The Influence of Estrogens, Progestins, and Pregnancy on the Liver

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I. Introduction

The central role which the liver plays in the chemical transformation and biological inactivation of estrogens and progestins in man is well known. In recent years there has been a further awareness that in this metabolic interaction, the hormones can act directly or indirectly on the liver to produce a variety of biological effects that may have both physiological and pathological significance. The purpose of the present review is to summarize the biological effects produced in the liver of man and ex-

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perimental animals by estrogens and progestins; in addition, the effects of pregnancy on the liver are reviewed because during normal gestation the mother and fetus are exposed to high concentrations of these hormones (J. Fishman *et al.*, 1962; Hytten and Leitch, 1964; Yousem and Strummer, 1964). Inclusion in this review of studies dealing with changes in the liver associated with pregnancy does not imply that these changes are attributable solely to the individual effects of estrogens and progestins. Although these steroid hormones can be implicated as causative agents in a number of pregnancy-induced alterations in liver function, their relation to other instances of such alterations must still be considered presumptive. The effects of pregnancy on the liver are therefore considered as independent phenomena, and their relationship to estrogens and progestins is analyzed where experimental data are available. The same considerations apply to certain hepatic effects associated with the clinical use of oral contraceptives containing synthetic estrogens and progestins.

In addition to the effects on the liver itself, certain alterations in the constitutents of circulating plasma are also reviewed here, in cases where such alterations are considered to be a secondary, but physiologically or clinically meaningful, manifestation of hormonal actions on the liver. The influences of estrogens and progestins on isolated enzymes or subcellular structures have not been considered to fall within the scope of this review unless they present tangible physiological significance. Other clinical and experimental aspects of steroid pharmacology pertinent to the present topic have been reviewed previously (Kappas and Palmer, 1963; J. Kottra and Kappas, 1967).

II. Anatomic Alterations

A. Liver Weight

An increase in the weight of the liver in pregnant animals has been well documented (Poo *et al.*, 1939; Kennaway and Tipler, 1947; Schwenk and Joachim, 1961). Liver weight increases progressively in pregnant rats, remains elevated in the early puerperium and during lactation, but declines and approaches control values about one month after delivery (Poo *et al.*, 1939; Schwenk and Joachim, 1961). On day 18 of gestation, the average weight of the liver in female rats weighing 150 gm at the time of impregnation was noted by Poo *et al.* (1939) to be 8.835 gm in comparison to 6.271 gm in nongravid control rats. An increase of comparable magnitude was also reported for pregnant mice (Kennaway and Kennaway, 1944). A large proportion of the weight increase in the rat can be accounted for by fractional increases in the content of water, phospholipids, protein, and ribonucleic acid (RNA) in the liver (Campbell and

Kosterlitz, 1949). Of these components, water appears to undergo the greatest increment.

Data concerning the weight of the liver in pregnant women are few. In a review of autopsy records, Combes *et al.*, (1963) found no significant difference in the liver weight of women in early and late pregnancy who died after brief illnesses. In surgical exploration of pregnant women with intrahepatic cholestasis of pregnancy the gross appearance of the liver was generally normal although one instance of slight enlargement was reported (Ikonen, 1964).

In experimental animals, administered estrogens (Griffiths *et al.*, 1941; Korenchevsky *et al.*, 1941; Gallagher *et al.*, 1966) as well as progesterone (Hines, 1967) bring about significant enlargement of the liver. For example, 2 weeks' exposure to diethylstilbestrol, estriol, or estradiol implanted subcutaneously in intact rats (Griffiths *et al.*, 1941) resulted in increments of liver weight ranging from 17 to 52%; and progesterone, when administered intraperitoneally for 9 days at 50–100 mg/kg/day, provoked comparable weight increases (Hines, 1967). No experimental data are available concerning the effect of estrogen-progesterone combinations on liver weight. Repeated exposure to relatively large doses of estrogens over a period of days seems to be essential for the induction of liver enlargement. A single intravenous administration of 0.5 mg of estradiol in mice (Thompson *et al.*, 1966) does not alter the liver weight significantly. This contrasts with the well-known effect of estrogens on one of their classical target organs, the uterus; a single physiological dose of estrogen, for example, induces in the uterus of immature or ovariectomized animals a rapid growth and enlargement characterized by hyperemia and imbibition of water (G. C. Mueller, 1957; Hechter and Halkerston, 1965). Such spurts of uterine growth are noticeable within hours after the administration of estrogen (Astwood, 1938), whereas the liver remains unaffected by such treatment.

B. Microscopic Alterations

The histological appearance of the liver in normal human pregnancy is generally unremarkable. In 17 pregnant women, from whom liver biopsies were taken during delivery (Ingerslev and Teilum, 1945), the only notable findings were variations in the size of the liver cells and nuclei, small lymphocytic infiltrations in portal areas in some specimens, scattered fat vacuoles near centrolobular areas, and hypertrophic Kupffer cells in sinusoids. On the whole, this histological picture varies little from the picture found in normal, nonpregnant humans. Similar conclusions have been reached by Nixon *et al.* (1947) and Dietel (1947). Thus, the histological entity described by Hofbauer (1908, 1911) as *Schwanger-*

schaftsleber, or "pregnancy liver," consisting of fatty infiltration, glycopenia, and cholestasis in the centrolobular area and purporting to be characteristic of the liver in pregnant women, is only of historical interest. It is clear now that such morphological findings were attributable to terminal events unrelated to pregnancy.

The effects of estrogens on the histological appearance of the liver may depend in part on the degree to which the functional status of the organ is affected. M. N. Mueller and Kappas (1964a) obtained percutaneous biopsies of the liver from 6 patients without evidence of liver disease before and after administration of estradiol in amounts (50–200 mg/day for 17–39 days) that produced impairment in hepatic metabolism of sulfobromophthalein (BSP) without overt signs of liver disease such as jaundice or pruritus. This study was conducted to evaluate the effects of estrogen on hepatic function, especially the hepatic handling of BSP. No alterations in histological appearance, detectable by light microscopy, were induced by such treatment. In a similar study, Kleiner *et al.* (1965) noted no alterations in liver biopsy specimens obtained from normal women after they had taken an oral contraceptive preparation for several months. Study of the tissues in the latter report included examination by light and electron microscopy as well as by histochemical stains for various phosphohydrolases.

On the other hand, significant histological alterations become readily noticeable in the liver of those women who develop overt clinical symptoms and signs of hepatic dysfunction as a result of estrogen therapy (Kreek *et al.,* 1967a) or after taking oral contraceptives containing various combinations of synthetic estrogenic and progestational hormones (Palva and Mustala, 1964; Larsson-Cohn and Stenram, 1965; Broake *et al.,* 1965; Haemmerli and Wyss, 1967). These patients generally present clinically with signs and symptoms of frank disturbances of hepatic function including jaundice, pruritus, delayed clearance of BSP from plasma, elevated serum alkaline phosphatase activity, and mild elevation of serum transaminase activities. Histologically, the liver in these individuals shows normal lobular architecture, bile thrombi in bile canaliculi, and few inflammatory infiltrates (Larsson-Cohn and Stenram, 1965). Hepatocellular damage, if present, is limited to centrolobular areas (Stoll *et al.,* 1965). On examination in the electron microscope, liver sections show dilatation of bile canaliculi, bile stasis, shortening and blunting of microvilli, and dilatation of endoplasmic reticulum, which often lacks ribosomes (Broake *et al.,* 1965; Larsson-Cohn and Stenram, 1965). These morphological findings are the well-known changes which have been shown to accompany the so-called intrahepatic cholestases of various etiologies, most prominently drug toxicity following such agents as chlor-

promazine and testosterone analogs (Schaffner and Popper, 1960; Albot *et al.*, 1965).

The incidence of jaundice following the ingestion of oral contraceptives has not been accurately established. Taking into consideration the large number of women (Swyer, 1967) currently receiving contraceptive steroids and the sporadic reports of complicating jaundice that have appeared in the literature (Carlström *et al.*, 1965; Haemmerli and Wyss, 1967; Ockner and Davidson, 1967), one can surmise that the incidence must be low.

A natural counterpart of the oral contraceptive-induced jaundice appears to be the so-called "idiopathic jaundice of pregnancy" or "intrahepatic cholestasis of pregnancy" (Svanborg, 1954; Moore, 1963; Ikonen, 1964; Haemmerli, 1966). This is a clinical entity occurring in late pregnancy and is characterized by jaundice, pruritus, BSP retention, and elevated serum activities for alkaline phosphatase and 5′-nucleotidase. Elevation of serum transaminase activities is usually slight. The syndrome often recurs during the course of subsequent pregnancies. Biopsies of the liver (D. F. Brown *et al.*, 1963; Kater and Mistilis, 1967) show the microscopic characteristics of an intrahepatic cholestasis essentially indistinguishable from the histological appearance of the liver in the cases of estrogen-induced jaundice described above.

It appears therefore that the development of such histological alterations may be a feature confined to those women in whom overt signs and symptoms of liver dysfunction, including jaundice and pruritus, are noted during the course of pregnancy or treatment with estrogens and progestins.

III. Nucleic Acid and Protein Metabolism

The uterus is exquisitely sensitive to estrogens. In an immature or ovariectomized animal, the uterus undergoes profound physical growth and chemical alterations following the administration of single, minute amounts of these hormones (G. C. Mueller, 1957). A large body of literature has accumulated concerning the specific effects of estrogens on nucleic acid and protein syntheses in this organ (G. C. Mueller, 1957; Gorski *et al.*, 1965; Hechter and Halkerston, 1965). The idea has found wide acceptance that estrogens act in the uterus to regulate gene activity, leading ultimately to the formation of specific RNA's (Barton and Liao, 1967; Trachewsky and Segal, 1967) and proteins, i.e., enzymes, to the activities of which the morphological expression of hormone action is attributable.

In contrast, published reports on the effects of estrogens on nucleic acid metabolism in the liver (Leslie, 1955) are few, a fact not surprising

in view of the very large amounts of these hormones required to induce rather trivial changes. Moreover, the biological significance of such changes may not be immediately apparent.

It has been noted that the mode of uptake and distribution of estrogens in the liver, in comparison to the uterus, show features which may possibly be characteristic of a tissue that is not a "target organ" of these hormones in the classic sense. One important distinction between these organs is reflected in their abilities to take up and retain estrogenic hormones from the circulation. Jensen and Jacobson (1962) showed that, in immature as well as in actively growing uteri, administered estradiol-6,7-^{3}H reaches a peak level of incorporation at a rate somewhat slower than in the liver, but that the rate of decay from the uterus is greatly delayed, indicating prolonged ability of this organ to take up and retain the hormone. Moreover, essentially all the retained label in the uterus is in the form of chemically unaltered hormone. In contrast, the initial uptake of labeled estradiol is much more rapid in the liver, but subsequent decay is also greatly accelerated. Furthermore, once taken up by the liver, the estradiol is apparently rapidly converted to its metabolites estrone and estriol. Other "nontarget" organs such as the muscle and the kidney display uptake–decay kinetics essentially similar to that of the liver.

A second difference is apparent in the intracellular localization of labeled estrogen in the liver and uterus. Differential centrifugation of homogenized tissues (King *et al.*, 1965a; Wotiz *et al.*, 1963) as well as radioautography (Inman *et al.*, 1965) have been employed to determine the sites of localization in these tissues. A substantial proportion of labeled estradiol (30–40%) administered to rats has been localized in the nuclei of uterine epithelium, whereas in the liver most of the label was recovered in the soluble fraction (King *et al.*, 1965a). The liver nuclei contained only about 5% of the total tissue radioactivity (King *et al.*, 1965a). It is of interest that in other organs that are hormonally highly responsive to estrogens, viz. the anterior pituitary and mammary tumors, over 60% of the label was found in the nuclei (King *et al.*, 1965a,b). These observations may imply that one of the special characteristics of a tissue that responds to estrogen with pronounced cell growth may be its ability to retain the hormone in an active form for an appreciable length of time, possibly at some estrogen-binding site (Gorski *et al.*, 1965; Eisenfeld and Axelrod, 1966) in the nucleus.

