EFFECT OF CHLOROQUINE ON THE INDUCTION OF RAT UTERINE PEROXIDASE BY AN ORAL CONTRACEPTIVE

BY

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DECLARATION

THE EXPERIMENTAL WORK DESCRIBED IN THIS THESIS WAS CARRIED OUT BY ME AT THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF GHANA, UNDER THE SUPERVISION OF PROFESSOR G.S. ASANTE.

I CERTIFY THAT THIS WORK HAS NOT PREVIOUSLY BEEN ACCEPTED FOR ANY DEGREE, NOR IS IT BEING CONCURRENTLY SUBMITTED IN CANDIDATURE FOR ANY OTHER DEGREE.

SIGNED: .....

CANDIDATE

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V I T A

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ABSTRACT

Uterine peroxidase has been induced in immature female rats with estradiol and anovlar 21. Anovlar 21 is a steroid contraceptive containing 4 mg. norethisterone acetate (a progestin), and 0.05 mg. ethinyl estradiol (an estrogen) per tablet. Maximum enzyme activity was found to occur 24 hours after oral administration of the contraceptive. The increased peroxidase activity caused by the combined steroids was inhibited by the administration of cycloheximide and puromycin (inhibitors of protein biosynthesis). Peroxidase is thus synthesised de novo on the influence of the steroids.

Chloroquine diphosphate has also been found to increase the activity of peroxidase, with the maximum activity occurring 18 hours after the drug has been injected intramuscularly. Cycloheximide has been found to prevent this stimulatory effect of chloroquine. Chloroquine was effective in increasing peroxidase activity in vivo over a dose range of 0-20 mg/kg. body weight. It however, did not affect peroxidase activity in vitro.

Chloroquine potentiated uterine peroxidase induction by anovlar 21. Chloroquine and/or anovlar 21 prevented the production of litter by adult female rats.

INTRODUCTION

The topic for this thesis is part of a major research project aimed at finding the possible interaction between steroid contraceptives and chloroquine in target tissues (Asante 1975). Steroid contraceptives are commonly called oral contraceptives or "the pill". This investigation is being made on the uterus which is the target tissue for the steroid contraceptives. The main object of the research is to find information which will promote understanding in the use of steroid contraceptives as birth control or family planning practice in Africa in general and Ghana in particular, where malaria is endemic and therefore chloroquine is extensively used for both prophylactic and therapeutic purposes.

Malaria in man is characterized by successive fevers, chills and sweat, nausea, anaemia and splenic enlargement. It is caused by the presence in the red blood cells of 4 closely related species of the protozoan Plasmodium. These are P. vivax, P. malariae, P. ovale and P. falciparum.

Since malaria is endemic in Ghana there is little benefit from radical cure. This is because the life cycle of the plasmodium is represented by a number of stages which vary in the degree of their susceptibility to chemotherapeutic treatment.

Primaquine which can act on the exo-erythrocytic stage as well as on the gametocyte is found to be unsuitable because of the multiple stages of the parasite at any time. Primaquine also causes hoemolytic anaemia in about 20% of Ghanaians. These are the people who have G6PD deficiency (Faulkner et al, 1968)

The recommended drugs for prophylactic supression of malaria are: Quinine, Chloroquine, Pyrimethamine, Daraprim, Chloroguanide, and Dapsone plus Pyrimethamine.

Effective doses of quinine may cause cinchonism in a large percentage of individuals. Instead it is usual to employ chloroquine in a dose of 300 mg base per week in adults, 150 mg in children and 75 mg in infants. Toxic effects are usually inconsequential. Apparently, confirmed cases of resistance have been encountered in areas outside, West Africa. The only necessary precaution required is to increase the dose to 600 mg weekly when the situation demands (WHO Technical Report 375).

Apparently chloroguanide (Paludrine) and pyremathamine (Daraprim) are less effective than chloroquine (Hendrickse, 1967). In fact reports of resistance to pyrimethamine and chloroguanide came from West Africa only a few years after the drugs were introduced.

Pyrimethamine and Dapsone - 125 mg : 100 mg - can be used for prophylactic purposes under the trade name of Maloprim. The combination may delay the onset of resistance and may be useful against parasites that are resistant against pyrimethamine alone. Dapsone in

large quantities however, causes haemolysis in individuals with G6PD deficiency. Rasbridge et. al., (1973) found that Glutathione (GSH) levels fell with dapsone treatment with a greater fall in G6PD deficient patients. In addition dapsone accelerated red blood cell ageing in normals while older cells were destroyed in G6PD deficient patients. There is a question as to the role of dapsone in depressing the formation of granulocytes (Ognibene, 1970).

For the clinical supression of malaria a quick acting drug needs to be used. These are quinine and chloroquine. In the supression of uncomplicated malaria, quinine is reserved for cases that are resistant to chloroquine. Chloroquine is therefore the drug that is most commonly used for the supression of falciparum malaria - 600 mg of the base followed by 300 mg six hours later the same day and once a day on two successive days. Pruritus is a common complaint of persons receiving therapeutic doses of chloroquine but this can be relieved by giving corticosteroids, [illegible]. Nieto-Caicedo (1956) has attributed the usefulness of chloroquine to its high potency against every species of Plasmodium without resistant strains being produced. This high potency permits a short treatment. There is also a high tissue fixation and slow excretory rate and degradation. Thus the case for the use of chloroquine as an anti-malarial agent is established.

There is then the need to have an insight into the importance of oral contraceptives in family planning practice. The Planned Parenthood

Association of Ghana (PPAG) was established in 1967. The founders of PPAG included a number of doctors who felt impelled to do something to alleviate the sufferings and high mortality rate of mothers in childbirth. They saw family planning as a means whereby mothers could be assisted to control the number of their children by spacing their birth and also to help couples decide on the number of children they wished to have. While this might lead to a reduction in the rate of population growth, it also aimed at improving the health and welfare of married couples and their children.

The pioneer of the National Family Planning Programme was Miss Edith Gates of the Pathfinder Fund, who visited the country in 1956 to explore the possibility of introducing the service. The Christian Council also came in in 1961 and this eventually led to the establishment of the PPAG.

It has been recognized that Africa has now reached a stage in her demographic evolution which is marked by an upward growth of its population and it is expected that a sharp rise may occur. The social and economic implications are of far reaching significance for our development programmes.

With the first PPAG clinic opened in the country in 1968, there had been 192 of the clinics by 1977 with 429,500 acceptors of the various methods. About 54% of these acceptors have been on the pill (Kwafo, 1977). It is gradually becoming a popular method of contraception.

The pill acts in such a delicate way that any departure from its recommended pattern of administration can lead to its becoming ineffective. As such it is necessary to find also the effect any commonly used drug will have on its action. A drug that is found to either increase or decrease the effectiveness of the pill will make an adjustment in its administration necessary. A reduction of the dose can lead to a reduction in the side effects of the pill which include weight gain from water retention, breakthrough bleeding, aggravation of an already existing cancer of the breast or cervix, and the development of fibroids (Kistner, 1969). An increase in the dose will reduce the risk of its being ineffective. Chloroquine as stated above, is a drug that is widely used in Ghana to prevent and treat malaria cases. It has recently been used as a local anaesthetic in surgery (Takyi, et al., 1972) in addition to the treatment of other diseases apart from malaria.

Chloroquine has been found to have many other side effects on the normal physiological properties of the individual. It is known to affect normal uterine physiology which is the same phenomenon by which the oral contraceptive works, and this is discussed later.

Since the pill is produced in parts of the world where the use of chloroquine is not so widespread, it cannot be assumed that a possible effect chloroquine would have on its action was taken into consideration. These considerations have therefore motivated this investigation.

There are a number of enzymes in the uterus whose activities are affected by the steroid contraceptive. Amongst these enzymes is the peroxidase which is suspected to be involved in effecting the contraceptive action of the pill. Thus the effect of chloroquine and an oral contraceptive, (Anovlar) on the activity of this enzyme has been chosen for the present study. Anovlar 21 was used for the study because it has been a popular oral contraceptive in this country.

LITERATURE REVIEW

THE UTERUS - Its Morphology

The uterus is a part of the female reproductive system which is mainly responsible for the implantation of the foetus with its subsequent development into a matured embryo before parturition.

As described by Bradbury (1973), it is a pear shaped organ with four parts, namely: the rounded upper end called the fundus, the isthmus, the cervix and the portio vaginalis which protrudes into the vagina.

The uterine wall has three cell layers: the outermost connective tissue covering, the myometrium which is a thick muscular lining with blood vessels, and the endometrium which is a mucous layer. The body of the uterus has both circular and longitudinal muscles, while the cervix is made up of circular muscles with dense collagen and elastic fibres which give it a firm consistency. The endometrium is lined by columnar epithelium with ciliated and secretory cells. The epithelium is more stratified towards the cervix, with thicker mucosa. The secretory cells secrete mucin. The superficial part of the epithelium is supplied blood by spiral arteries from the myometrium while the deeper region which does not change during the menstrual cycle has a separate blood supply.

In the uterus are a group of cells which are also important as far as uterine physiology is concerned. These are the uterine eosi-

nophils. There has been a review of these cells by Tchernitchin (1973). The eosinophils are located deep in the stroma of the mucosa or in connective tissue in the muscular layers. They have multilobed or ring shaped nuclei with aggregates of chromatin. The cytoplasm has a few Golgi bodies, lysosomes, microsomes and mitochondria. They have pseudopodium - like elongations of lower electron density.

Hyperestrogenic animals have more eosinophils, and these are found close to the plasma membrane. The area of juxtaposition is more in hyperestrogenic animals. Pinocytic vesicles are found close to these. The eosinophils do not exist in the uterus of the immature female animal, and the number of the eosinophils present is a function of the estrogen level of the animal (Tchernitchin. et. al., 1973). The eosinophils are diminished by the presence of progesterone. Eosinophilia producing factors such as histamine liberators and intra uterine devices enhance eosinophil levels (Tchernitchin, 1973). The presence of pseudopodia on eosinophils suggests their motility in the uterus. These eosinophils are attracted by estrogens to the uterus through a mechanism which is not quite well known.

The eosinophils act as phagocytes and are attracted to antigen-antibody sites (Ross et. al., 1966). Enzymes contained in eosinophils include cathepsin, acid and alkaline phosphatase and peroxidase. The blood is the immediate source of uterine tissue eosinophil.

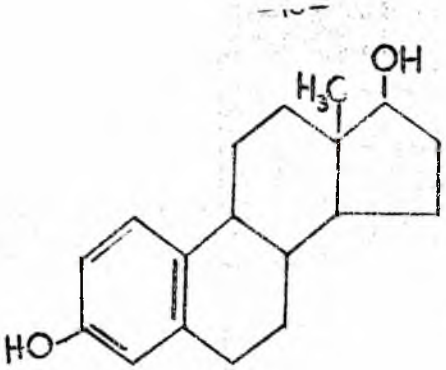
Disruption of the eosinophil occurs in estrus with the spilling of its cytoplasmic contents, and this may be responsible for some of the early effects of estrogen which include water imbibition and vascular permeability of the uterus (Tchernitchin, 1973).

UTERINE PHYSIOLOGY

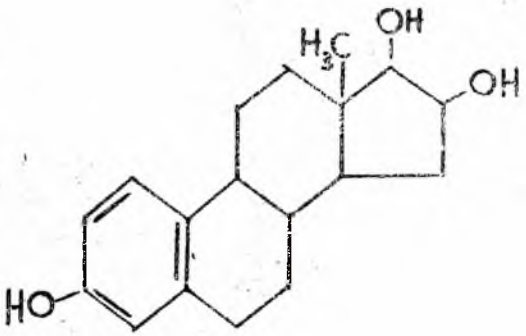
The major physiological activity that takes place in the uterus and its associated structures - the ovaries - is the production of ova and a sequence of events that ensure the implantation and development of a fertilized ovum. These processes are initiated by a group of steroid hormones (estrogens), produced by the gonads and adrenal cortex.

The major estrogen produced by the adrenal gland is estradiol (estra - 1, 3, 5 - triene - 3, 17 β diol). Structurally and functionally related to this estrogen are estrone and estriol. There is also a synthetic estrogen named diethylstilbestrol which is functionally related to estrodiol (fig. 1). Estradiol which is the most potent, is about 8 - 10 times more potent than estrone and 100 times that of estriol.

