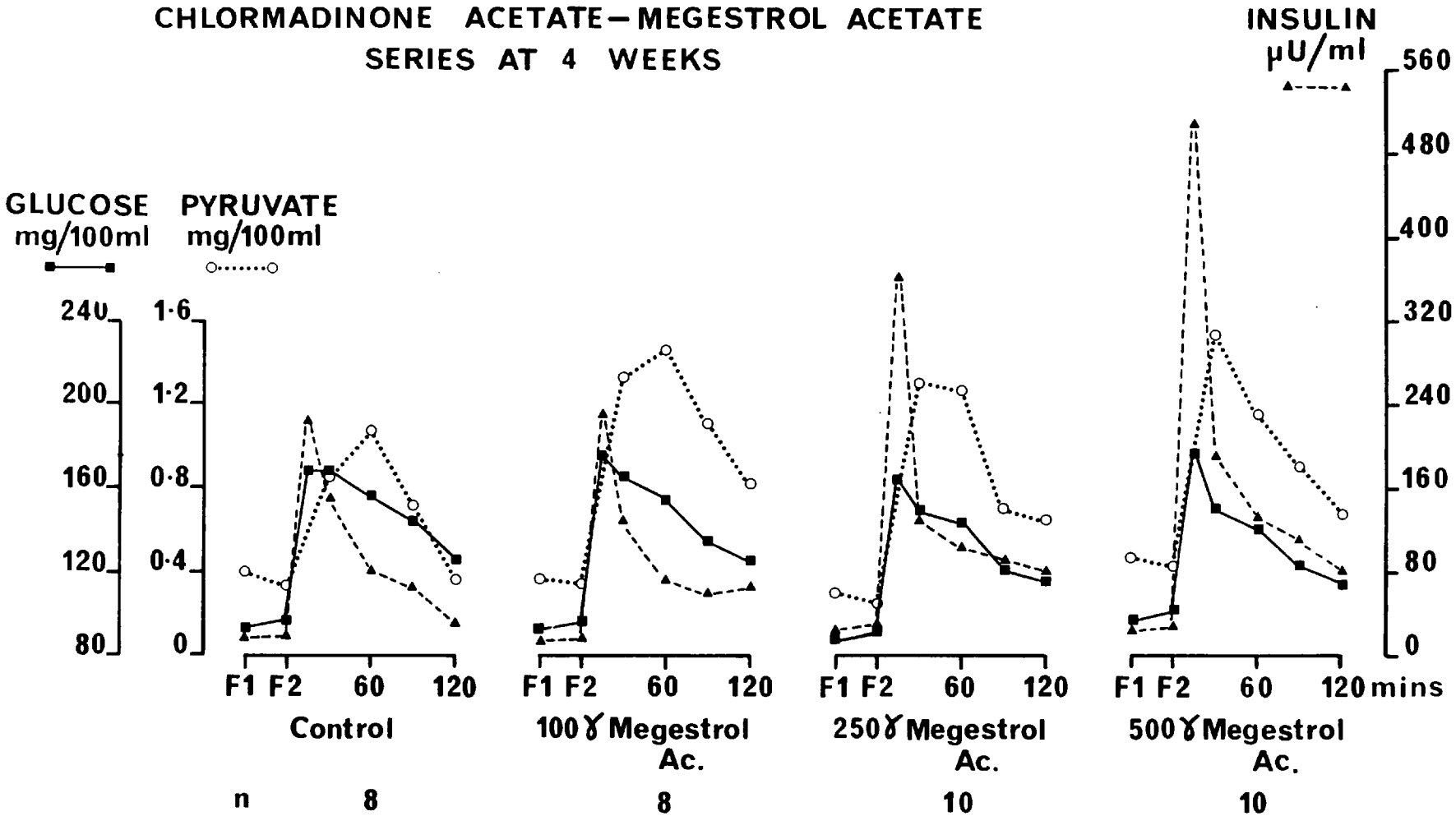


Fig. 10A

CHLORMADINONE ACETATE – MEGESTROL ACETATE SERIES AT 4 WEEKS



		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX 0.10 ml	Glucose mg/100ml	87 ± 2	95 ± 2	156 ± 4	156 ± 4	164 ± 4	144 ± 4	119 ± 5
Oil Control	Pyruvate mg/100ml	0.63 ± 0.07	0.45 ± 0.05	----------	0.75 ± 0.06	0.93 ± 0.14	0.62 ± 0.08	0.59 ± 0.08
N = 8	Insulin μU /ml	17 ± 1	17 ± 3	216 ± 18	93 ± 12	82 ± 11	58 ± 5	49 ± 8
OVX +100 μg	Glucose	87 ± 2 N.S.	92 ± 2 N.S.	170 ± 4 < .025	178 ± 5 < .005	169 ± 6 N.S.	152 ± 8 N.S.	129 ± 5 N.S.
Chlormadinone Acetate	Pyruvate	0.49 ± 0.05 N.S.	0.39 ± 0.05 N.S.	----------	1.05 ± 0.14 N.S.	1.28 ± 0.22 N.S.	0.85 ± 0.18 N.S.	0.59 ± 0.07 N.S.
N = 8	Insulin	26 ± 3 < .05	20 ± 2 N.S.	274 ± 74 N.S.	169 ± 22 < .05	87 ± 12 N.S.	83 ± 19 N.S.	59 ± 10 N.S.
OVX + 250μg	Glucose	87 ± 2 N.S.	92 ± 2 N.S.	170 ± 5 < .05	175 ± 6 < .05	164 ± 5 N.S	144 ± 5 N.S.	123 ± 4 N.S.
Chlormadinone Acetate	Pyruvate	0.58 ± 0.11 N.S.	0.49 ± 0.15 N.S.	----------	1.17 ± 0.14 < .05	1.49 ± 0.19 < .05	0.88 ± 0.11 N.S.	0.68 ± 0.15 N.S.
N = 9	Insulin	19 ± 4 N.S.	23 ± 3 N.S.	313 ± 51 N.S.	202 ± 27 < .05	113 ± 21 N.S.	69 ± 11 N.S.	46 ± 9 N.S.
OVX +500 μg	Glucose	85 ± 2 N.S.	93 ± 2 N.S.	185 ± 10 < .02	170 ± 8 N.S.	151 ± 7 N.S.	131 ± 5 N.S.	127 ± 3 N.S.
Chlormadinone Acetate	Pyruvate	0.41 ± 0.04 < .02	0.36 ± 0.03 N.S.	----------	1.53 ± 0.19 < .005	1.06 ± 0.28 N.S.	0.79 ± 0.14 N.S.	0.53 ± 0.05 N.S.
N = 9	Insulin	31 ± 3 < .05	32 ± 3 < .005	399 ± 59 N.S.	186 ± 43 N.S.	82 ± 12 N.S.	58 ± 5 N.S.	56 ± 5 N.S.

(N.S = not significant)

TABLE IX

Mean ± S.E.M.

(Units min)

O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for Chlormadinone Acetate series at 2 weeks.

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX O.10ml	Glucose mg/100ml	87 ± 2	95 ± 2	156 ± 4	156 ± 4	164 ± 4	144 ± 4	119 ± 5
Oil Control	Pyruvate mg/100ml	0.63 ± 0.07	0.45 ± 0.05	----------	0.75 ± 0.06	0.93 ± 0.14	0.62 ± 0.08	0.59 ± 0.08
N = 8	Insulin μU /ml	17 ± 1	17 ± 3	216 ± 18	93 ± 12	82 ± 11	58 ± 5	49 ± 8
OVX + 100 μg	Glucose	86 ± 3 N.S.	93 ± 3 N.S.	179 ± 12 N.S.	169 ± 7 N.S.	170 ± 6 N.S.	144 ± 4 N.S.	116 ± 3 N.S.
Megestrol Acetate	Pyruvate	0.46 ± 0.06 N.S.	0.33 ± 0.05 N.S.	----------	1.47 ± 0.19 < .005	1.33 ± 0.20 N.S.	1.00 ± 0.14 < .05	0.48 ± 0.06 N.S.
N = 8	Insulin	25 ± 4 N.S.	25 ± 3 N.S.	333 ± 69 N.S.	174 ± 36 N.S.	138 ± 26 N.S.	56 ± 7 N.S.	47 ± 9 N.S.
OVX + 250 μg	Glucose	77 ± 2 < .005	86 ± 2 < .01	178 ± 4 < .001	156 ± 4 N.S.	164 ± 8 N.S.	136 ± 7 N.S.	122 ± 4 N.S.
Megestrol Acetate	Pyruvate	0.43 ± 0.04 < .02	0.28 ± 0.03 < .01	----------	1.52 ± 0.17 < .005	1.25 ± 0.24 N.S.	0.78 ± 0.16 N.S.	0.42 ± 0.05 N.S.
N =10	Insulin	28 ± 3 < .005	29 ± 2 < .005	355 ± 30 < .005	118 ± 8 N.S.	104 ± 14 N.S.	88 ± 10 N.S.	87 ± 12 < .05
OVX + 500 μg	Glucose	87 ± 3 N.S.	95 ± 3 N.S.	183 ± 7 < .005	166 ± 8 N.S.	169 ± 9 N.S.	135 ± 5 N.S.	119 ± 2 N.S.
Megestrol Acetate	Pyruvate	0.57 ± 0.06 N.S.	0.45 ± 0.04 N.S.	----------	1.80 ± 0.17 < .001	1.33 ± 0.15 N.S.	1.07 ± 0.10 < .005	0.74 ± 0.10 N.S.
N = 8	Insulin	28 ± 4 < .02	33 ± 4 < .01	395 ± 62 N.S.	223 ± 63 N.S.	183 ± 26 < .05	109 ± 18 < .05	89 ± 8 < .005

TABLE IX A Mean ± S.E.M. O.G.T.T. Plasma glucose , insulin and blood pyruvate with significance levels for Megestrol Acetate series at 2 weeks.
(N.S. = not significant)

		F1	F2	15 min	30 min	60 min	90 min	120 min
VX 0.10 ml	Glucose mg/100ml	92 ± 3	97 ± 2	166 ± 11	166 ± 10	159 ± 4	146 ± 4	126 ± 3
Oil Control	Pyruvate mg/100ml	0.42 ± 0.02	0.32 ± 0.03	----------	0.84 ± 0.13	1.08 ± 0.17	0.72 ± 0.13	0.35 ± 0.04
N = 8	Insulin μU/ml	18 ± 3	19 ± 2	227 ± 42	134 ± 25	79 ± 11	64 ± 5	36 ± 6
OVX + 100 μg	Glucose	92 ± 1 N.S.	95 ± 2 N.S.	154 ± 8 N.S.	175 ± 9 N.S.	165 ± 3 N.S.	131 ± 7 N.S.	120 ± 2 N.S.
Chlormadinone Acetate	Pyruvate	0.53 ± 0.04 <.02	0.38 ± 0.04 N.S.	----------	0.80 ± 0.08 N.S.	1.12 ± 0.14 N.S.	0.84 ± 0.13 N.S.	0.58 ± 0.05 N.S.
N = 8	Insulin	14 ± 3 N.S.	17 ± 4 N.S.	157 ± 13 N.S.	136 ± 26 N.S.	81 ± 11 N.S.	46 ± 13 N.S.	33 ± 12 N.S.
OVX + 250 μg	Glucose	92 ± 2 N.S.	95 ± 1 N.S.	164 ± 8 N.S.	167 ± 6 N.S.	153 ± 3 N.S.	142 ± 5 N.S.	120 ± 4 N.S.
Chlormadinone Acetate	Pyruvate	0.45 ± 0.09 N.S.	0.34 ± 0.04 N.S.	----------	1.00 ± 0.09 N.S.	1.29 ± 0.16 N.S.	0.85 ± 0.16 N.S.	0.52 ± 0.09 N.S.
N = 9	Insulin	23 ± 3 N.S.	19 ± 2 N.S.	209 ± 19 N.S.	175 ± 31 N.S.	74 ± 7 N.S.	65 ± 7 N.S.	42 ± 7 N.S.
OVX + 500 μg	Glucose	91 ± 2 N.S.	98 ± 2 N.S.	191 ± 7 N.S.	166 ± 6 N.S.	147 ± 7 N.S.	137 ± 4 N.S.	126 ± 5 N.S.
Chlormadinone Acetate	Pyruvate	0.44 ± 0.04 N.S.	0.38 ± 0.03 N.S.	----------	1.49 ± 0.15 <.005	1.06 ± 0.18 N.S.	0.69 ± 0.10 N.S.	0.56 ± 0.06 <.01
N = 9	Insulin	22 ± 4 N.S.	21 ± 3 N.S.	321 ± 36 N.S.	163 ± 27 N.S.	81 ± 14 N.S.	79 ± 5 <.05	54 ± 5 <.05

TABLE IX B Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for Chlormadinone Acetate at 4 weeks.
(N.S. = not significant)

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX 0.10 ml Oil Control N = 8	Glucose mg/100ml	92 ± 3	97 ± 2	166 ± 10	166 ± 10	159 ± 4	146 ± 4	126 ± 3
	Pyruvate mg/100ml	0.42 ± 0.02	0.32 ± 0.03	----------	0.84 ± 0.13	1.08 ± 0.17	0.72 ± 0.13	0.35 ± 0.04
	Insulin μU /ml	18 ± 3	19 ± 2	227 ± 42	134 ± 25	79 ± 11	64 ± 5	36 ± 6
OVX + 100 μg Megestrol - Acetate N = 8	Glucose	91 ± 1 N.S.	96 ± 3 N.S.	176 ± 6 N.S.	166 ± 7 N.S.	153 ± 4 N.S.	134 ± 5 N.S.	125 ± 5 N.S.
	Pyruvate	0.37 ± 0.64 N.S.	0.35 ± 0.03 N.S.	----------	1.32 ± 0.12 <.02	1.47 ± 0.15 N.S.	1.10 ± 0.15 N.S.	0.82 ± 0.11 <.001
	Insulin	13 ± 2 N.S.	17 ± 3 N.S.	232 ± 37 N.S.	133 ± 33 N.S.	74 ± 13 N.S.	58 ± 14 N.S.	66 ± 13 N.S.
OVX + 250 μg Megestrol - Acetate N = 10	Glucose	89 ± 2 N.S.	91 ± 4 N.S.	165 ± 6 N.S.	149 ± 6 N.S.	143 ± 8 N.S.	121 ± 6 <.005	116 ± 5 N.S.
	Pyruvate	0.30 ± 0.03 N.S.	0.23 ± 0.01 <.02	----------	1.29 ± 0.15 <.05	1.27 ± 0.17 N.S.	0.73 ± 0.14 N.S.	0.65 ± 0.12 <.05
	Insulin	26 ± 3 N.S.	28 ± 3 <.05	363 ± 46 N.S.	129 ± 22 N.S.	104 ± 17 N.S.	90 ± 11 N.S.	82 ± 13 .05
OVX + 500 μg Megestrol - Acetate N = 8	Glucose	98 ± 2 N.S.	102 ± 2 N.S.	178 ± 9 N.S.	151 ± 6 N.S.	140 ± 6 <.025	122 ± 4 <.001	114 ± 5 <.05
	Pyruvate	0.47 ± 0.06 N.S.	0.42 ± 0.07 N.S.	----------	1.55 ± 0.10 <.001	1.16 ± 0.10 N.S.	0.92 ± 0.13 N.S.	0.66 ± 0.09 <.01
	Insulin	25 ± 4 N.S.	29 ± 2 <.05	506 ± 92 <.025	193 ± 27 N.S.	131 ± 23 N.S.	114 ± 14 <.05	82 ± 16 <.05

TABLE IX C Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for Megestrol Acetate series at 4 weeks. (N.S. = not significant.)

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX 0.10 ml Oil Control N = 8	586 ± 10	205 ± 9	2.80 ± 0.27	1.02 ± 0.27	347 ± 21	275 ± 14
OVX + 100 μg Chlormadinone Acetate N = 8	627 ± 16 <.05	260 ± 14 <.01	3.67 ± 0.52 N.S.	2.11 ± 0.50 N.S.	468 ± 72 N.S.	388 ± 70 N.S.
OVX + 250 μg Chlormadinone Acetate N =9	608 ± 14 N.S.	241 ± 13 <.05	4.10 ± 0.42 <.025	2.39 ± 0.33 <.01	553 ± 57 N.S.	426 ± 59 N.S.
OVX + 500 μg Chlormadinone Acetate N = 9	589 ± 18 N.S.	218 ± 16 N.S.	3.82 ± 0.53 N.S.	2.40 ± 0.47 <.05	514 ± 62 N.S.	385 ± 57 N.S.
60 Minute Areas.						
OVX 0.10 ml Oil Control N = 8	301 ± 5	111 ± 5	1.43 ± 0.13	0.54 ± 0.13	223 ± 11	187 ± 7
OVX + 100μg Chlormadinone Acetate N = 8	326 ± 7 <.05	142 ± 6 <.005	1.89 ± 0.26 N.S.	1.11 ± 0.24 N.S.	312 ± 53 N.S.	272 ± 52 N.S.
OVX + 250 μg	321 ± 7	138 ± 7	2.13 ± 0.26	1.27 ± 0.24	371 ± 46	324 ± 44

TABLE X

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas						
OVX 0.10 ml Oil Control N = 8	586 ± 10	205 ± 9	2.80 ± 0.27	1.02 ± 0.27	347 ± 21	275 ± 14
OVX + 100 μg Megestrol - Acetate N =8	613 ± 17 N.S.	242 ± 16 N.S.	4.21 ± 0.39 <.01	2.87 ± 0.38 <.001	520 ± 81 N.S.	420 ± 72 N.S.
OVX + 250 μg Megestrol - Acetate N = 10	588 ± 16 N.S.	244 ± 16 N.S.	3.90 ± 0.55 N.S.	2.79 ± 0.50 <.05	514 ± 40 <.05	376 ± 41 N.S.
OVX + 500 μg Megestrol - Acetate N = 8	601 ± 13 N.S	220 ± 19 N.S.	4.78 ± 0.23 <.001	3.00 ± 0.14 <.001	625 ± 49 <.05	485 ± 51 <.05
60 Minute Areas						
OVX + Oil Control (0.10 ml) N = 8	301 ± 5	111 ± 5	1.43 ± 0.13	0.54 ± 0.13	223 ± 11	188 ± 7
OVX + 100 μg Megestrol - Acetate N = 8	325 ± 11 N.S.	140 ± 8 <.02	2.30 ± 0.26 <.05	1.60 ± 0.27 <.05	372 ± 65 N.S.	322 ± 61 N.S.
OVX + 250 μg	310 ± 6	137 ± 6	2.28 ± 0.29	1.73 ± 0.27	331 ± 23	262 ± 22

TABLE X A

	Glucose Total Area(△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX +0.10 ml Oil Control. N = 8	600 ± 17	213 ± 16	3.15 ± 0.36	1.93 ± 0.32	382 ± 40	305 ± 46
OVX + 100 μg Chlormadinone Acetate N=8	589 ± 12 N.S.	211 ± 14 N.S.	3.24 ± 0.30 N.S.	1.71 ± 0.32 N.S.	328 ± 38 N.S.	261 ± 31 N.S.
OVX + 250 μg Chlormadinone Acetate N=9	592 ± 9 N.S.	211 ± 10 N.S.	3.58 ± 0.37 N.S.	2.47 ± 0.31 N.S.	412 ± 25 N.S.	334 ± 26 N.S.
OVX + 500μg Chlormadinone Acetate N=9	591 ± 14 N.S.	198 ± 18 N.S.	3.71 ± 0.36 N.S.	2.20 ± 0.29 N.S.	475 ± 45 N.S.	391 ± 49 N.S.
60 Minute Areas						
OVX + 0.10 ml Oil Control. N = 8	311 ± 11	118 ± 9	1.64 ± 0.17	1.02 ± 0.16	259 ± 33	220 ± 37
OVX + 100 μg Chlormadinone Acetate N=8	315 ± 9 N.S.	125 ± 9 N.S.	1.55 ± 0.13 N.S.	0.78 ± 0.14 N.S.	225 ± 21 N.S.	191 ± 19 N.S.
OVX + 250 μg Chlormadinone Acetate N=9	308 ± 5 N.S.	117 ± 6 N.S.	1.81 ± 0.15 N.S.	1.15 ± 0.17 N.S.	288 ± 21 N.S.	250 ± 23 N.S.
OVX + 500 μg Chlormadinone Acetate N = 9	318 ± 8 N.S.	121 ± 8 N.S.	2.20 ± 0.21 N.S.	1.45 ± 0.17 N.S.	355 ± 30 N.S.	310 ± 34 N.S.

TABLE X B Mean ± S.EM.(Units-min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Chlormadinone Acetate at 4 weeks. 'N.S. =not significant.'

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX 0.10 ml Oil Control. N = 8	600 ± 17	213 ± 16	3.15 ± 0.36	1.93 ± 0.32	382 ± 40	305 ± 46
OVX + 100 μg Megestrol - Acetate N = 8	586 ± 9 N.S.	204 ± 11 N.S.	4.48 ± 0.37 < .025	3.08 ± 0.39 < .05	385 ± 65 N.S.	318 ± 56 N.S.
OVX + 250 μg Megestrol - Acetate N =10	539 ± 15 < .02	174 ± 19 N.S.	3.72 ± 0.41 N.S.	2,79 ± 0.40 N.S.	520 ± 59 N.S.	441 ± 49 N.S.
OVX + 500 μg Megestrol - Acetate N = 8	546 ± 16 < .05	143 ± 21 < .05	4.16 ± 0.28 < .05	2.82 ± 0.32 N.S.	690 ± 72 < .005	570 ± 72 < .02
60 Minute Areas.						
OVX 0.10 ml Oil Control. N = 8	311 ± 11	118 ± 9	1.64 ± 0.17	1.02 ± 0.16	259 ± 33	220 ± 37
OVX + 100 μg Megestrol - Acetate N = 8	313 ± 7 N.S.	122 ± 7 N.S.	2.23 ± 0.19 < .05	1.53 ± 0.20 N.S.	257 ± 44 N.S.	223 ± 39 N.S.
OVX + 250 μg Megestrol - Acetate N = 10	289 ± 8 N.S.	106 ± 9 N.S.	2.03 ± 0.21 N.S.	1.57 ± 0.20 N.S.	338 ± 39 N.S.	281 ± 37 N.S.
OVX + 500 μg Megestrol - Acetate N = 8	298 ± 9 N.S.	96 ± 12 N.S.	2.34 ± 0.13 < .01	1.53 ± 0.17 N.S.	471 ± 60 < .05	411 ± 60 < .05

TABLE XC Mean ± S.E.M. (Units-min)

120 Minute and 60 Minute total and incremental areas with significance levels for Megestrol Acetate series at 4 weeks. (N.S. = not significant.)

