Steroid Hormones and the Pill

Two related symposia—"Physiological Control of Conception and Its Implications" (Sunday, 26 December) and "Mode of Action of Steroid Hormones" (29 and 30 December)—are among the most timely sessions that will be held at the AAAS meeting in Berkeley. (Many other sessions, but by no means all, are described briefly in the November *AAAS Bulletin*. The entire program will be published in the 4 December issue of *Science*.)

Birth Control Pill and Its Implications

For over 30 years endocrinologists have been able to control ovulation in mammals by administering certain steroid hormones. A little more than 10 years ago clinical testing of steroids as contraceptive agents was begun, and so successful were the tests that today several million women are using steroid pills to prevent pregnancy.

The pills contain compounds that are closely related chemically to the hormone progesterone. Progesterone is produced at one stage of the menstrual cycle in amounts large enough to prevent ovulation. The hormonal action is not directly on the ovary but is mediated through the hypothalamus, where the follicle-stimulating hormone that promotes ovulation is produced. Progesterone inhibits the production of follicle-stimulating hormone.

Early work with laboratory animals and later clinical studies showed that progesterone administered orally would inhibit ovulation. Large doses were required, however, and in the clinical studies these produced undesirable side effects and imperfect control of menstruation. It was not until more biologically active steroids which did not produce undesirable side effects became available that oral contraception became practical.

The progestational agents are structurally related to progesterone and fall into two classes—progesterone derivatives and 19-norsteroid derivatives. Both these compounds are widely used in the pills Provera (medroxyprogesterone acetate) and Enovid (norethynodrel). Several other similar substances are used in other trade preparations.

In the normal menstrual cycle, menstrual flow results from a series of physiological events which include ovulation. When ovulation is inhibited, as it is following fertilization and implantation of the ovum, menstrual flow is prevented. When oral contraceptives are used, a controlled menstrual flow can be made to occur even though there has been no ovulation. This is accomplished by withdrawing the drug for several days. Normally the progestational agent is taken orally for 20 days and is then withdrawn for 5 days, then taken again for 20 days.

A large-scale clinical test of oral contraceptives has been carried on in Haiti and Puerto Rico by Gregory Pincus and his co-workers at the Worcester Foundation for Experimental Biology. They have found that continued use of progestational agents effectively prevents pregnancy without affecting fertility. In fact, the evidence indicates that fertility is actually increased when an oral contraceptive is used for from 1 to 4 years and is then withdrawn.

Although some women experience undesirable symptoms, such as gastrointestinal disturbance and change in body weight, when they first begin to use oral contraceptives, on continued use the symptoms largely disappear. Concern that these steroids might produce pathological changes led to an extensive study of vaginal smears and cervical biopsies of women using oral contraceptives. The prevalence of smears indicative of pathological changes was no higher in women using oral contraceptives than in those not using them, and was significantly lower among the users than in women using vaginal contraceptives or intrauterine devices.

Although much meticulous research has been done on the medical aspects of oral contraception, there are many implications that have not been considered. The AAAS Committee on Science in the Promotion of Human Welfare has arranged a symposium to consider some of these implications. Margaret Mead, Walter Modell, and Gregory Pincus organized the symposium —"The Physiological Control of Contraception and its Implications"—to be held at the Berkeley meeting on 26 December. Modell will serve as chairman. The symposium will review the medical research that has already been done on the use of progestational agents for contraception, and will consider areas in which further research is called for. Pincus will review the medical research. Steven Plank of the Harvard School of Public Health will discuss the potential risks and benefits consequent to the widespread use of oral contraceptives and will indicate areas of needed research.

Martin B. Loeb, director of the School of Social Work, University of Wisconsin, will give a sociological assessment of the changes in sexual technology and social structure that are resulting from the new technique of contraception.

Some of the psychological implications of the problem will be considered by George H. Pollock, director of Research of the Institute for Psychoanalysis, Chicago. Pollock has been doing research on reproductive and productive need as contrasted with genital sexual gratification and its relation to contraception.

Rhoda Métraux of the American Museum of Natural History will discuss some anthropological aspects of the problem, and the session will conclude with a summing up of the symposium from a broad philosophical point of view by René Dubos of the Rockefeller University.