A. DNA

Campbell and Kosterlitz (1949, 1953) noted that in pregnant mice and rats, the deoxyribonucleic acid (DNA) content of the liver was slightly increased and that the increase exceeded by 10–15% the rise which might

be predicted (Campbell and Kosterlitz, 1950) from the increase in maternal body weight. The significance of this finding is not clear because of the heterogeneity of cell types in the liver and the lack of accurate information concerning their proliferative activity (e.g., mitotic indices). The following observations suggest that this small increase in DNA content may be the result of reticuloendothelial, rather than parenchymal, cell proliferation in the liver: (1) increased activity of the reticuloendothelial system in pregnant mice and rats (Nicol *et al.*, 1964), as determined by phagocytic activity; (2) stimulation of the hepatic reticuloendothelial system by estrogens (Kelly *et al.*, 1960; Nicol *et al.*, 1964), as determined by phagocytic activity as well as survival time against bacterial infection (but weak or no stimulation by progesterone (Nicol *et al.*, 1964, 1965)); (3) increase in the relative number of hepatic reticuloendothelial cells following estrogen administration (Kelly *et al.*, 1960); and (4) increased synthesis of DNA in the liver after estrogen treatment (Kelly *et al.*, 1960; Thompson *et al.*, 1966) and localization of a labeled DNA precursor solely in the reticuloendothelial cells, as demonstrated by radioautography (Kelly *et al.*, 1960).

B. RNA and Total Protein

A remarkable increase in the RNA content of the liver in pregnant rats, mice, and guinea pigs has been noted by Campbell and Kosterlitz (1949, 1953). In the rat, the rate of incorporatin of $^{32}P_i$ into RNA was also increased (Campbell and Kosterlitz, 1949), reaching a peak value near term, and returning to normal levels after parturition. The RNA content of the liver in nongravid animals generally parallels its protein content; during pregnancy, however, the increase in RNA exceeds the increase noted for protein (Campbell and Kosterlitz, 1953). The production of such "excess RNA" in the liver is apparently dependent on a viable placenta, since removal from the gravid rat, on days 14 or 15 of pregnancy, of ovaries, adrenals or pituitary, singly or in various combinations, did not affect the production of this RNA. When the placentae were removed, in addition to fetuses and various endocrine organs, a large proportion of the "excess RNA" was eliminated from the liver.

Estrogen appears to be responsible, at least in part, for the increased RNA content of the liver in pregnant animals. Estradiol administered to intact female rats for 14 days in doses of 20 and 200 μg/day resulted in a significant increase in liver RNA, whereas progesterone at 1–4 mg/day had no appreciable effect (Campbell *et al.*, 1953). Again, it appears that repeated administration of estrogens over a period of time (10–14 days) is a prerequisite for the hormone effect on liver RNA. A single dose of 0.5 mg of estrogen given to intact mice (Thompson *et al.*, 1966) caused

no alteration of RNA content during a period of 10 days. Further characterization of such estrogen-induced RNA has not progressed beyond the localization of labeled RNA precursors in subcellular fractions of the liver. Iwamoto *et al.* (1963) fractionated the homogenate of the liver obtained from estrogen-treated rats by means of differential centrifugation. They noted an enhancing effect of estrogen on the incorporation of $^{32}P_i$ into nuclear RNA and into a species of uncharacterized metabolically active RNA's present in the supernatant fraction (Kusakari *et al.*, 1963).

Total protein content of the liver in experimental animals has been shown to parallel the amount of protein ingested (Campbell and Kosterlitz, 1953). A slight but significant rise in the protein content of the liver appears to take place in pregnant rats (Campbell and Kosterlitz, 1949) placed on a stock diet. At moderate to high levels of protein consumption, the increase in the total protein becomes more pronounced (Poo *et al.*, 1940). This increase, however, is less than the increase in the total weight of the liver, so that the protein concentration, expressed as the amount of protein per unit weight of the liver, actually declines (Poo *et al.*, 1940). A relatively greater rise in the water content of the liver (Campbell and Kosterlitz, 1949) probably accounts for this phenomenon. It is obvious that such phenomena must be taken into consideration in expressing biochemical data, especially in choosing the reference for enzyme activities that are determined in crude homogenates.

The rate of incorporation of glycine-1-^{14}C (Burt and Dannenburg, 1965) and valine-1-^{14}C (Little and Lincoln, 1964) into the total liver protein is accelerated in pregnant rats. However, a single dose of estradiol (2 μg), which provokes a significant increase in the incorporation of valine-1-^{14}C into the uterine protein of ovariectomized rats, failed to affect the rate of protein synthesis in the liver (Little and Lincoln, 1964). No data are available concerning the effect of a more prolonged estrogen treatment.

C. Hepatic Enzymes

In view of the increments in RNA and protein content of the liver which take place during pregnancy and estrogen treatment, it is perhaps not surprising to find elevated activities and altered isozyme patterns of certain liver enzymes. Of particular interest in this regard is the estrogen effect on the level of glucose-6-phosphate dehydrogenase in the liver. Glock and McLean (1953) first noted a marked sex difference in hepatic glucose-6-phosphate dehydrogenase (G-6-PD) activity in the rat. The average activity of this enzyme, expressed in units per gram of liver, was more

than twice as high in the liver of females compared with males. In ovariectomized and adrenalectomized female and in castrated male rats, 3 weeks' treatment with estradiol at 10–20 μg/day resulted in an approximately 2- to 4-fold increase in the enzyme activity (Huggins and Yao, 1959). The activity of 6-phosphogluconate dehydrogenase, the enzyme that follows G-6-PD in the hexose monophosphate shunt pathway, was also enhanced by estrogens. It has recently been shown that the estrogen-induced increment in G-6-PD activity can be prevented by puromycin administered during the last 5 days of estrogen treatment (Hori and Matsui, 1967). McKerns and Kaleita (1960) and Marks and Banks (1960) independently made the interesting observation that certain C_{17}- or C_{20}-oxo steroids, such as pregnenolone and dehydroepiandrosterone, are potent *in vitro* inhibitors of G-6-PD in various mammalian tissues, e.g., rat adrenal gland, human erythrocytes, human liver, and rat liver. The inhibition appears to be of the noncompetitive type. At concentrations of 10^{-5} and 10^{-7} *M*, these steroids inhibited G-6-PD activities in crude preparations of human and rat livers (Marks and Banks, 1960) to the extent of 10–82%. Sulfation of dehydroepiandrosterone leads to loss of its inhibitory action (Tsutsui *et al.*, 1962).

Recently, Hori and Matsui (1967) have succeeded in separating the G-6-PD of the rat liver into 6 molecular forms, or isozymes, by electrophoresis in polyacrylamide gel and staining with a tetrazolium salt. The isozymes were designated A through F, in the order of increasing mobility toward the anode. An occasional diffuse seventh isozyme was also seen and named E′. The authors noted that the isozyme D had a consistently greater activity in the adult female than in the male rat. It was absent in the newborn rats of both sexes. When male rats were orchiectomized and treated with 0.2–20 μg of estradiol benzoate daily for 20 days, the hepatic G-6-PD activity rose markedly and approached or exceeded the activity noted in the female. Concomitantly, the isozyme D became more active, so that the zymogram now resembled the female pattern. Puromycin given to the rats during the last 5 days of estrogen treatment abolished the increment in the total enzyme activity as well as in the isozyme D. On the other hand, when female rats were treated with dehydroepiandrosterone, a C_{19} steroid related to the androgens, total hepatic activity of G-6-PD decreased and the isozyme D became faint, so that the zymogram now resembled that of a male rat. Although the possible direct inhibitory action of dehydroepiandrosterone at low concentration on the activity of hepatic G-6-PD was not rigorously considered, these results suggest that estrogens may play a role in controlling the synthesis of G-6-PD in the rat liver. They also raise the possibility of estrogen-

androgen interaction in regulating the activity (Willmer and Foster, 1965; Tepperman and Tepperman, 1963), and in determining the sex-linked phenotype, of hepatic G-6-PD.

A substantial amount of evidence has accumulated to implicate estrogens and progestins in the induction of another hepatic enzyme, δ-aminolevulinic acid (ALA) synthetase. This is the first enzyme in the biosynthetic sequence that converts glycine and succinyl coenzyme A to porphyrins and heme, and its activity is considered to be the rate-limiting step (Granick and Urata, 1963; Granick, 1966) in heme biosynthesis. In acute intermittent porphyria, an inborn error of metabolism in man which is transmitted as an autosomal dominant trait (Waldenstrom, 1957), the genetic defect is thought to result in an excessive production of ALA synthetase (Tschudy *et al.*, 1965; Nakao *et al.*, 1966). A significant but less marked increase in ALA dehydrase, a second enzyme in the biosynthetic chain, is also seen. Patients with this disorder experience episodic abdominal pain and various neurological symptoms and excrete in their urine a large amount of the porphyrin precursors, δ-aminolevulinic acid and porphobilinogen. In experimental animals, a disease resembling human porphyria can be produced by a variety of chemicals, e.g., 3,5-dicarbethoxy-1,4-dihydrocollidine (Granick and Urata, 1963) and allylisopropylacetamide (Goldberg and Rimington, 1955; Gray *et al.*, 1961). The treated animals show a greatly elevated hepatic ALA synthetase activity and excrete augmented amounts of urinary δ-aminolevulinic acid and porphobilinogen. The increase in ALA synthetase activity can be inhibited by actinomycin D, 5-fluorouracil, or puromycin (Marver *et al.*, 1966), a finding compatible with RNA-dependent *de novo* synthesis of this enzyme (Granick, 1963, 1966). Clinical and experimental aspects of porphyrias have been the subject of several recent reviews (Schmid, 1966; De Matteis, 1967).

The suggestion of an effect of sex hormones on porphyrin metabolism in man comes from a variety of clinical observations (C. J. Watson *et al.*, 1962; Levere, 1966; Zimmerman *et al.*, 1966). These include the high female preponderance of the disease despite its inheritance as an autosomal dominant trait; the frequency with which exacerbations of the disease occur in relation to puberty or, in some patients, to specific phases of the menstrual cycle or pregnancy; and the relapses in the disorder which can be evoked by the administration of synthetic estrogens and progestins (Welland *et al.*, 1964; Wetterberg, 1964; Burton *et al.*, 1967) as well as by such natural hormones as progesterone (Levit *et al.*, 1957). Conclusive evidence of a regulatory action of such steroids on porphyrin and heme synthesis has been recently obtained in studies utilizing chick embryo liver cells growing in primary culture (Granick, 1966; Granick

and Kappas, 1967a,b; Kappas and Granick, 1968). In this *in vitro* preparation, a variety of natural hormones including estradiol and progesterone—and particularly a number of C_{19} and C_{21} metabolites of the 5β-H type, such as pregnanediol, pregnanolone—have been shown to greatly stimulate porphyrin synthesis. The mechanism of this steroid action appears to involve the *de novo* formation of ALA-synthetase in liver cells. This steroid action has been shown to extend to embryonic erythroid cells as well (Levere *et al.*, 1967). The most potent inducers of ALA-synthetase are, interestingly, the metabolites of such precursor hormones as progesterone, rather than the hormones themselves. These metabolites are active in concentrations at least as low as 10^{-8} M, thus displaying potency in tissue culture which equals that of the most potent porphyria-inducing drugs known. Glucuronidated steroids are inactive and the porphyrinogenic action of free steroids can be blocked not only by the classical inhibitors of nucleic acid and protein synthesis, but by uridine diphosphate glucuronic acid (UDPGA) and by heme and other metalloporphyrins. A hypothetical model for steroid control at the genome level of porphyrin and heme formation has been proposed (Kappas and Granick, 1968) in which the rates of glucuronidation of steroids and of hydrolysis of the glucuronides by β-glucuronidase may be important determinants of the extent to which steroid inducers of ALA-synthetase may express their activity. It seems likely that steroids may be involved in the pathogenesis of hepatic porphyria in certain patients, particularly at times when their inactivation by the glucuronidation process becomes limited for some reason. It is of interest in this regard that (1) in certain strains of mice estrogens have been shown to increase the number and size of hepatic lysosomes (Achtelik, 1966) and to enhance the activity of β-glucuronidase (W. H. Fishman and Farmelant, 1953), an enzyme that catalyzes the hydrolysis of β-glucuronides including steroid glucuronides; and (2) various 5β-H steroids, including pregnanediol, are inhibitors of microsomal UDP-glucuronyltransferase activities (Hsia *et al.*, 1963b). Both processes could theoretically lead to enhancement of the inducing potential of endogenous steroids and thus facilitate enhanced heme formation in the liver.