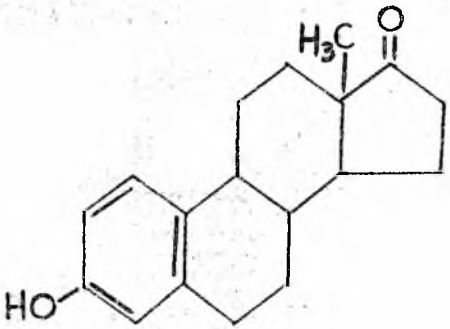
The estrogens on being secreted, are transported through the blood to the hypothalamus where they cause the production of a follicle stimulating hormone releasing factor (FSHRF). This factor acts on the anterior pituitary gland, causing the production of follicle stimulating hormone (FSH), which is transported to the ovaries to initiate the growth and maturation of the Graafian Follicles. The ovaries will then produce a luteotropic hormone which causes the



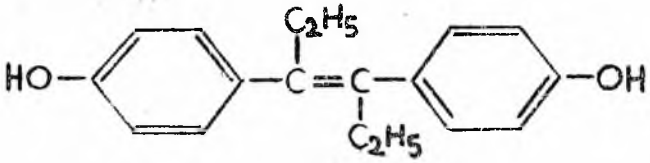
ESTRADIOL



ESTRIOL



ESTRONE



DIETHYLSTILBESTROL (SYNTHETIC)

FIG. 1: STRUCTURES OF SOME ESTROGENS

hypothalamus to produce a luteinizing hormone releasing factor. This factor will cause the pituitary gland to release the luteinizing hormone (LH). The LH, on reaching the ovaries causes the production of progesterone which is needed for the initiation and development of pregnancy. The LH also causes further estrogen production by the ovaries and finally the rupture of the ovary to release the matured ovum. Large amounts of estrogen will stop the production of FSH, therefore, the extra estrogen produced by the ovary will stop the FSH production; this is a possible mechanism for ensuring that only one ovum develops at a time (Kistner, 1969). The increasing levels of progesterone will, after ovulation, inhibit FSH and LH production and thus cut down the production of both estrogen and progesterone, both of which are needed to maintain the uterus for implantation and the further development of the fertilized ovum (Fig. 2). This condition will therefore start a degeneration of the uterine endometrial lining, the growth of which had been initiated by the initial estrogen production. Thus there is the menstrual flow. If there is fertilization however, further estrogen and progesterone production is taken over by placental stimulation (Roberts, 1973).

ESTROGEN UPTAKE BY THE UTERUS

The mode of uptake of the estrogens is another problem that has engaged the attention of various workers for some time.

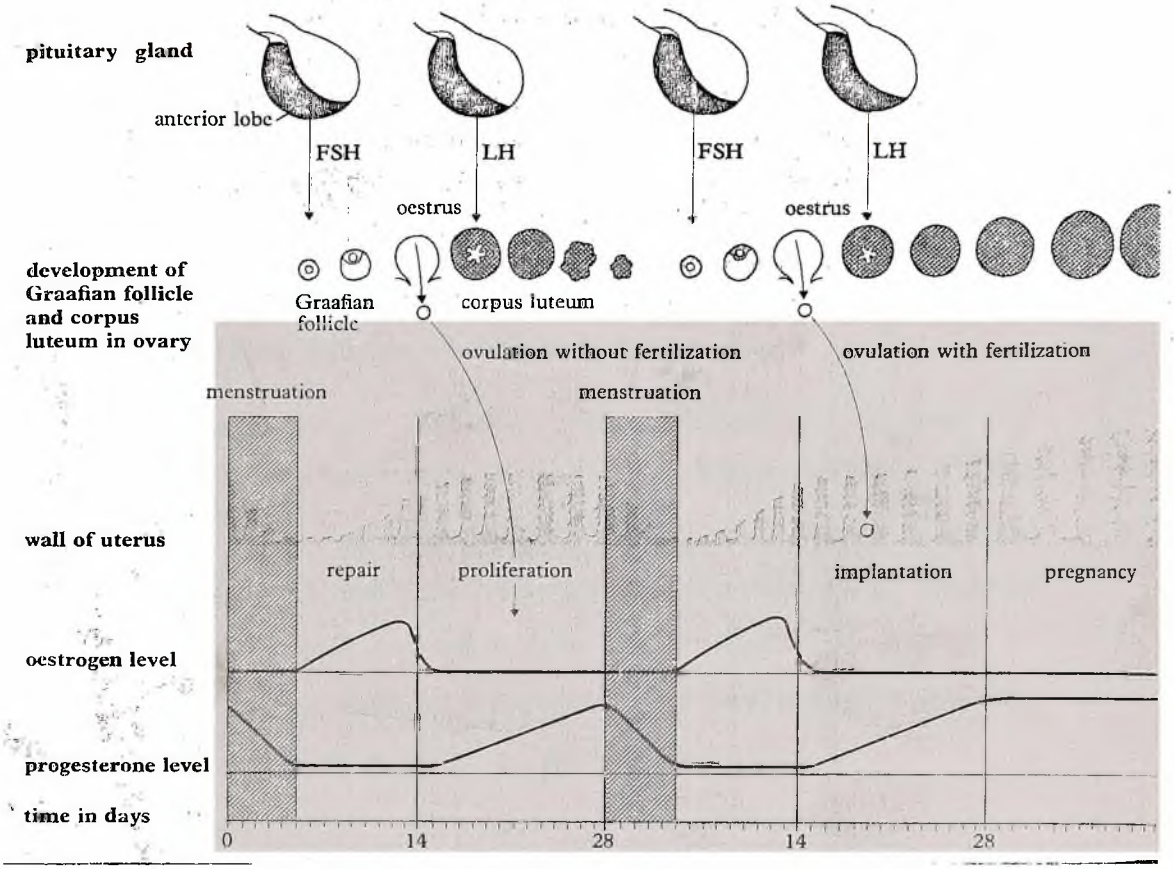


Fig. 2 : THE MENSTRUAL CYCLE. (Roberts, 1973.)

Spaziani (1975) found that estradiol is rapidly taken up and retained by the uterus, vagina and the pituitary gland without any conversion. Other tissues which take it up do not retain it. This is further supported by the work of Tchernitchin et. al. (1973), who found that between 5 and 20 minutes after estradiol injection the estrogen was found in most uterine cell nuclei, extracellular space and in eosinaphils of matured rats.

Jensen et. al. (1962) similarly found that estradiol was present in the uterus as such, while it was bound to protein in the liver six hours after estradiol injection. They further found that the uterus and vagina have a long period of retention and incorporation. The uterus does not seem to have the ability to oxidise estradiol to estrone and this suggests the possibility of estradiol having its effect without its being metabolised.

Gorski et al, (1968) found that at both high and low dose, 55% of injected estradiol was present in the nuclear fraction of uterine cells with 30% in the cytosol. The rest was in the microsomal and mitochondrial fractions.

Estradiol uptake by uterine tissue has a low activation energy. The uptake can take place at 20^{o}C and it is not saturatable even under hyperestrogenic conditions. However, estrogen retention in the uterus can be saturated (Jensen et al., 1968). The uterus was

found to have marked retention for estradiol. This retained estradiol will stay for over 6 hours after estradiol taken up by other tissues are depleted (Noteboom et. al., 1965). This latter view lends support to the possibility of some binding. Later work by Lyttle, et al. (1972) showed that the effect of estrogen treatment on a rat could remain for 24 hours.

The possibility of estradiol binding to ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) was ruled out by Noteboon et al., (1965) because ribonucleases do not release bound estradiol. Actinomycin D does not affect estradiol uptake either.

Work by Gorski et al. (1968) concluded that there was a specific binding protein. It is present in limited quantities and appears to have a strong affinity for biologically active estrogens. The cytosol has a similar protein receptor. Estrogens are therefore thought to travel through the blood stream with serum proteins to the uterus. It forms a 9.5S receptor - estrogen complex in the cytoplasm and goes to the nucleus where it binds to a 5S protein. The estrogen - protein complex (estroprotein) formed, is an activated form of the estrogen (Szego, 1953).

Jensen et al. (1968) found a 9.5S factor that was involved in the uptake of estrogen with the final formation of a 5S receptor complex in the nucleus. The transfer of the 9.5S to 5S complex is done readily at 37^{o}C but slowly at 2^{o}C. The process is therefore

energy dependent and may be enzymatic. The final fate of the 9.5S complex is not certain but there is the possibility of its either being destroyed or broken down to the 5S complex.

Treating chromatin with deoxyribonuclease (DNAse) and extraction with potassium chloride produced estrogen bound to a macromolecule of 5S sedimentation constant on a sucrose density gradient (Gorski et. al. 1968).

Spaziani (1975) later found that there was an initial binding of estradiol to cytoplasmic proteins, the most significant of which is an oligomer which sediments at 8S on a sucrose gradient. The binding is spontaneous and the bound estradiol resides in the nucleus

Shyamala et al., (1969) also found that on administration, estrogen moves into the cytoplasm and binds to a 9S protein. This complex moves into the nucleus, but binding in the nucleus is not specific. They had no evidence to conclude whether the 9S receptor entered the nucleus or not.

Tchernitchin (1972) found two binding systems in the uterus for estrogens: The first is a cytoplasmic receptor in the 105,000 g supernatant (8S - 5S) receptor system; and the second is the eosinophil. The eosinophil receptors have a high affinity, great specificity and a limited capacity for compounds with estrogenic activity. The 8S - 5S system is responsible for the genomic response

which leads to RNA and protein synthesis and uterine growth. Actinomycin D blocks this effect. Estradiol is the more active estrogen for this system. The eosinophil system takes care of the early responses such as water imbibition and histamine release as well as estrogen priming on which actinomycin D has no effect. Estrogen bound to eosinophil cannot be extracted with water but estrogen in other tissues are affected by water extraction.

Work by Gorski et. al. (1968) showed that the uptake of estradiol 17B is competitively inhibited by diethylstilbestrol but this uptake is not affected by estradiol 17 α.

Shyamala et. al. (1967) found that when whole uteri were incubated at 0°C there was an increased cytosol but little nuclear binding. Incubation at 37°C resulted in increased nuclear binding. Incubation at 0°C and then at 37°C resulted in increased estrogen in the nuclei and decreasing amounts in the cytosol. The 9.5S receptor in the cytosol was found to have disappeared when the uteri were incubated at 37°C. Though no explanation was offered for this observed phenomenon, it would be inferred from this observation that the temperature-dependent binding may be an adaptation for ensuring that the nucleus has a preference for the uptake of estrogen at the normal body temperature. It is not however, certain what adaptive feature this view of increased cytosol binding at low temperatures has.

With the establishment of the presence of uterine protein-estrogen-receptors, the next problem that engaged various workers' attention was the nature of the binding protein and more specifically the nature of the binding site. King (1974) reported that cells that are capable of responding to a particular steroid possess a carefully tailored protein that will recognize and bind that steroid, and which can discern very subtle changes in the structure of the steroid. These proteins are the receptor proteins. Jensen et al (1967) showed that the uptake of estradiol by rat uteri is destroyed by treating the tissue with sulfhydryl (SH) blocking agents such as N-ethyl-maleimide (NEM) and p-hydroxymercuri-benzoate (PHMB). Estradiol that was previously incorporated was released. This showed that SH groups of the protein receptor are essential to its ability to associate with estradiol.

Human endometrium has also been found to have proteins with SH groups that have a high affinity and specificity for estrogens. Thus uterine estrogen uptake occurs through a protein receptor with a binding site having SH groups which interract with the estrogens.

Work has been done on the fate of progesterone but this has been relatively recent. McGuire et al (1971) found a limited number of receptor sites in rat uteri for the binding of progestational hormones. Binding could occur in the cold and is therefore not energy dependent. It does not depend on cellular organization

either since binding has been demonstrated in tissue homogenates. Non-progestogens do not competitively inhibit this binding. Feil et al (1972) made similar observations in rats, mice, guinea pigs and rabbits. The receptor according to Milgrom et al. (1973) is very stable even in the absence of progesterone, and it has a half life of five days.

The progesterone binding sites have been found to be low in prepubertal rats but high in post-pubertal ones (Ladeinde et al. 1974). The human myometrial progesterone receptor is under a dual steroidal influence: Whereas estrogen increases the progesterone binding capacity, progesterone reduces this capacity. Nevertheless the plasma estradiol level does not affect the concentration of the myometrial progesterone binding sites. It is therefore the cytosol estradiol level that increases the binding capacity by increasing the concentration of the sites. The binding capacity has been found to be inversely correlated to both the plasma and cytosolic progesterone concentrations. The highest binding capacity of the progesterone sites was observed to occur during the proliferative phase of the menstrual cycle (Kontula 1975 & Janne et al. 1975).

The progesterone binding sites are possibly activated by estradiol (Ladeinde, 1974). Feil (1972) similarly found that estrogen treatment enhanced rat uterine tissues' responsiveness to

progestin. This observation suggests that estrogen regulates the uptake of progesterone by the uterus.

Intra-uterine copper produces an inhibition in the uptake of progesterone though it increases estrogen uptake in four weeks. Copper may therefore have an effect on these receptors which have been found to be specific for progestins. (Ladeinde et al, 1974). It was found however that estradiol reverses this effect of copper, and this further supports the work of Feil et al (1972).

SH groups have been found to affect the integrity of the receptor. NEM reduced the binding capacity (Feil et al, 1972). These workers further supported the protein nature of the receptor with the finding that pronase destroyed progesterone uptake while RNAse and DNAse had no effect.

The protein receptor has been found by Milgrom et al (1973) to be under dual control. There is a positive effect by estrogen which requires RNA and protein synthesis, which occurred within 6-24 hours. The negative control is that by progesterone which caused an inactivation of the sites. Nuclear receptors are inactivated faster than cytosol ones.