TABLE XD Animal weights. Chlormadinone acetate and Megestrol acetate series.
Mean ± S.E.M. (body weight in grams)

	Initial	2 week	4 week
OVX Oil Control	213 ± 5	245 ± 5	279 ± 5
OVX + 100 µg Chlormadinone acetate	213 ± 4	258 ± 5	280 ± 8
OVX + 250 µg Chlormadinone acetate	207 ± 5	247 ± 5	280 ± 8
OVX + 500 µg Chlormadinone acetate	208 ± 4	250 ± 8	274 ± 10
OVX + 100 µg Megestrol acetate	215 ± 5	258 ± 8	289 ± 8
OVX + 250 µg Megestrol acetate	212 ± 5	262 ± 6	281 ± 8
OVX + 500 µg Megestrol acetate	212 ± 6	266 ± 8	288 ± 6

These observations made for the control and Megestrol Acetate groups make it difficult to interpret glucose dissimilation during the O.G.T.T.; therefore these results must be looked at with some reservation.

At the 15 min interval all of the Chlormadinone Acetate groups had mean values significantly less than the control mean value; 100 μg ($p < .025$), 250 μg ($p < .05$) and 500 μg ($p < .02$).

The Megestrol Acetate groups also had mean values greater than the controls, however only the 250 μg and 500 μg groups had values that were significantly elevated over the control value, ($p < .001$) and ($p < .005$) respectively.

At the 30 min interval all Chlormadinone Acetate groups had mean values that were greater the control mean value, with the 100 μg and 250 μg groups reaching significance at ($p < .005$) and ($p < .05$) respectively. The Megestrol Acetate had mean values with a tendency to be higher than the controls but no values were significantly different from the control mean value.

At the 60, 90 and 120 minute intervals the mean values for both progestogens at all doses were not significantly different from the control mean value.

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS TABLES X+XA

120 Minute Areas:-

The total and incremental areas for all doses of Chlormadinone Acetate were greater than the respective control areas. The 100 μg Chlormadinone Acetate group had significantly elevated total($p < .05$) and incremental ($p < .01$) areas over the respective control areas. The 250 μg Chlormadinone Acetate group had a significantly elevated incremental area ($p < .05$) over that of the control area, the total area was not significantly different. The 500 μg Chlormadinone Acetate group total and incremental areas were not significantly different from the controls.

The total and incremental areas for all doses of Megestrol Acetate were greater though not significantly different from the respective control areas.

60 Minute Areas:-

The total and incremental areas for Chlormadinone Acetate at all dose levels were greater than the respective control areas. The 100 μg Chlormadinone Acetate group had total and incremental areas significantly greater than the control areas, ($p < .05$) and ($p < .005$) respectively. The 250 μg Chlormadinone Acetate group had a significantly elevated incremental area ($p < .02$), with the total area not significantly different from the control area.

The 500 μg Chlormadinone Acetate group had total and incremental areas that were not significantly different from the control areas.

The total and incremental areas for all dose levels of Megestrol Acetate were greater than the respective control areas. The 100 μg and 250 μg incremental areas were significantly greater than the control area ($p < .02$) and ($p < .01$) respectively. The total areas for these two groups were not significantly different from the control area. The 500 μg Megestrol Acetate group had a significantly elevated total area ($p < .02$) but incremental area was not significantly different in comparison to respective control areas.

BLOOD PYRUVATE AT 2 WEEKS FIGS. 9+10 TABLES 1X+1XA

Fasting:-

The mean fasting values for all dose levels of Chlormadinone Acetate were not significantly different from the control mean values. The mean values for 100 μg and 500 μg Megestrol Acetate groups were not significantly different from the control value, however the 250 μg group had a mean value that was significantly less than the control ($p < .01$).

DURING THE O.G.T.T.

Although the pyruvate pattern for the control group during the O.G.T.T. appears normal in comparison to previous control groups during these investigations; the interpretation of pyruvate data should be made with some reservation in view of the abnormal glucose tolerance in this group.

At the 30 min interval the mean values for all doses of Chlormadinone Acetate were greater than the control mean value. The 100 μg group did not attain significance, however both the 250 μg and 500 μg group mean values were significantly greater than the controls ($p < .05$) and ($p < .005$) respectively. The Megestrol Acetate groups at this time interval all had mean values that were significantly greater than the control mean value; 100 μg and 250 μg groups had ($p < .005$), and the 500 μg group ($p < .001$).

At the 60 min interval the mean values for all Chlormadinone Acetate groups were greater than the control mean values, however only the 250 μg dose was significantly elevated ($p < .05$). The mean values for the Megestrol Acetate groups were all greater than the control mean value, however no group value, attained significance.

At the 90 min interval, all mean values for the Chlormadinone Acetate groups were greater than the control mean value, but no group value was significantly greater than the control. The Megestrol Acetate groups all had mean values greater than the controls with the 100 μg and 500 μg group significantly greater than the control, ($p < .05$) and ($p < .005$) respectively.

At the 120 min interval the mean values for both progestogens, at all doses, were not significantly different from the control mean value.

120 Minute Areas:-

The total and incremental areas for all doses of Chlormadinone Acetate were greater than the respective control areas.

The 100 μg Chlormadinone Acetate group had areas that were not significantly different from control areas, however the 250 μg total and incremental areas were significantly greater than the control areas, ($p < .025$) and ($p < .01$) respectively. The 500 μg group, had a significantly greater incremental area ($p < .05$), but total area was not significantly different from the control area.

The total and incremental areas for all doses of Megestrol Acetate were greater than the respective control areas. The 100 μg Megestrol Acetate group had significantly elevated total ($p < .01$) and incremental ($p < .001$) areas over the respective control areas.

The 250 μg group had a significantly elevated incremental area ($p < .05$), total area for this group was not significantly different from the control area. The 500 μg group had significantly greater total and incremental areas than the respective control areas, ($p < .001$) for both areas.

60 Minute Areas:-

The total and incremental areas for all doses of Chlormadinone Acetate were greater than the respective control areas.

The 100 μg Chlormadinone Acetate group had areas that were not significantly different from the respective control areas. The 250 μg and 500 μg Chlormadinone Acetate groups had significantly elevated total and incremental areas over those of the respective control areas, ($p < .05$) for the two areas in both groups.

The total and incremental areas for all doses of Megestrol Acetate were greater than the respective control areas. The 100 μg and 250 μg Megestrol Acetate groups had total and incremental areas significantly greater than the control areas, ($p < .05$) for the two areas, in both groups. The 500 μg group had significantly greater total and incremental areas than the respective control areas, ($p < .001$), for the two areas in both groups.

PLASMA INSULIN AT 2 WEEKS FIGS. 9+10 TABLES 1X+1XA

Fasting:-

The mean values for the 100 μg and 250 μg Chlormadinone Acetate groups were not significantly different from the control mean value. The 500 μg Chlormadinone Acetate group had a significantly elevated mean value over that of the control ($p < .005$).

The 100 μg Megestrol Acetate group had a mean value not significantly different from the control mean value. The 250 μg and 500 μg Megestrol Acetate groups both had mean values significantly greater than the control value, ($p < .005$) and ($p < .01$) respectively.

DURING THE O.G.T.T.

As with the glucose and pyruvate results, the interpretation of insulin data should be made with some reservation because of the abnormal glucose tolerance.

At the 15 min interval the mean values for all doses of Chlormadinone Acetate were greater, though not significantly different from the control value. The mean values for the Megestrol Acetate groups were all greater than the control mean value.

The 250 μg Megestrol Acetate group had a mean value significantly greater than the control value ($p < .005$); however the 100 μg and 500 μg group values were not significantly different from the control.

At the 30 min interval, mean values for all doses of Chlormadinone Acetate were greater than the control value. The 100 μg and 250 μg group values were significantly greater than control ($p < .05$) for both groups; the 500 μg group was not significantly different from control. The Megestrol Acetate groups all had mean values greater than the control mean value, however no group was significantly different from the control.

At the 60 min , interval the mean values for all Chlormadinone Acetate groups were equal or greater than the control value but no group was significantly different from the control. The mean values for the Megestrol Acetate groups were all greater than the control value however only the 500 μg group was significantly greater than the control ($p < .05$).

At the 90 min interval, the mean values for the Chlormadinone Acetate groups were not significantly different from the control values. The Megestrol Acetate groups had values equal to or greater than the control value with the 500 μg group significantly greater than the control ($p < .05$).

At the 120 min interval the mean values for the Chlormadinone Acetate groups were not significantly different from the control values.

The Megestrol Acetate groups, 250 μg and 500 μg had significantly greater values than the control value ($p < .05$) and ($p < .005$) respectively.

MEAN O.G.T.T. PLASMA INSULIN AREAS AT 2 WEEKS
TABLES X + XA

120 Minute Areas:-

The total and incremental areas for all doses of Chlormadinone Acetate were greater than the respective control areas, however no area was significantly different from the control areas. The total and incremental areas for Megestrol Acetate at all dose levels were greater than the respective control areas. The total and incremental areas for Megestrol Acetate 100 μg group and the incremental area for the 250 μg groups were not significantly different from the respective control areas. The 250 μg Megestrol Acetate group total area and the 500 μg group total and incremental areas were significantly greater than the respective control areas, ($p < .05$) for both groups and all areas.

60 Minute Areas:-

The total and incremental areas for all doses of Chlormadinone Acetate were greater than the respective control areas, however no area was significantly different from the control areas. The total and incremental areas for all doses of Megestrol Acetate were greater than the respective control areas.

The 100 μg Megestrol Acetate group had total and incremental areas that were not significantly different from the control areas.

The 250 μg and 500 μg Megestrol Acetate groups had total and incremental areas that were significantly greater than the control areas, ($p < .05$) for both groups and all areas.

PLASMA GLUCOSE AT 4 WEEKS FIGS. 9A + 10A TABLES 1XB 1XC

Fasting:-

The mean fasting values for the two progestogens at all doses showed no tendency to be either greater or lower than the control mean value and no progestogen group value was significantly different from the control value.

DURING THE O.G.T.T.

The control group at this 4 week period demonstrated a normal O.G.T.T. pattern for the glucose in comparison to previous control groups shown in this investigation. The "humping" effect observed in the Megestrol Acetate groups at 2 weeks had also disappeared by this 4 week period and the O.G.T.T. pattern appeared normal.

The mean values for all doses of Chlormadinone Acetate, at all time intervals, were not significantly different from the control mean values, nor was there a tendency for the values to be greater or less than the control throughout the O.G.T.T.

The mean values for the 100 μg Megestrol Acetate group were not significantly different from the control mean values, nor was there a tendency for the progestogen values to be greater or less than the control values. The 250 μg and 500 μg Megestrol Acetate groups at the 15 min interval had mean values that were not significantly different from the control mean value.

At the subsequent time intervals of 30, 60, 90 and 120 minutes, the mean values for both these two groups had a tendency to be less than the control mean values. The 250 μg group 90 min value was significantly less than the control, the 60 min and 120 min values were not significantly different from the respective control values. The 500 μg group had significantly less mean values for the 60 min , ($p < .025$) 90 min ($p < .001$) and 120 min interval ($p < .05$) compared with control values.

MEAN O.G.T.T. GLUCOSE AREAS AT 4 WEEKS TABLES XB+X

120 Minute Areas:-

The total and incremental areas for all doses of Chlormadinone Acetate were not significantly different from the respective control areas, nor was there a tendency for the progestogen areas to be greater or less than the control areas.

The total and incremental areas for all doses of Megestrol Acetate had a tendency to be less than the respective control areas. The 100 μg Megestrol Acetate group had areas that were not significantly different from the control areas. The 250 μg group had a significantly less total area ($p < .02$) but not incremental area compared with control areas. The 500 μg group had total and incremental areas significantly less than the respective control areas, ($p < .05$) for both areas.

60 Minute Areas:-

The total and incremental areas for both progestogens had no tendency to be either greater or less than the respective control areas, and no area was significantly different from the respective control area.

BLOOD PYRUVATE AT 4 WEEKS : FIGS 9A+10A TABLES 1XB+ 1XC

Fasting:-

The mean values for all doses of Chlormadinone Acetate were not significantly different from the control mean value. The 100 μg and 500 μg Megestrol Acetate groups had mean values that were not significantly different from the control mean value, however the 250 μg group had a significantly lower value ($p < .02$) compared with control value.

DURING THE O.G.T.T.

At the 30 min interval the 100 μg Chlormadinone Acetate mean value was not significantly different from the control mean value. The mean values for the 250 μg and 500 μg Chlormadinone Aceate groups were greater than the control mean value, however only the 500 μg group attained significance ($p < .005$).

The Megestrol Acetate groups all had mean values significantly greater than the control mean value; 100 μg ($p < .02$), 250 μg ($p < .05$) and the 500 μg group ($p < .001$).

At the 60 min and 90 min interval no Chlormadinone Acetate group had a tendency for the mean values to be greater or less than the controls, with no value significantly different from the controls. The Megestrol Acetate groups had mean values that were greater than the controls, but no value was significantly different from the control mean values. At the 120 min interval, all Chlormadinone Acetate groups had mean values greater than the controls, however only the 500 μg group was significantly greater, ($p < .01$).

The Megestrol Acetate groups had mean values that were all significantly greater than the control mean value 100 μg ($p < .001$), 250 μg ($p < .05$) and 500 μg group, ($p < .01$).

MEAN O.G.T.T. PYRUVATE AREAS AT 4 WEEKS
TABLES XB + XC

120 Minute Areas:-

The total and incremental areas for all Chlormadinone Acetate groups had a tendency to be greater than the respective control area, however no area was significantly different from the respective control areas.

The total and incremental areas for all Megestrol Acetate groups were greater than the respective control area. The 100 μg group total and incremen tal areas were both significantly greater than the control areas, ($p < .025$) and ($p < .05$) respectively.

The 250 μg Megestrol Acetate group had total and incremental areas that were not significantly different from the control areas; the 500 μg group total area was significantly greater than the control area, ($p < .05$), but incremental area was not significantly different from the control area.

60 Minute Areas:-

The total and incremental areas for all Chlormadinone Acetate groups were not significantly different from the respective control areas, however there was a trend for the 250 μg and 500 μg groups to have areas greater than the control areas.

The total and incremental areas for the Megestrol Acetate groups were all greater than the respective control areas, however only the 100 μg and 500 μg group total areas were significantly greater than the control, ($p < .05$) and ($p < .01$) respectively.

PLASMA INSULIN AT 4 WEEKS FIGS. 9A + 10A TABLES 1XB+1X

Fasting:-

The mean values for all Chlormadinone Acetate groups were not significantly different from the control mean value. The Megestrol Acetate mean values for the 250 μg and 500 μg groups were significantly greater than the mean value ($p < .05$) for both groups; the 100 μg group mean value was not significantly different from control value.

DURING THE O.G.T.T.

At the 15 min interval the Chlormadinone Acetate mean values were not significantly different from the control value.

The Megestrol Acetate groups all had mean values greater than the control mean value with the 500 μg group significantly greater than the control, ($p < .025$).

At the 30 min interval the mean values for both progestogens at all dose levels were either approximately equal or greater than the control mean value, although no value was significantly different from the control value.

At the 60 min interval the mean values for both progestogens at all doses were not significantly different from the control mean value. The Megestrol Acetate 250 μg and 500 μg groups had mean values that tended to be higher than the controls.

At the 90 min interval the mean value for the 500 μg Chlormadinone Acetate group was significantly greater than the control mean value ($p < .05$), the 100 μg and 250 μg groups had mean values that were not significantly different from the control value. The mean values for the Megestrol Acetate groups had a tendency to be greater than the control mean value, however only the 500 μg group was significantly greater, ($p < .05$).

At the 120 min interval the mean value for the 500 μg Chlormadinone Acetate group was significantly

greater than the control mean value ($p < .05$), the 100 μg and 250 μg groups had mean values that were not significantly different from the control mean value.

The Megestrol Acetate mean values were greater than the control for all groups, with the 250 μg and 500 μg groups significantly greater than the control, ($p < .05$) for both groups; the 100 μg mean value was not significantly different from the control mean value.

MEAN O.G.T.T. INSULIN AREAS AT 4 WEEKS TABLES XB+XC

120 Minute Areas:-

The total and incremental areas for the Chlormadinone Acetate groups demonstrated a tendency to increase with increasing dose of steroid, however no area was significantly different from the respective control area. The total and incremental are as for the Megestrol Acetate groups also demonstrated a tendency to increase with increasing doses of steroid; the 500 μg group had significantly greater total and incremental area compared with control areas, ($p < .005$) and ($p < .02$) respectively. The 100 μg and 250 μg group areas were not significantly different from the control areas.

60 Minute Areas:-

The total and incremental areas for the Chlormadinone Acetate groups whilst demonstrating a tendency for the two areas to increase with increasing dose of steroid, no area was significantly different from the respective control area.

The total and incremental areas for the Megestrol Acetate groups also demonstrated a tendency to increase with increasing dose of steroid, with all areas greater than the respective control areas, however only the 500 μg group had areas that were significantly greater than the respective control areas, ($p < .05$) for both areas.

SUMMARY OF RESULTS FOR PROGESTERONE AND THE PROGESTOGEN SERIES.

GLUCOSE.

In general the administration of Progesterone, the 17 alpha OH progesterone derivatives, Chlormadinone acetate and Megestrol acetate and the 19 nor testosterone derivatives, Norethynodrel and Norethisterone acetate for periods of 2 and 4 weeks were without effect on mean fasting glucose levels.

The administration of Progesterone, Norethynodrel and Norethisterone acetate for periods of 2 and 4 weeks had no effect on glucose removal during an O.G.T.T. The two week glucose tolerance data for the progestogens, Megestrol acetate and Chlormadinone acetateis difficult to interpret because of the abnormal glucose tolerance in the control group. However after 4 weeks, the control group glucose tolerance data was normal and the Chlormadinone acetate was seen to have no effect on glucose tolerance, whilst Megestrol acetate improved glucose tolerance with increasing dose.

PYRUVATE.

The administration of Progesterone, Norethynodrel, Norethisterone acetate, Chlormadinone acetate and Megestrol acetate for periods of 2 and 4 weeks were without effect on mean fasting pyruvate levels.

The progestogens, Norethynodrel and Norethisterone acetate administered for 2 and 4 weeks caused significant elevations in O.G.T.T pyruvate levels. The administration of Chlormadinone acetate or Megestrol acetate for 2 weeks was seen to increase pyruvate levels in comparison to controls. However, since the control group glucose tolerance was abnormal, the interpretation of the pyruvate data should be made with reservation. After 4 weeks the control group glucose tolerance was normal.

The progestogens Chlormadinone acetate and Megestrol acetate at this 4 week period gave only marginal increases in O. G. T. T. pyruvate levels.
Progesterone administration for 2 and 4 weeks gave only marginal increases in O. G. T. T. pyruvate levels.

INSULIN.

In general the administration of Progesterone and Norethister one acetate for periods of 2 and 4 weeks were without effect on mean fasting insulin levels. The progestogens Megestrol acetate, Chlormadinone acetate and Norethynodrel after 2 weeks of administration had a tendency to increase the mean fasting insulin levels, however after 4 weeks of continued progestogen administration only the Megestrol acetate had a significantly elevated fasting insulin value. Progesterone Norethynodrel and Norethisterone acetate administration for 2 weeks had no significant effect on insulin response during an O. G. T. T.
After 4 weeks of Progesterone administration, there was a biphasic insulin response which was related to the dose of steroid. The low doses 0. 50mg and 1. 25 mg increased the insulin response and the higher doses 2. 50 mg and 5. 00 mg gave insulin responses equal to or slightly less than the control insulin response.
The administration of Norethisterone acetate for 4 weeks was without any major effect on insulin response during the O. G. T. T.
Norethynodrel at 4 weeks demonstrated a slight decrease in the insulin response during an O. G. T. T.
The progestogens Megestrol acetate and Chlormadinone acetate gave elevated insulin responses during the O. G. T. T., at both the 2 and 4 week intervals. The insulinogenic property displayed by these two progestogens appeared to be dose related, that is the insulin response became greater with increasing dose of progestogen.

DISCUSSION.

In general Progesterone, and the progestogens Norethynodrel, Norethisterone acetate and Chlormadinone acetate do not effect glucose tolerance in the ovariectomized rat. However after 4 weeks of high dose Megestrol acetate administration, there was a slight improvement in glucose tolerance. This finding is probably due to the effects of insulin, for the progestogen imparts a state of hyperinsulinism on the animal with elevations in the fasting levels and the insulin response during the O.G.T.T.

The administration of Chlormadinone acetate at the higher dose levels also increased the insulin response during the O.G.T.T. whilst Norethynodrel and Norethisterone acetate did not effect fasting insulin levels or the insulin response during an O.G.T.T.. The biphasic insulin response demonstrated by Progesterone with low doses increasing and higher doses having no effect on insulin response, is similar to the findings for Oestradiol benzoate seen in chapte 2 of this thesis. Therefore Progesterone as well as Oestradiol benzoate when administered in high doses, may inhibit the glucose trigger mechanism for insulin release from the islet as proposed by Hager et al (1972). However, Costrini and Kalkhoff (1971) and Hager et al (1972) have shown that the islets isolated from rats treated with 5.00 mg Progesterone have increased insulin content and a greater release of insulin in response to a glucose stimulus.

Beck (1969) working with monkeys demonstrated that insulin responses during an i.v. glucose tolerance test were increased in animals that had been treated with Progesterone, Norethynodrel, Norethisterone acetate and Chlormadinone acetate. The progestogen Megestrol acetate was not studied in these experiments. Beck noted that glucose removal during the tolerance test was only improvedin animals treated with Chlormadinone acetate. Progesterone, Norethynodrel, and Norethisterone acetate had no effect, or slightly impaired glucose tolerance.

Beck postulated from this work that the generation of a partial positive charge at C 5 in the steroid nucleus imparts insulinogenic as well as insulin resistance properties on the steroid; however with C6 unsaturation as occurs in the 17 alpha OH progester one derivatives, there is a reduction in the gluconeogenic effects of the steroid whilst the insulinogenic property is maintained. The findings in this section of the work would agree to a certain extent with Beck's theory. There is little to be found in the literature regarding the effects of Progesterone and the progestogens on glucose metabolism. Matute and Kalkhoff (1973) demonstrated that the livers of rats which had been receiving Progesterone have increased glycogen content in comparison with untreated controls, however gluconeogenesis from pyruvate was not markedly affected.