The complex nature of the relationship between endocrine status and clinical expressions of hepatic porphyria is apparent from the observations of Perlroth *et al.* (1965) that the symptoms of patients in whom exacerbations were associated with menstruation could be prevented by small amounts of oral contraceptive agents; in one patient, synthetic preparations of estrogen or progestin were effective when administered singly. Androgen therapy has also been reported to be effective in preventing cyclic attacks (Perlroth *et al.*, 1965; Schmid, 1966) of porphyria.

These paradoxical results suggest that there may be more than one mechanism whereby sex hormones exert their effects on porphyrin metabolism in man; or possibly that exogenous administration of certain steroids in small amounts may suppress endogenous production of larger amounts of more potent inducers of ALA synthetase. Recently, Tschudy *et al.* (1967) have reported that estradiol administered intravenously in amounts as small as 4 μg evoked in the livers of ovariectomized rats a "rebound" induction of ALA synthetase which followed an initial decline in its activity. They concluded that estrogen may not be a primary inducer of the enzyme but actually an inhibitor of its synthesis, and suggested that the "rebound" induction and subsequent oscillations of ALA synthetase activity which they observed may reflect the transient perturbing effect of the hormone on the equilibrium of the enzyme-synthesizing system in the liver.

Reports of the influence of estrogens, progestins, or pregnancy on a variety of other hepatic enzymes are to be found in the literature. During pregnancy, the specific activity of hepatic arginase increases (Roberge *et al.*, 1967) while the activity of glucose-6-phosphatase, expressed as activity per unit weight, declines (Burt, 1959). Progesterone or estrone administered for 5 days (Burt, 1959) does not affect hepatic glucose-6-phosphatase. The activity per unit weight of cysteic acid decarboxylase is about twice as high in the male compared with the female rat liver (Sloane-Stanley, 1949). This difference is abolished by ovariectomy, but is partly restored by estrone administered to ovariectomized rats. Pregnancy or estradiol inhibits the cortisol-induced synthesis of hepatic alanine transaminase in the rat (Harding *et al.*, 1966) and progesterone partially blocks this response. The physiological significance of these scattered observations is uncertain.

D. Plasma Proteins

This section of the review summarizes the variety of effects which pregnancy and estrogens exert on plasma proteins. A significant number of these proteins are now known to be synthesized in the liver; this number will undoubtedly increase as more plasma proteins are isolated and characterized and newer methods for demonstrating their sites of synthesis are developed. The plasma proteins of hepatic origin and the experimental methods employed to demonstrate their hepatic synthesis have recently been summarized by Schultze and Heremans (1966). Of the plasma proteins that are affected by pregnancy or estrogens, some have been shown to be manufactured largely or exclusively in the liver, and the alteration of their plasma concentration would be a composite reflection of the altered rate of their hepatic synthesis, release or degradation.

Such proteins include albumin, ceruloplasmin, transferrin, fibrinogen, and haptoglobin. For the rest of the plasma proteins discussed here, the sites of synthesis have not been clearly defined. These proteins include: thyroxine-binding globulin, transcortin, testosterone-binding protein, and an α_2-globulin that appears to be specific for pregnancy. Serum lipoproteins will be discussed in a later section.

A decrease in the total serum protein concentration during human pregnancy has been amply documented, and a summary of this phenomenon has been presented by Hytten and Leitch (1964). De Alvarez *et al.* (1961) showed that, in a large series of women in weeks 13–16 of pregnancy, the mean serum protein concentration was 5.264 gm per 100 ml as compared to the value of 7.182 gm in nonpregnant women. This represents a pregnancy-induced decline of approximately 22%. The protein concentration remained low during the remainder of pregnancy and was still significantly depressed in the sixth and seventh postpartum weeks. Most of the decrease in total protein could be accounted for by fractional reduction in the serum albumin concentration. γ-Globulin concentration appears to be slightly decreased or not affected at all (Hytten and Leitch, 1964). Administration of estrogen or estrogen-progestin contraceptive pills leads to similar but less marked effects on total serum protein and serum albumin (Musa *et al.*, 1967). Such a selective decrease in serum albumin appears to rule out hemodilution accompanying pregnancy or estrogen therapy as the primary factor in decreasing the serum protein concentration.

During electrophoretic studies of human serum on starch gel, Smithies (1959) noted a zone of protein migrating in the region of α_2-globulin, which was present in pregnant and puerperal women. In nonpregnant females, this protein was either absent or present in only trace amounts (J. C. Robinson *et al.*, 1966a). This "pregnancy zone" migrates between haptoglobin and slow α_2-globulins and is distinct from certain other serum proteins that are also elevated in concentration in pregnancy, viz. transcortin, thyroxine-binding globulin, aminopeptidase (Afonso and De Alverez, 1963, 1964), or alkaline phosphatase (J. C. Robinson *et al.*, 1966b). The appearance or augmentation of the pregnancy zone can also be induced by the administration of estrogen (Musa *et al.*, 1967) or a combination of estrogen and progesterone (Afonso and De Alvarez, 1963) or by trophoblastic disease (J. C. Robinson *et al.*, 1966a). It is of interest that MacLaren *et al.* (1959) found, by means of a modified Ouchterlony method, evidence for at least one and possibly two antigens that were present only in sera from pregnant women. Neither these antigens nor the pregnancy-specific serum α_2-globulin have been further characterized.

In normal pregnant women the serum levels of total copper and ceruloplasmin, the copper-binding protein, gradually increase, approaching twice the normal values near term (Lahey *et al.*, 1953; Sass-Kortsak, 1965). The copper content of the fetal liver is also elevated but declines to a normal level during the first year after birth (Gerlach, 1934). Administration of estrogen in man results in an elevation of the serum copper and ceruloplasmin levels (Johnson *et al.*, 1959; Doe *et al.*, 1967), and the degree of increase in ceruloplasmin appears to correlate with the amount of estrogen given (Musa *et al.*, 1965). A mean increment of 84.6% was reported (Musa *et al.*, 1967) following the administration of 0.5 mg of ethynylestradiol/day for 20 days to normal females in the reproductive age. Similar responses of serum copper and ceruloplasmin have been reported to take place in patients with Wilson's disease (Bearn, 1957; German and Bearn, 1961), a heritable disorder of copper metabolism characterized by a marked deficiency of serum ceruloplasmin. It is of note that, in patients with this disease, raising the serum ceruloplasmin concentration by administration of ethynylestradiol to the level approaching or exceeding normal values did not prove to be of therapeutic value (German and Bearn, 1961). It appears therefore that serum concentration of the copper-transporting protein is not in itself a determining factor in the pathogenesis of Wilson's disease.

The concentration of serum transferrin, measured as the total iron-binding capacity, is a significant indicator of iron metabolism in man. Because of its increase in the iron deficiency state (Ramsay, 1958) and because of substantial demands for iron imposed on pregnant women, the serum iron and iron-binding protein have received a considerable amount of attention by investigators. Progressive elevation of transferrin concentration during the latter part of pregnancy (Rath *et al.*, 1950; Ventura and Klopper, 1951) is now well substantiated, although there is no general agreement on response of the serum iron concentration (Rath *et al.*, 1950; Holly, 1953; E. H. Morgan, 1961). The increment in serum transferrin is apparently not related to iron deficiency since it was observed in women treated with ferrous gluconate during pregnancy (E. H. Morgan, 1961). It must be related to some factors other than estrogens since long-term estrogen treatment in men and women did not appreciably alter serum transferrin concentration (Musa *et al.*, 1967). Long-term effects of progesterone on serum transferrin levels have not been reported.

Pregnancy induces a significant rise in plasma fibrinogen levels in women (Phillips and Skrodelis, 1958; Gillman *et al.*, 1959) as well as in experimental animals (Schmidt *et al.*, 1927). In the dog, pregnancy stimulates hepatic synthesis of fibrinogen, as demonstrated by the

localization of fluorescent antifibrinogen antibody in hepatic parenchymal cells (Forman and Barnhart, 1964). Less than 5% of the parenchymal cell population in liver biopsies from nonpregnant dogs took up the fluorescent antibody, whereas 75–95% of the parenchymal cells of pregnant dogs showed intense fluorescence. Administration of estrogen (Phillips *et al.*, 1961) or estrogen–progestin combinations (Beller and Porges, 1967) to men and women and implantation of pellets of estradiol or testosterone in gonadectomized rats (Gillman *et al.*, 1958) all led to augmented plasma fibrinogen levels. The effects of hormones on a variety of other blood coagulation factors have recently been summarized by Beller and Porges (1967), and Fresh *et al.* (1956) have reported the elevation of a number of these factors in pregnant women.

Serum haptoglobin, the hemoglobin-binding glycoprotein that migrates in the region of α_2-globulins on starch gel electrophoresis (Jayle and Moretti, 1962), appears to be sensitive to the endocrine status of man. Its serum concentration is significantly higher in males than in females and, in females, is inversely related to the urinary estrogen level (Nyman, 1959). Estrogen treatment in women depresses its serum concentration (Borglin and Nyman, 1961; Musa *et al.*, 1967), while androgens have an opposite effect (Laurell and Skanse, 1963). Hemolysis, with release of free hemoglobin into the circulating plasma and formation of the hemoglobin-haptoglobin complex, depresses or abolishes its measurable serum level (Allison and Rees, 1957; Allison, 1958). In chronic as well as acute hepatocellular diseases, a marked depression of serum levels of haptoglobin is observed (Nyman, 1959). After a careful analysis of other synthetic parameters of the liver in patients with hepatocellular diseases, Nyman suggested that impaired metabolism of endogenous estrogens and enhanced rate of breakdown of haptoglobin partly accounted for the depressed serum haptoglobin level. The mode of action of estrogens in depressing its serum level is uncertain, but increased destruction of erythrocytes concomitant to estrogen therapy or activation of the reticuloendothelial system have been offered as possible explanations (Borglin and Nyman, 1961). It is paradoxical then that serum haptoglobin levels remain normal in pregnant women (Nyman, 1959).

