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Developments in jaundice

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JAUNDICE is a large subject, but two topical aspects are the potential hepatic toxicity of oral contraceptives, and the possible use of hepatic enzyme induction in therapy.

Hepatic effects of oral contraceptives

Oral contraceptives are usually prescribed as a combined pill containing 1–4 mg of a progestagen, and 0·05–0·15 mg of an oestrogen, taken for 3 weeks from the 5th day of each menstrual cycle. Less commonly sequential administration is used, when oestrogen is taken for the first 2 weeks, and oestrogen plus progestagen for the next week. These doses are small compared to the normal production of progesterone and oestradiol, for the corpus luteum of menstruation secretes 20–30 mg/day of the former and 1 mg/day of the latter, and in late pregnancy the placenta up to 300 mg, and 15–30 mg/day, respectively.

The progestagens used in contraceptive pills are synthetic and of two types (Fig. 1). Most are derivatives of testosterone but, lacking its C_{19} methyl group, are called 19-*norsteroids*. They include norethisterone (norethindrone), norethynodrel and ethynodiol; and lynoestrenol. The two other progestagens used, megestrol and chlormadinone, are derivatives of hydroxyprogesterone, and have a different structure. Either ethynyloestradiol or mestranol are used for the oestrogen component. None of these steroids are 17α-*alkyl*-substituted, but the 19-norprogestagens and these oestrogens do have *un*saturated hydrocarbon groups in the 17α position.

Unlike testosterone, 19-norprogestagens are partly converted to oestrogens *in vivo* (Bishop, 1968). About 10% of norethisterone acetate is metabolized to urinary 17α-ethynyloestradiol (Brown & Blair, 1960) which has twenty times the oestrogenic effect of oestradiol, and although Paulsen (1966) found that it produced gynaecomastia, other oestrogenic effects surprisingly are not marked. Megestrol and chlormadinone are not so metabolized (Cooper & Kellie, 1968).

Occurrence of impaired hepatic function

In 1964 Eisalo, Jarvinen & Luukkainen reported from Helsinki that twelve elderly post-menopausal

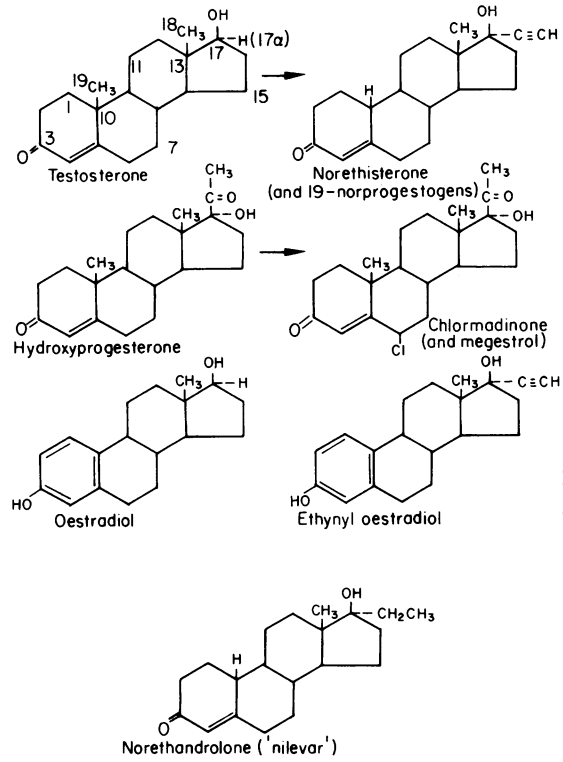

FIG. 1. The structure of some steroid molecules.

women developed raised serum transaminase levels and retention of bromsulphthalein (BSP) after 1 month's treatment with an oral contraceptive preparation for menstrual irregularities. Since then approximately 100 cases of jaundice have been recorded in the world literature, although this reaction probably occurs in less than 1 in 10,000 of the millions of women taking the pill (Schaffner, 1966). Initial symptoms of vague nausea and pruritus are followed by jaundice which is usually slight, but occasionally severe. Biochemically and histologically the picture is of a mixed hepatocellular and cholestatic lesion. This syndrome usually occurs in the first few weeks of treatment and rapidly regresses when the pill is withdrawn.