In comparison with controls, the elevations in pyruvate levels during an O.G.T.T. observed with Progesterone and progestogen administration are indicative that there is more glucose being metabolized along the glycolytic and/or the Hexose mono-phosphate shunt pathways. The elevations in pyruvate levels seen with Megestrol acetate and to a lesser extent with Chlormadinone acetate and Progesterone are possibly due to the effects of insulin, which is known to increase the de-novo synthesis and activate the glycolytic and Hexose mono-phosphate shunt enzymes.

The progestogens, Norethynodrel and Norethisterone acetate cause very marked elevations in O.G.T.T. pyruvate levels. However these two progestogens lack the insulinogenic properties of the other steroids investigated in this section, suggesting that another mechanism, besides insulin, is playing a role in the metabolism of glucose in animals treated with these 19 nor testosterone derivatives.

Paulsen (1965) and Paulsen et al (1962), has shown in the human, that Norethynodrel and Norethisterone acetate can both be metabolized to the oestrogenic steroid Mestranol. The conversion rate for these two progestogens to Mestranol is low and approximates to 2-3 μg equivalents of Oestradiol benzoate / mg of progestogen administered. The same author demonstrated that the 17 alpha OH progesterone derivatives were not converted to oestrogenic metabolites. Therefore one could speculate that Norethynodrel and Norethisterone acetate influence the metabolism of glucose in a manner similar to that for oestrogens, that is, increased activity of the glycolytic and/or the Hexose mono-phosphate shunt enzyme systems.

The possibility may also exist that the progestogens and Progesterone may be converted to a metabolite not oestrogenic in nature but which affects glucose metabolism in the rat.

In summary, Progesterone, Norethynodrel, Norethisterone acetate and Chlormadinone acetate are without major effect on glucose tolerance in the rat. Megestrol acetate at high dose levels was seen to improve glucose tolerance. Elevated pyruvate levels seen with Norethynodrel and Norethisterone acetate administration are possibly related to the oestrogenic properties of these progestogens, whilst insulin is considered to be the effector in the steroids, Progesterone, Chlormadinone acetate and Megestrol acetate.

CHAPTER 4

THE EFFECTS OF COMBINED OESTROGENIC AND PROGESTOGEN STEROIDS ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE OVARIECTOMIZED RAT.

INTRODUCTION.

In this section, the effects of combined gonadal and oral contraceptive steroids on glucose metabolism, pyruvate formation and insulin response during an O.G.T.T. were investigated.

The oestrogen - progestogen combinations were selected on the basis of those combinations which appear most frequently in combined O.C. therapy, with the exception of the combination of Oestradiol benzoate + Progesterone. The combinations used were Mestranol + Norethynodrel, Mestranol + Norethisterone Acetate, Mestranol Chlormadinone Acetate , Ethinyl oestradiol + Megestrol Acetate and Oestradiol benzoate + Progesterone.

The steroids were given in combination with each other rather than in sequence because the combined administration of these steroids in the human has been reported as having a greater proportion of abnormal carbohydrate tolerance tests associated with it compared with the sequential administration of these steroids, Adams&Oakley (1972) and Spellacy (1969.)
Therefore it would appearthat this type of steroid administration would be more likely to induce a change in the metabolism of the rat.

The doses employed for the particular oestrogen / progestogen were selected from the work presented in Chapters 2 and 3 on the basis that the steroid dose had an effect whether adverse or otherwise on glucose tolerance, pyruvate formation and insulin response during an O.G.T.T.

Fig.11

COMBINED OESTROGEN–PROGESTOGEN STEROID SERIES AT 2 WEEKS

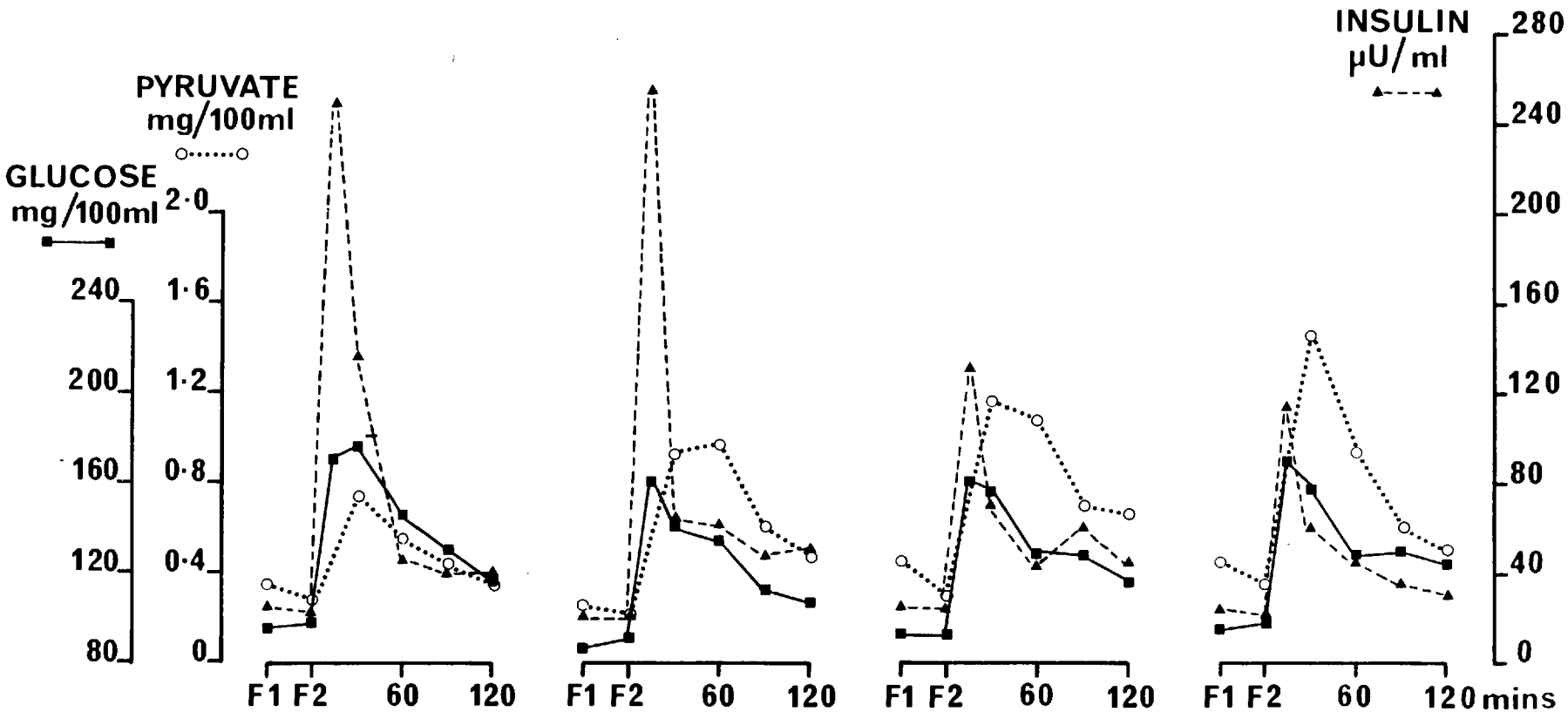


Fig. 11 A

COMBINED OESTROGEN–PROGESTOGEN STEROID SERIES AT 4 WEEKS

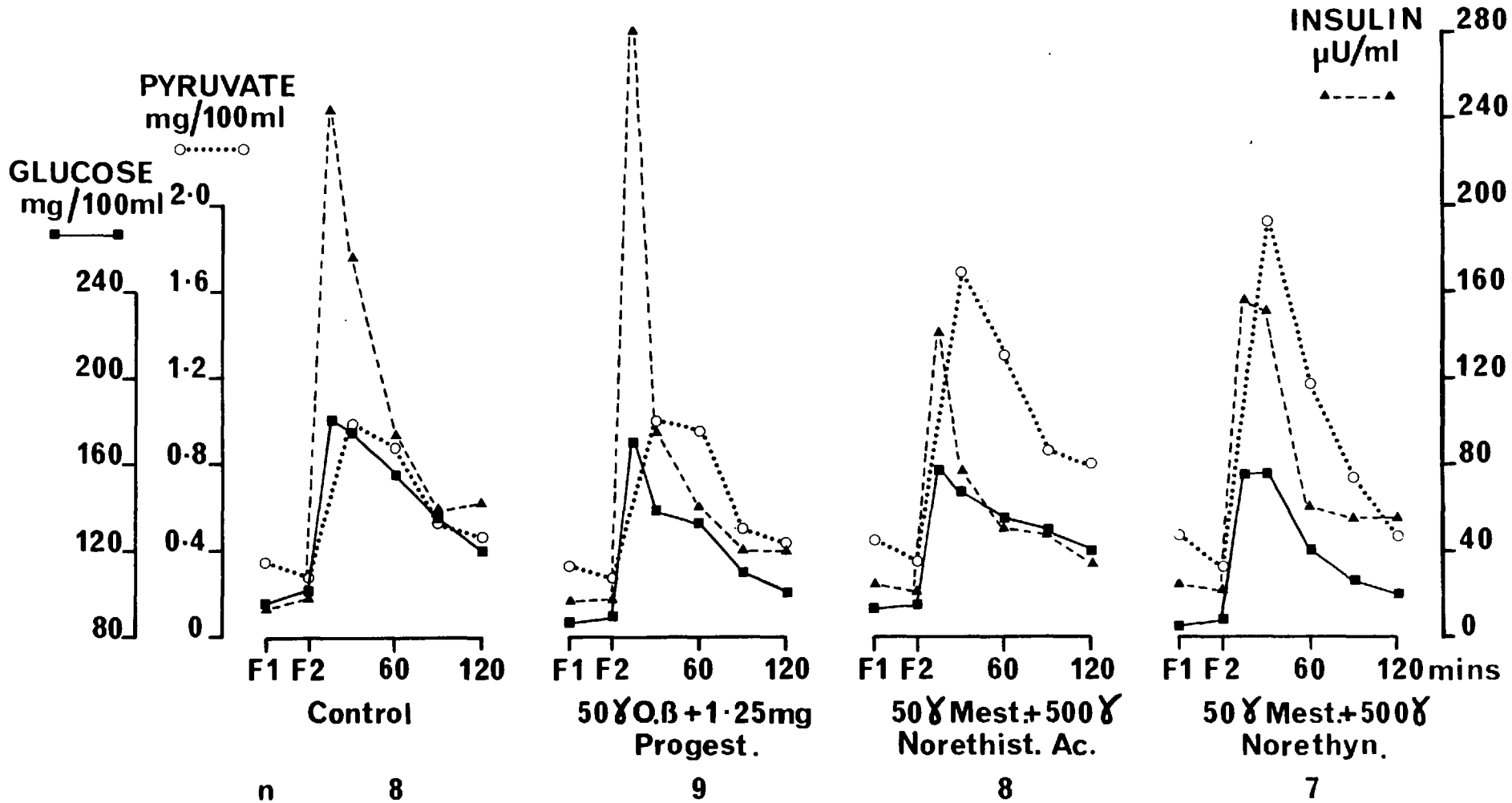


Fig. 12

COMBINED OESTROGEN–PROGESTOGEN STEROID SERIES AT 2 WEEKS

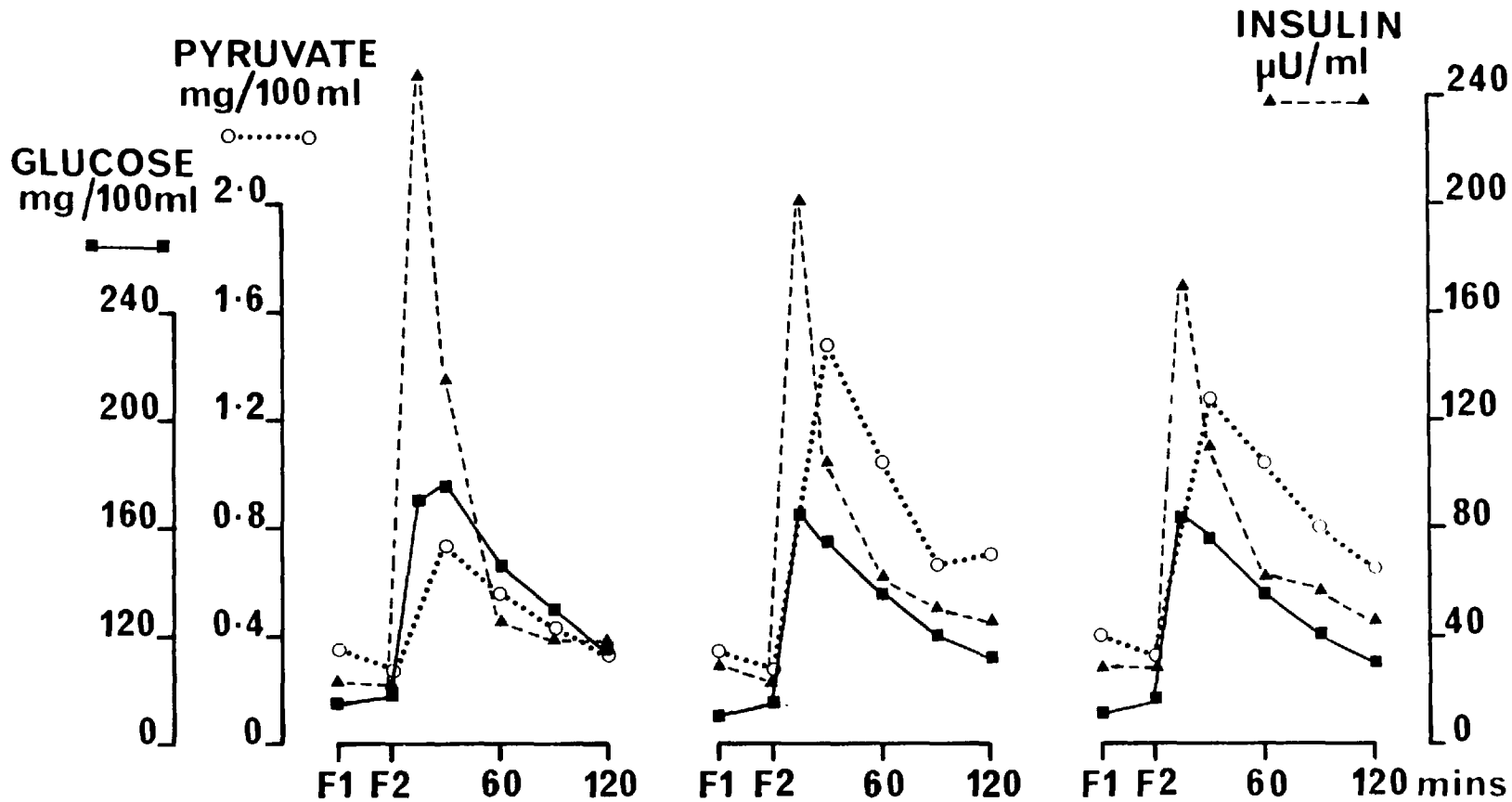


Fig. 12A

COMBINED OESTROGEN–PROGESTOGEN STEROID SERIES AT 4 WEEKS

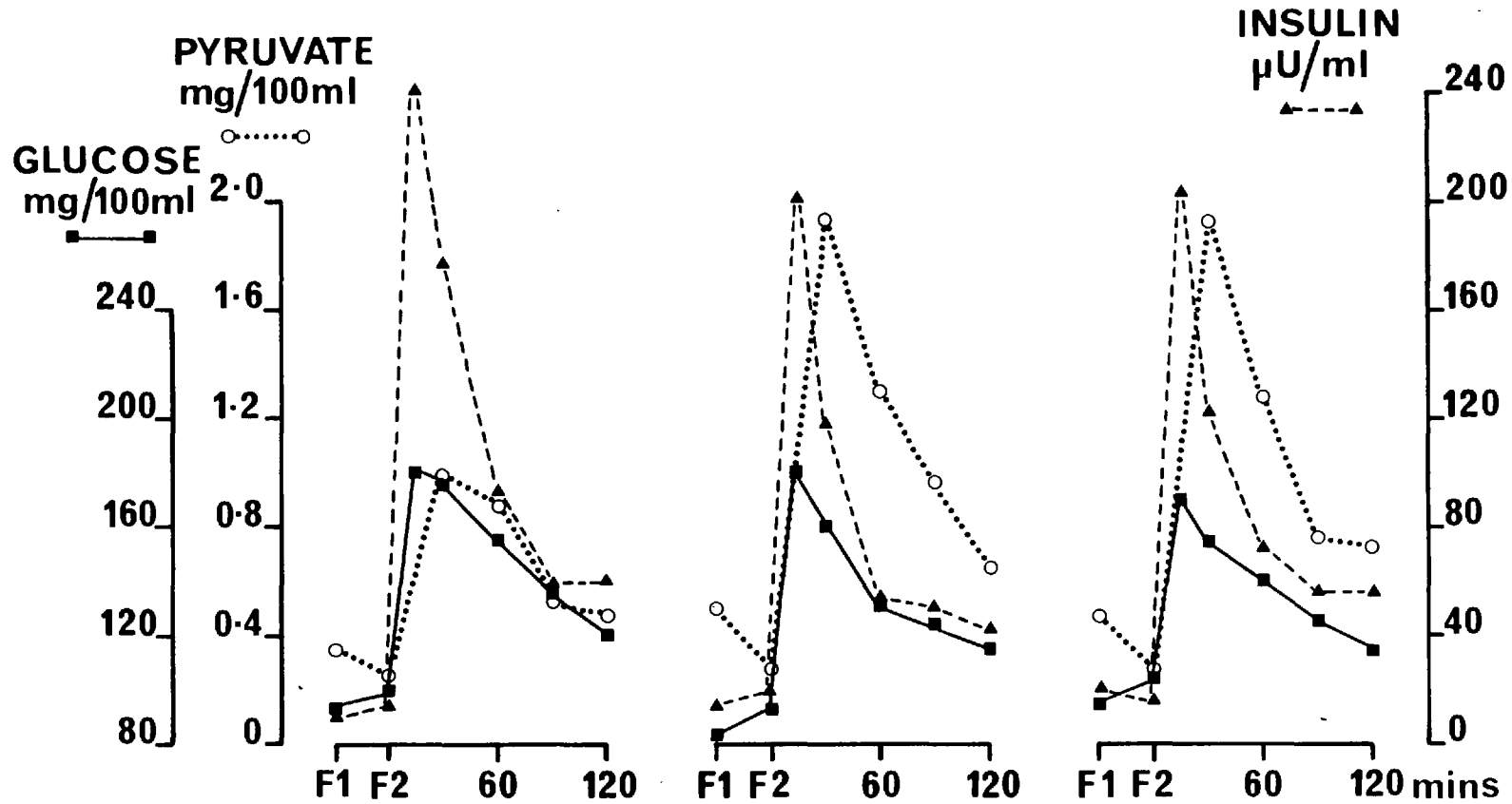


TABLE XI

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX + 0.10 ml Oil Control. N = 8	Glucose mg/100ml	94 ± 1	97 ± 1	169 ± 5	175 ± 6	147 ± 5	130 ± 4	114 ± 3
	Pyruvate mg/100ml	0.37 ± 0.09	0.28 ± 0.05	----------	0.74 ± 0.09	0.56 ± 0.11	0.42 ± 0.04	0.32 ± 0.03
	Insulin μU/ml	23 ± 1	21 ± 2	248 ± 38	136 ± 23	47 ± 5	40 ± 6	38 ± 2
OVX + O.B.+ Progesterone N = 10	Glucose	84 ± 2 <.001	89 ± 2 <.005	162 ± 7 N.S.	141 ± 6 <.005	135 ± 7 N.S.	112 ± 2 <.001	107 ± 3 N.S.
	Pyruvate	0.23 ± 0.02 N.S.	0.20 ± 0.01 N.S.	----------	0.91 ± 0.12 N.S.	0.97 ± 0.14 <.05	0.59 ± 0.12 N.S.	0.48 ± 0.09 N.S.
	Insulin	20 ± 3 N.S.	19 ± 3 N.S.	253 ± 57 N.S.	62 ± 9 <.05	59 ± 10 N.S.	47 ± 5 N.S.	49 ± 8 N.S.
OVX + Mestranol + Noreth-ist Ac N = 8	Glucose	94 ± 3 N.S.	92 ± 2 N.S.	160 ± 4 N.S.	157 ± 10 <.05	127 ± 6 N.S.	129 ± 8 N.S.	115 ± 3 N.S.
	Pyruvate	0.46 ± 0.08 N.S.	0.30 ± 0.04 N.S.	----------	1.17 ± 0.17 <.05	1.09 ± 0.18 <.05	0.70 ± 0.06 <.005	0.67 ± 0.11 <.05
	Insulin	24 ± 2 N.S.	25 ± 2 N.S.	130 ± 24 <.05	71 ± 5 <.05	44 ± 6 N.S.	61 ± 14 N.S.	45 ± 9 N.S.
OVX + Mestranol + Norethynodrel N= 8	Glucose	92 ± 3 N.S.	97 ± 3 N.S.	171 ± 3 N.S.	157 ± 9 N.S.	129 ± 7 N.S.	129 ± 8 N.S.	123 ± 8 N.S.
	Pyruvate	0.46 ± 0.04 N.S.	0.36 ± 0.06 N.S.	----------	1.46 ± 0.18 <.05	0.93 ± 0.21 N.S.	0.60 ± 0.11 N.S.	0.52 ± 0.16 N.S.
	Insulin	24 ± 4 N.S.	21 ± 1 N.S.	111 ± 9 <.05	59 ± 6 <.05	46 ± 7 N.S.	36 ± 4 N.S.	31 ± 4 N.S.
OVX + Ethinyl-O. + Megesrol Ac. N = 10	Glucose	90 ± 3 N.S.	95 ± 2 N.S.	169 ± 6 N.S.	155 ± 6 <.05	134 ± 5 N.S.	120 ± 4 N.S.	113 ± 2 N.S.
	Pyruvate	0.33 ± 0.03 N.S.	0.29 ± 0.04 N.S.	----------	1.48 ± 0.15 <.05	1.03 ± 0.08 <.005	0.66 ± 0.07 <.05	0.69 ± 0.11 <.05
	Insulin	28 ± 2 N.S.	25 ± 1 N.S.	201 ± 18 N.S.	103 ± 13 N.S.	61 ± 10 N.S.	50 ± 5 N.S.	46 ± 4 N.S.
OVX + Mestranol + Chlormad. Ac. N = 10	Glucose	92 ± 2 N.S.	97 ± 2 N.S.	163 ± 6 N.S.	154 ± 6 <.025	136 ± 10 N.S.	120 ± 5 N.S.	109 ± 4 N.S.
	Pyruvate	0.40 ± 0.04 N.S.	0.34 ± 0.03 N.S.	----------	1.28 ± 0.16 <.02	1.04 ± 0.14 <.025	0.81 ± 0.14 <.05	0.64 ± 0.06 <.001
	Insulin	28 ± 3 N.S.	29 ± 2 <.05	169 ± 25 N.S.	112 ± 20 N.S.	61 ± 13 N.S.	57 ± 6 N.S.	46 ± 4 N.S.