Progesterone metabolism takes place only after its binding (McGuire et al, 1971). It is this bound progesterone-receptor complex that moves into the nucleus to effect transcription after

which the complex is degraded(Milgom et al., 1973).

The effect of estrogens on uterine physiology at the molecular level has been a popular topic amongst many investigators for the past decade. Though initially there were differences of opinion as to whether protein or RNA synthesis preceded the other as an early estradiol effect on the uterus, the general view as at present seems to support an early RNA synthesis. This is then followed by the synthesis of proteins that are responsible for the macroscopic estrogen effects.

Hamilton et al (1965 & 1968) reported of the stimulation of nuclear ribosomal-RNA (r-RNA) synthesis by the uterus on estrogen administration. RNA polymerase activity is then stimulated next. Hamilton (1968) found that on estrogen stimulation, there was a binding of the hormone to chromatin in the nucleus. This binding caused r-RNA synthesis followed by an acceleration of ribosomal precursor synthesis. This was transported to the cytoplasm with messenger - RNA (m-RNA) attached. New polyribosomes accumulated in the cytoplasm and these have different amino acid incorporation properties than the older ones.

Trachewsky et al (1967) reported of an enhanced in vitro synthesis of guanine-rich and adenine-rich RNA which are poor in uracil and cytosine.

Billing et al., (1968) also found that the early effects of estradiol include the synthesis of all RNA fractions particularly r-RNA. This view is supported by Knowler et al (1971). They in addition found that the RNAs are synthesised at different rates with the synthesis of nuclear RNA occurring first.

Later work by DeAngelo et al. (1970), Barry et al (1971), Luck et al (1972) and Muuns et al (1971) all support the earlier observations that RNA synthesis preceded any other estrogen effects in the uterus at the molecular level. Glasser et al (1972) reported an increased RNA polymerase activity 30 minutes after an estradiol injection. Trachewsky et al (1967) concluded from his studies that estradiol may exert its influence at the transcription level.

Raynaud-Jammet et al (1971) studying the effect of ionic concentration on RNA synthesis induced by in vivo estrogen administration concluded that estradiol promotes the activity of a factor which activates nucleolar polymerase. The polymerase does not function in high ionic concentration.

Means et al (1966) have reported that a small number of specific RNA molecules are synthesised within a few minutes after estrogen administration and these molecules support the synthesis of specific proteins in early estrogen action. Within the first 30 minutes of estrogen administration there is a depression of protein synthesis but there is an increase after 2 hours, during which time RNA synthesis

falls. DeAngelo et al (1970) similarly reported of a lag phase in protein synthesis 40 minutes after estrogen administration, with a rise in one hour.

Noteboom et al (1963) had earlier detected an increase in rat uterine protein synthesis 2-4 hours after estrogen administration and this was thought to have a part to play in the early estrogen action on the uterus. The initial protein synthesised is of specific type but this is followed by an overall protein synthesis.

Barnea et al (1970) however found that the increased protein synthesis which occurs after 40 minutes declines by 4 hours. This view is supported by Spaziani (1975) who found that protein synthesis increases after one hour, lasting till three hours.

Hamilton (1964) had a slightly different view which says that there is an initial protein synthesis which initiates RNA synthesis. This sets the stage for the more extensive protein synthesis which is a characteristic uterine response to estrogen stimulation.

Estrogen treated uterus becomes increasingly permeable to water and electrolytes. Water uptake occurs within four hours and its solid content increases within 20 hours after estradiol injection (Szego et al, 1953). Estradiol will also stimulate sugar transport into the uterus (Spaziani, 1975). These are long term estrogen effects on the uterus and these have been found to be antagonised

by progesterone (Spaziani, 1975 and Szego, 1953). According to Szego estrogen will cause a general proliferation of the uterus resulting from protein synthesis. Progesterone opposes this effect, but in appropriate dose ratio the two will potentiate each other in uterine growth. He further found that uterine growth affected both endometrium and myometrium. Miller (1966) reports of antagonistic effects of estrogen and progesterone on adrenergic receptors of the uterus.

Edelman (1975) has summarized the general sequence of action of steriod hormones on their target tissues: The hormone is transported by the blood to the target tissue into which it penetrates. There is a stereospecific binding to high affinity protein receptors followed by a temperature sensitive activation of the steriod-receptor complex. There is then the attachment of the active complex to chromatin which causes an induction of RNA and protein synthesis. This will eventually result in the physiological expression of the induced protein (Fig. 3).

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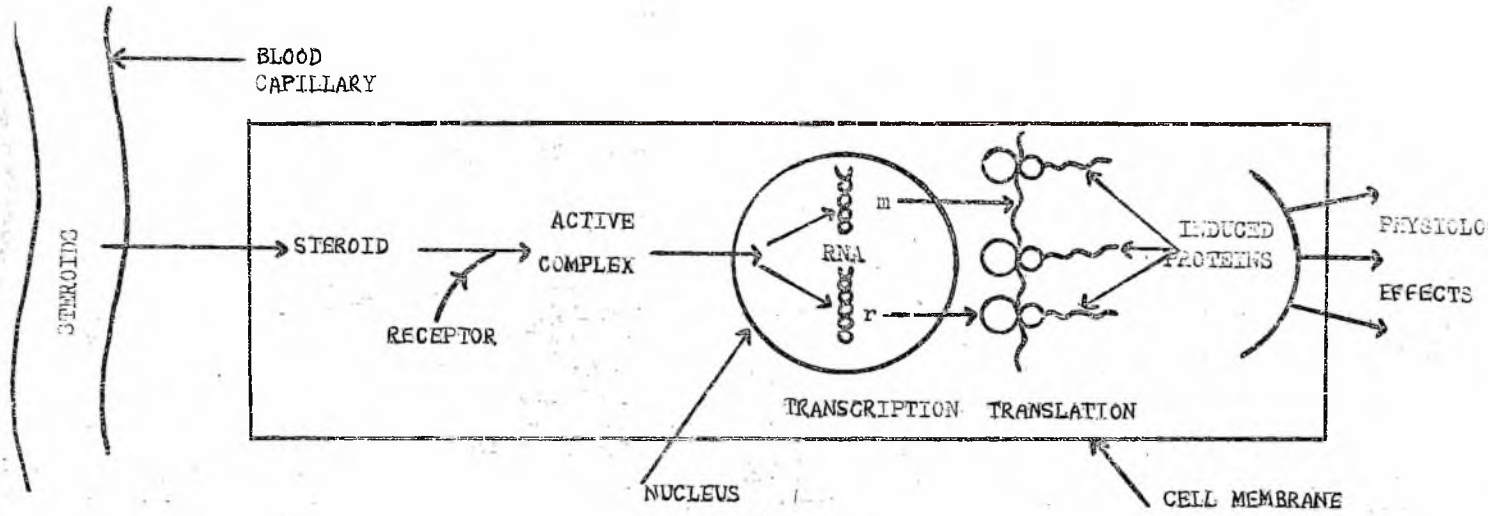


Fig. 3 Sequence of action of steroid hormones on target tissue. (Edelman, 1975)

CONTRACEPTION

The approved birth control methods in use today include certain practices, devices and chemical agents that have been found to be quite safe, effective and generally acceptable. Their effects should be reversible so that when conception or pregnancy is desired, fertility can be promptly restored by discontinuing the particular method in use.

Pregnancy occurs as a result of the union of a sperm from the male and an ovum from the female following copulation, and the subsequent implantation of the fertilised ovum in the uterus.

Birth control methods in practice at this time are basically to:

1) Prevent the union of ova and sperms by:
   a) the use of mechanical barriers eg. condoms, intra uterine devices and diaphrams which prevent the sperms from getting in contact with the ova;
   b) the use of chemical means to inactivate the sperms when shed. This involves the use of vaginal foams, jellies and creams. These may also provide some form of mechanical barrier.

c) the use of surgical methods to block the passage of ova into the uterus through the fallopian tube in the female, or the passage of sperms through the sperm duct in the males - Tubal ligation in females and vasectomy in males.

2) Create an unfavourable condition or environment for the full development of the ovum, prevent ovulation and also implantation. This latter method makes use of the steroid contraceptive which is commonly called "The Pill".

The pill has been found to be the most effective contraceptive method.

Pugh (1968) has reviewed the frequency of failure of various contraceptive methods being used by 100 women in a year, as shown in the table below;

Method	% Failure
The Pill	0 - 1.7
I.U.D.	2.5 - 5.5
Condoms, Caps Foams, Jellies	14
Coitus Interruptus	17
Safe Period (Rhythm)	38
Douche (Post coital)	40

The effect of the pill is made possible by the fact that it prevents ovulation, but if there is ovulation, implantation is prevented. This is made possible by the presence of estrogen and progesterone, which as has been discussed earlier, prevent the production of FSH and LH respectively when in large amounts and therefore prevent ovary development and it subsequent rupture (Kistner, 1969).

The pill is of two types:

a) combination type; which contains estrogen and progestin throughout the course of 20 or 21 tablets: and

b) Sequential type; which has estrogen in 14 - 16 tablets: followed by the combination in the rest of 7-5 tablets respectively.

There are many different brands of contraceptives, the differences depending on the estrogen and the progestin contents. Examples of some oral contraceptives are shown in Table 1.

TABLE 1: SOME ORAL CONTRACEPTIVES

Trade Name	Progestin	mg/ Tablet	Estrogen	mg/ Tablet	Total Steroids (mg).
Enovid 10	Norethynodrel	9.85	Mestranol	0.15	10.0
Norinyl	Norethindrone	2.0	Mestranol	0.10	2.1
Oracon	Dimethisterone	25.0	Ethinyles-tradiol	0.10	25.1
Ovral	Norgestrel	0.5	"	0.05	0.55
Provest	Medroxypro-gesterone	10.0	"	0.05	10.05
Ovulen	Ethynodiol diacetate	1.0	Mestranol	0.10	1.10
Anovlar 21	Norethisterone acetate	4.0	Ethinyles-tradiol	0.05	4.05
Eugynon	dinorgestrel	0.5	"	0.05	0.55

In the combination type the main action of the estrogen is to prevent ovulation (Pugh, 1968). The progesterone component alters the character of the cervical mucus, making it thus impermeable to sperms. Progesterone also produces a hypoplastic endometrium which is unsuitable for implantation. . . . . The sequential pills mainly inhibit ovulation with some secondary modification of the endometrium.

A WHO report (1971) has however emphasized that the mechanism of action of the contraceptive steroids was incompletely understood. This statement is still valid today. It is not certain, however, whether other effects of these compounds also contribute to their desired action, nor is there complete understanding of the mechanism of action of oral contraceptives that do not inhibit ovulation.

Other drugs are being developed that have similar actions (Pugh, 1968). Chlormadinone is a progestogen taken daily (300 mg) and it has almost 100% contraceptive action. It produces a sticky and thick cervical mucous that forms a barrier to sperm entry to the uterus. This drug however causes a lot of bleeding. The endometrium is also made hypoplastic.

There is also Deladroxate. This is given as a monthly injection of progesterone acetophenide (150 mg) on the 8th day of the menstrual period, and it has also been found to be very effective. The gestagen hormonal contraceptives are also monthly, three-or six-monthly inject-

tables which have progestin effects. (Llewellyn-Jones, 1978). There are oral forms of it as well.

There are other types of contraceptive that have lactose or iron interpolated.

One advantage of the pill is found in its use as a treatment for endometriosis which is a possible cause of infertility(Kistner, 1969).

The pill cannot be said to be without any disadvantages. Various workers, however, do not agree on whether it causes some disease conditions or it only aggravates already existing ones. Stoll et al. (1966) have detected hepototoxic effects caused by the progestin component. The hepatocellular damage is detected through a raised serum glutamate oxaloacetate transaminase (SGOT) levels. Larson-Cohn (1965) also found a raised serum glutamate pyruvate transaminase (SGPT) level as well as the level of SGOT and alkaline phosphatase. There was also bromosulphthalein retention. Similar findings were made by Carlstrom et al.(1965), Eisalo et al. (1965 & 1964) and Pava et al. (1964). Sotanieni et al. (1964) detected cholestasis which occurred as a result of hepatocanalicular damage. In all these cases the withdrawal of the pill caused a return to normal conditions. Pugh (1968) has also reported thrombosis in women with sickle cell disease. Urban et al. (1968) has attributed liver dysfunction to mestranol (an estrogenic component of the pill)

while norethinodrel (a progestogen) has no effect.

The frequency of benign breast disease has been negatively associated with the dose of norethisterone acetate while there is a positive trend between this progestin and hypertension (Royal Coll. Gen Pract., 1977).

Swyer et al. (1965) found no pathologically high values of SGOT, SGPT, alkaline phosphatase, bilirubin or bromosulphthalein retention in women on the pill. The same results have been shown by Borglin (1965), Linthorst (1964), Rice-Wray (1964), Swaab (1964) and Tyler (1964). One cannot therefore make a definite conclusion on the effect of the pill on liver function since there are these definitely divergent views on this topic. Work by Taylor (1971) has also shown that recurrent jaundice of pregnancy may be related to the presence of natural estrogens as well as the administration of steroid contraceptives.