Minor abnormalities of liver function and histology probably occur in a much higher percentage of women on the pill. Kleiner, Kresch & Arias (1965) used the infusion test of Wheeler *et al.* (1960) to measure both the hepatic storage capacity and the transport maximum (T_m) for bromsulphthalein. Both these are concerned in the hepatic excretion of this dye, and this test may detect changes in liver function when the routine BSP test (measurement of retention in blood 30–45 min after intravenous injection) is within normal limits. They found that contraceptive doses of norethynodrel and mestranol given for several months impaired BSP transport maxima in all nine normal women studied, storage capacities remaining normal (Fig. 2). The electron

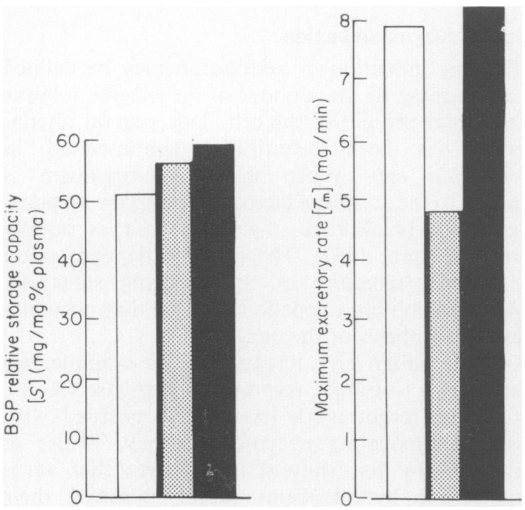

FIG. 2. The effect of norethynodrel and mestranol on BSP storage capacity and maximum excretory rates of normal women. Open columns, Before; stippled columns, norethynodrel + mestranol; solid columns, after.

microscope will also often show changes of cholestasis in the liver during contraceptive treatment, with dilated canaliculi, distortion and loss of canalicular microvilli, and increased numbers of abnormal mitochondria. Light microscopy is usually normal (Larsson-Cohn & Stenram, 1967).

Cause of abnormalities

It is uncertain whether the progestagen, the oestrogen component, or both are responsible. Combes *et al.* (1963) have shown selective impairment of the BSP transport maxima in the last trimester of pregnancy, when large amounts of progesterone and oestrogen circulate. When equivalently large amounts of oestradiol were given to thirty-one non-pregnant women, retention of BSP frequently occurred (Mueller, Kappas & Damgaard, 1964), while the serum transaminase levels and routine liver histology were unaffected. In rats, Gallagher, Mueller & Kappas (1966) have demonstrated abnormal BSP retention with oestradiol and mestranol, but progesterone and norethynodrel were inactive, as was progesterone in man (Kappas, 1968).

Further information comes from the relationship of contraceptive liver damage to recurrent intrahepatic cholestasis of pregnancy (Haemmerli, 1966). In this condition pruritus alone, or pruritus and moderate obstructive jaundice develop in the last trimester of succeeding pregnancies, returning to normal soon after delivery. Serum alkaline phosphatase levels are raised and retention of BSP occurs. Centrizonal cholestasis in needle liver biopsies is surprisingly mild. These women are particularly liable to develop jaundice with oral contraceptives, and they account for about half of the cases of such jaundice reported. The condition is commoner in Scandinavia and Chile, where the incidence of contraceptive jaundice is also highest (Orellana-Alcade & Dominguez, 1966). In addition, abnormalities of liver function without jaundice are probably commoner in women on the pill in these areas for serum transaminase levels are raised in up to 18% of pre-menstrual women in some Scandinavian series (Eisalo, Jarvinen & Luukkainen, 1965), but are normal in series from Mexico (Rice-Wray, 1964), Holland (Swaab, 1964), London (Swyer & Little, 1965) and Los Angeles (Tyler, 1964). There is presumably some genetic or environmental factor behind this geographical variation.