TABLE XI Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with with significance levels for combined Oestrogen - Progestogen steroid series at 2 weeks. (N.S. = not significant.)

TABLE XI A

		F1	F2	15 min	30 min	60 min	90 min	120 min
OVX + 0.10 ml Oil Control N = 8	Glucose mg/100ml	93 ± 3	102 ± 3	180 ± 10	177 ± 5	156 ± 7	136 ± 6	120 ± 5
	Pyruvate mg/100ml	0.35 ± 0.05	0.26 ± 0.01	----------	0.98 ± 0.15	0.88 ± 0.20	0.52 ± 0.06	0.44 ± 0.0
	Insulin μU/ml	14 ± 2	19 ± 4	245 ± 53	176 ± 36	93 ± 20	56 ± 7	62 ± 7
OVX + O.B. + Progesterone N = 9	Glucose	87 ± 2 N.S.	90 ± 1 < .001	171 ± 5 N.S.	138 ± 7 < .001	133 ± 6 < .05	110 ± 4 < .005	100 ± 5 < .01
	Pyruvate	0.32 ± 0.03 N.S.	0.27 ± 0.02 N.S.	----------	1.00 ± 0.08 N.S.	0.96 ± 0.15 N.S.	0.49 ± 0.06 N.S.	0.43 ± 0.(N.S.
	Insulin	18 ± 3 N.S.	17 ± 5 N.S.	279 ± 45 N.S.	97 ± 27 N.S.	62 ± 11 N.S.	39 ± 9 N.S.	41 ± 12 N.S.
OVX + Mestranol + Norethist Ac. N = 8	Glucose	93 ± 2 N.S.	93 ± 1 < .02	159 ± 4 N.S.	147 ± 8 < .01	136 ± 7 N.S.	128 ± 6 N.S.	118 ± 3 N.S.
	Pyruvate	0.45 ± 0.09 N.S.	0.34 ± 0.05 N.S.	----------	1.69 ± 0.14 < .005	1.29 ± 0.08 N.S.	0.86 ± 0.08 < .01	0.80 ± 0.11 < .05
	Insulin	16 ± 3 N.S.	15 ± 3 N.S.	144 ± 21 < .05	77 ± 7 < .05	54 ± 17 N.S.	49 ± 12 N.S.	35 ± 8 < .05
OVX + Mestrnol + Norethynodrel N = 7	Glucose	84 ± 2 < .02	87 ± 1 < .001	154 ± 3 < .05	152 ± 5 < .005	120 ± 5 < .005	107 ± 3 < .005	101 ± 3 < .01
	Pyruvate	0.49 ± 0.07 N.S.	0.32 ± 0.07 N.S.	----------	1.94 ± 0.18 < .001	1.18 ± 0.17 N.S.	0.74 ± 0.19 N.S.	0.47 ± 0.12 N.S.
	Insulin	24 ± 3 < .02	20 ± 4 N.S.	157 ± 17 N.S.	150 ± 28 N.S.	59 ± 13 N.S.	56 ± 12 N.S.	53 ± 4 N.S.
OVX + Ethinyl -O + Megestrol Ac. N =10	Glucose	79 ± 3 < .005	92 ± 4 < .05	179 ± 6 N.S.	159 ± 7 N.S.	132 ± 5 < .02	125 ± 4 N.S.	115 ± 3 N.S.
	Pyruvate	0.52 ± 0.10 N.S.	0.27 ± 0.04 N.S.	----------	1.92 ± 0.12 < .001	1.29 ± 0.10 N.S.	0.97 ± 0.10 < .005	0.64 ± 0.05 < .01
	Insulin	12 ± 2 N.S.	18 ± 2 N.S.	201 ± 18 N.S.	118 ± 21 N.S.	51 ± 7 N.S.	50 ± 9 N.S.	43 ± 8 N.S.
OVX + Mestranol + Chlormad. Ac. N = 10	Glucose	96 ± 3 N.S.	104 ± 2 N.S.	171 ± 4 N.S.	152 ± 7 < .02	139 ± 7 N.S.	124 ± 5 N.S.	116 ± 4 N.S.
	Pyruvate	0.48 ± 0.06 N.S.	0.27 ± 0.03 N.S.	----------	1.91 ± 0.10 < .001	1.28 ± 0.08 N.S.	0.78 ± 0.08 < .02	0.74 ± 0.10 < .05
	Insulin	21 ± 2 < .05	17 ± 3 N.S.	203 ± 50 N.S.	122 ± 19 N.S.	72 ± 7 N.S.	55 ± 8 N.S.	56 ± 7 N.S.

TABLE XI A Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate levels with significance levels for combined oestrogen-progestogen steroid series at 4 weeks. (N.S. = not significant.)

TABLE XII

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX + 0.10 ml Oil Control N = 8	574 ± 6	187 ± 6	2.07 ± 0.21	1.07 ± 0.15	337 ± 30	255 ± 31
OVX + O.B. + Progesterone N = 10	508 ± 12 <.05	154 ± 14 N.S.	2.72 ± 0.34 N.S.	1.94 ± 0.28 N.S.	295 ± 29 N.S.	222 ± 27 N.S.
OVX + Mestranol + Norethist. Ac N=8	534 ± 19 N.S.	169 ± 14 N.S.	3.45 ± 0.39 <.05	2.20 ± 0.45 <.05	203 ± 17 <.005	117 ± 16 <.005
OVX + Mestranol + Norethynodrel N=8	548 ± 25 N.S.	150 ± 27 N.S.	3.42 ± 0.58 N.S.	2.18 ± 0.40 <.05	253 ± 26 N.S.	152 ± 21 <.025
OVX + Ethinyl-O. + Megestrol Ac N=10	534 ± 8 <.005	155 ± 13 N.S.	3.60 ± 0.25 <.001	2.53 ± 0.21 <.001	317 ± 18 N.S.	217 ± 17 N.S.
OVX + Mestranol + Chlormad. Ac N=10	532 ± 15 <.05	144 ± 16 <.05	3.62 ± 0.37 <.05	2.26 ± 0.36 <.05	335 ± 28 N.S.	220 ± 31 N.S.
60 Minute Areas.						
OVX + 0.10ml Oil Control N = 8	313 ± 6	120 ± 6	1.21 ± 0.15	0.77 ± 0.12	255 ± 29	213 ± 30
OVX + O.B. + Progesterone N=10	276 ± 7 <.001	99 ± 7 <.05	1.45 ± 0.17 N.S.	1.06 ± 0.15 N.S.	209 ± 27 N.S.	172 ± 25 N.S.
OVX + Mestranol + Norethist. Ac. N= 8	284 ± 9 <.02	102 ± 7 N.S.	1.86 ± 0.24 <.05	1.24 ± 0.28 N.S.	147 ± 15 <.01	97 ± 13 <.01
OVX + Mestranol + Norethynodrel N = 8	293 ± 11 N.S.	94 ± 12 N.S.	2.09 ± 0.29 <.05	1.47 ± 0.20 <.05	128 ± 9 <.05	85 ± 10 <.05
OVX + Ethinyl-O + Megestrol Ac. N = 10	291 ± 7 <.05	102 ± 10 N.S.	2.14 ± 0.18 <.005	1.58 ± 0.14 <.005	214 ± 13 N.S.	164 ± 13 N.S.
OVX + Mestranol + Chlormad Ac. N= 10	289 ± 8 <.05	96 ± 9 <.05	1.97 ± 0.20 <.02	1.29 ± 0.19 N.S.	223 ± 23 N.S.	183 ± 19 N.S.

TABLE XII

Mean ± S.E.M. (Units - min) O.G.T.T. 120 Minute 60 Minute total and incremental areas with significance levels for combined Oestrogen - Progestogen steroid series at 2 weeks

(N.S. = not significant.)

TABLE XII A

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas.						
OVX 0.10 ml Oil Control N = 8	599 ± 16	191 ± 11	2.73 ± 0.30	1.68 ± 0.35	399 ± 61	325 ± 45
OVX + O.B.+ Progesterone N = 9	504 ± 13 <.001	145 ± 12 <.02	2.80 ± 0.17 N.S.	1.72 ± 0.19 N.S	339 ± 48 N.S.	266 ± 37 N.S.
OVX + Mestranol + Norethist Ac. N= 8	536 ± 16 <.02	164 ± 16 N.S.	4.39 ± 0.30 <.005	3.02 ± 0.29 <.02	258 ± 29 <.05	195 ± 29 <.05
OVX + Mestranol + Norethynodrel N =7	490 ± 7 <.05	142 ± 11 <.05	4.25 ± 0.59 <.05	2.99 ± 0.50 <.05	338 ± 40 N.S.	249 ± 36 N.S.
OVX + Ethinyl O. + Megestrol Ac. N = 10	546 ± 14 <.02	176 ± 10 N.S.	4.63 ± 0.20 <.001	3.57 ± 0.16 <.001	299 ± 33 N.S.	227 ± 28 N.S.
OVX + Mestranol + Chlormad -inone AC N =10	546 ± 16 <.05	132 ± 11 <.005	4.47 ± 0.18 <.001	3.38 ± 0.15 <.001	351 ± 31 N.S.	281 ± 34 N.S.
60 Minute Areas.						
OVX 0.10 ml Oil Control N = 8	326 ± 7	122 ± 6	1.55 ± 0.23	1.03 ± 0.25	305 ± 57	265 ± 45
OVX + O.B.+ Progesterone. N = 9	278 ± 7 <.001	98 ± 6 <.02	1.61 ± 0.06 N.S.	1.07 ± 0.06 N.S.	248 ± 34 N.S.	211 ± 28 N.S..
OVX + Mestra -nol + Norethisterone AC N=8	281 ± 9 <.005	95 ± 8 <.025	2.51 ± 0.17 <.005	1.82 ± 0.16 <.02	161 ± 13 <.05	131 ± 13 <.05
OVX + Mestra -nol + Norethy nodrel N = 7	273 ± 5 <.001	99 ± 6 <.025	2.69 ± 0.29 <.01	2.06 ± 0.22 <.01	226 ± 29 N.S.	186 ± 31 N.S.
OVX + Ethinyl O + Megestrol Ac. N =10	297 ± 9 <.05	113 ± 5 N.S.	2.70 ± 0.14 <.001	2.16 ± 0.13 <.001	204 ± 24 N.S.	168 ± 22 <.05
OVX + Mestranol + Chlormadinone Ac N =10	294 ± 8 <.02	87 ± 6 <.005	2.68 ± 0.12 <.001	2.14 ± 0.10 <.001	233 ± 34 N.S.	198 ± 35 N.S.

TABLE XII A Mean ± S.E.M. (Units - min) 120 Minute and 60 Minute total and incremental areas with significance levels for combined Oestrogen - Progestogen steroid series at 4 weeks.
(N.S. = not significant.)

TABLE X II B Animal weights Combined oestrogen - progestogen series.

Mean ± S.E.M. (body weight in grams)

	Initial	2 week	4 week
OVX Oil Control	211 ± 4	225 ± 4	251 ± 7
OVX + O.B. + Progesterone	212 ± 4	204 ± 4	220 ± 2
OVX + Mestranol + Norethisterone acetate	215 ± 5	187 ± 3	191 ± 8
OVX + Mestranol + Norethynodrel	210 ± 4	193 ± 5	197 ± 5
OVX + Ethinyl-O. Megestrol acetate	210 ± 5	185 ± 4	193 ± 5
OVX + Mestranol Chlormadinone acetate.	224 ± 6	186 ± 6	185 ± 5

COMBINED OESTROGEN - PROGESTOGEN SERIES:

PROCEDURE:-

Ovariectomized (OVX) rats weighing 200-250 gm were given sub-cutaneous injections of combined oestrogenic and progestogen steroids in the following doses and combinations.

1. 50 μg Oestradiol Benzoate (O.B.) + 1.25 mg Progesterone (P)
2. 50 μg Mestranol (M) + 500 μg Norethisterone Acetate (N.A.)
3. 50 μg Mestranol (M) + 500 μg Norethynodrel (N)
4. 50 μg Ethinyl Oestradiol (E.O.) + 500 μg Megestrol Acetate (M.A.)
5. 50 μg Mestranol (M) + 500 μg Chlormadinone Acetate (C.A.)

The steroid combinations were dissolved in 0.10 ml corn oil and the control animals received 0.10 ml oil only.

The animals were injected for 2 weeks after which the time the first O.G.T.T. was performed, the animals were then maintained for another 2 weeks at the end of which time, the second O.G.T.T. (4 week) was performed.

RESULTS:

PLASMA GLUCOSE AT 2 WEEKS FIGS 11+12 TABLES X1

Fasting:-

The mean fasting values for all combination groups with the one exception, the Oestradiol Benzoate + Progesterone group were not significantly different from the control mean value. The O.B. + Progesterone group had a mean value significantly less than the control mean value ($p < .005$).

DURING THE O.G.T.T.

At the 15 min interval, there was no combined group mean value that was significantly different from the control mean value.

At the 30 min interval, mean values for all combined groups were less than the control mean value. The (O.B.+P) group had a mean value significantly less than the control mean value ($p < .005$). The (M+N.A.) group and (E.O.+M.A.) groups both had mean values significantly less than the control mean value, ($p < .05$) for both groups. The (M+C.A.) group also had a mean value significantly less than the control mean value, ($p < .025$); the (M+N.A.) group mean value was not significantly different from the control mean value.

At the 60 min, 90 min and 120 min intervals, the mean values for all combined groups were less than the control mean value, however there was no significant difference between the combined group values and control values.

MEAN O.G.T.T. GLUCOSE AREAS AT 2 WEEKS TABLE X11

120 Minute Areas:-

The total and incremental areas for all combined groups were less than the respective control areas. The (O.B.+P) group and (E.O.+M.A.) group both had total areas significantly less than the control area ($p < .05$) and ($p < .005$) respectively, the incremental areas for the two groups were not significantly different from the control incremental area. The (M + C.A.) group had total and incremental areas that were significantly less than the respective control areas, ($p < .05$) for both areas. The remaining two groups (M + N.A.) and (M+N) groups had total and incremental areas that were not significantly different from the respective control areas.

60 Minute Areas:-

All combined groups had total and incremental areas that were less than the respective control areas.

The (O. B. +P) group had significantly lower total ($p < .001$) and incremental, ($p < .05$) compared with respective control areas. The (M + N. A.) group had a significantly lower total area ($p < .02$), but not incremental area when compared to control areas. The total and incremental areas for the (M+N) group were not significantly different from the control areas. The (E. O+M. A.) group had a significantly lower total area, ($p < .05$), but not incremental area in comparison to respective control areas. The (M+C. A.) group had total and incremental areas that were significantly lower than the respective control areas ($p < .05$) for both areas.

BLOOD PYRUVATE AT 2 WEEKS FIGS. 11+12 TABLE X1

Fasting:-

The mean fasting values for all combined groups were not significantly different from the control mean value.

DURING THE O. G. T. T.

At the 30 min interval the mean values for all combined groups were greater than the control mean value. The (O. B. +P) group mean value was not significantly different from the control mean value. The (M+N. A.) group, the (M+N) group and the (E. O+M. A.) group all had mean values that were significantly greater than the control mean value, ($p < .05$) for all groups. The (M+C. A.) group mean value was also significantly greater than the control mean value, ($p < .02$).

At the 60 min interval all groups had mean values greater than the control mean values. The (O. B. +P) and (M+N. A.) groups had mean values that were significantly greater than the control mean value, ($p < .05$) for both groups. The (M+N) group mean value was not significantly different from the control mean value. Finally the (E. O+M. A.) and (M+C. A.) groups had mean values greater than the control mean value, ($p < .005$) and ($p < .025$) respectively for the two groups.

At the 90 min interval the (O. B. +P) and (M+N) groups had mean values that were not significantly different from the control mean value. The (M+N. A.), (E. O. +M. A.) and the (M+C. A.) groups had mean values that were significantly greater than the control value ($p < .005$) for the first group and ($p < .05$) for the latter two groups.

At the 120 min interval the (O. B. +P) group and the (M+N) group had mean values that were not significantly different from the control mean value. The mean values for the (M+N. A.) (E. O+M. A.) and the (M+C. A.) groups were significantly greater than the control mean values, ($p < .05$) for the first two groups and ($p < .001$) for the latter group.

MEAN O. G. T. T. PYRUVATE AREAS AT 2 WEEKS TABLE X11

120 Minute Areas:-

The total and incremental areas for all groups were greater than the respective control areas. The (O. B. +P) group areas were not significantly different from the control areas. The (M+N. A.) group had total and incremental areas that were significantly greater than the respective control areas, ($p < .05$) for both areas. The (M+N) group had a significantly elevated incremental area ($p < .05$) compared with the control area, however the total area was not significantly different from the control area. The (E. O+M. A.) group had total and incremental areas significantly greater than the respective control areas, ($p < .001$) for both areas. Finally the (M+C. A.) group total and incremental areas were significantly greater than the respective control areas, ($p < .05$) for both areas.

60 Minute Areas:-

The total and incremental areas for all groups were greater than the respective control areas. The (O. B. +P) group areas were not significantly different from the control areas.

The (M+N. A.) total area was significantly greater than the control area, ($p < .05$), however the incremental area was not significantly different from the control area. The total and incremental areas for the (M+N) group were all significantly greater than the respective control areas, ($p < .05$) for both areas. The (E. O+M. A.) group, total and incremental areas were significantly greater than the respective control areas, ($p < .005$) for both areas. Finally the (M+C. A.) group had a significantly elevated total area ($p < .02$) but not incremental area in comparison to respective control areas.

PLASMA INSULIN AT 2 WEEKS FIGS. 11 + 12 TABLE XI

Fasting:-

The mean values for the groups (O. B. + P), (M+N. A.), (M+N) and (E. O+M. A.) were not significantly different from the control mean value . The (M+C. A.) group had a mean value that was significantly greater than the control mean value ($p < .05$).

DURING THE O. G. T. T.

At the 15 min interval, the mean values for the (O. B. +P), (E. O. +M. A.) and (M+C. A.) groups were not significantly different from the control mean value. The (M+N. A.) and (M+N) groups had mean values that were significantly less than the control value with ($p < .05$) for both groups.

At the 30 min interval, the mean values for the (O. B. +P), (M+N. A.) and (M+N) groups were significantly less than the control mean value ($p < .05$), for all groups. The (E. O+M. A.) and (M+C. A.) groups had mean values that were not significantly different from the control mean value. At the subsequent time intervals of 60, 90 and 120 minutes the mean values for all the groups at these times were not significantly different from the control mean values.

MEAN O.G.T.T. INSULIN AREAS AT 2 WEEKS TABLE X11

120 Minute Areas:-

The total and incremental areas for all groups had a tendency to be less than the respective control areas. The (M+N.A.) group, total and incremental areas were significantly lower compared with the respective control areas, ($p < .005$) for both areas. The (M+N) group had a significantly lower incremental area ($p < .025$) but not total area in comparison with respective control areas. The remaining groups had areas that were not significantly different from the control areas.

60 Minute Areas:-

The total and incremental areas for all groups were less than the respective control areas. The (M+N.A.) group had a total area and incremental area significantly less than the respective control areas, ($p < .01$) for both areas. The (M+N) group also had significantly lower total and incremental areas compared with control areas, ($p < .05$) for both areas. The remaining groups had areas that were not significantly different from the control areas.

PLASMA GLUCOSE AT 4 WEEKS F IGS. 11A + 12A TABLE X1A

Fasting:-

The mean values for the (O.B. +P), (M+N.A.) and (M+N) groups were significantly less than the control mean value; the first and last of these groups had a p value ($< .001$) and the second of the groups ($p < .02$) The remaining two groups (E.O+M) and (M+C.A.) had mean values that were not significantly different from the control mean values.

DURING THE O.G.T.T.

At the 15 min interval, the mean values for all groups had a tendency to be lower than the control mean value with the (M+N) group having a value significantly less than the control, ($p < .05$).

At the 30 min interval, all combined groups had mean values that were less than the control mean value. The (O.B.+P) was significantly less than the control, ($p < .001$) and the other groups that were significantly less were, (M+N.A.) ($p < .01$); (M+N)($p < .005$) and (M+C.A.) ($p < .02$)

At the 60 min interval all combined groups had mean values that were less than the control mean value. The groups that were significantly less than the control were the (O.B.+P) group ($p < .05$); (M+N) group ($p < .005$) and the (E.O.+M.A.) group ($p < .02$). The remaining groups were not significantly different from the control.

At the 90 min interval, the mean values for all combined groups were less than the mean value for the control. The groups (O.B.+P) and (M+N) had mean values that were significantly less than the control mean value ($p < .005$) for both groups- the remaining groups were not significantly different from the controls.

At the 120 min interval the mean values for the combined groups were less the control mean value. The groups (O.B.+P) and (M+N) had mean values that were significantly less than the mean value for the control group, ($p < .01$) for both groups.

MEAN O.G.T.T. GLUCOSE AREAS AT 4 WEEKS TABLE X11A

120 Minute Areas:-

The total and incremental areas for all groups were less than the respective control areas. The (OB.+P) group had significantly lower total, ($p < .001$) and incremental area, ($p < .02$) compared with respective control areas.

The groups (M+NA.), (M+N) and (E.O+M.A.) had total areas that were significantly less than the control total area with ($p < .02$); ($p < .05$) and ($p < .02$) respectively. The incremental are as for these three groups was not significantly different from the control area. Finally the (M+C.A.) group had significantly lower total, ($p < .05$) and incremental ($p < .005$) areas compared with the controls.

60 Minute Areas:-

The total and incremental areas for all groups were less than the respective control areas. The (O.B.+P) group had total and incremental areas that were significantly less than the control areas with ($p < .001$) and ($p < .02$) respectively for the two areas. The (M+N.A.) group had significantly lower total, ($p < .005$) and incremental ($p < .025$) areas compared with the control areas. The total and incremental areas for the (M+N) group were significantly less than the control areas ($p < .001$) and ($p < .025$) respectively for the two areas. The (E.O.+M.A.) group had a significantly lower total area ($p < .05$) but not incremental area compared with control areas. Finally the (M+C.A.) group had both total and incremental areas that were significantly greater than the control areas, ($p < .02$) and ($p < .005$) respectively..

BLOOD PYRUVATE AT 4 WEEKS FIGS. 11A + 12A TABLE X1A

Fasting:-

The mean values for all combined groups were not significantly different from the control mean value.