CHEMISTRY AND PHARMACOLOGY OF CHLOROQUINE

One needs to define the terminology employed with reference to the function of anti-malarials. This has been done by a WHO committee (WHO, 1963):

a) Suppressive treatment: This is a treatment aimed at suppressing or eliminating clinical symptoms and/or parasitaemia by the early destruction of erythrocytic parasites. It does not necessarily prevent or eliminate the infection. - Overt malaria may develop after drug withdrawal.

b) Radical treatment: Treatment to achieve radical cure. This implies the use of drugs which destroy the secondary tissue stages of the parasite.

c) Prophylaxis: This is a complete prevention of the erythrocytic infection by the administration of drugs that destroy either the sporozoites or the primary tissue forms of the malaria parasite.

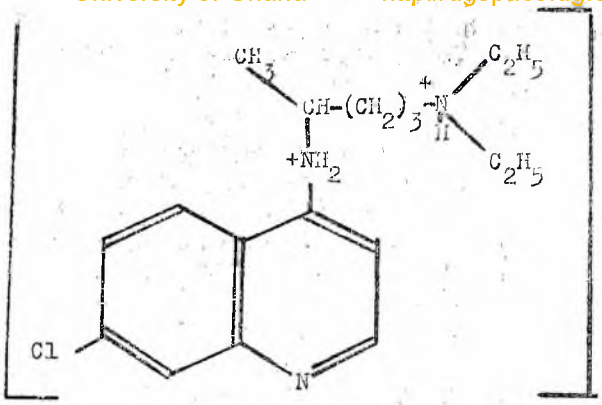
The pharmacology of chloroquine has been reviewed by many workers notably Olatunde (1971). Having such trade names as Avoclor,

Nivaquine, Resochin and Malarex, chloroquine is a potent schizonticidal anti-malarial agent. It is a drug of first choice in the treatment of an overt attack of falciparum as well as other species of malaria in man.

The benefits derived from this drug is not limited to the treatment of malaria alone. It is frequently employed in the treatment of certain collagen diseases such as rheumatoid arthritis (Goldman &Preston,1957). It also enjoys a good record for the treatment of extraintestinal amoebiasis (Conan 1948, and Lane 1951). It has also been found useful in the treatment of epilepsy (Burns, 1966). Takyi et al (1972) and Ijaduola (1977) report of its use as a local aneasthetic for surgery. The treatment of hypercalcaemia in sarcoidosis has also been accomplished with chloroquine (Hunt et al, 1963).

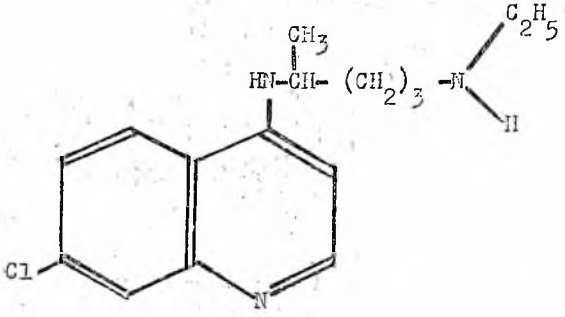
Chloroquine is a synthetic drug; it is a derivative of the 4-amino-quinolines with the formular: $C_{18}H_{20}ClN_3$. Chloroquine may exist in the salt form as the diphosphate or the sulphate (fig. 4). Chloroquine is dispensed as white tablets which are taken orally, or as solutions for injection. The tablets are odourless, have a bitter taste and slowly decolourise on exposure to light. Chloroquine is freely soluble in water, and an aqueous solution has a pH of about 4.7. It is however insoluble in alcohol.

Chloroquine diphosphate may be prepared by condensing 4-dicloroquinoline with 1-dimethylamino-4-aminopentane. The resulting base is

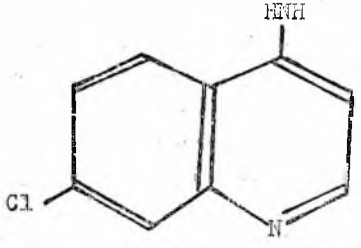


$2H\ PO_4^-$ or $SO_4^=$

CHLOROQUINE DIPHOSPHATE



Mono-de-ethylated Derivative



QUINOLINE NUCLEUS

Fig.4. CHLOROQUINE

treated with phosphoric acid in methanol (Surrey et al, 1946). The colourless crystals that separate have a melting point of 193° - 218°C.

There are three routes for chloroquine administration: oral, intramuscular and intravenous. When administered orally, adequate levels are reached within 2 hours while intramuscular administration takes 15 minutes to reach adequate levels. With intravenous administration the effect is immediate (Jelliffe, 1966). Since the oral route is too slow, the latter two methods are recommended in emergencies such as cerebral malaria (Gilles, 1966). The intravenous administration needs a little dilute solution given slowly if side effects such as convulsions, collapse and death are to be avoided. Chloroquine hydrochloride can be administered intravenously either by syringe or diluted with saline and given by infusion (Scott, 1950). This is a method of choice in falciparum treatment due to its high degree of therapeutic efficacy. This method of administration, however, causes some of the immediate side effects such as nausea, dizziness, vomitting, haziness of vision and drawsiness. Slow administration will also minimise the depression of systolic blood pressure--an effect of short duration. Goldman & Preston(1957) also reports of a better tolerance of hydroxychloroquine, but there could still be anorexia, nausea and difficulty in accommodation by the eye.

There is a similar report by Nieto-Caicedo (1956).

Chloroquine is a cumulative drug which reaches higher and higher levels in tissues in the course of prolonged usage (Chinyanga et al, 1971). It is therefore the total amount of the drug taken that is important and not the length of the period of administration. The normal oral dose of chloroquine for malaria treatment is 25 mg/kg body weight (Chinyanga et al, 1971). In the adult, the treatment is made up of an initial 600 mg chloroquine base followed by 300mg, 6-8 hours later on the same day, and on two successive days (Lewis, 1974). For prophylactic purposes 5 mg/kg body weight (Chinyanga et al, 1971) or 300 mg base (WHO, 1963) is taken once weekly. For the suppression of malaria a dose of 500 mg is administered on the same day of each week. For the treatment of an acute attack of malaria an initial dose of 1g is administered followed by 0.5g after 6-8 hours and on each of two consecutive days (Laurence, 1968). For an intravenous administration 0.5 mg/kg body weight is given slowly at a concentration of not more that 1 mg/ml. This is then followed immediately by an intramuscular injection of 4.5 mg/kg body weight (Jelliffe, 1966).

Chloroquine is rapidly and almost completely absorbed from the gut when taken by mouth (Loeb, 1946). Less than 10% is egested. The rate of absorption suggests that absorption takes place mostly in the stomach and upper small intestines. Olatunde (1971) suggests

that the efficiency of absorption from the gut does not call for parenteral administration except where such a method is marked by conditions like vomiting and unconsciousness.

Chloroquine has a very wide distribution in the body, having a strong affinity for melanin-containing cells and tissues (Sams &Epstein,1965). As such very high levels of the drug have been found in occular tissue in pigmented rats (Rubin, 1963). Other tissues that have been found to take up the drug include the liver, heart, kidney, spleen and the uterus (Knox & Owens1966, & Nelson & Fitzhurgh, 1948). In all these cases, most of the drug was reportedly stored unchanged Grundman et al, (1972) similarly found chloroquine to be present, according to the order of decreasing tissue concentration of the drug, in suprarenal gland, kidney, spleen, liver, lungs, ileocoecal gland, heart, muscle and brain. They found that in all the organs, with the exception of muscle and brain a maximum chloroquine content was attained between the 10th and 16th week during continuous daily administration over 24 weeks. This level stayed with no further change up to the 24th week. It was only in the muscle that the chloroquine concentration increased constantly during application of chloroquine over the whole period. Red blood cells containing chloroquine sensitive malaria parasites have been found to concentrate the drug to higher levels as compared to uninfected cells (Macomber et al, 1967). Chloro-

quine is stored in the liver parenchymal cells where high concentrations may build up, thus providing storage facilities to prolong drug action.

Chloroquine binds to plasma proteins to the extent of 55% (Berliner et al, 1948). Parker & Irvin (1952) have also shown that chloroquine has some affinity for the highly polymerised deoxypentose nucleate and nucleoprotein of beef spleen. It has actually been confirmed that chloroquine combines with DNA (Kurnich, 1956). This has led to the idea that such binding determines the distribution of the drug in vivo especially in tissues that take it up.

The metabolism of chloroquine has been investigated by Titus et al (1948). Two processes of detoxification were identified: The first involved conjugation to a uronic derivative, while the second was the successive removal of the side chain groupings down to the quinoline nucleus (fig. 4), which remained intact. The de-ethylated derivative has been found to be the major derivative (McChesney et al, 1954), but other intermediate products up to the quinoline group have been detected by Kuroda (1962).

The urinary excretion of chloroquine is slow and discontinuous (Zanca &Benatti, 1959) Three to five years after the last injection of chloroquine, patients were found excreting the drug in urine, and they were also found to have measurable concentrations in the blood

(Rubin et al, 1963). Knox &Owens(1966) similarly found that the slow excretion of chloroquine persists for years after its ingestion. This was supported by the findings of Burns (1966) that there is the possibility of chloroquine retinopathy years after the discontinuance of a chloroquine therapy. The proportion of drugs and metabolites excreted in the urine following daily administration of chloroquine to man has been determined by McChesney et al (1966) as 60% parent compound; 37% de-ethylated compound and 3% of the others.

MODE OF ACTION

It has been suggested that lactate production by the actively metabolising malaria parasites decreases the pH of the red blood cell. The decreased pH inside the cell will create a H^+ gradient across the cell membrane. This change in gradient across the cell membrane facilitates the entry of the drug (Peters, 1970). Upon reports that chloroquine could form a complex with certain porphyrin derivatives of haemoglobin, Macomber et al, (1967) suggested that it was actually the concentration of chloroquine by such a pigment in the parasite that formed the basis of the action of the drug. Homewood et al (1972) have postulated that chloroquine acts by initially raising the pH in the food vacuole of the parasite. This reduces the digestion of haemoglobin by the parasite and thus prevents its growth. In this connection they explain that the impotence of the drug on sporogonic

and exoerythrocytic stages of the parasite is due to the absence of digestive vacuoles.

Parker et al (1949) first reported the interaction of chloroquine with yeast RNA. They later showed that chloroquine interacts with nucleic acids when they demonstrated its strong binding with the highly polymerised deoxypentose nucleate and nucleoprotein of beef spleen (Parker &Irvin, 1952). Kurnick &Radcliffe (1962) found that the enzymic depolymerization of DNA is retarded or inhibited by chloroquine binding. The binding is a result of electrostatic forces involving two separate portions of the DNA molecule and the chloroquine (Cohen &Yielding, 1965). This formed the chloroquine-DNA complex.

Ciak &Hahn (1966) concluded from their studies that a molecular complex is formed with DNA and this makes DNA incapable of its own replication or the transcription of RNA. There is a rapid degradation of ribosomes, and protein synthesis inhibition occurs as a secondary effect.

OTHER EFFECTS OF CHLOROQUINE

Hellerman et al (1946) performed experiments which showed that chloroquine and other related compounds inhibit d-amino acid oxidase strongly at low concentrations of flavin adenine dinucleotide (FAD),

but only slightly at high FAD concentrations. This suggests an inhibition by competition with FAD for the protein.

On the effect of chloroquine on respiration Knox &Owens(1966) reported that it is a potent inhibitor of reduced nicotinamide adenine dinucleotide phosphate (NADPH) - cytochrome c reductase. Steroids inhibit the same step in the respiratory pathway (Yielding &Tomkins, 1959). Whitehouse (1963) and Whitehouse et al, (1965) however showed that Oxidative phosphorylation is insensitive to chloroquine.

In the treatment of dermatologic disorders Goldman &Preston(1957) reported of the drug's interference with the metabolic activities of hexokinase.

As indicated earlier chloroquine cannot be said to be without toxic side effects. It may occasionally give rise to headache, gastrointestinal disturbances and blurring of vision (Alving et al, 1948). Permanent loss of vision from retinopathy may be the most serious sequel of prolonged chloroquine therapy (Burns, 1966). Cases of severe muscular weakness (neuromyopathy) have been reported (Loftus, 1963 & Du Bois, 1956). Scott (1950) reports of the lowering of blood pressure on intravenous administration. Chinyanga et al (1972) have reported that chloroquine induces respiratory depression. This is also reported by Kjaer, (1955). There is difficulty in breathing and swallowing, analgesia and paresthesia of the face and neck. Chloroquine has an anticoagulant effect (Takyi et al, 1972). It was

found to cause bleeding at a 1% concentration. Low chloroquine concentrations have a specific antihistamine effect (Akubue, 1975). It is therefore not likely that the pruritic reaction in chloroquine sensitive persons is a result of histamine release. A similar report was made by Olatunde (1970). He stated that chloroquine interfered with the histamine induced contractions of isolated guinea pig ileum.