Mode of Action of Steroid Hormones

Another symposium, on the mode of action of steroid hormones, will be held in five sessions, on 29 and 30 December. In the first two sessions hormones of the adrenal cortex will be considered—glucocorticoids (morning and early afternoon) and aldosterone (late afternoon). On 30 December the morning session will consider the mode of action of the estrogens, and the early afternoon session, the

mode of action of the androgens. In the late afternoon two papers on progestational agents will be presented. H. A. Lardy of the University of Wisconsin has arranged the symposium and will chair the final session.

Early research on the steroid hormones was concerned with their isolation and synthesis and with their effects on the metabolism of whole organisms and on isolated tissues. Newer techniques, including the use of labeled compounds and specific inhibitors, have made it possible to identify specific metabolic reactions which are controlled by steroid hormones. Several of these hormones have been shown to induce the synthesis of enzymes, and the enzyme synthesis follows an increase in the synthesis of the three major types of RNA. The steroid-mediated induction is believed to reflect a hormonal influence on the mechanisms by which the information contained in the genetic code of DNA is used in the synthesis of RNA templates.

In one of the ten papers to be presented during the session on glucocorticoids, Francis T. Kenney and his coworkers will discuss their work on the mechanisms involved in the induction of hepatic enzymes by hydrocortisone. They have found that the tyrosine transaminase of the livers of adrenalectomized animals is reduced when the animals are subjected to severe stress. The level of the enzyme is selectively increased by administration of hydrocortisone. The decrease in the enzyme under stress is believed to be mediated by a hormone which stimulates the formation of a repressor which stops the RNA-directed polymerization of amino acids into the enzyme. Hydrocortisone initiates new RNA synthesis and thereby stimulates enzyme synthesis.

Allen Munck has studied the glucocorticoids and glucose interactions with liver, adipose tissue, and thymus cells. He investigated adipose tissue and thymus cell suspension in vitro and in vivo. He found that cortisol and corticosterone decreased glucose uptake by adipose tissue and also the incorporation of radioactive glucose into lipid, protein, and other fractions of rat thymus. Since cortisol decreases the lactic acid output of thymus cells, and also depresses slightly the levels of glucose-6-phosphate, Munck believes that the hormone acts either by inhibiting phosphorylation of glucose at the level of hexokinase or by decreasing the rate of transfer of glucose to the phosphorylating site.

A third paper to be presented at the session on glucocorticoids is "Regulation of gluconeogenesis by adrenal steroids and other factors," by Paul Ray and his co-workers. They have investigated the role of hydrocortisone in enhancing gluconeogenesis in rats. One enzyme that is involved in gluconeogenesis is phosphoenolpyruvate carboxykinase. When this enzyme is specifically inhibited, carbohydrate precursors such as aspartate accumulate in large amounts. Administration of hydrocortisone leads to a further increase in aspartate. Ray and his associates conclude that hydrocortisone facilitates delivery of carbohydrate precursors to the liver.

In his paper "A unified theory of glucocorticoid action, relationship to liver metabolism," Richard W. Schayer proposes that the effects of glucocorticoids on liver metabolism may be due to interference by the steroids with