In the serum of man as well as experimental animals, there are several distinct proteins which possess a high affinity for specific hormones and the apparent function of which is to bind the hormones and transport them. The specificity and the degree of this affinity differentiate the properties of these proteins from such nonspecific carriers as serum albumin. In the bound form, the hormones are thought to be essentially devoid of biological activity. Corticosteroid-binding globulin (CBG) or transcortin, thyroxine-binding globulin (TBG), and testosterone-binding

protein are the specific proteins that are pertinent to the present discussion. Although these proteins are present in minute quantities in the serum, TBG (Tata, 1961) and CBG (Muldoon and Westphal, 1967) have been isolated and partially characterized. Testosterone-binding protein has recently been shown to be a molecular entity distinct from CBG (Pearlman *et al.*, 1967). The sites of synthesis of these proteins have not yet been explored. During human pregnancy, a significant increase in the binding capacity of serum for thyroxine (Dowling *et al.*, 1956), cortisol (Slaunwhite and Sandberg, 1959), and testosterone (Pearlman *et al.*, 1967) takes place, presumably indicating the augmented concentration, respectively, of TBG, CBG, and testosterone-binding protein. The increment in these proteins progresses during pregnancy and reaches a peak during the third trimester. Augmented serum concentrations of TBG, CBG (Musa *et al.*, 1965, 1967; Katz and Kappas, 1967), and testosterone-binding protein (Pearlman *et al.*, 1967) can also be produced by exogenous estrogens. The observation that the total serum concentration of thyroxine (Danowski *et al.*, 1950), corticosteroids (Bayliss *et al.*, 1955; Birke *et al.*, 1958), and testosterone (Meeker, 1966) are all elevated in pregnant women may have a physiological relevance to the augmented levels of their respective binding proteins in the serum, as pregnancy is generally thought to produce no clinical signs of overproduction of these hormones.

E. Serum Enzymes

A number of serum enzymes undergo significant alterations during pregnancy and after the administration of estrogens and progestins. These changes include elevation and depression of their total activities and, in certain of those enzymes existing in multiple molecular forms, alterations in the isozyme patterns. Leucine aminopeptidase, cholinesterase, ornithine transcarbamylase, and alkaline phosphatase are discussed in the present section in some detail because the serum levels of these enzymes have been extensively studied and correlated with the clinical status of the liver and because the hepatic origin of these enzymes is either confirmed or under consideration.

Leucine aminopeptidase (LAP), a proteolytic enzyme which hydrolyzes free N-terminal leucine or a related amino acid residue from peptides or amides, is widely distributed in human tissues and is found in normal sera. Its activity in sera from pregnant women is markedly elevated (Green *et al.*, 1955) and reaches at term a peak activity of approximately 3 times the nonpregnant value (Bressler and Forsyth, 1959). Using L-leucyl-β-naphthylamide as substrate multiple molecular forms of serum LAP have been demonstrated by starch gel electrophoresis (Smith

et al., 1962; Smith and Rutenburg, 1963). Normal sera exhibit a single zone of activity generally in the α_2-globulin region (Kowlessar *et al.*, 1960; Smith *et al.*, 1962), with a mobility similar to LAP in the extracts of human liver (Smith and Rutenburg, 1963). In patients with various diseases involving the pancreas or the hepatobiliary system, the rise in total serum LAP activity is associated with the rise in the isozyme in this α_2-globulin region as well as other new zones of activity (Kowlessar *et al.*, 1960; Smith *et al.*, 1962).

It now appears that the increase in total LAP activity in pregnancy sera is largely attributable to the *de novo* appearance during pregnancy of at least two aminopeptidase isozymes that are distinct from the isozymes noted in pancreatic or hepatobiliary diseases. These pregnancy-specific isozymes possess substrate specificities distinct from the "normal" LAP in the α_2-globulin region and can be separated from the latter by starch gel electrophoresis and stained with L-leucyl-β-naphthylamide as they share with the latter the affinity for this substrate (Page *et al.*, 1961). However, the "pregnancy" isozymes have a high specificity for oxytocin (the octapeptide hormone from the posterior pituitary gland) and for L-cystine-di-β-naphthylamide (Page *et al.*, 1961), substrates for which the normal LAP demonstrates negligible activity. It seems therefore that the pregnancy-specific aminopeptidases are in fact the "oxytocinase," a plasma enzyme capable of inactivating oxytocin or vasopressin, which is present in the sera of pregnant women (Müller-Hartburg *et al.*, 1959; Titus *et al.*, 1960), but not in the sera of non-pregnant women or women receiving estrogens or progestins (J. C. Robinson *et al.*, 1966a). This oxytocinase has also been called cystine aminopeptidase (Page *et al.*, 1961) because of its high affinity for the peptide bond adjoining a half-cystine residue with a free amino group (Müller-Hartburg *et al.*, 1959). The serum oxytocinase activity gradually increases during pregnancy and reaches a peak value near term (Titus *et al.*, 1960). A marked decline in plasma oxytocinase in a small group of women in early labor was reported by Hilton and Johnson (1959), and the possible physiological implication of the activity of this enzyme in relation to the onset of labor was suggested. This finding, however, was not confirmed by Titus *et al.* (1960) in a study of a larger number of pregnant women.

The placental origin of serum oxytocinase has been suggested on the basis of a number of clinical observations summarized by Page *et al.* (1961) and the presence of the enzyme activity in the extracts of human placenta. However, in contrast to the pregnancy-associated serum alkaline phosphatase isozymes, which show a strict correspondence in electrophoretic mobility with the enzyme from placental extracts (Boyer, 1961),

the pregnancy-associated oxytocinase isozymes from serum show no correspondence with the isozymes from placenta (Beckman *et al.*, 1966). The site of origin of pregnancy-specific serum aminopeptidase, oxytocinase, is therefore still an unanswered question.

Serum nonspecific cholinesterase, or pseudocholinesterase, is a hydrolytic enzyme of hepatic origin, the activity of which is a sensitive indicator of the synthetic capacity of the liver (Kunkel and Ward, 1947). The circulating serum enzyme in man can be rapidly and almost quantitatively depleted by irreversible inhibition with diisopropyl fluorophosphate (DFP) administered parenterally in doses that do not produce significant parasympathomimetic effects (Comroe *et al.*, 1946). The rate of regeneration of serum cholinesterase subsequent to such depletion would be the measure of the rate of its hepatic synthesis (and release) and has been shown to be markedly depressed in patients with cirrhosis of the liver (Kunkel and Ward, 1947). In patients with chronic liver disease, therefore, the serum cholinesterase activity is generally depressed and parallels the serum albumin concentration, carrying with it approximately the same clinical significance (Hunt and Lehmann, 1960). The fall in serum cholinesterase activity in pregnant women has been documented by several investigators (Pritchard, 1955; Wetstone *et al.*, 1958). The serum enzyme activity declines progressively during gestation at a rate roughly approximating the decline in serum albumin concentration (Wetstone *et al.*, 1958). No cholinesterase inhibitor was found in the sera from pregnant women. It is likely, therefore, that the depressed serum cholinesterase activity and serum albumin concentration both indicate the diminished rate of synthesis of these proteins in the liver during pregnancy. The effects of estrogens or progestins on the cholinesterase-regenerating system described above would be of interest, in view of the recent report (Robertson, 1967) that various preparations of oral contraceptives containing synthetic estrogens and progestins depress the serum cholinesterase activity of young women.

A very large number of reports have appeared concerning the activity of nonspecific alkaline phosphatase in human serum and the alterations of the enzyme activity in various diseases. The clinical applications of the determination of serum alkaline phosphatase have been periodically reviewed (O. Bodansky, 1961; Wilkinson, 1965; Posen, 1967). The elevation of alkaline phosphate activity in the serum of pregnant women has been repeatedly documented during the past three decades and the literature on this subject is now extensive. Unlike the sera from pregnant rats (Weil, 1941) and sheep (Auchinachie and Emslie, 1933) in which no increase in alkaline phosphatase activity is noted, the mean enzyme activity in

human serum gradually increases during gestation (M. Bodansky, 1939; Young *et al.*, 1946), so that at the time of delivery the level is approximately twice that seen in nongravid women or women in early pregnancy. The tissue of origin of the increment in serum alkaline phosphatase in various disease states, especially those affecting the hepatobiliary system, has been a source of controversy for many years. The problem has by no means been resolved at the time of this review, although a few reports that strongly suggest the liver as the source of enzyme in hepatobiliary diseases have appeared (Polin *et al.*, 1962; Sebesta *et al.*, 1964).

The augmented serum alkaline phosphatase activity in pregnant women has evoked various speculations concerning its tissue of origin. Alteration in bone metabolism (M. Bodansky, 1939) and release from placenta (Klees and Frenzel, 1960) have been offered as explanations. In recent years substantial experimental evidence has accumulated to support the probable placental origin of this increment in the enzyme activity. Beck and Clark (1950) utilized taurocholate inhibition of alkaline phosphatase (O. Bodansky, 1937) to distinguish serum alkaline phosphatase of placental origin. They noted that the serum enzyme from women in late pregnancy was relatively resistant to taurocholate at a concentration of 6.25 mM, a property resembling that of placental alkaline phosphatase, which is also taurocholate-resistant (Clark and Beck, 1950). Boyer (1961) separated serum alkaline phosphatases into various isozymic components by means of electrophoresis in starch gel. Two zones of enzyme activity, designated A and B, were shown to appear after week 15 of pregnancy. The electrophoretic mobility of A and B zones corresponded in each case to the alkaline phosphatase zone produced by the *n*-butanol extracts of the serum donor's placenta. The activity of the A and B zones represented approximately half of the total serum activity, sufficient to acount for most of the pregnancy-induced increment. Finally, immunochemical methods (Birkett *et al.*, 1966) have provided further support for the placental origin of the increase in serum alkaline phosphatase level observed in pregnancy.

Although the above evidence is definitely in favor of the placenta as the principal site of origin of the serum enzyme during pregnancy, this evidence cannot explain the frequent increase in serum alkaline phosphatase activity which is observed in nongravid women receiving estriol or estradiol in amounts approximating or exceeding those produced daily during normal gestation (M. N. Mueller and Kappas, 1964a). This increase in alkaline phosphatase activity is usually mild and accompanied by a significant reduction in the hepatic excretory capacity for administered BSP, a finding also frequently observed in pregnant women (Combes

et al., 1963; Tindall and Beazley, 1965). A similar elevation in the enzyme activity has been reported in normal women of reproductive age receiving various estrogen-progestrin combinations as contraceptive agents (Eisalo *et al.*, 1964, 1965; Larsson-Cohn, 1965). That such alterations in liver function take place with significant frequency in healthy women with no known history of hepatobiliary disease who are receiving estrogens suggests the production by estrogens of some type of biochemical abnormality involving the excretory surface of the liver. In experimentally produced extrahepatic cholestasis in rats, the alkaline phosphatase activity of the canalicular surface of the plasma membrane is considerably augmented, whether measured by histochemical methods on liver sections (Goldfischer *et al.*, 1962) or by quantitative enzyme assay on isolated plasma membrane fragments (Emmelot *et al.*, 1964). Similar, although less marked, histochemical findings have been obtained in rats (Goldfischer *et al.*, 1962) but not in men (Scherb *et al.*, 1963) treated with norethandrolone, a steroid which evokes excretory defects in the liver. Of interest is the recent observation of Kreek *et al.* (1967c), who noted slight rise in the mean value of serum 5′-nucleotidase activity in six normal young women receiving ethynylestradiol. In the liver, 5′-nucleotidase is also located in the plasma membrane (Wachstein and Meisel, 1957; Song and Bodansky, 1967), and the elevation of its activity in serum is considered to be highly specific for hepatic disorders, particularly those with intrahepatic cholestasis (Hill and Sammons, 1967).

It is probable therefore that the increase in the activity of serum alkaline phosphatase in estrogen-treated human subjects is related to the functional alterations involving the hepatic excretory surface and that, in certain cases of normal pregnancy and in cases in which pregnancy is complicated by overt clinical hepatic dysfunction (e.g., intrahepatic cholestasis of pregnancy), such alkaline phosphatase of nonplacental origin may make a significant contribution to the total increment in its serum activity.

Reichard *et al.* (1961) noted a slight increase in the activity of ornithine transcarbamylase (OTC) in the sera of approximately 15% of women during normal pregnancy. This urea cycle enzyme is located almost exclusively in the liver and the elevation of its activity in the serum is thought to be a sensitive and specific indicator of liver cell damage (R. W. Brown and Grisolia, 1959; Reichard, 1961). Placenta and other tissues from the female reproductive tract contain very little OTC activity (Reichard *et al.*, 1961). Significant and often pathological elevation of serum OTC activity has also been reported in a small series of healthy women during the first month after initiation of oral contraceptive therapy (Brohult and Westgren, 1965).