He observed that chloroquine has a wide variety of direct spasmolytic effects on smooth muscle. Chinyanga et al (1972) corroborate this with their observation that chloroquine causes a depression of muscle contraction. This occurs because chloroquine has a depressant effect on neuromuscular transmission and finally blocks it.

On the uterus and uterine physiology, there have been various reports on the toxic effects of chloroquine. Nelsen &Fitzhurgh(1948) fed albino rats for two years with chloroquine and the observations they made include the presence of brown uteri. There was a slight increase in the uterine fat. Kurantsin-Mills et al (1972) observed that low doses of chloroquine increase the magnitude of contractions of estrogen primed guinea pig uterus in vitro. This declined with higher dose. Engmann et al (1974) reported that rats given chloroquine injections for 6-20 days had aborted foetuses. The tendency to abort increased with a prolonged treatment. More established pregnancy is however difficult to be dislodged. A similar observation has been reported by Bentsi-Barnes (1977) in a human female.

It is estimated that out of every four or five pregnancies in Ghana, one ended in abortion. The Korle-Bu Hospital is reported to treat about 3,000 abortion cases yearly, half of these resulting from spontaneous abortion or miscarriage (Ampofo, 1977). The effects of chloroquine cannot be ruled out as part of the cause of this high incidence of abortion in Ghana, bearing in mind its wide usage and its toxic effects discussed above.

UTERINE PEROXIDASE AND ESTROGEN INACTIVATION

The bulk of estrogen as well as other steroid hormones' breakdown occurs in the liver. What happens to the estrogens that get into the uterus for the functions that have been discussed above? How are they inactivated when their action should cease before they are transported to the liver? Answers to these questions were partially found by Lucas et al, (1955). These workers found that estrogen stimulation of the uterus produced a remarkable increase in peroxidase activity in it.

Peroxidase (donor: H_2O_2 oxidoreductase - EC 1.11.1.7) is a haemoprotein (Archer et al, 1965). It is present in both plant and animal tissues. The most well known plant in which it occurs is the horseraddish. In the animal kingdom (Rytomoa &Teir,1961) report of the presence of peroxidase in mammals, and four main types of peroxidase have been identified. These are: a) Lactoperoxidase (b) Myeloperoxidase (c) Hepatic peroxidase and (d) Labile thyroid gland peroxidase. Mammals whose tissues have had peroxidase isolated from them include rats, mice, hamsters, guinea pigs and human beings(Lyttle &DeBombre,1977).The highest peroxidase activity has been demonstrated in tissues that exhibit cell renewal. These include the stomach, spleen, uterus and the lungs (Neufeld et al, 1958). The others are the intestines (Rytomoa et al, 1961) and the vagina (Lyttle et al, 1977). Peroxidase in these

tissues have been found to be associated with eosinophils (Archer &Hirsch,1963); Fahini 1970 & Brokelman 1969), epithelium (Brokelmann, 1969), rough endoplasmic reticulum of the endometrium (Churg &Andersen,1974), thyroid follicle (Strum et al, 1970) and rat liver Kupfer cells (Fahini 1970).

Horseraddish peroxidase has received a lot of attention amongst workers. It is reddish brown in slightly acid solution and has methae-moglobin type absorption spectra with four bands at 645, 583 and 498nm (Keilin et al, 1937). The alkaline form has its bands at 583 and 549 nm. These spectral characteristics show the presence of a ferric ion. When treated with sodium thiosulphate which reduces the haematin, the peroxidase solution turns red and the spectral bands shift. H_2O_2 does not affect the valency of the iron. This is because sodium thiosulphate will still produce reduced haematin spectra after the peroxidase solution has been treated with H_2O_2. Similar properties have been found for milk and eosinophil peroxidase haematin (Archer et al, 1965).

Rat uterine peroxidase has a molecular weight of 40,000, with a half life of 4 hours (McNabb et al 1975). It works at a pH optimum of 7.4(Jellinek &Irwin,1962) and has a $Km(H_2O_2)$ of 4.5×10^{-4} M (Archer et al, 1965).

The action of peroxidase has been found to be affected by a number of substances. These include inorganic ions and compounds,

phenolic groups and other organic compounds. Hollander &Stephens (1959) reported that horseraddish peroxidase is stimulated by H_2O_2 and Mn^{2+} and requires phenol as a co-factor. It is inhibited by CN^-, Cu^{2+} and catalase. Azide was found to have no effect on horseraddish but it inhibited 30% of uterine peroxidase activity. Phenolic substances have been generally found to stimulate peroxidase reactions (Klebanoff, 1961). The phenol is thought to be converted to the quinone before its effect is shown (Klebanoff, 1961). Thyroxine will stimulate adrenaline and nor-adrenaline, uric acid and ascorbic acid oxidation by peroxidase. NADH and NADPH oxidation are similarly effected. The reactions are inhibited by potassium cyanide, sodium azide, excess H_2O_2 (Klebanoff, 1959), cysteine and other heavy metal enzyme inhibitors (Akazawa &Conn,1958). Catalase has also been found to inhibit peroxidase reactions (Williams-Ashman et al, 1959 & Klebanoff, 1960). Ascorbic acid is also reported to inhibit H_2O_2 generation by peroxidase (Jollinck &Fletcher,1970).

Peroxidase ordinarily catalyses electron transfer from donors to H_2O_2 with the donors undergoing a free radical and related reactions (Mason et al, 1957). Peroxidases have been found to act as oxidases in various reactions. Swedin &Theorell(1940) concluded from their studies that peroxidase could act differently with different substrates. This came as a result of the ability of the enzyme to oxidise dioxymaleic acid with the spontaneous production of H_2O_2.

Neufeld et al (1958) later found that peroxidase may substitute for oxidase in oxidative metabolism of proliferating tissue. Peroxidase may acquire oxidase activity under certain conditions as found in the oxidation of dihydroxyfumarate, aerobic hydroxylation of aromatic compounds (Mason et al, 1957) and aerobic oxidation of reduced nucleotides in the presence of dichlorophenol. This reaction that has been effected with horse-raddish peroxidase occurs in the presence of Mn^{2+} (Hollander et al, 1959). Jellinck &Irwin (1962) reported that uterine homogenates with a high NADH oxidase activity had no peroxidase. This is just the converse of the report by Neufeld et al (1958) that tissues with a high peroxidase content have a low cytochrome oxidase concentration. Jellinck &Irwin (1961) reported that horseraddish peroxidase acts as an aerobic oxidase which can utilize tryptophan to produce H_2O_2. Estradiol, which is a phenolic compound, is capable of hydrogen transport for NADH and NADPH oxidation catalysed by peroxidases. Kenten and Mann (1953) reported that in the presence of Mn^{2+}, peroxidase oxidises oxalate, oxaloacetate and ketomalonate, with the formation of H_2O_2. Indole acetic acid is also oxidised by peroxidase (Machlachlan &Waygood 1956).

The importance of peroxidase in uterine physiology started to come to light when in 1955 Lucas et al reported that upon estrogen stimulation the uterus produced a remarkable increase in peroxidase

activity. The magnitude of the response depended on the concentration of the hormone, and the enzyme activity was maximum 72 hours after the estradiol injection. The work was done with ovariectomised rats which had a very low peroxidase activity.

Lyttle &Jellinck(1972a&b) have shown that peroxidase activity which is either very low in or absent from immature rat uterus is induced by estrogen or physiological doses of preganant mare serum gonadotrophin. These findings are further supported by McNabb &Jellinck (1974) who similarly found that peroxidase activity increases on estrogen treatment. Anderson et al (1975) found that administration of estradiol and diethylstilbesterol induced the synthesis of peroxidase in the vagina, cervix and uterus. The synthesis is specific since it is neither induced by testosterone nor progesterone. They further went on to state that peroxidase is a consistent marker for estrogen dependent tissues. The cycle of peroxidase synthesis and secretion coincides with the estrus cycle of mature rats and that of immature or ovariectomised rats after estradiol or diethylstilbestrol injection. A similar result came from the work of Lucas et al (1955) which showed that human endometrial scrappings contain peroxidase activity. The level of enzyme activity varies with the stage of the menstrual cycle. Lyttle &DeSombre(1977) have supported

this further by finding that uterine peroxidase is a good marker for estrogen stimulation of growth. They found further that there is a growth related increase in peroxidase concentration to growth responsive tissues of the uterus and vagina. Rat mammary tumours that are estrogen dependent have also been found to produce peroxidase (DeSombre et al, 1975).

Lyttle &Jellinck(1972a) established that estradiol-induced peroxidase activity persisted for only 24 hours while the activity reached maximum in 20 hours after estradiol injection (Lyttle &DeSombre 1977b). Churg et al (1974) also reported from histochemical studies that peroxidase production occurs 24-48 hours after estradiol injection.

There have been various reports that uterine peroxidase can degrade estrogens in vitro to inactive water soluble compounds. Klebanoff et al (1960) reported that estradiol is thus oxidised by peroxidase. This has been shown to occur in the presence of NAD, NADP and thyroxine. H_2O_2 can be formed if it is not present, and used in the reaction which is inhibited by catalase, and accelerated by an added H_2O_2. Jellinck &Irwin(1961&'62) and Klebanoff et al (1961) report of the conversion of estrone and estradiol by peroxidase to water soluble compounds. The reaction involves an initial H_2O_2 generation. There is a naturally occurring peroxidase reaction which increases the binding of estradiol to uterine tissue covalently (Brokelmann, 1969).

Lyttle &Jellinck(1972a&b)) have also found that the peroxidase is able to convert estradiol to water soluble products in vitro. Peroxidase increases estradiol binding to other high molecular weight substances in the presence of H_2O_2.(Jellinck et al, 1971). They went on to suggest that any estrogen that forms a complex with a receptor protein is protected from degradation till its role is accomplished. Degradation may then occur after its release.

Since estrogen inactivation occurs with H_2O_2 as a co-substrate there is the need for an adequate and periodic supply of H_2O_2 in the system. The body has various H_2O_2 generating systems that could cater for this need.

One process that has been proposed by Jellinck et al (1970) makes use of glutathione (GSH) in the presence of oxygen. The reaction is inhibited by cyanide and ascorbic acid:

$$2\ GSH + O_2 \xrightarrow{PEROXIDASE} GS\text{-}SG + H_2O_2$$

Klebanoff (1965) has also implicated G6PD and NADH/NADPH in another process:

$$NADP + \text{Glucose6-Phosphate} \xrightarrow{G6PD} NADPH + H^+ + \text{6-Phospho-gluconate.}$$

$$NADPH + H^+ + O_2 \xrightarrow{PEROXIDASE} NADP + H_2O_2.$$

A third process involves glucose oxidase (West et al, 1966):

$$\text{Glucopyranose} + O_2 \xrightarrow{\text{GLUCOSE OXIDASE}} \text{Gluconolactone} + H_2O_2.$$

A mechanism for the degradation of estrogen in vitro has been suggested (Jellinck et al, 1970):

$$EOH + H_2O_2 \xrightarrow{\text{PEROXIDASE}} (EO)^{\bullet} + H_2O + HO^{\bullet}.$$

$$(EO)^{\bullet} + GSH + HO^{\bullet} \xrightarrow{\text{Non Enzymic}} GS\text{-}EO + H_2O.$$

GS-EO is thought to be the water soluble inactivated estradiol (E). There is however no evidence for in vivo inactivation of estrogen in the uterus.

The ability of peroxidase to degrade estrogens in vitro has led to the suggestion that the peroxidase induction in the uterus by excess estrogen may be a possible adaptive mechanism to limit the duration of estrogen action in the uterus (Brokelman, 1969 and McNabb &Jellinck, 1974).

Based on the above reports and deductions, peroxidase activity is being implicated in the effective action of the oral contraceptive, through the degradation of the estrogens present in the uterus. This action will therefore prevent ovum development and ovary rupture, both being processes that are necessary for conception.

Uterine peroxidase has also been suspected to have a similar bactericidal action in the uterine fluid as exhibited by salivary-, neutrophil- and lacto-peroxidase (Churg &Andersen, 1974). Anderson et al (1975) have also suggested a possible spermicidal role for peroxidase in the uterus.

EXPERIMENTAL PROCEDURES

MATERIALS

Animals: 21-28 day old immature female albino Holtzmann rats weighing between 40 and 100 g were used. These were obtained from the Biochemistry and Animal Science Departments, Legon.

Reagents: Estrodiol 17β, cycloheximide, puromycin, chloroquine diphosphate and bovine serum albumin were obtained from Sigma Chemical Company, U.S.A.; Anovlar 21 made by Schering Chemicals Ltd., Sussex England, was obtained from local chemists shop. All other reagents of analytical grade were purchased from BDH Chemicals and M & B Ltd., England.

Unless otherwise stated in the text:

Chloroquine	refers to	chloroquine diphosphate,
Estradiol	refers to	estradiol 17β and
Anovlar	refers to	anovlar 21.