DURING THE O.G.T.T.

At the 30 min interval the only group whose value was not significantly different from the control value was the (O.B.+P) group, the other remaining groups had mean values that were greater and highly significant when compared to control mean value. The significance levels were as follows; (M+N.A.)($p < .005$) (M+N)($p < .001$;)

(E. O. +M. A.) ($p < .001$) and finally (M.+C. A.) ($p < .001$)

At the 60 min interval the mean values for all combined groups were greater than . the mean value for the contro l, however none were significantly different from the control value.

At the 90 min interval the (M+N. A.) (E. O. +M. A.) and (M. +C. A.) groups had mean values that were significantly greater than the control mean value, ($p < .01$) and ($p < .005$) and ($p < .02$) respectively. The remaining groups had mean values that were not significantly different from the control mean value.

At the 120 min interval the (M+N. A.), (E. O. +M. A.) and the (M+C. A.) groups had mean values that were significantly greater than the mean control values with ($p < .05$), ($p < .01$) and ($p < .05$) for the three groups respectively. The remaining groups had mean values that were not significantly different from the control mean value.

MEAN O. G. T. T. PYRUVATE AREAS AT 4 WEEKS TABLE X11A

120 Minute Areas:-

The total and incremental areas for all groups were greater than the respective control areas. The (O. B. +P) group had total and incremental areas that were not significantly different from the control areas. The total and incremental areas for the (M+N. A.) group were significantly greater than the control areas, ($p < .005$) and ($p < .02$) respectively, for the two areas. The (M+N) group had significantly elevated total and incremental areas compared with respective control areas, ($p < .05$) for both areas. The two groups (E. O. +M. A.) and (M+C. A.) had total and increm ental areas that were significantly greater than the respective control areas, ($p < .001$) for the two areas, in both groups.

60 Minute Areas:-

The total and incremental areas for all groups were greater than the respective control areas. The (O. B. +P) group had total and incremental areas that were not significantly different from the respective control areas. The (M+N. A.) group had significantly elevated total, ($p < .005$) and incremental, ($p < .02$) areas compared with the respective control areas.

The (M+N) group had significantly elevated total and incremental areas compared with control areas ($p < .01$) for both areas. The two groups (E. O+M. A.) and (M+C. A.) had very significantly elevated total and incremental areas compared with the respective control areas, ($p < .001$) for both areas in both groups.

PLASMA INSULIN AT 4 WEEKS FIGS. 11 A + 12 A TABLE X1A

Fasting:-

The mean values for all groups were not significantly different from the control mean value.

DURING THE O. G. T. T.

At the 15 min interval the only group that had a mean value significantly different from the control mean value was the (M+N. A.) group which had a value less than the control, and a p value of $< .05$.

At the subsequent time intervals 30, 60, 90 and 120 minutes, the mean values for all the combined groups were less than the control mean value, and with the exception of the (M+N. A.) group, 30 and 120 min. mean values which were significantly less than the control values, ($p < .05$) for both times; none of the other values were significantly different from the controls.

MEAN O.G.T.T. INSULIN AREAS AT 4 WEEKS TABLE X11A

120 Minute Areas:-

The total and incremental areas for all groups were less than the respective control areas. The only group that had significantly lower total and incremental areas, ($p < .05$) for both areas, was the (M+N.A.) group, all the other groups had areas that were not significantly different from the respective control areas.

60 Minute Areas:-

The total and incremental areas for all groups were less than the respective control areas. The (M+N.A.) group had significantly lower total and incremental areas ($p < .05$) for both areas, compared with respective control areas. The (E.O+M.A.) group had a significantly lower incremental area ($p < .05$) but not incremental area, compared with control areas. The remaining groups had areas that were not significantly different from control areas.

SUMMARY OF RESULTS FOR COMBINED SERIES:

GLUCOSE.

The mean fasting glucose values after 2 weeks of combined oestrogen progestogen were in general not significantly different from the control mean values, with the exception of the (oestradiol benzoate + Progesterone) group which had a mean value significantly lower than that of the control mean value. After 4 weeks combined oestrogen progestogen administration there were lower mean fasting glucose levels in all groups except the (Mestranol +Chlormadinone acetate) group.

After 2 and 4 weeks of combined oestrogen progestogen administration, the glucose mean values for all groups were less than the respective control mean values during an O.G.T.T., indicating a faster rate of glucose removal.

PYRUVATE.

After 2 and 4 weeks of combined oestrogen progestogen administration ther was no apparent trend for fasting pyruvate values to be either greater or less than the control fasting mean value.

All combined groups with the exception of the Oestradiol benzoate + Progesterone group demonstrated significant increases in pyruvate levels during an O.G.T.T. after 2 and 4 weeks of steroid administration.

INSULIN.

After 2 and 4 weeks of combined oestrogen progestogen administration there was no apparent trend for mean fasting values to be either greater or less than the control mean value.

After 2 and 4 weeks of combined oestrogen progestogen administration the insulin response to an oral glucose challenge was approximately the same or significantly less in the groups (Mestranol + Norethynodrel) and (Mestranol + Norethisterone acetate) in comparison with the control insulin response.

DISCUSSION

Combined oestrogen - progestogen administration is seen to improve carbohydrate tolerance in the ovariectomized rat. This effect is more pronounced after 4 weeks of steroid administration, when the fasting blood glucose levels and mean glucose areas are lower for the combined steroid groups in comparison with the respective control values.

Studies in the human with combined O.C. are conflicting with Spellacy et al (1970), Wynn and Doar (1966) and (1969) and Gershberg, Javier and Hulse (1964) demonstrating that carbohydrate tolerance is markedly impaired whilst Taylor and Kass (1968) and Danowski et al (1968) demonstrated no significant alteration in glucose tolerance.

The finding of improved glucose tolerance in the absence of hyperinsulinism seen with combined oestrogen-progestogen administration in these animal experiments is possibly due to the oestrogen component; for Progesterone and the Progestogens when administered alone at the same dose levels employed in the combined preparation are seen to be without major effect on glucose tolerance. Oestrogen administration to rats increases the incorporation of glucose into liver and peripheral tissue glycogen, Griffiths et al (1941) and Waalas (1952 a + b) and also its metabolism via the Hexose mono-phosphate shunt pathway, Gilmour and McKerns (1966). Animals receiving combined oestrogen progestogen preparations attain much greater pyruvate levels than do animals which receive the same oestrogen or progestogen singly at the same dose level (chapter 2+3).

This finding in the absence of an elevated insulin response would suggest that the oestrogens and progestogens administered in combination, have a synergistic action on the mechanisms involved in the formation and/or the removal of pyruvate.

Hagerman (1962) has shown that liver slices from pregnant rats have an increased glycolytic activity with increased formation of pyruvate from glucose. The same worker also demonstrated that these liver s had a reduction in the incorporation of pyruvate into glucose, Matute and Kalkhoff (1973) have shown that rats treated with a combination of Oestradiol benzoate and Progesterone have a significant reduction in gluconeogenesis from the 3C precursors alanine and pyruvate. This inhibition of gluconeogenesis may be related to the lower O.G.T.T. glucose levels seen in the animals receiving combined oestrogen-progestogen preparations presented in this section of the work.

The progestogens, Norethynodrel and Norethisterone acetate may potentiate the oestrogen action on liver and peripheral enzyme systems involved with glucose metabolism, by virtue of their conversion to the oestrogenic steroid Mestranol, Paulsen (1965). This conversion would thereby effectively increase the levels of circulating oestrogen in these animals.

However, Chlormadinone acetate and Megestrol acetate cannot be implicated with such a mechanism, for they are reported by Paulsen not to be converted to oestrogenic steroids. Megestrol acetate when administered alone, has been demonstrated to slightly improve carbohydrate tolerance. Chlormadinone acetate whilst having no effect on carbohydrate tolerance, shares with Megestrol acetate the property of causing marginal elevations in O.G.T.T. pyruvate levels. These findings for the two progestogens are attributed to their insulinogenic properties However in this series of experiments insulin levels were found not to be significantly elevated in the combined oestrogen-progestogen groups that contained either of these two 17 alpha OH progesterones, or the 19 nor testosterone derivatives.

Hager et al (1971) has shown that high doses of Oestradiol benzoate administered to rats, inhibits insulin secretion from the isolated islets of these animals, a finding which he postulated to be due to oestrogen interfering with the glucose tirgger mechanism for insulin secretion. However Green and Taylor (1972) have shown that isolated islets of pregnant rats are more sensitive to a glucose stimulus than are the islets from normal non-pregnant females. Costrini and Kalkhoff, (1971), have demonstrated increased insulin secretion from the isolated islets of rats treated with a combination of Oestradiol benzoate and Progesterone. The role of insulin in the combined oestrogen-progestogen studies presented in this thesis has not been clearly defined, but it would appear that the oestrogen component has an inhibiting effect on pancreatic islet cell function. The possibility also exists that the insulin response is lower

in these animals receiving insulinogenic progestogens in the combined oestrogen-progestogen groups, because of the lower blood glucose levels in these groups.

The progestogens may also alter the metabolism of glucose in an indirect manner by prolonging the duration and effects of the oestrogen component by competing for the same enzymes involved with the metabolism of these steroids. Evidence for this comes from the work of Gram and Gillette (1969) which showed that Oestradiol and Progesterone are metabolized by the same hepatic microsomal enzymes.

Therefore in summary, the administration of combined oestrogen and progestogen steroids improve glucose tolerance in the rat, an effect which is possibly related to an increased metabolism of glucose by mechanisms sensitive to the oestrogen component and a reduction in gluconeogenesis.

CHAPTER 5

THE ROLE OF THE ADRENAL GLAND IN MEDIATING ELEVATED PYRUVATE LEVELS DURING OESTRADIOL BENZOATE ADMINISTRATION TO THE RAT

SECTION 1 — THE EFFECT OF 50 μg OESTRADIOL BENZOATE ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE ADRENALECTOMIZED - OVARIECTOMIZE RAT.

SECTION 11 — THE EFFECT OF CORTICOSTERONE AND CORTICOSTERONE WITH OESTRADIOL BENZOATE ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE ADRENALECTOMIZED - OVARIECTOMIZED RAT.

INTRODUCTION.

It has been demonstrated in earlier chapters of this thesis that Oestradiol benzoate, Mestranol, Ethinyl oestradiol and the progestogens, Norethynodrel, Norethisterone acetate and to a lesser extent for Megestrol acetate and Chlormadinone acetate all gave elevations in blood pyruvate levels during an O.G.T.T.

Previous investigations by Doar, Wynn and Cramp (1969) have shown that blood pyruvate levels are elevated in fasting and during an O.G.T.T. in patients receiving corticoid therapy, obesity and women using oral contraceptives. Doar (1969 M.D. Thesis) showed further that these elevations were due to an increase in the formation of pyruvate and not due to a decrease in its rate of removal. Because of the similarity in the blood pyruvate pattern of women using oral contraceptives with those of patients receiving glucorticoid therapy and because of the known effects of oral contraceptives, in particular the oestrogen component causing an elevation in circulating levels of cortisol, (Sandberg and Slaunwhite 1959. and Sandberg, Rosenthal and Slaunwhite 1969 .) it is suggested that the adrenal corticoids may be involved in causing elevations in blood pyruvate levels.

The progestogens Norethynodrel, Norethisterone acetate, Megestrol acetate and Chlormadinone acetate have not been previously implicated with regard to causing elevations in O.G.T.T. blood pyruvate levels. Therefore, it would appear that the progestogen component of the oral contraceptive must also be considered if a mechanism is to be elucidated in the complex oestrogen progestogen interaction with the adrenal and the role it has in bringing about an alteration in glucose and pyruvate metabolism.

The purpose of this section therefore is to investigate the role of the adrenal gland in gonadal steroid induced hyperpyruvtaemia.

Oestradiol benzoate administration for a period of 2 weeks and a dose level of 50 μg was selected on the basis that this dose and duration of treatment has been shown to affect glucose and pyruvate metabolism.

Fig.13

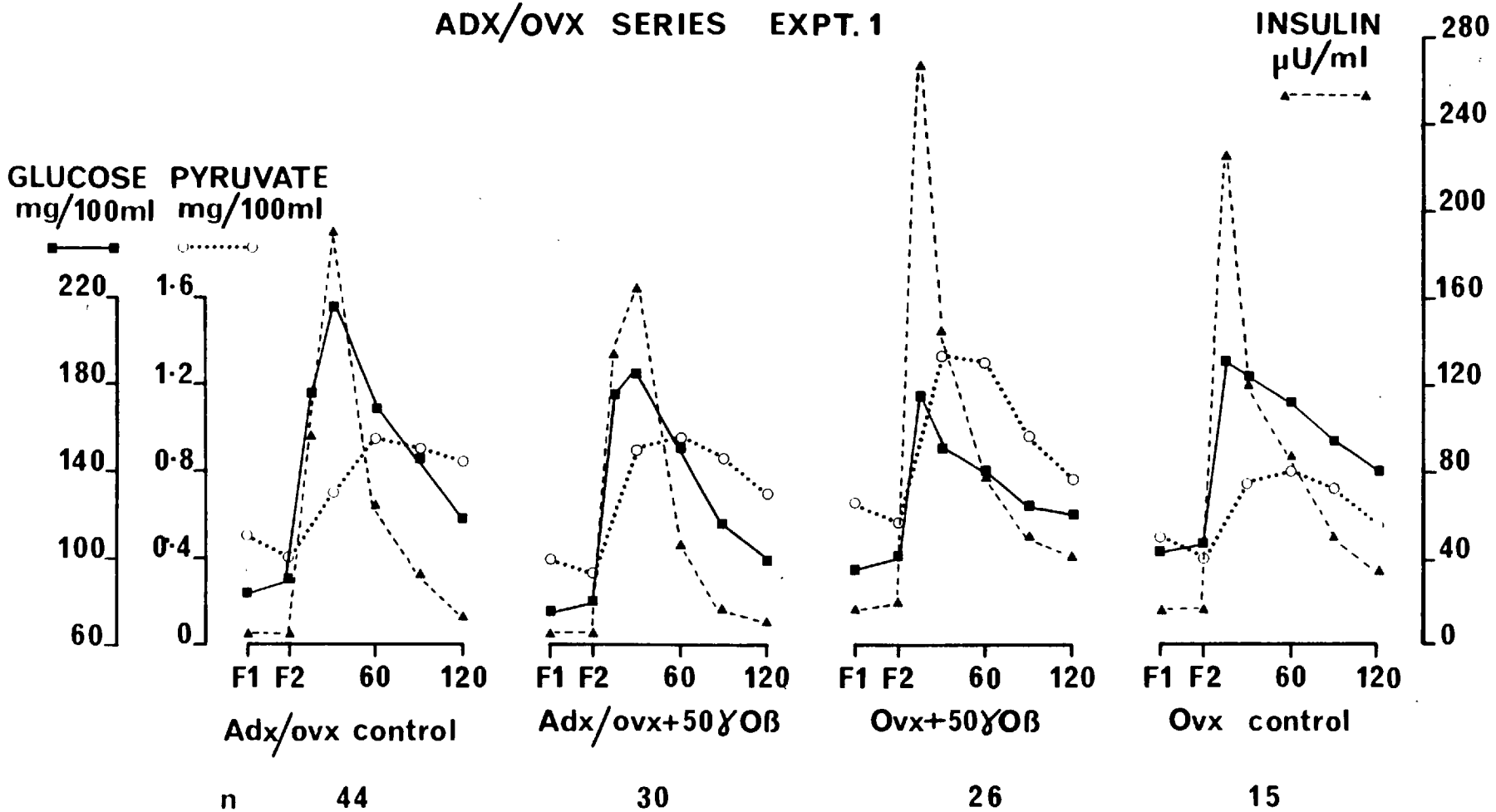
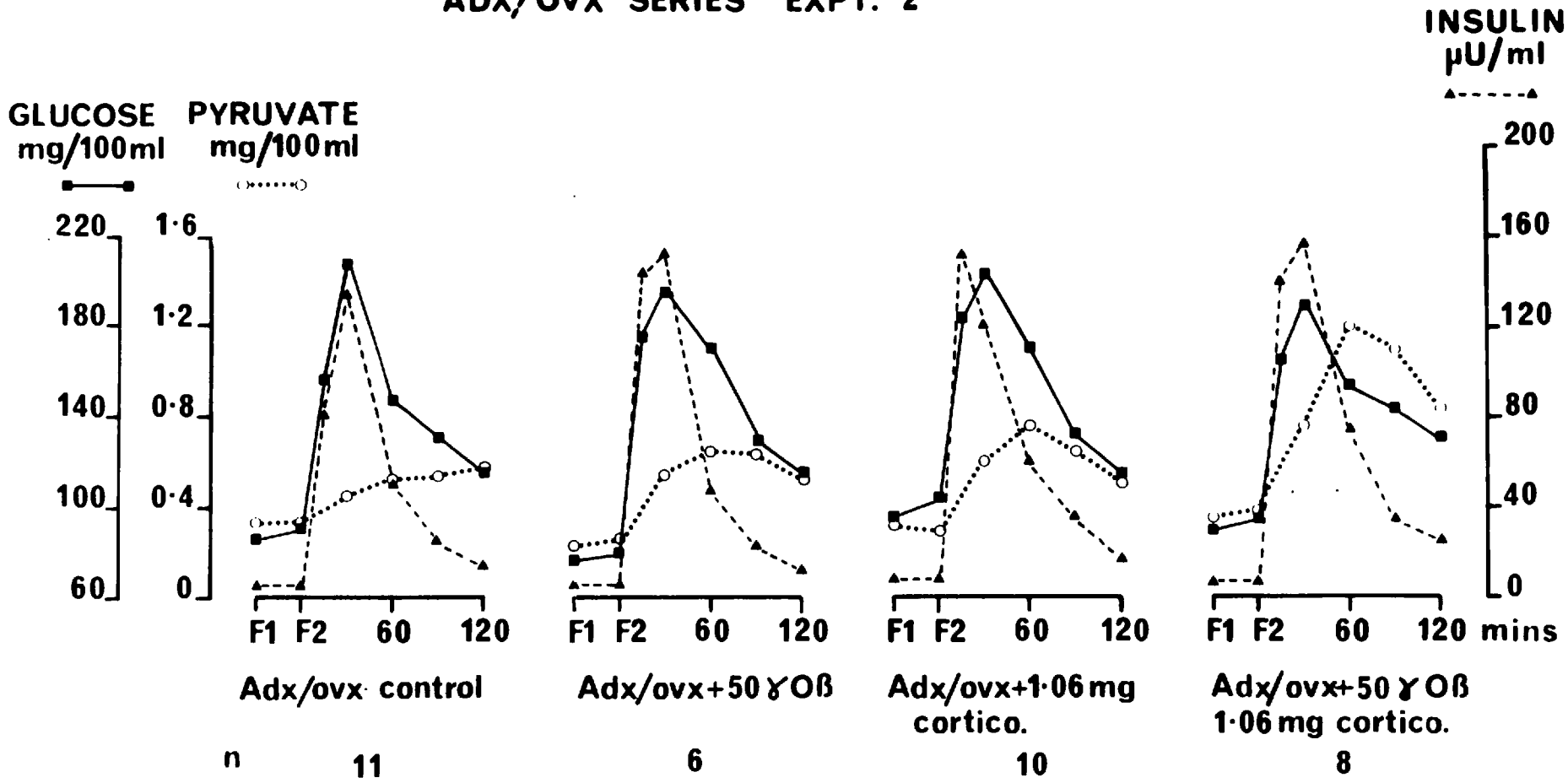

ADX/OVX SERIES EXPT. 1
GLUCOSE mg/100ml
PYRUVATE mg/100ml
INSULIN µU/ml
220
180
140
100
60
1·6
1·2
0·8
0·4
0
280
240
200
160
120
80
40
0
F1 F2 60 120
Adx/ovx control
Adx/ovx+50γOß
Ovx+50γOß
Ovx control
n 44 30 26 15

Fig. 13A
ADX/OVX SERIES EXPT. 2



		F1	F2	15 Min	30 Min	60 Min	90 MIN	120 Min
ADX/ OVX	Glucose mg/100ml	85 ± 2	91 ± 2	177 ± 5	218 ± 5	168 ± 7	143 ± 5	119 ± 4
Oil Control	Pyruvate mg/100ml	0.51 ± 0.04	0.39 ± 0.03	----------	0.78 ± 0.04	0.96 ± 0.06	0.90 ± 0.06	0.84 ± 0.06
N = 44	Insulin μU/ ml	4 ± 1	4 ± 1	96 ± 10	189 ± 19	63 ± 7	32 ± 3	15 ± 1
ADX / OVX	Glucose	76 ± 3	80 ± 3	174 ± 6	185 ± 8	151 ± 9	117 ± 6	101 ± 6
		<.001	<.001	N.S.	<.001	N.S.	<.001	<.01
+50 μg O.B.	Pyruvate	0.40 ± 0.03	0.34 ± 0.02	----------	0.91 ± 0.06	0.95 ± 0.07	0.88 ± 0.07	0.70 ± 0.07
		<.05	N.S.		<.02	N.S.	N.S.	N.S.
N = 29	Insulin	3 ± 1	4 ± 1	133 ± 18	163 ± 27	46 ± 8	17 ± 3	10 ± 3
		N.S.	N.S.	N.S.	N.S.	N.S.	<.005	<.005
OVX +50 μg O.B.	Glucose	97 ± 3	101 ± 2	179 ± 5	151 ± 5	139 ± 4	126 ± 4	120 ± 4
		<.001	<.001	N.S.	<.001	<.005	<.01	N.S.
	Pyruvate	0.65 ± 0.04	0.54 ± 0.04	----------	1.34 ± 0.10	1.29 ± 0.08	0.96 ± 0.06	0.72 ± 0.08
		<.025	<.001		<.001	<.005	N.S.	N.S.
N = 25	Insulin	18 ± 2	21 ± 10	267 ± 27	145 ± 19	75 ± 8	50 ± 7	40 ± 5
		<.001	<.001	<.001	N.S.	N.S.	<.005	<.001
OVX + 0.10ml	Glucose	104 ± 2	107 ± 2	191 ± 5	184 ± 7	174 ± 6	152 ± 4	139 ± 6
		<.001	<.001	N.S.	<.005	N.S.	N.S.	<.01
Oil Control.	Pyruvate	0.51 ± 0.04	0.41 ± 0.03	----------	0.74 ± 0.05	0.79 ± 0.08	0.73 ± 0.08	0.52 ± 0.03
		N.S.	N.S.		N.S.	N.S.	N.S.	<.005
N = 15	Insulin	16 ± 2	17 ± 2	227 ± 27	122 ± 14	87 ± 12	49 ± 8	36 ± 6
		<.001	<.001	<.001	N.S.	N.S.	<.02	<.001
OVX Oil Control	Glucose	N.S.	N.S.	N.S.	<.001	<.001	<.001	<.01
versus	Pyruvate	<.05	<.005	----------	<.001	<.001	<.05	<.05
OVX + 50 μg O.B.	Insulin	N.S.	N.S.	N.S.	N.S	N.S.	N.S.	N.S.