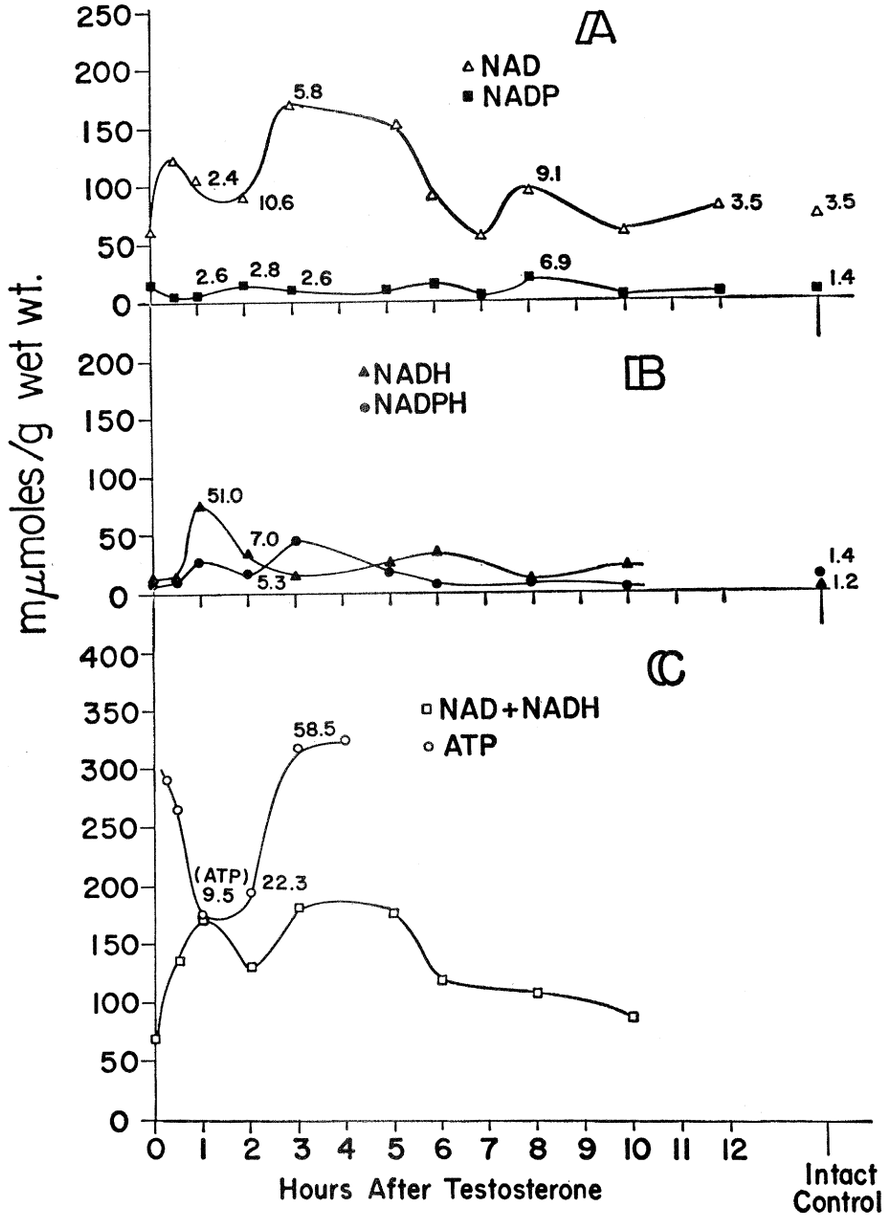

Fig. 1. (*A*) Oxidized (NAD, NADP) and (*B*) reduced (NADH, NADPH) pyridine nucleotide concentrations in prostates of castrate rats given one 150-μg injection of testosterone and killed at the indicated intervals after injection, and in prostates of intact control rats (points at right end of the curves). (*C*) Concentrations of oxidized plus reduced NAD and of ATP. The values (not shown) for prostatic concentration of ATP in intact control rats and in castrate controls, respectively, are 765 and 649 mμmole/g. The standard error of the mean is indicated beside each point where three or more determinations were made. Each determination was made in duplicate from prostatic tissue from five or six rats.

the synthesis of the intrinsic dilator, histamine, with a consequent change in blood flow through liver capillaries. Several lines of evidence support this hypothesis—for example, the fact that stressors which increase synthesis of histamine suppress corticoid-induced metabolic changes.

The first paper in the second session of the symposium is "Stimulation of RNA and protein synthesis, in vivo, in the rat by aldosterone," by Harold E. Williamson and his co-workers. They have found that the antinatriuretic action of aldosterone is inhibited by actinomycin D, an antibiotic which inhibits the DNA-directed synthesis of RNA. They have shown, further, that the synthesis of renal RNA is stimulated by aldosterone, and that this synthesis is inhibited by actinomycin D. Their observations are consistent with the hypothesis that aldosterone stimulates the synthesis of DNA-directed RNA, which, in turn, stimulates the ribosomal synthesis of an enzyme which is involved in the transport of sodium by the renal tubules.

Geoffrey Sharp and Alexander Leaf have studied the mode of action of aldosterone on sodium transport in toad bladders in vitro. With this organ they have studied the increase in sodium transport which is induced by aldosterone. They have found that the hormone is bound in the tissue in two ways, but that the physical presence of the hormone is not required at the time its physiological response is elicited. They have obtained evidence which indicates that the hormonal effect is to increase the permeability of the mucosal surface to sodium.

Studies on the identification of the molecular receptor for aldosterone have been carried out by Darrell D. Fanestil. Using tritium-labeled aldosterone he has found that aldosterone is bound in the nuclei of kidney cells, and he suggests that it is there that the hormone exerts its influence on the control of sodium transport.