METHODS

A. PREPARATION OF REAGENTS FOR:

1. Treatment of animals:

   a) Saline - 0.9%; 0.9g sodium chloride was dissolved in, and made up to 100ml with water.

b) Estradiol - 0.01 mg/ml; 1 mg estradiol was dissolved in 2ml methanol and made up to 100 ml with 0.9 saline.

c) Anovlar 21 - 4.0 mg norethisterone acetate and 0.05 mg. ethinylestradiol per tablet.

d) Chloroquine - 2 mg/ml; 0.2g of the diphosphate salt was dissolved in saline and made up to 100 ml.

e) Cycloheximide - 0.5 mg/ml; 1mg was dissolved in 2ml saline.

f) Puromycin - 0.4 mg/ml; 1mg was dissolved in 2.5 ml saline.

2. Enzyme extraction and assay:

a) Sucrose - 0.32M with 1mM $MgCl_2$; 5.35g sucrose and 0.1016g $MgCl_2$ were dissolved in water and made up to 500 ml

b) Ortho-Dianisidine - 1%; 0.01g was dissolved in 1ml methanol. This was prepared fresh and kept in the dark.

c) Hydrogen peroxide - 0.3mg/ml; 0.1ml 30% H_2O_2 was made up to 100 ml with water.

d) Sodium phosphate buffer - 0.01M, pH6.0; 7.164 g disodium monohydrogen phosphate and 3.121g sodium dihydrogen phosphate were each dissolved in 100ml water separately. 6.15ml of the disodium monohydrogen phosphate solution was then added to 43.85ml of the sodium dihydrogen phosphate solution. The mixture was made up to 100 ml with water.

3. Protein determination:

a) 2% sodium carbonate in 0.1N sodium hydroxide; 0.4g sodium hydroxide and 2g sodium carbonate were dissolved in water and made up to 100 ml with water.

b) 0.5% copper sulphate in 1% sodium potassium tartrate; 1g copper sulphate and 2g tartrate were each dissolved in 100 ml water. Equal volumes of the two solutions were then mixed.

c) Standard bovine serum albumin - 0.2mg/ml; 2mg was dissolved in 10 ml water. Serial dilutions were made for a standard curve.

d) Alkaline coper tartrate; solution (a) was added to solution (b) in the ratio of 50:1. This was prepared fresh.

e) Folin Ciocâlteau reagent; Stock diluted with water 1:1.

B. GENERAL METHODS

The animals were put into groups of three in cages to receive the various treatment described below. After the treatments the uteri from animals in the same group were dissected out and pooled for peroxidase extraction and assay.

Where anovlar was being administered, each animal was given one tablet in an aqueous suspension by a medicine dropper.

Chloroquine, estradiol, cycloheximide and puromycin were administered by an intramuscular injection in 0.9% saline. This dose, based on the work of Lyttle et al (1972), is shown in the table below:

Treatment	Dose/100G Body WT	Volume (ML)
Estradiol	2ug	0.2
Chloroquine	2mg	0.2
Cycloheximide	120ug	0.2
Puromycin	100ug	0.25
Saline (control)	0.9%	0.2

Fifteen to twenty rats were used to study the effect of each treatment, and the mean results are reported in the work.

The following investigations have been carried out on the animals.

1) Estrogens and chloroquine on uterine peroxidase activity:- Respective groups of rats were given the following treatments daily for five days: Control; estradiol; anovlar 21, chloroquine. The animals were sacrificed 20 hours after the fifth day's treatment.

2) Cycloheximide and puromycin on the action of estradiol:- Single doses of estradiol were given to each animal. One group was given cycloheximide while another group was given puromycin thirty minutes after the estradiol administration. This was done for

five days and the animals were sacrificed after the last treatment.

3). Cycloheximide on the action of chloroquine:- Single chloroquine and cycloheximide injections were given simultaneously to the same animals. These were then sacrificed 18 hours later.

4) Time course activity study for chloroquine and anovlar:- Chloroquine or anovlar were respectively administered to two groups of rats. Three rats from each group were killed at 6 or 12 hourly intervals between 0 and 60 hours after the treatment.

5) Effect of different chloroquine doses on peroxidase activity: Five groups of rats were given the following doses of chloroquine in terms of mg/kg body wt. 0, 5, 10, 15 and 20. The animals were sacrificed 18 hours later.;

6) Effect of chloroquine on peroxidase activity in vitro:- One millilitre portions of peroxidase extracts were incubated at room temperature for 1 minute with 0.3 ml chloroquine solution of different concentrations. The chloroquine concentrations used were 0.00; 0.03; 0.06 and 0.12 mg/ml. After the incubation period the 1.30 ml incubated extract was assayed, adjusting the reaction mixture to a final volume of 3.00 ml. with water.

7) Combined administration of anovlar and chloroquine:- Four groups of rats were given the following treatments respectively: Control;

chloroquine; anovlar; anovlar and chloroquine. Three animals from each group were sacrificed at time intervals of 5, 12, 18, 24 and 36 hours after the treatments.

Each of the above treatments has been followed by enzyme extraction from the pooled uteri from each treatment group, peroxidase assay and protein determination as described below:

B1 - Enzyme Extraction

The method used for the extraction of peroxidase from the uterus was according to that of Lyttle et al (1972). The animals were killed after the respective treatment periods by cervical dislocation. The uteri were immediately dissected out. The dissected uteri were freed of all fat, blotted and weighed individually. Uteri from the same treatment group were then pooled for the extraction of the enzyme. The pooled uteri were homogenised for 3 min in a 0.32M sucrose solution containing 1mM Mg^{2+}. The tissue concentration was 50mg/ml. A 'jencons' auto homogeniser was used. The homogenate was filtered through cheese cloth. The filtrate was then diluted to a 0.25M sucrose concentration with water. An equal volume of 0.32M sucrose was carefully layered underneath. This was then centrifuged at 700xg to remove the nuclear fraction (Widnell et al, 1964). The resultant supernate was used for the peroxidase assay. The enzyme extraction was done in the cold.

B2 - Peroxidase Assay

The assay for peroxidase activity was done according to the method of Klebanoff (1965). The reaction mixture was made up as follows:

Water	--	0.35 ml
1% ortho-dianisidine	--	0.50 ml
Sodium phosphate buffer pH 6.0	--	0.30 ml
Hydrogen peroxide (0.3mg/ml)	--	0.30 ml
Enzyme extract	--	2.00 ml
Total volume	=	3.00 ml

The increase in optical density at 460 nm was followed. The enzyme activity was determined in units; a unit of activity being the amount of enzyme causing an increase in absorbance of 0.001 per minute.

B3 - Protein Determination

The determination of the protein content of the extract was done by the method of Lowry et al (1951). Five millilitres of alkaline copper tartrate was added to 1ml of the extract and mixed thoroughly. This was allowed to stand at room temperature for 10 minutes. Exactly 0.5ml Folin-Ciocalteau reagent was added after this time, with immediate shaking. This was then left in the dark for 30 minutes, after which

the absorbance was read at 750 nm. Bovine serum albumin was used to plot a standard curve after serial dilutions of the albumin solution has been treated similarly.

B4 - Mating Experiments

Adult female rats that had littered once, were put in isolation from males for 14 days after they had been weaned of their litter. They were then grouped in fours to receive treatment as in '7' above. The treatments were given daily for five days. Males were introduced into the cages after the fifth day's treatment. Each cage containing two females on the same treatment had one male. The treatment were continued for a further 8 days. The males were removed after the 8th day's treatments and the females were observed for the production of litter for the next 31 days. The gestation period for rats is 21 - 23 days (Farris, 1950).

RESULTS

A - Effect of estradiol, anovlar 21 and chloroquine on uterine Peroxidase Activity

Single doses of the above treatments were given daily to the rats for five days. The peroxidase activities in the uterine homogenates were then determined.

Table 2: Uterine peroxidase activity in the presence of estradiol, anovlar 21 and chloroquine

Treatment Group	Av. Uterine wt. (Mg)	Enzyme units/ml	Enzyme Activity (units/mg tissue)	Enzyme Activity (units/mg protein)	% increased Activity *
Control	36.5	5.30	0.121	11.48	0
Estradiol	71.2	10.62	0.267	21.24	85
Anovlar	92.7	11.92	0.264	14.91	30
Chloroquine	34.0	7.73	0.106	15.10	32

*Based on units/mg protein.

There has been an increase in the peroxidase activities in the uteri of rats from all the treated groups as compared with the control group. Specific activities show that estradiol gave a marked increase in peroxidase activity (21.24 units/mg protein) while chloroquine and anovlar gave about 15 units/mg protein. Specific activity for the control was 11.48 units/mg protein.

B. Effect of Cycloheximide and puromycin on the stimulation by estradiol of uterine peroxidase

Daily injections of estrodiol were administered to three groups of rats for 5 days. Thirty minutes after the estradiol administration, cycloheximide was given to rats in one group while puromycin was given to those in a second group. The control group were injected with saline. Uterine peroxidase activities determined are shown in Table 3.

Table 3: Cycloheximide and puromycin effects on the induction of Uterine peroxidase by estradiol

Treatment Group	Av. uterine wt. (mg).	Enzyme units/ml	Enzyme activity(units/ mg tissue)	Enzyme Activity(units/ mg protein)	% Activity change *
Control	36.5	5.30	0.121	11.48	0
Estradiol	71.2	10.62	0.267	21.24	+85
Estradiol + Cycloheximide	54.7	2.69	0.056	5.27	-54
Estradiol + Puromycin	64.7	3.02	0.067	5.02	-56

*Based on units/mg protein

The results show an inhibition of the effect of estradiol by the protein synthesis inhibitors. There was 54% inhibition by cycloheximide and 56% inhibition by puromycin.

C. Effect of cycloheximide on the action of chloroquine

Two groups of rats were given a single dose of chloroquine. One group had a simultaneous injection of cycloheximide. Uterine peroxidase activities were then determined.

Table 4: Cycloheximide Effect on the stimulation by Chloroquine of uterine peroxidase

Treatment group	Av. uterine wt. (mg)	Enzyme units/mg	Enzyme Activity (units/mg tissue)	Enzyme Activity (units/mg protein)	%Acvitity Change *
Control	38.6	1.70	0.084	14.36	0
Chloroquine	45.2	4.10	0.210	30.67	+114
Chloroquine + Cycloheximide	39.2	1.40	0.072	11.24	-22

*Based on units/mg protein

These results have also shown that cycloheximide inhibited the stimulation by chloroquine of the uterine peroxidase activity. There was about 22% inhibition by cycloheximide. (Puromycin was not available for use in this experiment).

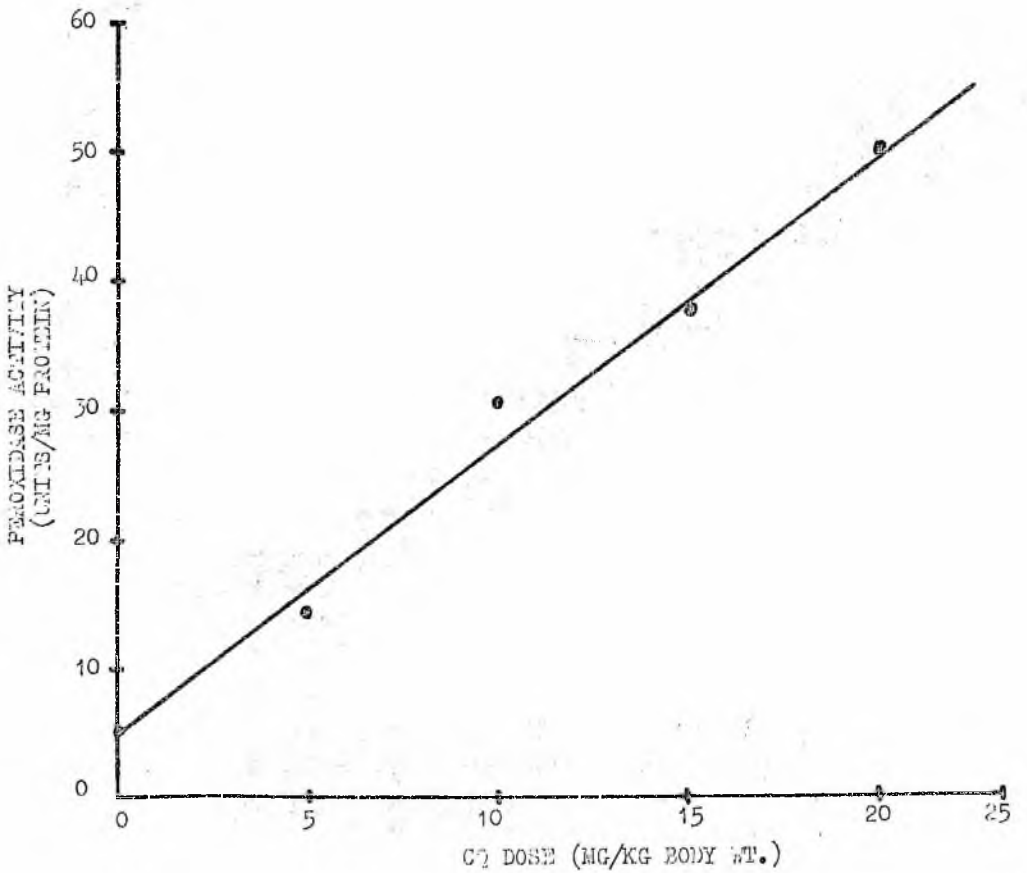
D. Effect of different Chloroquine doses on Peroxidase Activity

Chloroquine was administered to rats at a dose range of 0.20 mg/kg body wt., the animals killed after 18 hours, and peroxidase activity determined.