IV. Carbohydrate Metabolism

Study of carbohydrate metabolism in the liver during pregnancy or after treatment with estrogens and progestins has been limited to the synthesis and breakdown of glycogen and other related phenomena generally involving study of the response to administered glucose, glucagon, or insulin. Even so, the published results are widely variable and at times contradictory, perhaps because of the many different experimental conditions under which the studies have been carried out because of the intimate relationship between hepatic carbohydrate metabolism and the influences of other hormones, such as the catecholamines, glucocorticoids, insulin, and glucagon, the secretion and activity of which in turn are affected by the physiological status of the experimental subjects and, in the cases of glucocorticoids and insulin, by estrogens themselves. No systematic attempt to investigate the effects of pregnancy, estrogens, or progestins on hepatic carbohydrate metabolism has as yet been made; there are, however, a number of scattered observations of alterations in the metabolism of carbohydrate in the liver.

A. Hepatic Glycogen and Glycogenesis

Quantitative data on the glycogen content of the liver in pregnant women are not available. Early claims by Hofbauer (1908, 1911) that glycopenia was one of the features of the so-called "pregnancy liver" have been largely discredited by subsequent investigators (Ingerslev and Teilum, 1945; Dietel, 1947), who noted no histochemical evidence for glycopenia in biopsy specimens obtained from pregnant women. The latter findings remain to be substantiated by chemical determinations. Indirect information concerning the hepatic glycogen content in pregnant women comes from the study of glycogen mobilization following parenteral administration of glucagon (Burt, 1957). The hyperglycemic response of fasted pregnant women to 0.02 mg of glucagon per kilogram of body weight, considered to be sufficient for practically complete mobilization of hepatic glycogen, was comparable to that of nonpregnant subjects. In view of the specificity of the action of glucagon on the liver in the production of hyperglycemia (Behrens and Bromer, 1958), the author concluded that the hyperglycemic responses in pregnant women were inconsistent with hepatic glycopenia in pregnancy.

Extraction and quantitative determination of hepatic glycogen in experimental animals have produced results that are contradictory for reasons that are not immediately apparent. In pregnant dogs, a significant reduction in hepatic glycogen content was reported by Schmidt *et al.* (1927). In nonfasting rats on days 18 and 20 of pregnancy, Campbell

and Kosterlitz (1949) noted a mean hepatic glycogen content of 0.86 gm/0.25 gm of DNA in contrast to 2.55 gm/0.25 gm of DNA in nongravid controls. In a more recent study (Hagerman, 1962), a decrease of lesser magnitude, expressed in terms of dry weight of the liver, was obtained for rats on days 20 and 22 of gestation, with no apparent impairment in the ability of the liver to incorporate added glucose into glycogen *in vitro*. On the other hand, a report of a significant increase in hepatic glycogen content, expressed in terms of wet weight of the liver, in fasted as well as in glucose-treated rats on day 17 of pregnancy has appeared (Burt and Kimel, 1957). Comparison of these data is difficult because of the differences in the nutritional history of the animals, analytical methods employed, and expression of the results obtained.

There now appears to be a general agreement on the increase in hepatic glycogen content following the administration of estrogen to experimental animals (Griffiths *et al.*, 1941; Walaas, 1952; Ingle, 1959) despite some contradictory reports (Gaunt *et al.*, 1939; Talaat *et al.*, 1965). Estradiol, estriol, or diethylstilbestrol given as subcutaneous implants (Griffiths *et al.*, 1941) or relatively large doses of estradiol, estrone (Walaas, 1952), or diethylstilbestrol (Janes and Nelson, 1942) all augment the glycogen content of the liver in rats. The increase amounts to approximately 3- to 4-fold over the control value and appears to be related to the duration of estrogen treatment (Janes and Nelson, 1940). No concomitant alteration in the muscle glycogen content is noted. Intact pituitary and adrenal glands are necessary for the estrogen-induced increment in hepatic glycogen content (Janes and Nelson, 1942; Long, 1942), and it has been suggested that the glycogenic effect of estrogens is an indirect one, mediated through the release of adrenal cortical hormones. However, Ingle (1959) demonstrated that the glycogenic action of estrogen was largely restored in adrenalectomized rats by treatment with adrenal cortical extract and that the mean hepatic glycogen content in these animals greatly exceeded that of adrenalectomized rats given adrenal cortical extracts but no estrogen. He proposed that the adrenal cortical hormones have a "permissive role" in estrogen-induced hepatic glycogenesis, viz. that the glycogenic action of estrogen on the liver is manifest only in the presence of adrenal cortical hormones, either endogenous or exogenously supplied. Further physiological complexity is suggested by the following observations: (1) Estrogens prolong the biological half-life of adrenal cortical steroids (Yates and Urquhart, 1962; Tait and Burstein, 1964) possibly by decreasing the rate of their metabolism in the liver; and (2) large amounts of estrogens increase the insulin concentration of the rat pancreas and induced hypertrophy as well as hyperplasia of the islets of Langerhans in hypophysectomized rats with subtotal pancreatectomy

(Houssay, 1960). Actual increase in the plasma insulin concentration has recently been demonstrated (Spellacy and Carlson, 1966) using an immunoassay in women taking an estrogen-progestin combination. In view of the glycogenic action of corticosteroids as well as of insulin, the possibility of their contribution to the estrogen-induced hepatic glycogenesis cannot be ruled out.

B. Glycolysis

A systematic exploration of the activities of the glycolytic enzymes in the liver of pregnant animals is yet to be initiated. In a partial study, Burt and Pulliam, (1962) found slightly increased total activity of phosphorylase, as determined by the rate of release of glucose 1-phosphate from glycogen at pH 5.7, in the liver of rats on day 17 or 18 of gestation. The specific activity of this enzyme, however, showed no significant difference from nonpregnant animals. By means of *in vitro* studies using liver slices from rats on days 20 and 22 of pregnancy, Hagerman (1962) obtained the following results, expressed in terms of wet weight: (1) increased rate of utilization of added glucose; (2) increased rate of production of lactate and pyruvate from the slice; and (3) decreased rate of conversion of added pyruvate to glycogen, glucose, and CO_2. The overall results were suggestive of an enhancement in the glycolytic capacity of the liver in pregnancy with a concomitant reduction in oxidative metabolism.

C. Peripheral Utilization of Glucose

It is beyond the scope of the present review to summarize the extensive literature on the altered tolerance to a glucose load and the general resistance to the effects of insulin developing during human pregnancy. Significantly greater hyperglycemic responses to oral and, less frequently, to intravenous administration of glucose (Welsh, 1960; Benjamin and Casper, 1966) are observed during late stages of pregnancy, with a widely varying frequency that depends in part on the selection of subjects and the criteria of abnormality. Such diminished tolerance to administered glucose is noted despite the higher fasting concentration of plasma insulin and the enhancement of plasma insulin response to a given hyperglycemic stimulus (Spellacy and Goetz, 1963; Bleicher *et al.*, 1964) during gestation. The clinical implication of these findings, with particular reference to the "diabetogenic" effects of pregnancy, has been discussed by numerous authors (Burt, 1960; Kyle, 1963; Bleicher *et al.*, 1964; Hytten and Leitch, 1964; Kalkhoff *et al.*, 1964). It is of note that essentially similar findings have been observed in young women receiving oral contraceptives containing estrogens and progestins (Wynn and Doar, 1966; Spellacy and

Carlson, 1966; Posner *et al.*, 1967). In addition, Wynn and Doar (1966) noted frequent increase in the blood pyruvate level in these women, an alteration similar to that noted during glucocorticoid administration (Henneman and Bunker, 1957). It appears possible, therefore, that some of the changes in carbohydrate metabolism induced by pregnancy or by pharmacological doses of gestational hormones may be mediated through the action of other hormone(s), e.g., corticosteroids. This latter action may be related in part to the general inhibitory effect of estrogens on hepatic enzymes that metabolize and inactivate drugs and hormones including corticosteroids.

V. Lipid Metabolism

In the previous section, we noted the complex physiological relationships that exist by means of which pregnancy and gestational hormones could exert their effect on hepatic carbohydrate metabolism. Such complexities probably apply also to the present topic, as the metabolism of lipids is now well known to be modified by the actions of the pituitary, thyroid, and adrenal hormones (Paoletti, 1964), and the secretion or biological activity of these hormones are in turn affected by pregnancy (Hytten and Leitch, 1964) and estrogens.

A. Hepatic Lipids

Employing histochemical stains such as Sudan III for tissue lipids, Ingerslev and Teilum (1945) and Dietel (1947) noted slightly more pronounced, but nonpathological, accumulation of fat vacuoles in centrolobular areas in liver biopsy specimens taken from pregnant women. Extension of these findings into quantitative determination of hepatic lipid content in pregnant women is yet to be carried out. The total lipid content of the liver in pregnant rats, measured as the material extractable with solvents such as ether or petroleum ether and expressed as fraction of the wet-organ weight, does not differ significantly from that of nonpregnant animals (A. F. Morgan and White, 1950; Shipley *et al.*, 1953). However, because of the increase in the weight of the liver during pregnancy, the total lipid content per organ would also be increased in parallel with the increment in the liver weight which usually ranges from 30 to 40% in late pregancy. This appears to hold true also for the hepatic contents of cholesterol (Schwenk and Joachim, 1961; Dannenburg *et al.*, 1964), triglycerides, and phospholipids (Dannenburg *et al.*, 1964). Thus in pregnant rats, the total lipid content ranges from 2.6 to 7.2% of the liver weight, and the mean values for each of the lipid components reported are: 2.15 mg of cholesterol per gram of liver on day 20 of gestation;

5.34 mg of triglycerides per gram of liver and 1.74 mg of phospholipid phosphorus per gram of liver on day 17 of gestation.

There are only a few published studies dealing with the conversion of labeled acetate or pyruvate into various hepatic lipids in pregnant animals, and the available data are at variance with each other. Schwenk and Joachim (1961) observed that the *in vivo* conversion of acetate-1-^{14}C into hepatic cholesterol, expressed as counts per milligram of cholesterol, did not change appreciably during pregnancy in the rat, whereas its incorporation into fatty acids was significantly increased. Campbell and Kosterlitz (1949) noted a substantial increase in the uptake of $^{32}P_i$ into the phospholipid phosphorus of the liver by rats in late pregnancy and concluded that the turnover rate of the phospholipid phosphorus was increased. Upon removal of the fetuses, with the placentae left intact, the rate of incorporation of $^{32}P_i$ into phospholipids declined to the control value, suggesting the possible role of the metabolic demands of gestation in augmenting the turnover of hepatic phospholipids. Hagerman reported an almost 2-fold increase in the *in vitro* utilization of added pyruvate 2-^{14}C to form lipids by liver slices from pregnant rats. Conversion of acetate-1-^{14}C *in vitro* into the "unsaponifiable fraction" of the hepatic lipids, which is a heterogeneous collection of water-insoluble lipid components including higher molecular weight alcohols such as cholesterol, was reported by Dannenburg *et al.* (1964) to be elevated 5 times in pregnant rats. An even greater increase in the utilization of this lipid precursor for the synthesis of fatty acids—mostly the fatty acids of the triglycerides and phospholipids—was noted by the same authors. Thus, in the pregnant rat, the rate of lipid synthesis appears to be generally increased, although the extent of the reported increase is widely variable.