The concluding paper of the aldosterone session will be given by Grace M. Fimognari. She has been concerned with the role of oxidative phosphorylation on the action of aldosterone in sodium transport by isolated toad bladder. Her research suggests that aldosterone acts by stimulating a step, or steps, in the trichloroacetic acid cycle at a point between condensing enzyme and α-ketoglutarate dehydrogenase.

The third session of the symposium will be chaired by E. A. Doisy of St. Louis University, who pioneered research on the estrogens more than 35 years ago. Elwood V. Jensen, who will give the first paper in this session, has used estradiol labeled with tritium to study the estrogen receptors in target tissues. Hormone-responsive tissues such as the uterus and vagina contain a substance that binds estradiol strongly. The interaction of the estrogens with receptor sites and the subsequent uterotrophic action appear to take place without chemical transformation of the hormone molecule.

Clara Szego's research leads her to conclude that the characteristic metabolic responses to estrogen are secondary to alterations in the availability of metabolites resulting from histamine-induced expansion of the microcirculation. Evidence for this is the local liberation of histamine during the early action of estrogen in the uterus, and the similarity of histamine and estradiol in effecting accumulation of water by the uterus and the incorporation of C^{14}-labeled amino acids into uterine proteins.

Using tritium-labeled estradiol, Jack Gorski and his collaborators have studied the receptor in rat uterus. They have found that the receptor is a large protein of molecular weight about 200,000, and they estimate that there is only 6×10^{-3} microgram of this protein in one immature rat uterus. Shortly after the interaction of estrogen and the receptor, protein synthesis is stimulated. The increase in protein synthesis is not affected by actinomycin D, a finding which suggests that the hormone is acting at a translational level beyond the step of DNA-controlled RNA synthesis.

Phosphovitin is a phosphoprotein, present in egg yolk and in the plasma of hen's blood, which forms a complex with ferric iron. The protein is normally absent from the blood of cockerels, but its formation can be induced by injecting the cockerel with an estrogen such as diethylstilbestrol. Olga Greengard has studied the induction of phosphovitin in cockerels and has found that the synthesis is inhibited if actinomycin is injected along with the hormone. This indicates that the hormone action lies between DNA and the synthesis of RNA.

Not only diethylstilbestrol but the urine of pregnant women increases the phosphovitin content, and consequently the iron content, of the plasma of cockerels. This provides a simple qualitative test for estrogens, since the difference between the very low iron concentration in the plasma of uninjected cockerels and the concentration in cockerels injected with material containing more than 4 micrograms of estradiol can be seen with the naked eye upon the addition of an iron reagent.

The first paper in the session on the mode of action of the androgens is on the regulation of ribonucleic acid and protein biosynthesis by androgens. Charles D. Kochakian and his coworkers have investigated the effects of castration and subsequent administration of androgen on the RNA content of mouse kidneys. Testosterone propionate enhances the incorporation of orotic acid into the pyrimidines of the RNA of mouse kidney.

Another paper in this session is entitled "NAD biosynthesis and redox rearrangement as an early part of androgen action." Carl Ritter and his co-workers at the University of Pennsylvania have studied the NAD:NADH relationship in the prostates of castrated rats following the injection of testosterone. Some of their observations are recorded in Fig. 1. Within half an hour after the administration of testosterone the concentration of NAD doubles; it then falls, and there is a corresponding rise in NADH. The hormone seems to have less effect on the concentrations of NADP and NADPH, though the tissue concentration of NADPH is elevated about fourfold 3 hours after injection of the hormone. The concentration of adenosine triphosphate (ATP) decreases at first and then rises to a stable maximum 3 hours after injection of testosterone into the castrated rats. Ritter and his associates believe that, since actinomycin D and puromycin do not inhibit the NAD response, the androgen activates processes independent of its activation of RNA and protein synthesis, and that such activation is a redirection of energy metabolism toward the more efficient production of ATP.

Two papers will comprise the last session of the symposium. Walter Wiest of Washington University will discuss his research on progesterone and pseudopregnancy in the rat, and Gregory Pincus will report on the work that he and his co-workers at the Worcester Foundation for Experimental Biology have done on the steroidal control of fertility in mammals, the topic on which he is also reporting in the symposium on the physiological control of conception and its implications.