Kreek *et al.* (1967) treated seven patients with previous pruritus or jaundice of pregnancy with 0·5–1·5 mg of 17α-ethynyloestradiol alone for up to two weeks, but did not try a progestagen. This dose is ten times that in the pill, but in six control women it produced only nausea. In the seven patients their previous symptoms in pregnancy were reproduced, liver function was variably impaired, and one patient became temporarily jaundiced.

Progestagens

In most studies, administration of progestagens has had little effect. Adlercreutz & Ikonen (1964) gave the progestagen lynoestrenol to a patient with previous pruritus of pregnancy without change, but two progestagen and oestrogen contraceptive combinations induced jaundice before and after. In the original twelve patients of Eisalo *et al.* (1964) lynoestrenol was inactive, and Borglin (1965) had similar results. In other studies, however, the 19-norprogestagens without oestrogens have been shown to impair liver function and rarely to cause jaundice (Perez-Mera & Shields, 1962). In a recent

report, remarkably prolonged and deep jaundice developed in two sisters, one treated with norethisterone alone, the other with norethisterone plus ethynyloestradiol (Somayaji *et al.*, 1968).

Other 19-norsteroids, such as methyltestosterone and norethandrolone, are well known to impair BSP excretion (Marquardt *et al.*, 1961) and to cause cholestatic jaundice. These steroids have an *alkyl* group in the 17α-position (Fig. 1), which increases their cholestatic action (Gallagher *et al.*, 1966). There is some evidence that the similar but unsaturated substitution groups in the 17α-position in 19-norprogestagens do likewise, and Eisalo, Heino & Rasanen (1968), for example, have recently found that the 19-norprogestagen lynoestrenol induced more abnormal liver function tests than did the progestagen megestrol, which has a different structure.

Kleiner *et al.* (1965) suggested that progestagens are the hepatotoxic component of the pill, for they found that oestradiol (2·5 mg/day) for 10 days had no effect on the liver function of normal women, even on the BSP transport maxima. But they used smaller doses of oestrogen than Mueller *et al.* (1964), and did not actually test the progestagen alone. These results, and those of Urban, Frank & Kern (1968) are better interpreted as showing that synthetic oestrogens impair liver function more than the natural steroids. In support of this, the oestrogenic effects of ethynyloestradiol are much more marked than those of oestradiol.

It is possible that the combination of oestrogen and a progestagen, particularly a 19-nor compound, may increase their individual toxicity. Eisalo *et al.* (1968) found this, lynoestrenol plus mestranol further impairing the liver function tests which had been altered by lynoestrenol alone. Alternatively the metabolism of 19-norprogestagens to the very active ethinyloestradiol (Brown & Blair, 1960) may fully explain the hepatic toxicity of progestagens.

Significance of toxicity

The current evidence, therefore, is that minor abnormalities of liver function occur quite commonly, but frank jaundice is rare, except in certain areas where there may be increased susceptibility as shown by the frequent occurrence of intrahepatic cholestasis of pregnancy. Both are probably caused by oestrogens. Progestagens, particularly 19-nor compounds, may be occasionally responsible and may also have an additive effect with the oestrogen. All these lesions are reversible, and must be considered benign, but long-term studies of liver function in women taking the pill are required to determine both the incidence and significance of these effects, especially in the British population.

Oral contraceptives should not be given to patients with a previous history of jaundice or pruritus of pregnancy, nor to patients with hereditary hepatic excretory defects, nor with any of the different varieties of intrahepatic cholestasis. It is probably best not to prescribe them to patients with hepatitis or other liver disease within recent months, although opinions on this are divided (Ockner & Davidson, 1967).

Contraceptive pills which have a progesterone-derived progestagen such as megestrol or chlormadinone, rather than the testosterone-derived 19-nor-progestagens may be safer, and the long term contraceptive use of chlormadinone alone (Martinez-Manautou *et al.*, 1967) may prove to produce even less liver damage.

Hepatic enzyme induction

Enzyme induction or adaptation may be defined as an increase in the amount of an enzyme relative to the total protein of the cell. This general phenomenon has been beautifully demonstrated in *Escherichia coli*. When this micro-organism is exposed to the substrate lactose, the enzyme responsible for its breakdown, β-galactosidase, is rapidly induced (Cohn, 1957). The reaction depends upon the specific structure of the inducing substrate, which may inhibit a genetic repressor that normally prevents synthesis of the enzyme.