B. Plasma Lipids and Lipoproteins

In human plasma the major portion of the lipids is transported in association with specific proteins in the form of lipid–protein complexes, or lipoproteins, which are characterized by typical electrophoretic mobility, density distribution, and immunological properties (Putnam, 1965; Scanu, 1965; Frederickson and Lees, 1966). According to density and flotation rate in the ultracentrifuge, human plasma lipoproteins have been generally classified into five major fractions. They are, in order of increasing density, chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and free fatty acids. The latter are present in plasma in association with albumin. In general, the trend in the lipoprotein composition with increasing density is the increase in the content of protein, phospholipids and cholesterol esters, with concomitant decrease in the content of triglycerides. Electro-

phoretically, chylomicrons and VLDL have a mobility in the region of α_2-globulin, and LDL and HDL in the region of β_1-globulin and α_1-globulin, respectively.

Because of the ready availability of human plasma for investigative purposes, a large number of studies have been conducted concerning the changes in serum lipids during pregnancy and after treatment with various estrogens. In view of the important role played by the liver in the synthesis (Radding *et al.*, 1958; Marsh and Whereat, 1959; De Jong and Marsh, 1968) of both the protein and the lipid moieties of the plasma lipoproteins, their alterations associated with pregnancy and estrogen treatment are briefly summarized in this section. It must be emphasized, however, that two other extrahepatic sites, the intestine and the adipose tissue, play prominent roles in the metabolism of plasma lipoproteins (Scanu, 1965) and that alterated plasma lipoprotein patterns may often be a composite indication of metabolic changes taking place in more than one site.

Total lipid content of the serum shows a progressive rise during the latter part of human pregnancy and reaches a peak value immediately before delivery (von Studnitz, 1955; De Alvarez *et al.*, 1959). Thus, the mean serum lipid content, determined as the material extractable with a chloroform–methanol mixture, rose from 650–700 mg/100 ml found during early pregancy to over 1000 mg/100 ml near term. The control value in nonpregnant subjects was 711 mg/100 ml. The serum concentrations of phospholipids, free and esterified cholesterol (W. C. Watson, 1957; De Alvarez *et al.*, 1959; Svanborg and Vikrot, 1965), triglycerides, and free fatty acids (De Alvarez *et al.*, 1967) generally began to increase during the second trimester and reached maximum values near term. All values began to decline following delivery, but after 1 week postpartum were still higher than those noted for control subjects.

These alterations in the serum lipids are reflected to some extent in the changes in the serum lipoproteins as determined by means of ultracentrifugation (Gofman *et al.*, 1954), electrophoresis (von Studnitz, 1955; W. C. Watson, 1957; Mullick *et al.*, 1964), column chromatography (Aurell and Cramér, 1966), or ethanol fractionation (Russ *et al.*, 1954) of sera. Gofman *et al.* (1954) noted in a serial study of 9 pregnant women that, during the second trimester, the most significant alteration in the serum lipoprotein pattern was an increase of HDL concentration amounting to approximately 25%, and that this value did not change appreciably during the remainder of gestation. In the third trimester, as much as a 40% increase in the concentration of LDL (S_f 0–20) and more marked rise in VLDL (S_f 20–400) were observed. Aurell and Cramér (1966) obtained comparable results on chromatographic separation of sera from women in

the third trimester of pregnancy. Inasmuch as the bulk of the serum lipid components are carried in association with these two lipoprotein fractions, especially with LDL, such alterations would explain the augmented serum concentration of all of the lipid components seen in late pregnancy. Electrophoretic studies of serum lipoproteins generally have been limited to separation into two major classes, α- and β-lipoproteins, with filter paper as supporting medium. The increase in β-lipoproteins is more pronounced during pregnancy (von Studnitz, 1955), and the ratio of concentrations of β- to α-lipoproteins has been noted to increase steadily during the latter part of gestation (Oliver and Boyd, 1955; W. C. Watson, 1957; Mullick *et al.*, 1964). These findings are in general compatible with the ultracentrifugal data obtained by Gofman *et al.* (1954). In the pregnant rat, the alterations in serum lipids appear to be similar to those in women. Knobil *et al.* (1957) noted elevated concentrations of serum total lipids, cholesterol, and phospholipids during late gestation in the rat, extending from day 19 to day 20. On the third postpartum day, the concentrations of these lipids in serum were restored to normal values.

In 1951, Gofman and his associates (H. B. Jones *et al.*, 1951) reported that in human males of 25 years or more the mean serum concentration of LDL (S_f 12–20) was significantly higher as compared with females, and that in females this lipoprotein fraction increased slowly and by the age of 50–60 reached the values noted in the young male. The concentration of HDL fractions (Delalla *et al.*, 1954) or the amount of cholesterol associated with them (Barr *et al.*, 1952; Havel *et al.*, 1955) was generally higher in female sera. After the menopause, the following alterations take place in the serum lipoprotein pattern of women (R. W. Robinson *et al.*, 1957; Heiskell *et al.*, 1961) so that the distinction from the pattern seen in male sera becomes no longer apparent: (1) increase in total serum cholesterol concentration; (2) relative increase in LDL or the amount of cholesterol associated with LDL; and (3) increase in total cholesterol:phospholipid ratio. Barr and his associates (Barr *et al.*, 1952; Russ *et al.*, 1955) demonstrated that estrogen reversed this serum lipoprotein pattern in men and postmenopausal women (Eilert, 1953; R. W. Robinson *et al.*, 1957) with a resultant decrease in serum cholesterol concentration, decrease in cholesterol:phospholipid ratio, and relative increase in HDL (Danemann *et al.*, 1960). The relationship between serum lipoproteins and experimental atherosclerosis and the therapeutic use of estrogens and related drugs in clinical atherosclerosis has been discussed elsewhere (Hess, 1964; Marshall, 1964).

Several reports on the influence of various estrogen-progestin contraceptives on serum lipoproteins have appeared. In a small series of women, Aurell *et al.* (1966) noted elevated levels of LDL, cholesterol, phospho-

lipids, and triglycerides, while no significant change was observed by Brody *et al.* (1966). However, in a group of 102 women who had received various preparations or oral contraceptives for a period of more than 3 months, Wynn *et al.* (1966) observed elevation of serum concentrations of VLDL, LDL, cholesterol, and triglycerides. The alterations in the serum lipoprotein pattern were somewhat similar to those observed in late pregnancy and were toward a "male" lipoprotein pattern. It is of note that estrogens or progesterone alone (Oliver and Boyd, 1956; Svanborg and Vikrot, 1966) do not in general produce such changes in serum lipoprotein pattern, although the long-term effects of these steroids administered singly to young women have not been clearly established. The possible androgenic action of the synthetic progestins used in the contraceptives or the interaction of estrogens with progestins may be of significance (Wynn *et al.*, 1966) in the production of altered serum lipoprotein pattern.

C. Estrogens and Hepatic Lipogenesis

Despite the extensive studies that have been carried out in experimental animals concerning the effects of estrogens and related compounds on serum lipid concentrations, relatively little effort has been expended toward elucidating the relationship between these hormones and hepatic lipid biosynthesis. The possible role of estrogen in influencing biosynthesis in the liver has been suggested by the following observations in the rat: (1) Female rats have a significantly higher serum concentration of cholesterol than male rats (Fillios, 1957; Boyd, 1961) and convert acetate-1-^{14}C to a greater extent to serum cholesterol (Fillios *et al.*, 1958). (2) Serum concentration of cholesterol shows a fluctuation with the estrous cycle (Fillios *et al.*, 1958). (3) Acetate-to-cholesterol conversion is reduced in ovariectomized rats, and this conversion is enhanced by estradiol, but not by progesterone, in gonadectomized rats (Fillios *et al.*, 1958).

Although these findings suggest that estrogens increase the hepatic synthesis of cholesterol, further studies have yielded widely varying and conflicting results. Thus, Fillios (1957) and Boyd (1961) noted that ovariectomy resulted in an actual elevation in the serum concentration of cholesterol, and Perry and Bowen (1958) reported no significant alteration in the rate of utilization of labeled acetate *in vitro* by liver slices from ovariectomized rats. Estradiol given at the rate of 1 mg/day for 10 days to female rats had no appreciable effect on the rate of conversion of labeled acetate to hepatic cholesterol by liver slices *in vitro*, while conversion of the label to hepatic fatty acids was enhanced (Perry and Bowen, 1958). In a recent study, Fewster *et al.* (1967) found that intact male rats treated with 1.7 mg of estradiol benzoate per day for 5 days had

a diminished incorporation of the label from acetate-1-^{14}C *in vivo* into free and esterified cholesterols, glycerides, and phospholipids of the liver. Estrogen treatment has been shown to have an even more variable influence on the concentration of serum lipids in the rat (Moskowitz and Wissler, 1961; Boyd, 1962).

Valid comparison of these data is not possible, because the effects of estrogens on lipid metabolism in experimental animals vary widely and depend on the dose and duration of the drug given and the age, sex, diet, and species of the animals employed. Experimental results are probably comparable only within identical sets of conditions selected from these variables, and it is under such well-defined experimental contexts that most of the work bearing on the influence of estrogens and related compounds on the serum lipid concentrations and the production of atherosclerosis has been carried out. This latter subject, which is beyond the province of the present review, has been covered in detail by a number of authors (Pick *et al.*, 1959; Hess, 1964; Marshall, 1964).

In certain animals hepatic fatty infiltration, or pathological accumulation of droplets of triglycerides in the cytoplasm of parenchymal cells, can be readily induced by means of diet or chemicals (Hartroft, 1963; Rouiller, 1964; Taft, 1964; Lombardi, 1965). The controversial subject concerning the origin of the triglycerides in experimental fatty liver has been reviewed by Scanu (1965). It was observed by György and his associates (György *et al.*, 1947; György and Rose, 1949) that estrogens, when administered to rats placed on choline-deficient and low-protein diets, had a significant lipotropic effect, viz. that the hormones reduced the fat accumulation in the liver and the progression into cirrhosis. The livers of animals with this type of nutritional hepatic disorder have been shown to contain significantly diminished amounts of phospholipids (Artom, 1952; Di Luzio and Zilversmit, 1959) and to incorporate $^{32}P_i$ at an enhanced rate into total phospholipids (Artom, 1952; Di Luzio and Zilversmit, 1959) and into phosphatidylcholine (Bowser *et al.*, 1961), a choline-containing phospholipid (lecithin). The latter phenomenon is thought to be due to compensatory increase in the rate of phospholipid synthesis from endogenous choline and ethanolamine (Artom, 1952) in response to overall diminution in hepatic phospholipids. Estradiol propionate given at 0.1 mg twice a week during the period of exposure to a choline-deficient diet inhibited fat accumulation in the rat liver and increased the hepatic phosphatidylcholine concentration only slightly, without affecting the rate of incorporation of $^{32}P_i$ into this phospholipid (Bowser *et al.*, 1961). It was concluded that the lipotropic effect of estradiol was mediated through a mechanism other than the synthesis or availability of choline.

VI. Metabolism of Drugs and Cholephilic Substances

In vertebrates, many endogenous metabolic end products and foreign chemicals undergo modifications in the liver (Williams, 1959, 1963; Remmer, 1965; Gillette, 1966; Conney, 1967) that have two principal biological consequences: (1) some of the lipid-soluble compounds are rendered more hydrophilic and amenable to excretion via the kidneys and the intestinal tract; and (2) the biological activities of these compounds are altered, resulting frequently, but not always, in the diminution or abolition of the pharmacological, toxic or other properties that characterize the parent compounds. Many, but not all, of the enzymes that catalyze the chemical conversion of metabolites and drugs are located in the membranous portion of the hepatic endoplasmic reticulum, and according to the chemical reactions catalyzed, they belong to two broad classes: (1) those enzymes that take part in the conjugation of the chemicals with various hydrophilic compounds, e.g., glucuronic acid, glycine, glutathione; and (2) those enzymes that take part in the direct conversion of these chemicals, e.g., by various oxidative, reductive, or hydrolytic reactions. The disposal of endogenous metabolites or administered drugs can therefore be influenced by alterations in the activity of the drug-metabolizing enzymes, availability of substrates for the chemical modifications, or alterations in the transport functions of the hepatic parenchymal cells.