TABLE XIII Mean ± S.E.M. O.G.T.T. Plasma glucose, insulin and blood pyruvate with significance levels for Expt. 1 ADX/OVX series.
(N.S. = not significant.)

		F1	F2	15 Min	30 Min	60Min	90 Min	120 Min
ADX/ OVX	Glucose mg/100ml	88 ± 2	91 ± 2	158 ± 9	210 ± 11	147 ± 10	132 ± 9	115 ± 7
Oil Control	Pyruvate mg/100ml	0.34 ± 0.04	0.29 ± 0.04	----------	0.43 ± 0.05	0.52 ± 0.07	0.54 ± 0.07	0.58 ± 0.07
N = 11	Insulin μU / ml	4 ± 1	4 ± 1	80 ± 22	132 ± 34	49 ± 16	26 ± 4	15 ± 2
ADX / OVX	Glucose	77 ± 5 <.05	80 ± 6 <.05	183 ± 17 N.S.	200 ± 10 N.S.	160 ± 23 N.S.	125 ± 12 N.S.	116 ± 8 N.S.
+ 50 μg O.B.	Pyruvate	0.21 ± 0.03 <.05	0.25 ± 0.04 N.S.	----------	0.69 ± 0.16 N.S.	0.74 ± 0.17 N.S.	0.71 ± 0.15 N.S.	0.53 ± 0.16 N.S.
N = 6	Insulin	3 ± 2 N.S.	3 ± 1 N.S.	145 ± 48 N.S.	152 ± 39 N.S.	47 ± 19 N.S.	21 ± 6 N.S.	13 ± 3 N.S.
ADX/ OVX	Glucose	98 ± 4 <.05	103 ± 3 <.01	185 ± 10 N.S.	204 ± 10 N.S.	170 ± 11 N.S.	133 ± 6 N.S.	120 ± 5 N.S.
+ 1.06 mg	Pyruvate	0.34 ± 0.04 N.S.	0.29 ± 0.02 N.S.	----------	0.61 ± 0.05 <.02	0.76 ± 0.06 <.02	0.64 ± 0.06 N.S.	0.55 ± 0.05 N.S.
Cortictósterone N = 10	Insulin	8 ± 3 N.S.	8 ± 2 <.05	150 ± 29 N.S.	118 ± 30 N.S.	60 ± 12 N.S.	36 ± 12 N.S.	16 ± 3 N.S.
DX/ OVX	Glucose	91 ± 3 N.S.	93 ± 4 N.S.	170 ± 12 N.S.	186 ± 12 N.S.	144 ± 20 N.S.	142 ± 9 N.S.	129 ± 8 N.S.
1.06 mg Cort	Pyruvate	0.35 ± 0.08 N.S.	0.36 ± 0.07 N.S.	----------	0.82 ± 0.09 <.001	1.19 ± 0.12 <.001	1.09 ± 0.20 <.01	0.80 ± 0.12 N.S.
costerone + 0 μg O.B.	Insulin	6 ± 2 N.S.	6 ± 1 N.S.	139 ± 34 N.S.	157 ± 43 N.S.	74 ± 19 N.S.	36 ± 8 N.S.	24 ± 6 N.S.
N = 8								

TABLE XIII A Mean ± S.EM. O.G.T.T. Plasma glucose, insulin and blood pyruvate levels with significance levels for Expt 2 ADX/OVX series
(N.S. = not significant)

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120. Minute Areas.						
ADX/ OVX Oil Control N = 44	639 ± 12	271 ± 14	3.22 ± 0.18	1.62 ± 0.14	295 ± 21	279 ± 22
ADX / OVX +50 μg O.B. N = 29	566 ± 19 <.001	247 ± 24 N.S.	3.24 ± 0.18 N.S.	1.89 ± 0.15 N.S.	261 ± 32 N.S.	246 ± 33 N.S.
OVX + 50μg O.B. N =25	556 ± 10 <.001	151 ± 7 <.001	4.22 ± 0.20 <.001	2.04 ± 0.20 N.S.	389 ± 30 <.02	313 ± 26 N.S.
OVX + Oil Control N = 15	655 ± 16 N.S.	226 ± 14 N.S.	2.72 ± 0.16 N.S.	1.13 ± 0.20 N.S.	363 ± 29 N.S.	297 ± 26 N.S.
OVX Oil Control versus OVX + 50 μg O.B.	<.001	<.001	<.001	<.001	N.S.	N.S.
60 Minute Areas.						
ADX/ OVX Oil Control N = 44	358 ± 7	176 ± 8	1.41 ± 0.08	0.61 ± 0.06	222 ± 18	210 ± 18
ADX / OVX + 50 μg O.B. N=29	322 ± 10 <.005	161 ± 12 N.S.	1.56 ± 0.09 N.S.	0.87 ± 0.08 N.S.	213 ± 29 N.S.	206 ± 30 N.S.
OVX + 50 μg O.B. N= 25	297 ± 6 <.001	95 ± 5 <.001	2.26 ± 0.13 <.001	1.17 ± 0.12 <.001	285 ± 22 <.05	243 ± 20 N.S.
OVX Oil Contr - ol N = 15	347 ± 9 N.S.	132 ± 9 <.005	1.35 ± 0.08 N.S.	0.52 ± 0.10 N.S.	253 ± 22 N.S.	220 ± 21 N.S.
OVX Oil Control versus OVX +50 μg O.B.	<.001	<.001	<.001	<.001	N.S.	N.S.

TABLE XIV Mean ± S.E.M.(Units - min) O.G.T.T. 120 Minute and 60 Minute total and incremental areas with significance levels for Expt 1 ADX/OVX series. 'N.S. = not significant

TABLE XIV A

	Glucose Total Area (△)	Glucose Incremental △	Pyruvate Total △	Pyruvate Incremental △	Insulin Total △	Insulin Incremental △
120 Minute Areas						
ADX/OVX Oil Control. N= 11	596 ± 21	231 ± 19	1.93 ± 0.15	0.75 ± 0.26	258 ± 74	241 ± 74
ADX/OVX + 50 μg O.B. N = 6	602 ± 35 N.S.	284 ± 53 N.S.	2.52 ± 0.48 N.S.	1.51 ± 0.31 N.S.	260 ± 53 N.S.	247 ± 51 N.S.
ADX/OVX +1.06 mg Corticosterone N=10	636 ± 25 N.S.	224 ± 17 N.S.	2.42 ± 0.14 <.05	1.28 ± 0.16 N.S.	285 ± 53 N.S.	253 ± 49 N.S.
ADX/OVX +1.06 Corticosterone + 50 μg O.B. N = 8	610 ± 40 N.S.	239 ± 23 N.S.	3.59 ± 0.49 <.005	2.45 ± 0.48 <.005	289 ± 53 N.S.	268 ± 52 N.S.
60 Minute Areas.						
ADX/OVX Oil Control N = 11	333 ± 14	150 ± 14	0.84 ± 0.07	0.26 ± 0.09	159 ± 33	151 ± 34
ADX/OVX + 50 μg O.B. N = 6	342 ± 17 N.S.	182 ± 24 N.S.	1.18 ± 0.24 N.S.	0.68 ± 0.17 N.S.	211 ± 51 N.S.	204 ± 51 N.S.
ADX/OVX +1.06 Corticosterone N = 10	357 ± 15 N.S.	151 ± 11 N.S.	1.13 ± 0.07 <.01	0.56 ± 0.08 <.02	196 ± 39 N.S.	180 ± 37 N.S.
ADX/OVX + 1.06mg Corticosterone + 50 μg O.B. N = 8	319 ± 21 N.S.	134 ± 14 N.S.	1.59 ± 0.15 <.001	0.87 ± 0.15 <.005	228 ± 45 N.S.	216 ± 44 N.S.

TABLE XIV A Mean ± S.E.M. (Units-min) O.G.T.T 120 Minute and 60 Minute total and incremental areas for Expt. 2 ADX/OVX series with significance levels.
N S = not significant.)

TABLE XIV B

Animal weights; adrenalectomized series

Expt 1.

Mean ± S.E.M. (body weight in grams)

	Initial	2 week
ADX/OVX Oil Control	225 ± 7	236 ± 5
ADX/OVX + 50 μg O.B.	220 ± 4	221 ± 5
OVX + 50 μg O.B.	222 ± 5	229 ± 6
OVX Oil Control	238 ± 4	258 ± 5

Animal weights; adrenalectomized series

Expt 2

Mean ± S.E.M.

ADX/OVX Oil Controls	225 ± 4	230 ± 6
ADX/OVX + 50 μg O.B.	227 ± 12	221 ± 13
ADX/OVX + Corticosterone	235 ± 5	264 ± 5
ADX/OVX + Corticosterone + 50 μg O.B.	228 ± 12	237 ± 14

PROCEDURES;

The investigation was comprised of two experiments, the first of which used Ovariectomized (OVX) or Adrenalectomized - Ovariectomized (ADX/OVX) animals which received either 0.10 ml corn oil only or 50 μg Oestradiol benzoate (O.B.) in 0.10 ml corn oil, daily for 2 weeks, at the end of which time an O.G.T.T. was performed.

The second experiment used ADX/OVX animals which received either a maintenance dose of corticosterone, 50 μg O.B., or a combination of corticosterone and O.B. The control animals received oil only. The animals were treated for 2 weeks with steroid or oil before having the O.G.T.T.

Animals in these two experiments that were moribund or had evidence of adrenal tissue at autopsy were not included in the series.

EXPERIMENT 1

THE EFFECT OF 50 μg O.B. ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE ADX/OVX RAT

RESULTS:

PLASMA GLUCOSE FIG 13 TABLE X111

Fasting:-

The mean fasting plasma glucose value in the ADX/OVX control group was significantly lower than the mean value for the OVX control group ($p < .001$) and the OVX + 50 μg O.B. group ($p < .001$).

The mean value for the OVX control group was not significantly different from the OVX + 50 μg O. B. group.

DURING THE O. G. T. T.

It can be seen that there is a marked change in the glucose pattern with adrenalectomy and with O. B. treatment. The ADX animal demonstrates a much higher peak glucose value and a rapid rate of removal of glucose in comparison to the intact adrenal control group. Oestradiol administration tends to have a glucose lowering effect.

At the 15 min interval, there was no significant difference in mean values between any of the groups.

At the 30 min interval the glucose mean values were significantly less in the groups ADX/OVX + 50 μg O. B., OVX + 50 μg O. B. and OVX controls compared with the ADX/OVX oil control group; ($p < .001$), ($p < .001$) and ($p < .005$) respectively for the three groups.

At the 60 min interval there was no significant difference in the mean values between the ADX/OVX control, OVX controls and ADX/OVX + 50 μg O. B. groups, however the OVX + 50 μg O. B. group had a significantly lower value ($p < .005$).

At the 90 min interval there was no significant difference between ADX/OVX control mean value and the OVX control mean value. The ADX/OVX + 50 μg O. B. and OVX + 50 μg O. B. groups had significantly lower mean values than the ADX/OVX control group; ($p < .001$) and ($p < .01$) respectively.

At the 120 min interval the OVX controls had a mean value significantly greater than the ADX/OVX control mean value ($p < .01$). The ADX/OVX + 50 μg O. B. value was significantly lower than the ADX/OVX controls ($p < .001$).

The ADX/OVX control mean value was not significantly different from the OVX+50μg O. B. group.

In the two groups of animals with adrenals intact, OVX controls and OVX +50 μg O.B; there was no significant difference in mean values for fasting; however during the O.G.T.T. at all time intervals except for the 15min interval the mean values were lower for the OVX+ 50 μg O.B. group ($p < .001$) for the 30, 60 and 90 min intervals and ($p < .01$) for the 120 min interval.

MEAN O.G.T.T. GLUCOSE AREAS TABLE X1V

120 Minute Areas:-

There was no significant difference in total and incremental areas between the ADX/OVX control group and the OVX control group.

The ADX/OVX + 50 μg O.B. and OVX+50μg O.B. groups had significantly lower total areas, ($p < .001$) for both groups compared with the respective areas for the ADX/OVX control groups. The OVX+ 50μg group incremental area was significantly less than the ADX/OVX control area ($p < .001$), however ADX/OVX + 50 μg O.B. group incremental area was less though not significantly different from the ADX/OVX control area.

60 Minute Areas:-

The ADX/OVX+50 μg O.B. group had a total area that was significantly less than the ADX/OVX control area, ($p < .005$) however the incremental area although lower was not significantly different from the control area. The OVX+50 μg O.B. group total and incremental areas were significantly lower than the respective ADX/OVX control areas ($p < .001$) for both areas. The OVX control areas although less than the respective ADX/OVX control areas only the incremental area was significantly less ($p < .005$).

Finally, in comparing the OVX control total and incremental areas with OVX + 50 μg O.B. areas, it is observed that O.B. treatment significantly lowers the 120 minute and 60 minute total and incremental areas ($p < .001$) for all areas.

BLOOD PYRUVATE FIG. 13 TABLE X111

Fasting:-

There was no significant difference between the mean value for ADX/OVX control group and OVX control mean value. The OVX+50μg group had a mean value significantly greater than the ADX/OVX control value, ($p < .001$).

DURING THE O.G.T.T.

The ADX/OVX control mean values throughout the O.G.T.T. were all greater than the respective OVX control mean values, however with the exception of the 120 min value, ($p < .005$), the values for the two groups were not significantly different.

There was no significant difference between the mean values for the ADX/OVX control group and the ADX/OVX +50μg O.B. group at the 60, 90 and 120 min intervals, however the 30 min ADX/OVX + 50 μg O.B. group had a mean value significantly greater than ADX/OVX control value, ($p < .02$).

The OVX+50 μg O.B. group mean values in comparison to the ADX/OVX control mean values had significantly elevated 30 and 60 min interval values ($p < .001$) and ($p < .005$) respectively, the 90 and 120 min values were not significantly different.

In comparing the OVX controls with the OVX+50 μg O. B. group, the latter group demonstrated significant elevations in fasting value, ($p < .005$) and during the O. G. T. T. 30 min ($p < .001$), 60 min ($p < .001$) and ($p < .05$) for both the 90 and 120 minute values.

MEAN O. G. T. T. PYRUVATE AREAS TABLE X1V

120 Minute Areas:-

The total and incremental areas for the ADX/OVX+50μg O. B. and OVX control groups were not significantly different from the respective ADX/OVX control areas. The OVX+50μg O. B. group total and incremental areas were greater than the ADX/OVX control areas, however only the total area was significantly greater, ($p < .001$).

60 Minute Areas:-

The total and incremental areas for the OVX controls were not significantly different from the respective ADX/OVX control areas. The ADX/OVX+50μg O. B. group total and incremental areas were greater than the ADX/OVX control areas but were not significantly different from the ADX/OVX control areas.

The OVX+50μg O. B. group had total and incremental areas significantly greater than the respective ADX/OVX control areas, ($p < .001$) for both areas.

In comparing the OVX controls and OVX+50μg O. B. group, the latter demonstrated significantly elevated 120 minute and 60 minute total and incremental areas over the respective OVX control areas, ($p < .001$) for all areas.

PLASMA INSULIN FIG 13 TABLE X111

Fasting:-

The ADX/OVX controls had a significantly lower mean value than the mean values for the OVX controls and OVX+50 μg O. B. groups ($p < .001$) for both groups. There was no significant difference between ADX/OVX controls and ADX/OVX+ 50 μg O. B. mean values, nor was there any significant difference between OVX controls and OVX + 50 μg O. B. group mean values.

DURING THE O. G. T. T.

At the 15 min interval, the OVX controls and OVX + 50 μg O. B. groups had mean values significantly greater than the ADX/OVX control mean value, ($p < .001$) for both groups.

There was no significant difference between the OVX control and OVX + 50 μg O. B. group mean values.

At the 30 min and 60 min intervals there was no significant difference between the mean values for any of the groups and the respective mean value for the ADX/OVX control group.

At the 90 min interval, the mean value for the ADX/OVX control was significantly less than the mean value for the OVX controls ($p < .02$) and OVX+50 μg O. B. ($p < .005$), but was significantly greater than the value for the ADX/OVX + 50 μg O. B. group, ($p < .005$).

At the 120 min interval, the mean value for the ADX/OVX control was significantly less than the mean value for OVX controls, ($p < .001$) and OVX + 50 μg O. B. ($p < .001$), but was significantly greater than the mean value for the ADX/OVX + 50 μg O. B. group ($p < .005$).

MEAN O.G.T.T. INSULIN AREAS TABLE X 1V

120 Minute Areas:-

The total and incremental areas for the OVX control and OVX + 50 μg O.B. groups were greater than the respective ADX/OVX control areas, howe ver only the OVX + 50 μg O.B. total area was significantly greater than the ADX/OVX control, area ($p < .02$), the other areas were not significantly different. The ADX/OVX + 50 μg O.B. group had total and incremental areas less than the respective ADX/OVX control areas, however no area was significantly different.

60 Minute Areas:-

The total and incremental areas for the OVX control and OVX + 50 μg O.B. group were greater than the respective ADX/OVX control areas, howe ver only the OVX + 50 μg O.B. group total area was significantly greater than the control area, ($p < .05$), the other areas were not significantly different. The ADX/OVX + 50 μg O.B. group had total and incremental areas less than the respective ADX/OVX control areas, however no area was significantly different.

In comparing the OVX control and OVX + 50 μg O.B. groups total and incremental areas with each other there was no significant difference in the 120 and 60 minute areas.

EXPERIMENT 2:

THE EFFECT OF CORTICOSTERONE AND CORTICOSTERONE WITH OESTRADIOL BENZOATE ON PLASMA GLUCOSE, INSULIN AND BLOOD PYRUVATE LEVELS DURING AN O.G.T.T. IN THE ADX/OVX RAT.

PROCEDURE:-

Corticosterone was chosen for the maintenance of the adrenalectomized-ovariectomized animal since this steroid constitutes 80% of the glucocorticoids secreted from the adrenal of the rat. The maintenance dose of corticosterone, 1.06 mg, was based on the steady state secretion rate of this steroid in the female rat Glenister D. W. and F. W. Yates.(1961)

RESULTS:-

PLASMA GLUCOSE FIG 13A TABLE X111A

Fasting:-

The mean value for the ADX/OVX + 50 μg O.B. group was significantly lower than the ADX/OVX oil control mean value, ($p < .05$). The mean value for the ADX/OVX + corticosterone group was significantly greater than the ADX/OVX controls, ($p < .01$). There was no significant difference between the mean values forthe ADX/OVX controls and the ADX/OVX + corticosterone + O.B. group.

DURING THE O.G.T.T.

There was no significant difference between the mean values for the ADX/OVX controls and the mean values for the other 3 groups at all time intervals.

MEAN O.G.T.T. GLUCOSE AREAS TABLE X1VA

120 Minute Areas:-

The total and incremental areas for the ADX/OVX oil control group were not significantly different from the respective areas for the other 3 groups.

60 Minute Areas:-

The total and incremental areas for the ADX/OVX oil control group were not significantly different from the respective areas for the other 3 groups.

BLOOD PYRUVATE LEVELS FIG. 13A X11A

The blood pyruvate levels measured in this experiment were lower than the expected values for ADX/OVX animals observed in experiment 1 of this series. However the general pattern of pyruvate formation during the O.G.T.T. was similar for the two experiments.

Fasting:-

There was no significant difference between the mean value for the ADX/OVX controls and the mean values for the other 3 groups.

DURING THE O.G.T.T.

The mean values for the ADX/OVX + 50 μg O.B. group were all greater than the respective mean values for the ADX/OVX controls during the O.G.T.T., however no value was significantly greater than the control value.

The ADX/OVX + corticosterone group had 30 and 60 min mean values that were significantly greater than the respective ADX/OVX control mean values, ($p < .02$) for both values.

The 90 min and 120 min values were not significantly different from the controls.

The ADX/OVX + corticosterone + O. B. group had significantly elevated mean values for the 30, 60 and 90 min intervals compared with the respective control mean values, the 120 min interval value was not significantly different from the control value. The levels of significance for the 30 and 60 min values was ($p < .001$) and the 90 min interval ($p < .01$).

MEAN O.G.T.T. PYRUVATE AREAS TABLE X1VA

120 Minute Areas:-

The total and incremental areas for the ADX/OVX + 50 μg O. B. group were greater than the respective ADX/OVX control areas, however the areas were not significantly different from the control areas. The ADX/OVX + corticosterone group total and incremental areas were greater than the respective ADX/OVX control areas, however only the total area was significantly greater than the control area, ($p < .05$).

The ADX/OVX + corticosterone + O. B. group total and incremental areas were significantly greater than the respective ADX/OVX control areas, ($p < .005$) for both areas.

60 Minute Areas:-

The total and incremental areas for the ADX/OVX + 50 μg O. B. group were greater than the respective ADX/OVX control areas, however no area was significantly different from the control area.

The ADX/OVX + corticosterone group had significantly elevated total and incremental areas in comparison with ADX/OVX control areas with ($p < .01$) and ($p < .02$) respectively for the two areas.

The ADX/OVX + corticosterone + O. B. group had significantly elevated total and incremental areas in comparison with ADX/OVX control areas, ($p < .001$) and ($p < .005$) respectively for the two areas.

PLASMA INSULIN FIG 13 A TABLE X11A

Fasting:-

There was no significant difference in mean fasting values between the ADX/OVX controls and the ADX/OVX + 50 μg O. B. group or the ADX/OVX + Corticosterone + O. B. group. However theADX/OVX + Corticosterone group had a significantly elevated mean value over the control ADX/OVX group mean value ($p < .05$).

DURING THE O.G.T.T.

There was no significant difference between the mean values for the ADX/OVX control group and the other 3 groups at the respective time intervals during the O.G.T.T.:

MEAN O.G.T.T. INSULIN AREAS TABLE X1V A

The 60 minute and 120 minute total and incremental areas for all 3 groups were not significantly different from the respective ADX/OVX control areas.

SUMMARY OF RESULTS FOR ADRENALECTOMY SERIES:-

GLUCOSE:-

Adrenalectomy significantly lowers the mean fasting glucose levels and Corticosterone given as replacement therapy to ADX animals returns the fasting glucose levels to normal. Oestradiol benzoate (O.B.) administration has a tendency to lower the mean fasting glucose levels in both ADX and adrenal intact animals.