In mammalian liver, tryptophan, for example, has been shown to induce tryptophan pyrollase (Knox, 1962), but recent work has been concerned with hepatic detoxicating enzymes. Conney, Miller & Miller (1956) first showed in rat liver that some carcinogenic hydrocarbons rapidly induced their own metabolism, but were inactive when added *in vitro*. The amino acid analogue, ethionine, prevented this response, which suggested that enzyme protein synthesis was occurring. A large number of drugs including some hypnotics, anti-convulsants, analgesics and insecticides (Remmer, 1964) have now been shown to induce many hepatic enzymes which metabolize toxic metabolites and drugs, and which are localized to the microsomal cell fraction. Surprisingly, this response is non-specific. Seen with the electron microscope the membranes of the cytoplasmic smooth endoplastic reticulum increase greatly in number during the induction (Remmer & Merker, 1963), these membranes corresponding to the microsomes of the biochemist.

Relation to bilirubin metabolism

An early effect of this induction is an increase of the hepatic haem-containing cytochrome enzyme P.450, which is involved in microsomal oxidation and electron transport during drug detoxication (Remmer *et al.*, 1966). If the isotopically labelled

precursor of bile pigments, glycine, or better, delta aminolaevulinic acid, is given to animals, the labelled bilirubin excreted in bile from a few minutes to 24 hr later is known as the 'early labelled' bilirubin (Israels *et al.*, 1963). Part of this comes from the breakdown of P.450, and to a lesser extent from other liver haem compounds (Schwartz, 1967). Schmid, Marver & Hammaker (1966), after giving for 6 days the powerful enzyme inducer phenobarbitone to rats, found a six-fold increase in the concentration of hepatic P.450, and a considerable increase of the early labelled bilirubin.

Glucuronyl transferase, which conjugates bilirubin, is also microsomal, and although in general glucuronidating enzymes are induced less than other detoxicating enzymes, Catz & Yaffe (1962) demonstrated, in newborn more than adult mice, an increase of hepatic bilirubin-conjugating activity following phenobarbitone. Roberts & Plaa (1967) found that phenobarbitone-treated rats excreted bilirubin loads more rapidly than controls, and with tied bile ducts the reflux of conjugated bilirubin into blood was increased.

Use of inducers in therapy

Yaffe *et al.* (1966), therefore gave phenobarbitone to an infant with unconjugated hyperbilirubinaemia, in whom glucuronyl transferase was probably deficient. Two periods of treatment with 45 mg/day led to a marked fall in the serum bilirubin levels. Crigler & Gold (1966) gave 30 mg/day to a severely hyperbilirubinaemic infant with kernicterus with a similar result, the fall in serum bilirubin levels being associated with an increased faecal excretion of bile pigment (Crigler & Gold, 1967), which would be expected to follow induction of the deficient transferase. But recently Robinson *et al.* (1967) studied the same patient, and found that phenobarbitone increased his hepatic endoplastic reticulum, but surprisingly, the production of early labelled pigment was only slightly raised.

We have treated with phenobarbitone a 16-year-old boy with unconjugated hyperbilirubinaemia and defective menthol excretion. His plasma bilirubin level of 8 mg/100 ml fell to less than 2 mg/100 ml over a 10-day period (Thompson & Williams, unpublished observations 1968) (Fig. 3). It is possible, therefore, to improve the unconjugated jaundice of some patients, but there will be no effect, however, if the enzyme is absent from the liver of man in the most severe forms of congenital unconjugated hyperbilirubinaemia, nor in the homozygous strain of Gunn rat, in spite of an increase in the endoplastic reticulum (De Leon, Gartner & Arias, 1967). The response to an inducer may be an indication of whether individual microsomal enzymes are present or genetically absent.