Fig. 5: Peroxidase Activity Versus Chloroquine Dose.

The figure shows a gradual and definite increase in uterine peroxidase activity caused by chloroquine over the dose range of 0-20 mg/kg body weight.

FIGURE 5



E. Effect of chloroquine on peroxidase activity in vitro

Uterine extracts with peroxidase activities were incubated with different concentrations of chloroquine and the peroxidase activities were then determined. The results are shown in Table 5.

Table 5: In vitro effect of chloroquine on peroxidase activity

[CQ] (mg/ml)	Expt.	Enzyme (units/ml)	Enzyme activity (units/mg. protein)
0.00	1	0.80	18.18
0.03		0.80	18.18
0.06		0.82	18.64
0.12		0.80	18.18
0.00	2	1.20	13.95
0.03		1.00	11.63
0.06		1.40	16.28
0.12		1.00	11.63
0.00	3	0.30	5.00
0.03		0.32	5.33
0.06		0.32	5.33
0.12		0.34	5.67

It is evident from the three experiments that chloroquine does not have any significant effect on peroxidase activity in vitro. In each of the three experiments, peroxidase extracts incubated with chloroquine gave similar values as their respective controls.

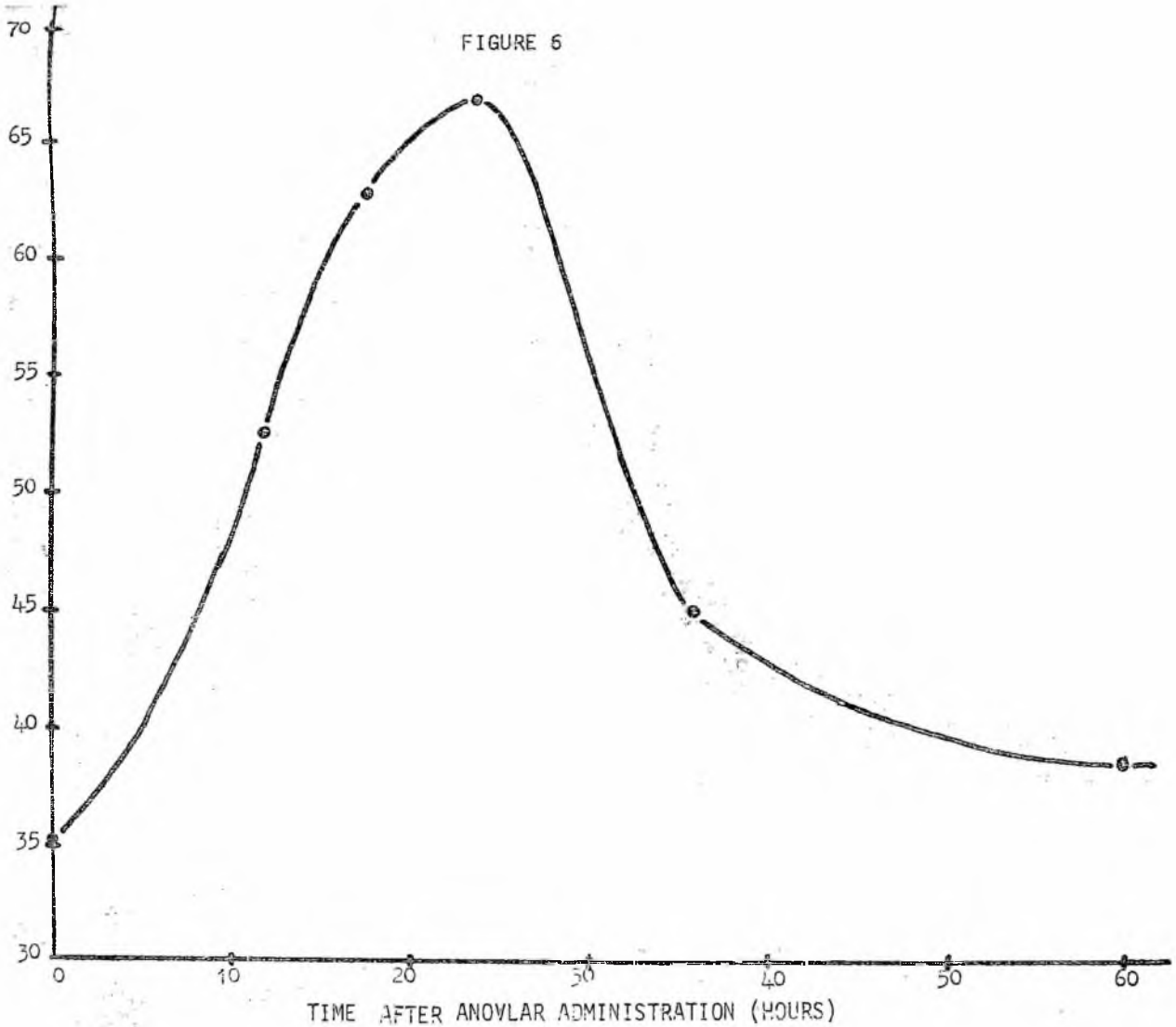
F_1 - Time of response to anovlar and duration of effect

Single doses of anovlar were given to a group of 18 rats. Three rats from the group were killed at 6 or 12 hourly intervals over a period ranging from 0 - 60 hours. Uterine peroxidase activities were determined.

Fig. 6: Peroxidase Activity: Action of Anovlar with time.

The uterine peroxidase activity reaches maximum around 24 hours after anovlar administration.

FIGURE 6



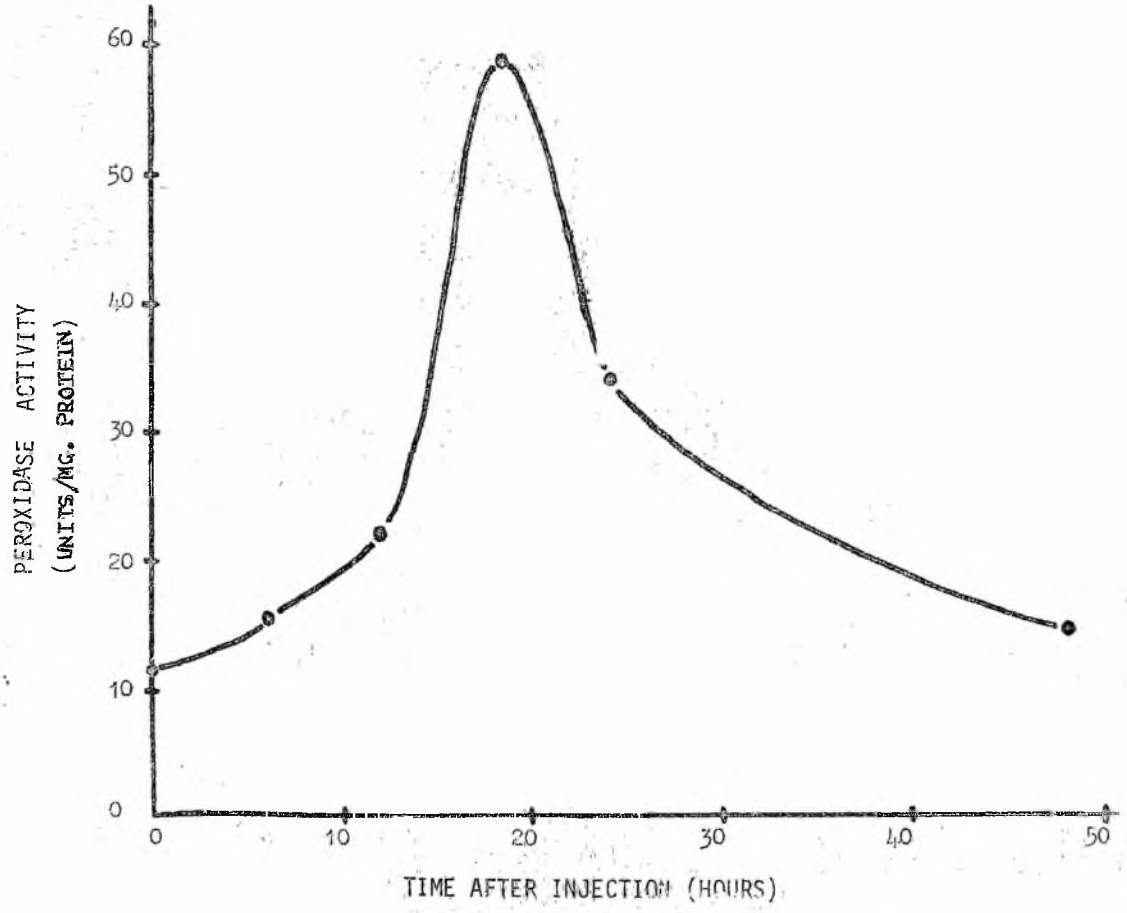
F_2 - Time of response to Chloroquine and duration of effect

Single doses of chloroquine were given to a group of 18 rats. Three rats from the group were killed at 6 or 12 hourly intervals over a period ranging from 0 - 50 hours. Uterine peroxidase activities were then determined.

Fig. 7: Peroxidase Activity: Action of Chloroquine with Time.

The uterine peroxidase activity reaches maximum around 18 hours after chloroquine administration.

FIGURE 7



G - Combined administration of anovlar and chloroquine

Four groups (each containing about 20 rats) were respectively given the following treatments:

Control; chloroquine; anovlar; anovlar with chloroquine. Three animals from each group were killed at given time intervals ranging from 6 - 36 hours. Peroxidase activities were determined.

Fig. 8: Effect of Chloroquine and/or Anovlar on Uterine Peroxidase Activity.

The results confirm the periods of peak activity for anovlar and chloroquine to be around 24 and 18 hours respectively. Also the combined treatmant produced a higher peroxidase activity than chloroquine or anovlar given alone. Tables 6 and 7 show increase of peroxidase activity and efficacy of treatment over control animals,, respectively.

FIGURE 8

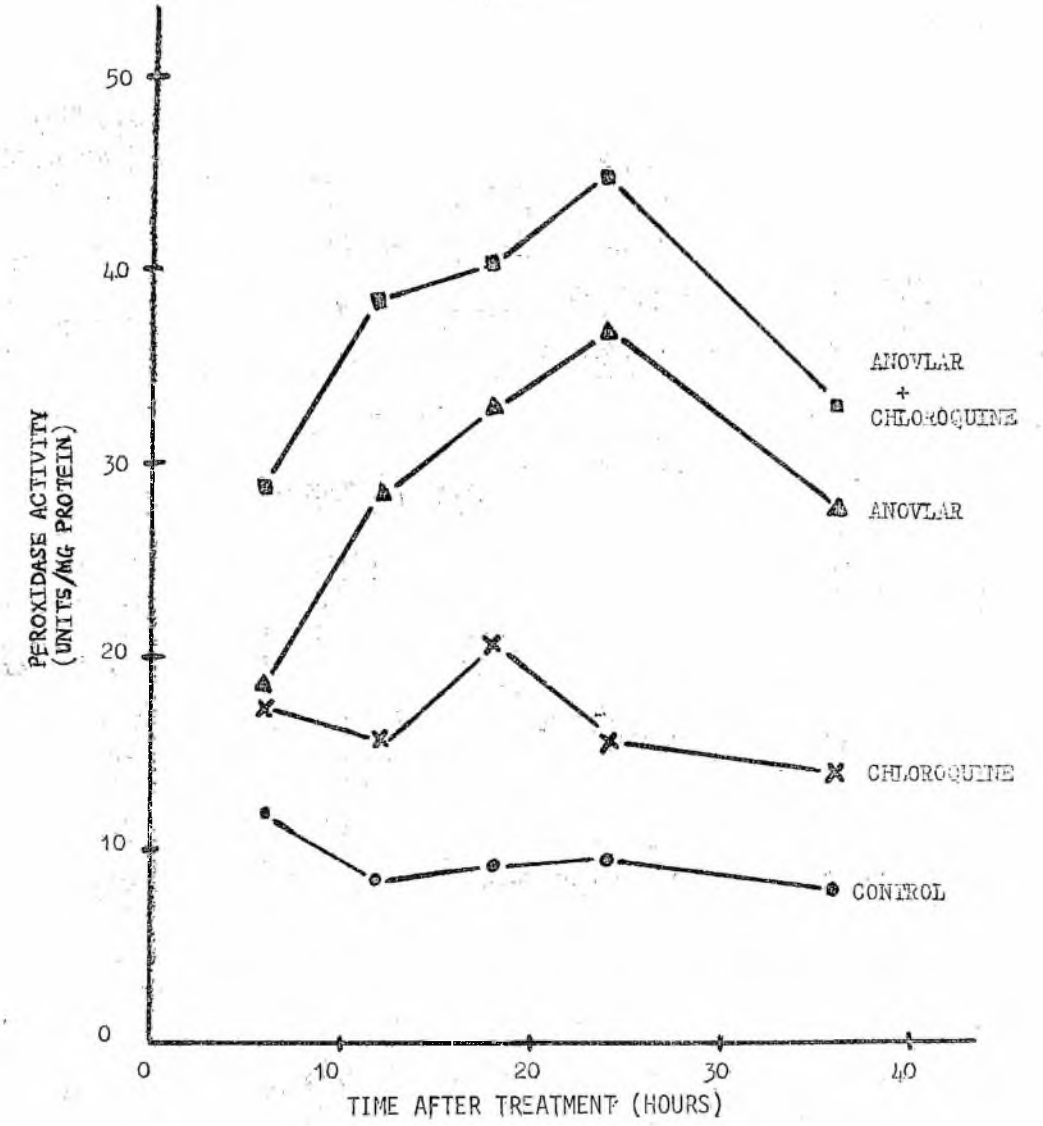


Table 6: Degree of increase in peroxidase activity

Time	Chloroquine	Anovlar	Chloroquine & Anovlar
6	1.44	1.56	2.42
12	1.90	3.40	4.60
18	2.27	3.62	4.44
24	1.61	3.85	4.66
36	1.77	3.53	4.21
Average	1.80	3.20	4.10

This table gives an average of 1.8-fold increase in peroxidase activity for chloroquine, 3.2-fold for anovlar and 4.1-fold for the combined treatment. The peroxidase activity for the control experiment is taken to be 1.00.