The untreated ADX animal tends to have a greater peak glucose value after a glucose challenge in comparison with untreated adrenal intact animals; however the rate of glucose removal appears to be the same.

Oestradiol benzoate administration tends to decrease the mean glucose values during an O.G.T.T., however the rate of glucose removal in animals treated with O.B. is only significantly increased in animals with adrenals intact. Adrenalectomized animals given replacement corticosterone therapy have mean glucose values during an O.G.T.T. similar to ADX animals not receiving replacement corticosterone. The administration of O.B. to ADX animals receiving corticosterone replacement does not effect mean glucose levels during an O.G.T.T.

PYRUVATE:-

Adrenalectomy appears not to have any major effects on mean fasting pyruvate levels. Oestradiol-benzoate administration to adrenal intact animals increased the mean fasting pyruvate level over that of the adrenal intact control, but was without effect in the ADX animal.

The administration of a replacement dose of corticosterone to ADX animals did not effect fasting pyruvate levels, nor did O. B. and corticosterone together have any significant effect on the fasting pyruvate levels in these animals.

There was no significant difference between the mean pyruvate values of the ADX controls and the ADX+50 μg O. B. and adrenal intact oil controls during an O. G. T. T.

The administration of O. B. to animals with adrenals intact or ADX animals receiving corticosterone replacement therapy increased mean pyruvate levels during an O. G. T. T.

On close analysis of the pyruvate levels during an O. G. T. T. , there was an indication that O. B. may have a direct effect on elevating pyruvate levels, since the 30 minute pyruvate level is slightly elevated in ADX O. B. treated animals with or without corticosterone replacement.

INSULIN:-

Adrenalectomy significantly lowers the mean fasting insulin levels and replacement corticosterone administration attempts to return the fasting values of these animals to normal. Administration of O. B. to ADX, ADX+corticosterone or adrenal intact animals has no effect on fasting insulin levels.

During an O. G. T. T. the insulin response is brisk, though there is an apparent inappropriate secretion of insulin in response to the more elevated glucose levels seen in the ADX animals during the early part of the O. G. T. T.

The insulin response to an oral glucose challenge in animals with adrenals intact was greater than the insulin response of ADX animals. Corticosterone given as replacement therapy to ADX animals had no significant effect on insulin response during an O.G.T.T. The administration of O.B. to animals with adrenals intact, ADX and receiving corticosterone, or ADX and receiving oil only, had no significant effect on insulin responses during an O.G.T.T.

DISCUSSION

The observations made in this section of work show that adrenalectomy significantly lowers the levels of glucose both in the fasting state and during an O.G.T.T. The lower fasting values for glucose in the adrenalectomized (ADX) animal have also been reported by Dunn, Chenoweth and Schaeffer (1969) and are most probably due to a decrease in gluconeogenesis either from the reduced availability of 3-carbon precursors or a depression of one or more enzymatic steps involved in the conversion of lactate or pyruvate to triose phosphate.

The workers Henning, Sieffert and Seubert (1963) and Shrago et al (1963) have shown that the activities of the important gluconeogenic enzymes, pyruvate carboxylase and phospho-enol pyruvate carboxykinase are reduced in the livers of ADX rats.

However Friedmann, Goodman and Weinhouse (1965) and Dunn et al (1969) have shown that gluconeogenesis from 3-carbon precursors is not effected by adrenalectomy. Ray, Foster and Lardy (1964) suggest that the primary action of glucocorticoids is to direct metabolites toward carbohydrate synthesis, that is to increase peripheral tissue protein and fat catabolism.

Another possibility for the lowered fasting glucose values is that there is less glucose made available from the liver of the ADX animal by glycogenolysis since Friedmann et al (1965) has shown depressed glycogenesis from Hexose in the livers of ADX rats.

Whilst fasting blood glucose levels are lower in the ADX animal, there is an elevation in the glucose levels during the early phase of the O.G.T.T. in these animals compared to the levels for adrenal intact animals. This may be due to insulin, for the secretion of insulin appears to be inappropriate for the plasma glucose levels that are reached. A similar finding has been made by Malaisse et al (1967) using isolated islets from ADX rats; he demonstrated reduced insulin secretion with a glucose stimulus compared with the response for adrenal intact animals. The islet insulin content was not effected by adrenalectomy and he concluded that the sensitivity of the islet of the ADX animal was reduced. Other factors which may be involved in elevating the levels of glucose during the early phase of an O.G.T.T. in the ADX rat is a smaller glucose pool size, reported by Dunn et al (1969) or a reduction in the ability of the animal to incorporate glucose into glycogen reported by Friedmann et al (1965).

The administration of Oestradiol benzoate (O.B) to the ADX or adrenal intact animal lowers the glucose levels during the O.G.T.T. without increasing the insulin response. The animals with adrenals intact and receiving O.B. remove glucose at a much faster rate than ADX animals receiving O.B. The metabolism of glucose along the glycolytic and / or the Hexose mono-phosphate shunt pathways is apparently unaffected by adrenalectomy since pyruvate levels during the O.G.T.T. are normal in comparison with the adrenal intact oil control animals, Ashmore and Weber (1968) supports this latter point by stating that the activity of the glycolytic enzymes are unaffected by corticoids.

The pyruvate levels during the O.G.T.T. for the adrenal intact animal receiving O.B. are much greater than the levels for the ADX animals receiving the same dose of O.B. This finding is in accord with the direct or secondary actions of oestrogens on the liver and peripheral tissue enzymes involved in glucose metabolism, both for glycogen deposition and glycolysis proposed earlier in this thesis. However the presence of the adrenal gland or in particular the corticoids are required for oestrogens to exert their full effect on the glycolytic and / or Hexose mono-phosphate shunt pathways. Evidence for this latter point is found when ADX animals given a replacement dose of corticosterone have only marginally increased pyruvate levels during the O.G.T.T. in comparison with the ADX oil controls or ADX with O.B., however when corticosterone replacement and O.B. are administered together there was significant elevations in the pyruvate levels during the O.G.T.T.

Whether the role of corticoids on the maintenance or activation of the enzymes concerned in pyruvate formation is a direct one or not is unknown, it may be that their effect is secondary mediated through insulin. Insulin is known to effect the de novo synthesis and activation of the enzymes concerned with glycolysis, lipogenesis and the metabolism of glucose via the Hexose mono-phosphate shunt pathway. Adrenalectomy has been shown to markedly lower the fasting insulin levels in comparison; with the adrenal intact controls, whilst corticosterone given as replacement therapy has a tendency to restore fasting insulin levels to normal. Oestradiol benzoate is without effect on the fasting insulin levels.

Therefore the maintenance of fasting insulin levels in the adrenal intact and ADX plus corticosterone treated animals is important in providing adequate, or normal levels of the enzymes involved with glucose metabolism.

Therefore in summary, it is evident that an intact adrenal, or the presence of corticosterone is required for Oestradiol benzoate to exert its effect on glucose metabolism and pyruvate formation during an O.G.T.T. in the ovariectomized rat.

CONCLUSION

CONCLUSION.

During the course of this thesis a discussion has been presented at the end of each section which has attempted to explain and elucidate a mechanism by which the oestrogens, Progesterone and the progestogens exert their effects on glucose metabolism in the ovariectomized rat.

In general, alterations have occurred in glucose tolerance and pyruvate formation after 2 weeks of steroid therapy and these effects were still evident after 4 weeks., of continued therapy.. The insulinogenic properties of steroids whilst evident at 2 weeks often became more pronounced after 4 weeks of continued steroid therapy.

The oestrogens, Oestradiol benzoate, Mestranol and Ethinyl oestradiol when administered to rats in pharmacological doses improve glucose tolerance and elevate the levels of pyruvate during an O.G.T.T., without significant alterations in the insulin response. However a lower dose of Oestradiol benzoate, 5 μg daily is shown to impair glucose tolerance. This latter finding is considered to be due to increased corticosterone levels causing insulin antagonism in the peripheral tissue and activation of gluconeogenic enzymes in the liver. The increased glucose removal with concomitant elevations in pyruvate levels seen with oestrogen treatment suggest that glucose is metabolized at a greater rate in these animals than in the controls.
Since there is no hyperinsulinism in these oestrogen treated animals it is postulated that oestrogens have a direct action on the activation and synthesis of the enzymes involved in glycogen synthesis and the metabolism of glucose via the glycolytic and Hexose mono phosphate shunt pathways.

The liver is regarded as being the main site involved in the metabolism of an oral glucose load since it has been estimated that some 60% of the glucose load is metabolized by this organ.

Another factor also considered as contributing to improved glucose tolerance is the indirect effect of oestrogens, whereby their activation of the rate limiting enzyme in the metabolism of L tryptophan, tryptophan oxygenase increases the levels of the tryptophan metabolite, quinolinic acid, a potent inhibitor of gluconeogenesis.

Studies in the adrenalectomized animal show that the presence of the adrenal gland or in particular corticosterone is required for Oestradiol benzoate or the oestrogenic steroids to exert their full effect on glucose metabolism and the formation of pyruvate during an O.G.T.T.

The adrenalectomized animal receiving Oestradiol benzoate daily for 2 weeks has lower O.G.T.T. glucose levels and an increased thirty minute O.G.T.T. pyruvate level, at the remaining time intervals the pyruvate levels are no different from the adrenalectomized controls.

Oestradiol benzoate administration to adrenal intact animals improves glucose tolerance and elevates the O.G.T.T. pyruvate levels.

Corticosterone given as replacement therapy to adrenalectomized animals is without effect on glucose tolerance, however O.G.T.T. pyruvate levels are elevated in comparison to the adrenalectomized control. Administration of Oestradiol benzoate with corticosterone replacement markedly increases the O.G.T.T. pyruvate levels.

The exact role of the adrenal in explaining these observations is unclear, however its maintenance of a sensitive pancreatic islet is considered to be important.

Studies using Progesterone, the 19 nor testosterone derivatives Norethynodrel and Norethisterone acetate and the 17 alpha hydroxy progesterone derivatives, Chlormadinone acetate and low doses of Megestrol acetate, have shown that these steroids do not effect glucose tolerance significantly.

However 4 weeks of high dose Megestrol acetate administration is seen to improve glucose tolerance.

Ther is marked elevations in O.G.T.T. pyruvate levels for the 19 nor testosterone derivatives and to a lesser extent for Progesterone and the 17 alpha hydroxy progesterone derivatives.

It is considered that elevations in pyruvate levels seen in these animals treated with Progesterone, or steroids from either of these two classes of progestogens is possibly due to two seperate mechanisms The mechanism by which the 19 nor testosterone derivatives influence O.G.T.T. pyruvate levels is attributed to their in vivo conversion to Mestranol, the metabolic effects of which have been discussed earlier. The mechanism by which Progesterone and the 17 alpha hydroxy progesterone derivatives affect O.G.T.T. pyruvate levels is attributed to their insulinogenic properties, for insulin is known to increase the de novo synthesis and activation of the enzymes involved in glycolysis and the Hexose mono phosphate shunt pathways.

Another factor also considered is that progestogens may possibly be converted to other steroid metabolites which possess properties that affect the metabolism of glucose and pancreatic islet cell function.

Studies in ovariectomized rats receiving oestrogens and progestogens in combination, demonstrate lower fasting and O.G.T.T. glucose levels compared with controls; this is particularly noticeable after 4 weeks of steroid administration.

The combined oestrogen progestogen groups demonstrate marked elevations in O.G.T.T. pyruvate levels.

In general, the insulin response during the O.G.T.T. for the combined oestrogen progestogen groups is not significantly different from the controls. However groups receiving the 19 nor testosterone derivatives with an oestrogenic steroid have reduced insulin responses during the O.G.T.T. in comparison with the controls.

These findings for the combined oestrogen progestogen groups of lower O.G.T.T. glucose levels and elevated pyruvate levels, in the absence of hyperinsulinism suggests that oestrogen is the dominant steroid in the combination and that the progestogen may have a synergistic action with the oestrogen component.

The mechanisms by which this synergism may occur is firstly, in the case of the 19 nor testosterone derivatives by virtue of their conversion to Mestranol, they effectively increase the circulating levels of the oestrogenic steroid. The second way in which this synergistic effect may result, is that both the 19 nor testosterone and the 17 alpha hydroxy progesterone derivatives, compete with the oestrogen component for the same hepatic microsomal enzymes involved in their metabolism.

Therefore, the progestogens may indirectly increase the circulating levels of oestrogenic steroids and prolong their effect on carbohydrate metabolism.

In conclusion, it is evident that the dosage and particular steroid is able to influence carbohydrate tolerance in the ovariectomized rat.

In general, the oestrogens improve glucose tolerance, elevate O.G.T.T. pyruvate levels and are without major effect on insulin levels. The adrenal is seen to have an important role in the mechanism involved in oestrogen mediated hyperpyruvataemia.

Progesterone is seen to be without effect on glucose tolerance and to cause slight elevations in O.G.T.T. pyruvate levels and insulin response.

The 19 nor testosterone derivatives were without effect on glucose tolerance and O.G.T.T. insulin response but increases the O.G.T.T. pyruvate levels. The 17 alpha hydroxy progesterone derivatives increase the O.G.T.T. insulin response but are without major effect on glucose tolerance except for high dose levels of Megestrol acetate which is seen to slightly improve glucose tolerance. The O.G.T.T.

pyruvate levels were seen to be increased slightly by these progestogens.

Combined oestrogen progestogen administration improves glucose tolerance, elevates O.G.T.T. pyruvate levels and has little effect or slightly lowers insulin responses during the O.G.T.T.

REFERENCES

Adams, P.W. & Oakley, N.W. (1972). Oral contraceptives and Carbohydrate metabolism: Clinics in Endocrinology and Metabolism, Vol 1 697

Adams, P.W. & Wynn, V. (1972) The effects of a progestogen oral contraceptive Megestrol acetate on carbohydrate and lipid metabolism. J of Obst. and Gynaecology of the British Commonwealth, 79, 744.

Albano, J., Ekins, R.P., Maritz, G. & Turner R.C. (1972). A sensitive and precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta. Endocrinol, 70, 487

Ashmore, J.& Weber, G. (1968). Hormonal control of carbohydrate metabolism in liver. In Carbohydrate Metabolism and its Disorders, Vol 1, ed Dickens, F., Randle, P.J.& Whelan, W.J. Acad press

Bastenie, P.A., Franckson, J.M.,& Conrad, V. (1953). Effect of cortisone on carbohydrate metabolism, measured by glucose assimilation coefficient. J Clin Endocrinol & Metab, 13, 865

Beck, P (1969) Effects of gonadal hormones and contraceptive steroids on glucose and insulin metabolism. In Metabolic Effects of Gonadal Hormones and Contraceptive Steroids ed Salhanick, H.R., Kipnis, D.M. & Vande. Wiele, R.L. 97 New York, Plenum press. page 97

Beck, P. (1970) Comparison of the metabolic effects of Chlormadinone acetate and conventional contraceptive steroids in man. J. of Clinical Endocrinology, 30, 785.

Ben-Dyke, R. (1971) Diurnal variation of oral glucose tolerance in volunteers and laboratory animals. Diabetologia, 7, 156.

Benjamin, F & Casper, D.J. (1966) Alterations in carbohydrate metabolism induced by Progesterone in cases of endometrial carcinoma and hyperplasia. Am. J. Obs. & Gynecol., 104. 448.

Biebuyck, J.F. & Alberti, K.G.M.M. (1971) The effect of anaesthetic agents on carbohydrate metabolism in the rat. Medical Research Society. Communications, Oct.

Braidman, I. P. (1972) PhD Thesis University of London St. Mary's Hospital Medical School.

Braidman, I.P. &Rose, D.P (1971a) Effects of sex hormones on three glucocorticoid inducible enzymes concerned with amino-acid metabolism in rat liver. Endocrinology, 89, 1250.

Buchler, D. & Warren, J.C. (1966) Effects of estrogen on glucose tolerance.
Amer. J. Obst. & Gynecol. 95, 479.

Burke, C.W. (1970) The effects of oral contraceptives on cortisol metabolism.
J. Clin Path. 23, Supplement3, 11

Burns, T.W. et al (1953) Studies on the interdependent effects of stress and the adrenal cortex on carbohydrate metabolism in man.
J. Clin. Invest. 32, 781.

Cahill, G.F., Ashmore, J., Renold, A.E. & Hastings, A.B. (1959) Blood glucose and the liver.
Amer. J. Med. 26, 264.

Costrini, N.V. & Kalkhoff, R.K. (1971) Relative effects of pregnancy estradiol and progesterone on plasma insulin and pancreatic islet insulin secretion.
J. Clin. Invest., 50, 992.

Cramp, D.G. (1967) New automated method for measuring glucose by glucose oxidase.
J . Clin. Path. 20, 910.

Cramp, D.G. (1968) Automated enzymatic fluorimetric method for the determination of pyruvic and lactic acids in blood.
J . Clin. Path. 21, 171.

Danowski, T. S. et al (1968). Glucose tolerance prior to and during therapy with contraceptive steroids. Clin. Pharmacol. and Therapeutics, 9, 223

Daly, J. R., Elstein, M & Murray, J. (1968). Chlormadinone acetate a new departure in oral contraceptives Excerpta. Medica. Foundation. ed by G. A. Christie published for Syntex Pharmaceuticals.

Davidson, M. B. & Holzman, G. B. (1973) Role of growth hormone in the alteration of carbohydrate metabolism in women taking oral contraceptives. J. Clin Endocrinol & Metab 36, 246.

Di Paola, G. et al (1968) Oral contraceptives and carbohydrate metabolism. Amer. J. Obst. & Gynecol, 101, 206.

Di Paola, G., Robin, M & Nicholson, T. (1970). Estrogen therapy and glucose tolerance test. Amer. J. Obst. & Gynecol. 107, 124.

Doar, J. W. H., Wynn, V & Cramp, D. G. (1969) Studies on venous blood pyruvate and lactate levels during oral and intravenous glucose tolerance tests in women receiving oral contraceptives. In, Metabolic effects of Gonadal hormones and Oral contraceptive steroids. Ed by Salhanick, H. A., Kipnis, D. M. & Vande Wiele, R. L. New York. Plenum press. page 178.

Doar, J. W. H. (1969) M. D. Thesis. Alexander Simpson Laboratories for Metabolic Research. St. Mary's Hospital Medical School. London.

Doe, R. L., Zinneman H. H., Funk, E. B. & Ulstrom, R. A. (1960) Significance of the concentration of non-protein bound plasma cortisol in normal subjects. Cushings Syndrome, Pregnancy and during Estrogen therapy. J. Clin. Endocrinol, 20, 1484.

Dorfman, R. (1968) Chlormadinone acetate a new departure in oral contraceptives. Excerpta. Medica Foundation, ed by G. A. Christie. published for Syntex Pharmaceuticals.

Dunn, A., Chenoweth, M & Schaeffer, L. D. (1969) Effect of adrenalectomy on glucose turnover, the Cori cycle and gluconeogenesis from alanine Biochim. Biophys. Acta., 177, 11.

Fenichel, R. L. Purse, R. M. Alburn, H. E. & Edgren, R (1969) Effects of oral contraceptives and their components on glucose tolerance in rats.
Acta. Endocrinol. 62, 438.

Fraenkal-Conrat, H. C. et al (1941) Mechanism of action of Estrogens on insulin content of the pancreas of rat; Proc. Soc., Exper. Biol. & Med. 48, 333.

Franckson, J.R.M. et al. (1953) Observations sur le diabete steroide experimental du rat.
Acta. Endocrinol. 14, 153.

Frantz, A.G. & Rabkin, M.T. (1965) Effects of estrogen and sex difference on secretion of growth hormone.
J. Clin. Endocrinol. 25, 1470

Foglia, V.C.; Shuster, N. & Rodriguez,R.R. (1947) Sex and diabetes.
Endocrinology, 41, 428

Foglia, V.C., Pentos, J.C. & Cardeza, A.F. (1954) Accion de las hormonas sexuales sobre los isoletes y la diabetes de ratas hipofisoprivas con pancreatectomia parcial.
Rev. Soc. Arg. Biol. 30 101.

Friedmann, B., Goodman, E.H. & Weinhouse, S. (1965) Dietary and hormonal effects on gluconeogenesis in the rat.
J. Biol. Chem. 240, 3729.

Gershberg,H., Javier, Z. & Hulse, M. (1964) Glucose tolerance in women receiving an ovulatory suppressant.
Diabetes. 13, 376.

Gershberg, H. et al. (1969). Effects of medroxy progesterone acetate on serum insulin and growth hormone levels in diabetics and potential diabetics.
Obst.. & Gynecol. 33, 383

Gershberg, H., et al (1967) Improvement of glucose tolerance with estrogen treatment in maturity onset diabetics. Diabetes. 16 525.

Gemzell, C.A. (1952) Increase in the formation and the secretion of A.C.T.H. in rats following administration of oestradiol monobenzoate. Acta. Endocrinol. 11, 221.

Gilfrich, H.J. et al (1969) Norethisterone oeanthate, investigation of a long acting parenteral contraceptive. Deutsche Mediziniche Wochenshrift, 94, 2473.

Gilmour, K.E. & McKerns, K.W. (1966) Insulin and estrogen regulation of lipid synthesis in adipose tissue. Biochim. Biophys. Acta. 116, 220

Goberna, R . et al. (1971) Effect of the synthetic progestin chlormadinone acetate on the insulin response to glucose in normal and sub-totally pancreatectomized rats. Endocrinology, 89 , 974.

Glenister, D.W. & Yates, F . E. (1961). Difference in the rate of disappearance of corticosterone-4-^{14}C from plasma of intact rats. Further evidence for the influence of hepatic $\Delta 4$ steroid hydrogenase activity on adrenal cortical function. Endocrinology, 68, 747.

Goldman, J.A., Ovadia, J.L. & Eckerling, B. (1968) Effect of progesterone on glucose tolerance in women. Israel J. of Medical Sciences, 4, 878.

Goldman, J. A. & Ovadia, J. L. (1969) The effect of estrogen on intravenous glucose tolerance in women. Amer. J. Obst. & Gynecol., 103, 172.

Goldman, J. A. et al (1971) Blood glucose and plasma insulin levels with ethynodiol diacetate oral contraceptive. J. Obstet. & Gynaecology of the British Commonwealth, 78, 255.

Gram, T. E. & Gillette (1969) The role of sex hormones in the metabolism of drugs and other foreign compounds by hepatic microsomal enzymes. In Metabolic Effects of Gonadal Hormones and Oral Contraceptive Steroids. Ed by Salhanick, E. A., Kipnis, D. M. & Vande Wiele, R. L. New York, Plenum Press. Page 86.