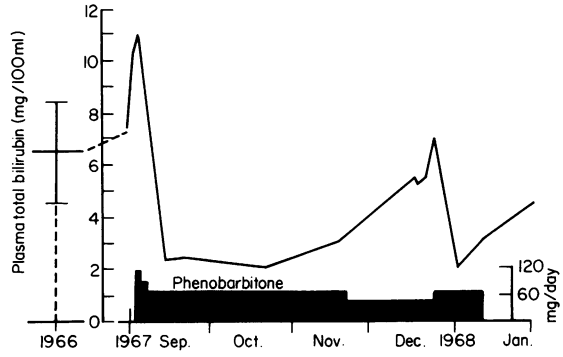

FIG. 3. The effect of phenobarbitone on the plasma bilirubin level of a boy with congenital unconjugated hyperbilirubinaemia.

The physiological unconjugated hyperbilirubinaemia of the newborn may be due to a deficiency of glucuronyl transferase (Odell, 1967). Hart *et al.* (1962) using rabbits, and Catz & Yaffe (1962), mice, demonstrated an increase of glucuronyl transferase activity in the livers of neonates born to animals treated with phenobarbitone during pregnancy. Trolle (1968) surveyed retrospectively the infants of mothers treated with phenobarbitone during pregnancy for epilepsy or pre-eclampsia, and found significantly less neonatal jaundice (serum bilirubin level of more than 10 mg/100 ml) in both premature and infants of normal weight compared with control infants born to mothers not so treated. He also found that 5–15 mg phenobarbitone given daily to infants after birth significantly reduced the incidence of jaundice. Whether it is possible to delay the exchange transfusion of rhesus-incompatible infants by such enzyme induction remains to be established.

Thompson & Williams (1967) reported slower reduction of plasma bilirubin levels in a small series of women with chronic intrahepatic cholestasis treated with phenobarbitone (Fig. 4). This does not occur in extrahepatic obstruction (Thompson & Williams, unpublished observations 1968), nor perhaps in biliary atresia (Cunningham, Kelley & Peters, 1968). Although Metge *et al.* (1964) found reduced activity of hepatic glucuronyl transferase in cirrhosis, this has not been confirmed. Thompson & Williams (1967) suggested that an increase of glucuronyl transferase activity to above normal levels may increase the concentration of conjugated bilirubin in hepatic cells, and so increase excretion into the bile canaliculi. The fall in plasma bilirubin levels in their patients is surprising, since bilirubin production from haem compounds is expected to be increased through induction of the hepatic haem enzymes.

The study of hepatic enzyme induction in man is difficult, for he is probably exposed to many inducers

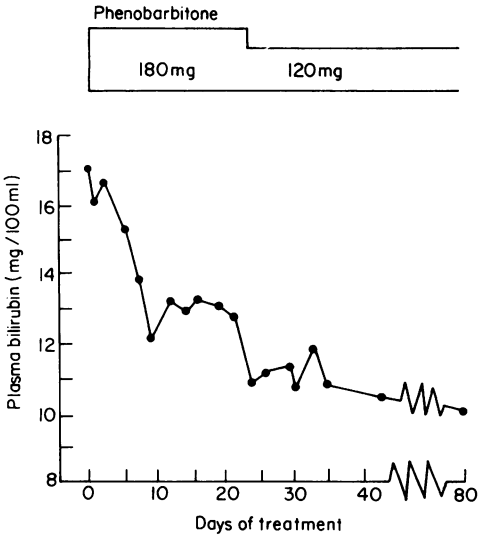

FIG. 4. The effect of phenobarbitone on the plasma bilirubin level of a woman with primary biliary cirrhosis.

in daily life, including drugs, insecticides, and even caffeine in food. In rats, the bedding of their cages may induce enzymes (Vesell, 1967). Another difficulty is a pronounced species variation (Remmer, 1968). Knowledge of how drugs which induce their own metabolism may produce tolerance to their action and to other drugs given simultaneously is scanty.

Therapeutic enzyme induction may also be dangerous, for a few drugs such as carbon tetrachloride, are metabolized to toxic products in the liver. In rats, treatment with phenobarbitone increases the hepatic toxicity of carbon tetrachloride (Marshall & McLean, 1968). Finally, long continued induction might even produce resistance to the drug or perhaps depression of enzyme activity so that clinical use should be approached with caution.

Acknowledgment

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