Table 7 : Comparative effects of chloroquine and/or anovlar treatments (based on the action of anovlar as 100%)

Time of Treatment	Anovlar	Chloroquine	Chloroquine + Anovlar
6	100	92	155
12	100	56	134
18	100	63	124
24	100	42	122
36	100	50	117
Average		61	130

The table shows that over the 36 hours period, chloroquine gave an average of 61% of the efficiency of anovlar in increasing peroxidase activity whereas the combined treatment of anovlar and chloroquine produced an average of 130%. Thus chloroquine potentiates the effect of anovlar 21. This effect appears more pronounced at the initial stages of the treatment.

H - Mating Experiments

Adult female rats that had littered once were prepared as described under general methods. They were divided into 4 groups and given appropriate treatments before and during mating. The males were removed on the last day of -treatment and the females were observed for litter production within the next 31 days. Table 11 shows the results of this experiment.

Table 8: Effect of Chloroquine and/or Anovlar on Litter Production

Treatment	No. of rats	No. that littered	Total No. of Litter
Control	8	7	27
Chloroquine	8	2	6
Anovlar	8	0	Nil
Chloroquine plus Anovlar	8	0	Nil

Within the period of observation which exceeded the gestation period of rats (21 - 23 days), almost all the rats of the control group as well as two of those that were given chloroquine, had litter. Animals in the other treatment groups did not have any litter. The number of litter shown is the respective litter size for each group.

DISCUSSION

The effect of estradiol on rat uterine peroxidase activity has been investigated by a number of workers. Lucas et al (1955), McNabb et al (1972) and Lyttle et al (1972) have detected stimulation of peroxidase activity in the rat uterus on estrogen administration. The peroxidase activity is normally very low or absent in the uterus of immature female rats (Lyttle et al, 1972 a & b). The present investigation has initially confirmed the stimulation effect under our laboratory conditions. The crude nuclear free extracts have in all cases been used for the assays.

In line with the previous reports, estradiol was found to induce peroxidase activity in the immature rat uterus. The presence of some peroxidase activity in the uterus of control animals is probably a result of the age of the young rats used. Protein synthesis inhibitors such as puromycin and cycloheximide caused an inhibition of the stimulation effect by estradiol. Cycloheximide caused a 54% inhibition while puromycin caused a 56% inhibition. Lyttle et al (1972) detected an almost 100% inhibition with actinomycin D and cycloheximide. These results therefore show a de novo enzyme synthesis induced by the estrogen in the rat uterus. The degree of inhibition shows that the enzyme has a high protein turnover rate in the uterus. The high turnover rate could be a general property of uterine protein, because the

uterus has been known to exhibit a rapid cell renewal (Neufeld et al, 1958), and a high protein turnover rate will be necessary for quick repair of the uterine tissue.

The study of the action of estrogens on the uterus has been further extended by using the estrogen containing oral contraceptive, Anovlar 21. Similarly, this contraceptive (the pill) was found to increase peroxidase activity in the rat uterus. This observation is analogous to induction of enzyme synthesis due to ethinyl estradiol in the pill which is a synthetic derivative of estradiol. It will, however, be observed that the level of increase in peroxidase activity by anovlar 21 (30%) is lower than that by estradiol (85%), Table 2. The pill contains the progestin, noresthisterone acetate which has an anti-estrogenic effect (Spaziani, 1975) and could therefore have antagonized the action of the estrogen in the pill, thus causing a low stimulation of the peroxidase activity. This antagonism was evident in spite of the fact that the pill has a higher level of estrogen derivative than the estradiol 17β given (each pill has 0.05mg ethinyl estradiol while each rat was given 0.002 mg estradiol 17β). Another factor for the lower effect of the pill could be the mode of administration; the pill was given orally whereas estradiol 17β was given by injection. The amount absorbed from the gut will be an important factor.

A similar -investigation as reported above has been done with chloroquine phosphate. This antimalarial drug was also found to cause about 32.1% increase in peroxidase activity in the rat uterus, compared to a 30% increase caused by the pill. The increase in peroxidase activity due to chloroquine has been investigated with cycloheximide. In this experiment, chloroquine caused a 114% increase in peroxidase activity over the control. The stimulatory effect by chloroquine was completely suppressed by cycloheximide which further caused about a 22% inhibition (Table 4). Though the stimulation by chloroquine is similar to that obtained by estradiol, it cannot be given the same interpretation. This is because chloroquine is known to inhibit DNA replication and transcription and consequently protein synthesis (Ciak et al, 1966). Thus the increase in peroxidase activity caused by chloroquine cannot be said to be a direct induction of enzyme synthesis. An interpretation can however be made indirectly by way of the possible effect of chloroquine on a system that may cause peroxidase synthesis in the uterus. Chloroquine has been found to increase estrogen binding to estrogen receptors in the uterine cytosol (Anato-Dumelo, 1979). Thus chloroquine may cause more bound estrogen to be available in the uterus, and this may be a contributory factor to the increase in the enzyme activity.

On uterine weight chloroquine has not caused any appreciable change under the conditions of the experiment. Nelson &Fitzhurgh(1948) observed that chloroquine increases slightly the fat content of the uterus.

This might not have any detectable effect on the uterine weight since fat has a low density. In any case in these experiments, most fats were removed from the uteri. Both estradiol and anovlar, on the other hand, were found to cause large increases in uterine weight. Oral contraceptives have been known to cause salt and water uptake and retention by the uterus (Kistner, 1969).

The present investigation has employed only physiological levels of chloroquine. It was found that chloroquine has consistently caused an increase in peroxidase activity over a dose range of 0 - 20mg/kg. wt. Within this physiological range, peroxidase activity was directly proportional to the dose of chloroquine administered.

Enzyme extracts with known activities have been incubated with chloroquine *in vitro* and re-assayed. This has been done to find out whether chloroquine will affect isolated enzyme molecules.

The *in vitro* activity of peroxidase has been known to be affected by a wide range of both organic and inorganic substances (Hollander, et al, 1959, Klebanoff, 1959, Klebonoff, 1966 and Jellinck *et al*, 1970). The results obtained showed that chloroquine does not affect peroxidase activity *in vitro*. This observation confirms the possibility that chloroquine has no direct activation effect on peroxidase activity *per se* even though *in vivo* experiments show that chloroquine increases peroxidase activity. Also cycloheximide (a protein synthesis inhibitor) caused an effective inhibition of chloroquine stimulation of peroxidase

in vivo. The action of chloroquine on peroxidase activity in the uterus will therefore not be one of a direct activation of enzyme molecules, but may be rather indirectly involved in the enzyme synthesis.

The activity pattern of peroxidase induced by both anovlar and chloroquine have been studied. Lyttle *et al*, (1972 & 1977) reported that estradiol-induced peroxidase activity reached a maximum in 20 hours and persisted for 24 hours. The present studies showed that anovlar-induced peroxidase activity reached a maximum in 24 hours. Chloroquine stimulation of the peroxidase reached its peak in 18 hours. This observation gives the impression that chloroquine may have a faster effect than anovlar in stimulating peroxidase activity. The difference in time of action could be traced firstly to the mode of administration. Anovlar has been given by mouth while estrogen and chloroquine were injected. This in itself will cause differences in the time of response by the target organ. The other factor is the presence of the anti-estrogenic progestin component of the anovlar. The times of response of chloroquine and estradiol (18 and 20 hours respectively) are essentially similar. The differences in the solubility and ionisation of the molecules in water as well as permeability could have affected their transport and therefore the period of response by the target organ. Chloroquine being a more soluble molecule than estradiol will be expected to be transported faster as well as being taken up more readily.

Assuming that peroxidase is important in the contraceptive action of the pill, then the present findings suggest that on the basis of peroxidase activity the effective duration of the contraceptive action of the pill in the rat uterus reaches maximum in 24 hours and falls thereafter. These results are further confirmed by the results in Fig 8 where the stimulation effects of anovlar and/or chloroquine were determined simultaneously. When the combined anovlar and chloroquine treatment was administered to the animals, the resultant peroxidase activity was higher than that produced by either drug/treatment given alone. The pattern of peroxidase activity with time, follows that of anovlar, reaching maximum around 24 hours (Fig. 8). A high peroxidase activity is obtained within a very short period after administration. The maximum peroxidase activity obtained on anovlar alone in 24 hours is 35 units/mg. protein. Assuming this value to be the peroxidase activity required for an effective contraceptive action, the combined treatment

attains this level of activity in 10 hours. This means that maximum contraceptive action, in the case of the combined treatment, occurs earlier than when anovlar is given alone. Also whereas in the case of anovlar the high peroxidase activity stays on for a very short time and falls thereafter, with the combination, the effect persists for about 22 hours. Thus the period for the contraceptive action of peroxidase is greatly enhanced with the combined treatment. It is therefore demonstrated that chloroquine potentiates the peroxidase induction action of anovlar to an average level of 130%. The degree of potentiation is not additive. Chloroquine causes a 1.8-fold increase, anovlar a 3.2-fold increase and the combination a 4.1-fold increase in peroxidase activity (Table 6). This non-additive potentiation suggests that similar mechanism and receptor sites might be involved in the peroxidase stimulation by anovlar and chloroquine. The implication is that when the receptor sites are saturated, no more chloroquine or estrogens from anovlar would be taken up. Such a situation would then produce a non-additive potentiation.

In the case of individual treatment where receptor sites might not be saturated, each treatment could show its maximum effect. If different and independent receptor sites were involved, then each component of the combined treatment would have elicited its maximum effect separately thus producing an additive result.

The significance of the results discussed above have been tested in mating experiments. Production of litter by the rats given anovlar or chloroquine or both, has been prevented in each of these cases. The case with anovlar can be said to be a definite contraception. Chloroquine is known to cause the abortion of foetuses when the drug is taken during early pregnancy (Engmann, et al, 1974). The absence of litter in the experiments where chloroquine or its combination was administered showed that chloroquine has a contraceptive effect. In the mating experiments, 6 out of the 8 rats which were given chloroquine did not produce any litter (Table 8). This result which represents 75% of the chloroquine treated animals seems to correlate that on the comparative effect of chloroquine, based on the action of anovlar, in enhancing peroxidase activity. In this case, chloroquine showed an average of 60% of the efficiency of anovlar (Table 7). The enhancement of peroxidase activity by both anovlar and chloroquine seems to be related to the biochemical basis for the observed contraceptive action.

CONCLUSIONS

1. Induction of peroxidase synthesis by estradiol and its inhibition by some protein synthesis inhibitors has been confirmed.

2. Induction of peroxidase activity by the contraceptive, Anovlar 21 is demonstrated.

3. Chloroquine increases the peroxidase activity in immature rat uterus. This enhancement is inhibited by cycloheximide.

4. Anovlar - induced peroxidase activity reaches its maximum in 24 hours while chloroquine causes peroxidase activity to reach a maximum in 18 hours.

5. Peroxidase activity has been shown to be a function of chloroquine dose (*in vivo*) over a range of 0-20 mg/kg. body weight.

6. Chloroquine does not affect peroxidase activity *in vitro*.

7. Chloroquine potentiates anovlar's effect in inducing peroxidase activity.

8. Chloroquine, anovlar or their combination prevents the production of litter by adult female rats.

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