A. Conjugation with Glycine and Glucuronic Acid

Benzoic acid administered to man is conjugated chiefly with glycine (Quick, 1931, 1936) and excreted by the kidney as hippuric acid. This conjugation appears to take place primarily in the liver as evidenced by the marked reduction in urinary excretion of hippuric acid following oral ingestion of sodium benzoate by patients with hepatic insufficiency. The condensing enzyme, which requires coenzyme A, is located in the mitochondria of beef liver (Schachter and Taggart, 1954). In pregnant women, hippuric acid excretion is markedly impaired in late gestation (Neuweiler, 1939; Sloman, 1943; Laqueur and Ovacik, 1946). Following the intravenous injection of 1.77 gm of sodium benzoate, pregnant women in the third trimester excreted an average of 0.43 gm of hippuric acid in 1 hour in contrast to the mean excretion of 0.768 gm by nonpregnant women. The factors that influence the rate of hippuric acid formation, including the mobilization of glycine, have been summarized by Williams (1959). The enhancement of urinary excretion of amino acids (Wallraff *et al.*, 1950; Miller *et al.*, 1954), especially of glycine (Christensen *et al.*, 1957), and the marked diminution in the plasma concentration of glycine

(Christensen *et al.*, 1957) noted during pregnancy may be related to the impaired hippuric acid formation by pregnant women. The specific effects of estrogens and progestins on this conjugation process in man have not been investigated.

Glucuronide biosynthesis is catalyzed by the microsomal enzyme(s), uridine diphosphate (UDP)-glucuronyltransferase, and is one of the major chemical reactions by which endogenous or foreign compounds are metabolized. This enzyme catalyzes the transfer of the glucuronic acid moiety from UDP-glucuronic acid (UDPGA) to a variety of substrates (amines, phenols, carboxylic acids, etc.) and forms an N- or O-glucuronide link. The biology of UDP-glucuronyltransferase has been given in a monograph recently (Dutton, 1966; Schmid and Lester, 1966; Jayle and Pasqualini, 1966). There has accumulated evidence for the presence in the liver of several UDP-glucuronyltransferases with distinct substrate specificities. This evidence has been compiled and critically reviewed by Dutton (1966).

The influence of pregnancy on the glucuronidation process in man has not been studied. Shibata *et al.* (1966) have examined the UDP-glucuronyltransferase activity in the liver homogenate of pregnant rats, using bilirubin as substrate. During days 15 and 21 of gestation, the enzyme activity rose to 170% of the values obtained in nonpregnant controls and declined gradually after delivery. This finding is especially interesting in view of the generally inhibitory effects which certain steroids produced in large amounts during pregnancy are known to exert on the activity of hepatic UDP-glucuronyltransferase. Thus, Inscoe and Axelrod (1960) found that chronic administration of estradiol to male rats resulted in a significantly reduced activity of hepatic microsomal UDP-glucuronyltransferase measured with *o*-aminophenol as substrate. Much earlier Lathe and Walker (1958) had shown that the formation of bilirubin glucuronide by rat liver slices was inhibited when sera from pregnant women, cord blood, or newborn infants were added to the incubation medium. A variety of steroids also inhibit bilirubin conjugation by rat liver slices (Lathe and Walker, 1958) and the activity of UDP-glucuronyltransferase in rat (Hsia *et al.*, 1960, 1963a) and guinea pig (Hsia *et al.*, 1963b) liver microsomes, measured with *o*-aminophenol, *p*-nitrophenol, and 4-methylumbelliferone as substrates.

Of particular interest with respect to pregnancy is pregnanediol (5β-pregnane-3α,20α-diol), which appears to be a competitive inhibitor of UDP-glucuronyltransferase in microsomal preparations (Hsia *et al.*, 1963a,b). Pregnanediol glucuronide is also an active inhibitor of this enzyme *in vitro* (Hsia *et al.*, 1963b; B. Jones, 1964) and, at lower concentrations, of the transport of conjugated direct-reacting bilirubin from

liver slices into the incubation medium (Bevan *et al.*, 1965). Pregnanediol has been isolated from the sera of pregnant women (Hsia *et al.*, 1960) and newborn infants (Holton and Lathe, 1963) and identified as one of the active principles responsible for the inhibitory action of pregnancy sera. Its 20β-isomer, 5β-pregnane-3α,20β-diol, which is also a competitive inhibitor of UDP-glucuronyltransferase, has also been isolated and purified (Arias *et al.*, 1964) from the milk of nursing mothers whose infants were suffering from the clinical syndrome of unconjugated hyperbilirubinemia associated with breast feeding (Arias *et al.*, 1963; Newman and Gross, 1963). The role of pregnanediol and its 20β-isomer in the pathogensis of neonatal jaundice is still uncertain. Arias and Gartner (1964) administered 5β-pregnane-3α,20β-diol to 4 infants at a dose level of 0.33 mg/kg/day, and in 2 infants aged 6–8 days, marked unconjugated hyperbilirubinemia developed after 4 to 5 days of steroid treatment. This promptly remitted following the withdrawal of the steroid. However, in a larger series of infants, Ramos *et al.* (1966) observed no significant effects of administered pregnanediol or its 20β-isomer on serum bilirubin levels. It is obvious that detailed understanding of glucuronide metabolism at the clinical level must await further clarification of the problems concerning the multiple forms of hepatic UDP-glucuronyltransferase (Dutton, 1966), the kinetic properties of the enzyme(s) with respect to various substrates including the steroid hormones themselves (Slaunwhite *et al.*, 1964), and the relationship between glucuronidation and the secretory mechanism in the overall hepatic disposal of substrates such as bilirubin (Lathe, 1967). Advances in these areas of investigation have been severely hampered by the recalcitrance of the enzyme to easy solubilization procedures and by its lability once solubilization is achieved.

B. Oxidative Reactions

The hepatic microsomal membranes, which are vesicular fragments of the endoplasmic reticulum, contain enzymes referred to as mixed-function oxidases (Mason *et al.*, 1965) which are responsible for various oxidative conversions of many chemicals and drugs (Gillette, 1966; Conney, 1967), including steroid hormones (Conney *et al.*, 1965; Conney, 1967). These enzyme-catalyzed reactions, which require TPNH and molecular oxygen, bring about hydroxylation, N-dealkylation, O-dealkylation, sulfoxidation, N-oxidation or phosphothionate oxidation of drugs, with or without concomitant alterations in their pharmacological or toxic properties (Remmer, 1965). Although there are some suggestions for multiplicity of the mixed-function oxidases (Gillette, 1966), all oxidative reactions catalyzed by hepatic microsomes seem to be function-

ally dependent on a CO-binding microsomal cytochrome (D. Y. Cooper *et al.*, 1965; Cammer *et al.*, 1966; Schenkman *et al.*, 1967), called cytochrome P-450 (Omura and Sato, 1962). This heme protein is so named because of the absorption peak at 450 mμ which is exhibited by its reduced CO-complex.

It has long been recognized (Holck *et al.*, 1937) that the pharmacological action of barbiturates, which are metabolized and inactivated in the liver via hydroxylation of the alkyl side chains (J. R. Cooper and Brodie, 1957), lasts longer in adult female rats compared with male animals. This sex difference is peculiar to the rat and is not seen with consistency in other animals (Holck *et al.*, 1937; Quinn *et al.*, 1958). In female rats, the sleeping time following the administration of hexobarbital is approximately 4 times as long as that in the male, and the clearance of the drug from female plasma is markedly delayed (Quinn *et al.*, 1958). The rate of metabolism of hexobarbital by microsomal preparations of female rats is moreover only about one-fifth the rate in the male; and the administration of testosterone to female rats enhances, and of estradiol to male rats depresses, the enzyme activity (Quinn *et al.*, 1958). Similar findings are noted for the hepatic oxidation of phosphothionates such as the dimethoxy ester of benzotriazine dithiophosphoric acid (DBD) and ethyl *p*-nitrophenyl thionobenzenephosphonate (EPN) (Murphy and DuBois, 1958). The hepatic hydroxylase activity for a number of steroids, viz. testosterone, estradiol, estrone, progesterone, and dehydroepiandrosterone, is also higher in adult male rats (Jellinck and Lucieer, 1965; Heinrichs *et al.*, 1966; Kuntzman *et al.*, 1966) than in immature or adult female rats.

In an *in vitro* study, Tephly and Mannering (1968) have shown that both estradiol and progesterone, together with a number of other steroid hormones, are competitive inhibitors of microsomal oxidase(s) for ethylomorphine and hexobarbital in the rat. This finding is compatible with the spectrophotometric demonstration of the interaction of estradiol and a variety of other drugs including hexobarbital microsomal cytochrome P-450 (Schenkman *et al.*, 1967). In studies in which steroid hormones are administered to rats, the following results have been reported. Chronic treatment of male rats with progesterone decreases the capacity of the liver to oxidize DBD and EPN (Murphy and DuBois, 1958), while exerting no influence on hexobarbital metabolism *in vitro* (Juchau and Fouts, 1966). Chronic administration of norethynodrel, on the other hand, induces a significant enhancement of *in vitro* metabolism of hexobarbital. It is of interest that treatment of equal duration with an oral contraceptive agent containing norethynodrel and mestra-

nol, results in a significant reduction of hexobarbital oxidation, in contrast to the increase observed with norethynodrel alone (Juchau and Fouts, 1966).

Whether some of the sex hormone-induced alterations in hepatic drug oxidation in the rat are related to changes in the amount of microsomal mixed-function oxidases, which can be induced by a number of drugs (Ernster and Orrenius, 1965; Conney, 1967), has not been rigorously examined, although the shortening of the duration of action of barbiturates is considered in general to be indicative of an increase in the level of hepatic microsomal enzymes that inactivate them (Conney *et al.*, 1960; Conney, 1967). Such a steroid-induced effect on the liver, whether mediated through direct inhibition or change in the amount of the oxidative enzyme(s), could have important implications in relation to problems of drug metabolism during pregnancy or the long-term use of synthetic hormones for suppression of ovulation or other purposes (Crawford and Rudofsky, 1966).

C. Transport into Bile

Hepatic parenchymal cells have the capacity to take up and secrete into the bile a number of substances at concentrations exceeding their plasma levels. These substances, both endogenous and foreign, have been designated "cholephils" by Hargreaves and Lathe (1963). They include bilirubin, bile acids, certain organic dyes (BSP, indocyanin green, rose bengal, fluorescein, etc.), antibiotics (novobiocin and methicillin), and steroid hormones. Bilirubin is conjugated primarily with glucuronic acid (Billing and Lathe, 1956; Schmid, 1956; Talafant, 1956), and bile acids with glycine or taurine (Bremer, 1956), before secretion into bile, while BSP is secreted intact or conjugated with glutathione (Javitt *et al.*, 1960; Combes and Stakelum, 1961). Indocyanin green and rose bengal are excreted intact (Cherrick *et al.*, 1960; Kubin *et al.*, 1960). In general, the rate of clearance of the cholephils from plasma, whether they are infused or endogenously produced, is considered to depend on the following biological processes: (1) uptake into the parenchymal cells; (2) metabolic conversion (if any) in the cell; and (3) secretion into the bile canaliculi. No resorption or active secretion of these substances is thought to take place within the distal portion of the bile duct system although this question has been experimentally resolved (Arias, 1966a). The metabolism and transport of various cholephilic substances, of which bilirubin and BSP have received the most intensive investigation, have recently been reviewed by several authors (Leevy, 1961; Hargreaves and Lathe, 1963; Combes, 1964; Wheeler, 1965; Arias, 1966b; Schmid, 1966). The present section of the review summarizes the evidence for both pregnancy-

and steroid-induced alterations in hepatic disposal of certain of these substances.