Green, I. C. & Taylor, K. W. (1972) Effects of pregnancy in the rat on the size and insulin secretory response of the islets of Langerhans. J. of Endocrinol. 54, 317.

Griffiths, M., Marks, H. P. & Young, F. G. (1941) Influence of oestrogens and androgens on glycogen storage in the fasting rat. Nature, 147, 359.

Grosvenor, C. E. & Turner, C. W. (1959) Effect of estrogen upon thyroxine secretion rate in intact female rats. Proc. Soc. Exptl. Biol. & Med. 101, 194.

Hager, D., Georg, R. H., Leitner, J.W. & Beck, P. (1971) Insulin secretion and content in isolated rat pancreatic islets following treatment with gestational hormones. Endocrinology, 91, 977

Hagerman, D. D. (1962) Metabolism of tissues from pregnant diabetic rats in vitro. Endocrinology, 70, 88.

Henning, H. V., Sieffert, I & Seubert, W. (1963) Cortisol induzierter austieg der pyruvatcarboxylase aktivat in der rattenleber. Biochim. Biophys. Acta, 77, 345.

Hilf, R. et al. (1972) Response of uterine glucose-6-phosphate dehydrogenase isoenzymes to estrogens. Endocrinology, 91, 280.

Hori, S. H. & Matsui, S. (1970) Effects of hormones on hepatic glucose-6-phosphate dehydrogenase of rat. J. Histochem. Cytochem, 15, 530.

Ingle, D. J. (1941) Diabetogenic effect of stilboestrol in force fed normal and partially depancreatized rats. Endocrinology, 29, 838.

Ingle, D.J., Sheppard, R., Evans, J .S. & Kuizenga, M. H. (1945) Comparison of adrenal steroid diabetes and pancreatic diabetes in the rat. Endocrinology, 37, 341.

Ingle, D. J., Nezamis, J. E. & Prestrud, M.C. (1947).
The effect of diethylstilboestrol upon alloxan diabetes in the male rat.
Endocrinology, 41, 207.

Ingle, D .J., Beary, D.F. & Purmalis, A. (1953) . Comparison of the effect of 11 β hydroxy progesterone and of 11 β 17 α dihydroxyprogesterone upon the glycosuria of the partially pancreatectomized rat.
Metabolism, 2, 510.

Javier, Z., Gershberg, H. & Hulse, M. (1968) Ovulatory suppressants, estrogens and carbohydrate metabolism.
Metabolism , 17, 443.

Kalkhoff, R.K., Jacobson, M., & Lemper, D. (1970) Progesterone prgnancy and the augmented insulin response.
J. Clin. Endocrinol. 31, 24.

Kalkhoff, R.K. (1972) Effect of oral contraceptive agents and sex steroids on carbohydrate metabolism.
Annual Review of Medicine, 23, 429.

Kekki, M.,& Nikkila, E.H. (1971) Plasma triglyceride turnover during use of oral contraceptives.
Metabolism, 20, 878.

Keller, N., Richards, V. I & Yates, E. (1969) Protein binding and the biological activity of corticosteroids. In vivo induction of hepatic and pancreatic alanine amino transferases by corticosteroids in normal and estrogen treated rats. Endocrinology, 84, 49.

Kipnis, D. M. (1969). Insulin antagonism and diabetes mellitus. In Diabetes ed by J. Östman Excerpta. Medica. Foundation.

Kissebah, A., Harrigan, P. & Wynn, V. (1973) Mechanism of hypertriglyceridaemia associated with contraceptive steroids. Hormones and Metabolic Research (In press)

Kitay, J. I. (1963a) Effects of estradiol on pituitary adrenal function in male and female rats. Endocrinology, 72, 947.

Kitay, J. I. (1963b) Pituitary and adrenal function in the rat after gonadectomy and gonadal hormone replacement. Endocrinology, 73, 253.

Kitay, J. I, Coyne, M. D., Nelson, R. & Newsom, W. (1966) Relation of the testis to adrenal enzyme activity and adrenal corticosterone production in the rat. Endocrinology, 78, 1061

Kizer, J. S., Vargas-Gordon, M., Brendel, K & Bressler, R. (1970) The in vitro inhibition of insulin secretion by diphenyl-hydantoin. J. Clin. Invest. 49, 1942.

Krebs, H. A. (1970) In Pathogenesis of Diabetes Mellitus eds. Cerasi, E & Luft, R., New York Wiley press. page 315.

Laarson-Cohn, U., Tengstrom, B. and Wide, L. (1969) Glucose tolerance and insulin response during daily continuous low dose oral contraceptive treatment. Acta. Endocrinol, 62, 242.

Lewis, J. T., Foglia, V. G. & Rodriguez, R. R. (1950). The effects of steroids on the incidence of diabetes in rats after sub total pancreatectomy. Endocrinology 46, 111.

Malaisse, W. J., Malaisse-Lagae, F., McCraw, & Wright, P. H. (1967) Insulin secretion in vitro by pancreatic tissue from normal, adrenalectomized and cortisol treated rats. Proc. Soc. Exptl. Biol & Med. 124, 924

Matute, M. L. & Kalkhoff, R. K. (1973) Sex steroid influence on hepatic gluconeogenesis and glycogen formation Endocrinology, 92, 76.

Maw, D. S. J. & Wynn, V. (1972) The relation of growth hormone to altered carbohydrate metabolism in women taking oral contraceptives. J. Clin Path. 25, 354.

McKerns, K. W., Coulomb, B. Kaleita, E. & de Renzo, E. C. (1958) Some effects if in vivo administered estrogens on glucose metabolism and adrenal cortical secretion in vitro. Endocrinology 63, 709.

McKerns, K. W. (1961) Direct action of insulin on perfused liver. Recent Progress in Hormone Research 17, 567.

McKerns, K. W. &Clynes, R. (1961) Sex difference in rat adipose tissue. Metabolism 10, 165.

McKerns, K. W. & Bell, P. H. (1960). The mechanism of action of estrogenic hormones on metabolism. Recent Progress in Hormone Research, 60, 130.

McIntyre, I. (1969). The intestinal control of insulin secretion - an historical introduction. Diabetes. ed by J. Ostman. Excerpta. Medica. Foundation.

Meyer, R. K. &Clifton, K.H. (1956) Effect of diethylstilboestrol induced tumorigenesis on the secre tory activity of the rat anterior pituitary gland. Endocrinology, 56, 686.

Paulsen C. et al (1962) Inherent estrogenicity of norethindrone and norethynodrel. Comparsion with other synthetic progestins and progester one. J. Clin. Endocrinol. 22; 1033.

Paulsen. C. (1965) Progestin metabolism; special reference to estrogenic pathways. Metabolism, 14, 213.

Peterson, R. E., Nokes, G., Chen, P. S. & Black, R. L. (1960) Estrogens and adrenal function in man J. Clin. Endocrinol, 20, 495.

Peterson, W. F., Steel, M. W. and Coyne, R. U. (1966) Analysis of the effect of ovulatory suppressants on glucose tolerance. Amer J. Obst. & Gynecol., 95, 484.

Plager, J. E., Shmidt, K. G. and Staubitz, W. J. (1964) Increased unbound cortisol in the plasma of estrogen treated subjects. J. Clin Invest. 43, 1066.

Posner, N. A. et al (1968a) Oral contraceptives and intravenous glucose tolerance. I. Data noted early in treatment. Obstet. & Gynecol. 29, 79.

Posner, N. A. et al (1968b) Oral contraceptives and intravenous glucose tolerance. II Long term effects. Obstet. & Gynecol. 29, 87.

Pi Sunyer, F. & Oster, S (1968). Effect of an ovulatory suppressant on glucose tolerance and insulin secretion. Obstet. & Gynecol., 31, 482

Pyorala, K. Pyorala, T. and Lampinen. V., (1967) Sequential oral contraceptive treatment and intravenous glucose tolerance. Lancet ii, 776.

Ray, P. D., Foster, D. O. & Lardy, H. A. (1964) Mode of actions of glucocoricoids. I. Stimulation of gluconeogenesis independent of synthesis de-novo of enzymes. J. Biol. Chem 239, 3396.

Ray, P. D., Foster, D. O. & Lardy, H. A. (1966). Paths of carbon in gluconeogenesis and lipogenesis. Inhibition by L. tryptophan of hepatic gluconeogenesis at the level of phosphoenol pyruvate formation. J. Biol. Chem. 241, 3904.

Richards, A. H. & Hilf, R. (1972). Influence of pregnancy, Lactation and involution on glucose 6 phosphate dehydrogenase and lactate dehydrogenase isoenzymes in the rat mammary gland. Endocrinology Vol 1, 287

Rock, J., Pincus, G. &Garcia, C. R. (1956) Effects of certain 19 nor-steroids on reproductive processes in animals Science 124, 890.

Rodriguez, R. R. (1950) Prevention of diabetes in forced fed-rats under prolonged diethylstilboestrol administration. Proc. Soc. Exptl. Biol&Med, 73, 317.

Rodriguez, R. R. (1954) Alloxan diabetes in the rat. Recovery following estrogen treatment Endocrinology, 55, 1.

Rose, D. P. (1966) The influence of oestrogens upon tryptophan metabolism in man. Clin. Sci. 31, 265.

Rose, D.P. & Toseland, P.A. (1973) Urinary excretion of quinolinic acid and other trytophan metabolites after deoxypyridoxine or oral contraceptive administration.
Metabolism, 22, 165.

Sandberg, A.A. & Slaunwhite, W.R. (1959) Transcortin a corticosteroid binding protein of plasma. II Levels in various conditions and the effects of estrogens. J . Clin. Invest. 38, 1290.

Sandberg, A.A., Rosenthal, H.G. & Slaunwhite, W.R. (1969) Certain metabolic effects of estrogens. In Metabolic effects of gonadal hormones and oral contraceptive steroids.
Ed by Salhanick, H.A., Kipnis, D.M. &Vande Wiele, R.L.
Page 367.

Schreibman, P.H. (1968) Alterations in carbohydrate and lipid metabolism by a progestin. Diabetes, 17, Supplement 1, 341.

Shrago, E, Lardy, H.A.,Nordlie, R.C. & Foster ,D.O. (1963) Metabolic and hormonal control of phospho-enol-pyruvate carboxykinase and malic enzyme in rat liver.
J. Biol. Chem. , 238, 3188.

Simon, S. et al (1967) Effect of medroxyprogesterone acetate upon stimulated release of growth hormone in man.
J. Clin. Endocrinol., 27, 1633.

Spellacy, W.N. (1969) A review of carbohydrate metabolism and the oral contraceptives.
Amer. J. Obst. & Gynecol., 104, 448.

Spellacy, W.N. et al (1970a) Medroxyprogesterone acetate and carbohydrate metabolism. Fertility and Sterility, 21, 457

Spellacy, W.N. et al (1970b) Glucose, insulin and growth hormone studies in long term users of the oral contraceptives. Amer. J. Obst. & Gynecol., 106, 173.

Spellacy, W.N., Buhi, W.C. & Birk., A.S. (1972a) The effectof estrogens on carbohydrate metabolism. Glucose, insulin and growth studies in 171 women ingesting premarin, mestranol and ethinyl oestradiol. Amer. J. Obst. & Gynecol., 114, 378.

Spellacy, W.N., Buhi, W.C. & Birk, S.A. (1972b) The effect of the progestogen ethynodiol diacetate on glucose, insulin and growth hormone after 6 months treatment. Acta. Endocrinol. 70, 373.

Taalat, M et al (1965a) Effect of sex hormones on the carbohydrate metabolism in normal and diabetic women. Archiv. Int.Pharmacodyn. 154, 235.

Taalat, M et al (1965b) Effects of oestradiol dipropionate on the glucose tolerance and insulin sensitivity curves in normal and ovariectomized female rabbits. Archiv. Int. Pharmacodyn. 153, 290.

Taylor, M.B. & Kass, M.B. (1968) Effect of oral contraceptives on glucose metabolism. Amer. J. Obst. & Gynecol., 102, 1035.

Thompson, R.G., Rodriguez, A., Kowarski, A &Blizzard, R.M. (1972) Growth hormone. Metabolic clearance rates, integrated concentrations and production rates in normal adults and the effect of prednisone. J. Clin. Invest., 51, 3193.

Veneziale, C. M. , Walter, P., Kneer, N. &Lardy, H. A. (1967) Influence of L. tryptophan and its metabolites on gluconeogenesis in the isolated perfused liver Biochemistry 6: 2129.

Vermeulen, A., Daniels, R &Thiery, M. (1970) Effects of oral contraceptives on carbohydrate metabolism Diabetologia 6, 519.

Waalas O. (1952a) Effect of estrogens on the glycogen content of the rat liver. Acta. Endocrinol, 10, 193.

Waalas, O., Waalas , E. &Loken, F. (1952b) Effect of oestradiol monobenzoate on the metabolism of rat uterine muscle. Acta Endocrinol 10, 201

Waine, H., Frieden, E. H. Caplan H. I . & Cole, T. (1963) Metabolic effects of enovid in rheumatoid patients. Arthritis and Rheumatism 6, 796

Wallace, E. Z &Carter, A. C. (1960) Studies on the mechanism of plasma 17 hydroxycorticosteroids elevations induced in man by estrogens. J. Clin Invest, 39, 601.

Weber, G., Lea. M. A., Fisher, E. A. & Stamm, N. B. (1966) Regulatory pattern of liver carbohydrate metabolizing enzymes. Insulin as inducer of key glycolytic enzymes. Enzymol biol. Clin. 7, 11.

Weber, G., Lea, M. A., Herd-Convery, H. J. and Stamm, N. B. (1967) Regulation of gluconeogenèsis and glycolysis; Studies of mechanisms controlling enzyme activity. Adv Enzyme Regulat. 5, 261.

West, K. M. (1957) Comparison of the hyperglycaemic effects of glucocorticoids in human beings. Diabetes, 6, 168.

Winegrad, A. I. Shaw, W. N., Lukens, F. D. S. & Stadie, W. D. (1959) Effects of prolactin in vitro on fatty acid synthesis in rat adipose tissue. J. Biol. Chem. 234. 3111.

Wynn, V&Doar, J. W. H. (1966) Some effects of oral contraceptives on carbohydrate metabolism. Lancet ii 615.

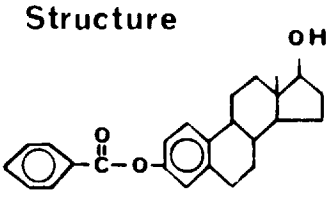
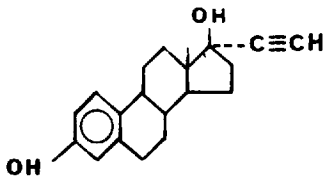
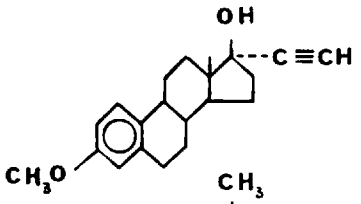
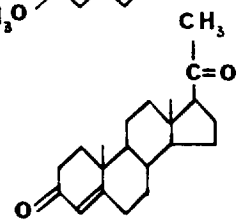
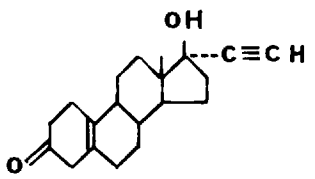
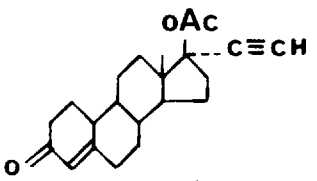
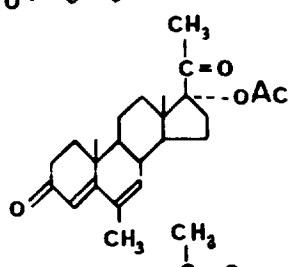
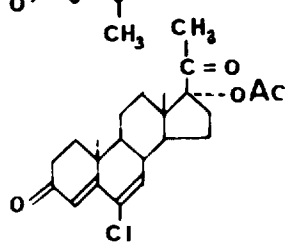
Wynn, V.& Doar, J. W. H. (1969) Some effects of oral contraceptives on carbohydrate metabolism Lancet ii 761

Yen, S. S. C. & Vela, P. (1968) Effects of contraceptive steroids on carbohydrate metabolism. J. Clin Endocrinol, 28, 1564

Yen, S. S. C. & Vela, P. (1969) Carbohydrate metabolism and long term use of oral contraceptives. J. Reproductive Medicine. 3, 25.

APPENDIX I

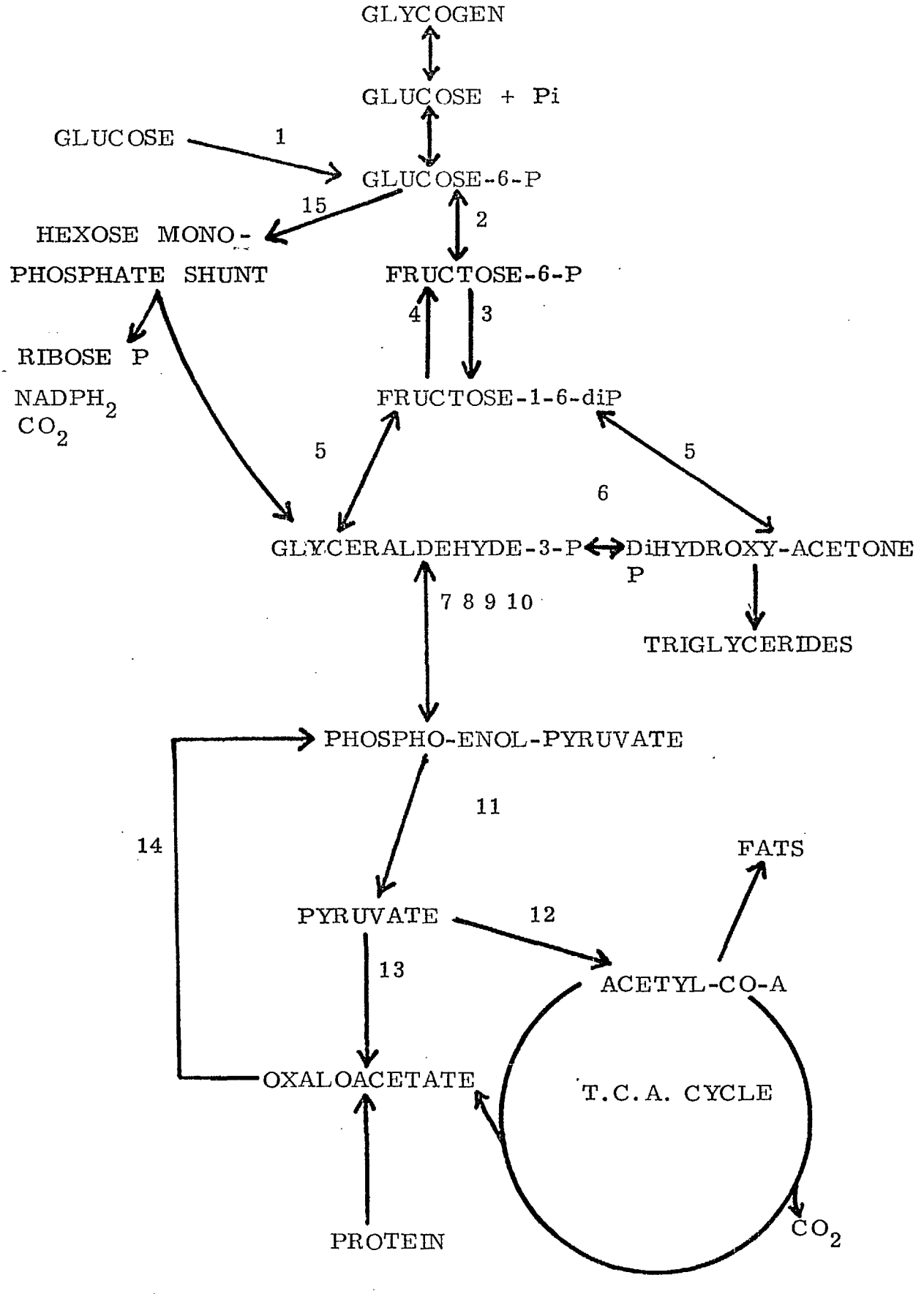
Gonadal Steroids Investigated In This Study

Structure	Trivial Name
	Oestradiol Benzoate
	Ethinyl-Oestradiol
	Mestranol
	Progesterone
	Norethynodrel
	Norethindrone Acetate (Norethisterone Acetate)
	Megestrol Acetate
	Chlormadinone Acetate

STEROID NOMENCLATURE.

Trivial name.	Systematic name.
Oestradiol-benzoate.	1, 3, 5(10) Estratriene-3, 17β diol-3, benzoate.
Ethinyl-oestradiol.	17α Ethinyl 1, 3, 5(10) estratrien-3, 17β diol.
Mestranol.	17α Ethinyl 1, 3, 5(10) estratrien-3, 17β diol-3 methyl ether.
Progesterone.	4- Pregnene- 3, 20 dione.
Norethynodrel.	17α Ethinyl-17β hydroxy-5(10)-estrene-3-one.
Norethisterone acetate.	17α Ethinyl 17β acetoxy estrene-3-one.
Megestrol acetate.	17α Acetoxy-6-methyl pregn-4, 6 diene 3, 20-dione.
Chloramadinone acetate.	17α Acetoxy-6-chlor-pregn-4, 6 diene 3, 20-dione.
Corticosterone.	11β 21 Dihydroxy-4-pregnene-3, 20 dione.

APPENDIX II



(Figures on pathways refer to enzymes, the identity of which may be found on the following page.)

ENZYME NOMENCLATURE

1. Hexokinase	EC 2.7.1.1
2. Glucosephosphate isomerase	EC 5.3.1.9
3. Phosphofructo kinase	EC 2.7.1.11
4. Fructose 1 6 diphosphatase	EC 3.1.3.11
5. Fructosediphosphate aldolase	EC 4.1.2.13
6. Triosephosphate isomerase	EC 5.3.1.1
7. Glyceraldehydephosphate dehydrogenase	EC 1.2.1.12
8. Phosphoglecerate kinase	EC 2.7.2.3
9. Phosphoglycero mutase	EC 2.7.5.3
10. Phosphopyruvate hydratase	EC 4.2.1.11
11. Pyruvate kinase	EC 2.7.1.40
12. Pyruvate dehydrogenase	EC 1.2.2.2
13. Pyruvate carboxylase	EC 6.4.1.1
14. Phospho enol pyruvate carboxykinase	EC 4.1.1.32
15. Glucose 6 phosphate dehydrogenase	EC 1.1.1.49