In pregnant rats Shibata *et al.* (1966) measured the maximum transfer rate (T_m) of bilirubin into bile by continuous infusion of bilirubin in amounts exceeding the T_m value (Weinbren and Billing, 1956). Despite known elevation in the activity of hepatic UDP-glucuronyltransferase, measured with bilirubin as substrate, the rats in various stages of pregnancy showed the values of bilirubin T_m similar to those of nonpregnant controls. It was concluded that in the rat the secretion of conjugated bilirubin into bile is the rate-limiting step in the overall transport of bilirubin from plasma to bile and that this secretory process is unaffected by pregnancy. In pregnant women, the serum concentrations of bilirubin and the results of the standard BSP test, which measures the retention of the dye in plasma 45 minutes after a single intravenous administration of a fixed amount of dye, generally fall within values considered to be in the normal range. The mean values for these tests, nevertheless, are slightly higher in pregnant women (Combes *et al.*, 1963; Tindall and Beazley, 1965). Blood flow through the liver appears to be unchanged during pregnancy (Munnell and Taylor, 1947).

Studies involving the infusion of cholephilic substances have resulted in a better understanding of the alterations in secretory activity of the liver during pregnancy than can be obtained by using the simpler, more conventional clinical tests. In 1933, Soffer reported that plasma clearance of an infused loading dose of bilirubin was significantly delayed in pregnant women during the latter half of gestation. The transient nature of this pregnancy-induced diminution in hepatic capacity to dispose of bilirubin was also noted. By means of the constant infusion method developed by Wheeler *et al.* (1960a,b), Combes and his associates (1963) studied the quantitative aspects of BSP removal by the liver in a group of pregnant women. This infusion technique permits estimation of both the storage and secretory capacities of the liver for BSP and provides considerably more sensitive indices of alterations in hepatic metabolism of the dye than is possible by the conventional single injection BSP test. The authors noted a mean increase of 122% in the relative hepatic storage capacity for BSP and a consistent and significant decrease in the maximum transport rate (T_m) for dye excretion into bile. The mean value for the T_m in nonpregnant women was 8.5 mg/minute; in women in the latter half of pregnancy it was 6.2 mg/minute, or an average of 27% less than the control value. Tindall and Beazley (1965) used compartment analysis (Richards *et al.*, 1959) based on multiple sampling of plasma following a single intravenous injection of BSP in pregnant women. The rates were expressed in milligrams of BSP transferred per minute per 100 mg of the

dye in the compartment from which the transfer was taking place. In late pregnancy the authors noted: (1) a slight increase in the rate of hepatic uptake of BSP from plasma; (2) a marked reduction in the rate of excretion of the dye from the liver cell into the bile; and (3) an increased rate of reflux of the dye into plasma. The latter phenomenon made quantitatively only a minor contribution in the overall transport of the dye. Although these two studies measured different kinetic parameters of dye transport, they produced results that are in substantial agreement. They indicate that pregnancy is associated with a significant impairment of the excretory capacity of the liver for BSP, noticeable either at saturating or nonsaturating concentration of the dye in the hepatic compartment. Recovery of the excretory capacity of the liver to a normal level takes place rapidly following delivery (Combes *et al.*, 1963).

Women with intrahepatic cholestasis of pregnancy develop BSP retention and jaundice with elevation of serum activities for alkaline phosphatase and 5′-nucleotidase, but little evidence for hepatocellular necrosis. Although these chemical findings as well as histological alterations in the liver compatible with intrahepatic cholestasis suggest a hepatic defect primarily in the transport capacity, few investigations bearing on this aspect of liver function have been made, particularly during the anicteric stage of the disease. In a possible example of such an investigation, Adlercreutz *et al.* (1967) studied a young woman with a history of recurrent jaundice during several successive pregnancies. Between the sixth and eighth lunar months of gestation, the concentrations of conjugated estradiol, estrone, and estriol in her bile decreased even though she was not icteric, suggesting impaired hepatic excretion of steroids into the biliary system despite the continued capacity of the liver to handle bilirubin disposal in an apparently normal manner. With the subsequent development of jaundice near term, the biliary concentration of estrogens underwent a further, and marked, decline. In a recent study of 13 patients with intrahepatic cholestasis of pregnancy Kater *et al.* (1967), employing an abridged modification of Wheeler's method, noted a significant diminution in the relative storage capacity and T_m of the liver for BSP in comparison with women in whom the pregnancy was uncomplicated. These results are not unexpected, but the surprisingly high values reported for the values of T_m for BSP in their subjects with uncomplicated pregnancy are difficult to interpret.

A hormonal basis for the pathogenesis of intrahepatic cholestasis of pregnancy has been repeatedly suggested (Svanborg and Ohlsson, 1959; Ikonen, 1964; Kappas, 1967, 1968). The course of the disease, viz. the characteristic onset of jaundice during late pregnancy and the prompt subsidence of all signs and symptoms upon termination of pregnancy

(Haemmerli and Wyss, 1967), has an obvious correlation with the extent of maternal exposure to endogenously produced estrogens and progestins. Furthermore, of the cases on record of women who developed jaundice following oral contraceptive therapy, the number of those who also have a history of intrahepatic cholestasis of pregnancy appears to be more than coincidental. Of 23 reported cases of contraceptive-induced jaundice with a history of pregnancy and known gestational course, more than half were noted to have previously had intrahepatic cholestasis of pregnancy (Haemmerli and Wyss, 1967). If one takes the incidence of *all* cases of jaundice during pregnancy to be less than 0.1% as reported by Haemmerli (1966), the significance of such an association is apparent. Finally, when patients with a known history of intrahepatic cholestasis of pregnancy where given oral contraceptives (Broake *et al.*, 1965; Elliot and Hendry, 1965) or a synthetic estrogen (Kreek *et al.*, 1967a,c) they developed all the signs, symptoms and laboratory findings of intrahepatic cholestasis, which subsided on withdrawal of the drugs. Thus, although the evidence still remains circumstantial, the argument that the hormonal changes peculiar to late gestation may be etiologically related to the intrahepatic cholestasis of pregnancy seems compelling.

The specific hormonal basis for this functional abnormality appears most likely to be attributable to the estrogens produced during pregnancy rather than to other steroids. Gallagher *et al.* (1966) have examined the effects of a variety of natural and synthetic steroids on BSP metabolism in the rat. Impaired hepatic disposal of the dye was produced by estradiol as well as by some of its metabolites, including estrone and estriol, following 10 days' treatment at 1 mg/day. Estradiol did not alter hepatic glutathione (GSH) content or BSP-GSH conjugating activity. At the same dose and duration of treatment, a variety of other natural steroids of the C_{19}, C_{21}, and C_{24} types (testosterone, progesterone, cortisol, bile acids, etc.) did not affect BSP metabolism. Kreek *et al.* (1967b) have also observed that rats treated with ethynylestradiol show, in addition to delayed clearance of BSP, a 50% reduction in bile flow and a decrease in the biliary excretion of the administered estrogen.

Estriol and estradiol, when given to man in amounts approximating those produced in late normal pregnancy (M. N. Mueller and Kappas, 1964a,b), also induce retention of BSP within a few days of treatment. Utilizing the constant-infusion techniques of Wheeler and associates (1960a,b), a regular and pronounced decline in hepatic secretory T_m for the dye was observed without predictable change in hepatic relative storage capacity for the dye. Multiple sampling of plasma following a single injection of BSP in estrogen-treated women (L. L. Kottra and Kappas, 1966) showed a significant delay in the later or "excretory" por-

tion of the decay curve, a finding compatible with impairment in transfer of the dye from the liver cell into the bile. The appearance of excessive amounts of conjugated BSP in plasma during estrogen treatment is consistent with an increase in the rate of reflux of the dye back into the plasma from the liver as observed in pregnancy.

The standard BSP test in women taking oral contraceptives reveals only occasional abnormal retention of the dye in plasma (Eisalo *et al.*, 1965; Kleiner *et al.*, 1965; Larsson-Cohn, 1965), although the incidence of this abnormality appears to be related in part to the amount of the contraceptive steroids (Allan and Tyler, 1967) ingested. However, Kleiner *et al.* (1965), using infusion techniques, determined the relative storage capacity and T_m of the liver for BSP in 9 women who had been taking an oral contraceptive preparation containing norethynodrel and mestranol at ovulation-suppressing doses and showed that after several months of either cyclic or continuous treatment, the mean hepatic T_m for BSP was reduced to 31–60% of control values. The storage capacity remained unchanged. In 2 patients from whom the drug was withdrawn, T_m values were normal 7 days later. The inference can be drawn that application of the infusion method generally to the study of BSP metabolism in such women would reveal a much higher incidence of hepatic abnormalities than is detected by the standard BSP test.

In summary, it can be stated that consistent and reversible alterations are produced in hepatic function late in human pregnancy and during treatment with estrogens in amounts approximating their daily production during pregnancy or with estrogen-progestin mixtures currently used in many ovulation-suppressing preparations. These alterations are characterized primarily by impairment in the transfer process of cholephilic substances from the hepatic compartment into the bile. The uptake of cholephils into the liver cells and their intracellular metabolism are usually unaffected or, if affected, do not appear to alter significantly the overall process of transport from the peripheral circulation into the biliary system. The rate of the latter process is limited by the excretory capacity of the liver, which is a function of the canalicular surface of the parenchymal cells. Thus, the transport abnormalities evoked in the circumstances indicated often resemble those observed in diseases characterized by congenital defects involving the excretory process, such as the Dubin-Johnson syndrome in man (Wheeler *et al.*, 1960b; Arias, 1961). The recurrent appearance of clinically apparent liver dysfunction characterized largely by intrahepatic cholestasis in certain pregnant women and the ease with which a similar abnormality can be reproduced in these women with administeded estrogens further suggest an intrinsic susceptibility of the liver in such subjects to the inhibitory

effects of certain hormones on the excretory mechanism. The reported instances of jaundice following oral contraceptive therapy may also be an exaggerated expression of the consistent, if clinically inapparent, excretory defect produced by such hormones in young women, and this form of jaundice and the intrahepatic cholestasis of pregnancy probably share a common etiologic basis.

VII. Concluding Remarks

The present review has summarized a number of the major biological actions of estrogens and progestins in the liver of man and experimental animals as well as certain alterations in the functional state of this organ which are associated with pregnancy. We have not considered the effects of estrogens or progestins on isolated liver cell systems, on isolated enzymes, on the natural history of certain primary liver diseases (e.g., hepatitis), or on the injurious sequelae of experimentally administered hepatotoxins. Nevertheless, the diversity of the biological effects described provides an indication of the potential importance of the liver as a site of action of estrogens and progestins, at least in the pharmacological situation as in pregnancy, and of the possible existence of a spectrum of as yet unidentified but clinically significant hepatic actions of these hormones.

Three aspects of the discussions presented in the review merit reemphasis: (1) the majority of the biological actions exerted by estrogens and progestins in the liver accompany the clinical or experimental use of potent synthetic hormones, or in the case of natural hormones, of amounts which generally approximate those produced in late gestation; (2) certain biological effects may not necessarily be attributable to the actions of the hormones themselves but to those of their metabolites or of nonovarian hormones or other biologically active substances the secretion of which may be evoked by estrogens and progestins; and (3) certain population groups, for genetic or other reasons, may be particularly susceptible to some of the deleterious actions of estrogens and progestins.

The heterogeneity of the material presented makes impossible an overall generalization of the clinical implications of the effects of estrogens, progestins, and pregnancy on the liver. However, it may be of importance to note that a variety of observations discussed in the preceding sections indicate a frequent inhibitory influence of pregnancy and gestational hormones on the capacity of the liver to dispose of endogenous metabolites or administered drugs by secretory or metabolic mechanisms. From the clinical point of view therefore it is necessary to be particularly aware of these steroid or pregnancy effects on hepatic function and to anticipate the possibility of atypical metabolism or disposal of certain drugs as a

result. The relevance of this consideration to problems of therapeutics in relation to pregnancy or the long-term clinical use of estrogens and progestins is evident.

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