A STUDY OF SOME ASPECTS OF LIVER GROWTH IN THE PREGNANT RAT

Research Topic

presented as requirement for the Degree

of M.Sc. in Biochemistry

by

PAMELA BRUNT, B.Sc.

(Session 1964/65)

Department of Biochemistry,
University of St. Andrews,
St. Andrews, Fife.

October, 1966

ACKNOWLEDGMENTS

The author would like to express her sincere thanks to Dr. G.A.J. Goodlad for all his help and encouragement throughout this work.

Thanks are also due to Dr. M.C. Macgregor and Mr. J. Mackie for help with electron-microscopy, to Mr. E. Carstairs for duplicating diagrams and photography, and to Mrs. McAlister for the typing.

C O N T E N T S

CONTENTS (Contd.)

INTRODUCTION

1. Nitrogen Metabolism in Pregnancy

1(i) Overall changes in N metabolism.

An increased retention of dietary nitrogen has been shown to accompany pregnancy in animals and in humans (Boyne, Chalmers and Cuthbertson, 1953; Hunscher, Donelson, Nims, Kenyon and May, 1933; Mitchell, Carroll, Hamilton and Hunt, 1931; Jakobsen, 1957). These data show that, although the net rate of protein synthesis by the adult mammal is greatly increased during pregnancy, there are appreciable variations between species. The rat, for instance, synthesises about ten times as much protein per unit time per unit body weight as does the human. Blaxter (1964) calculated from published data that the mg. N retained per day per kg. body weight in late pregnancy for rat, human, pig and ox were 430, 55, 34 and 80 respectively.

What is known about the internal deployment of the increased nitrogen retained? Penzes (1959) found that two thirds of the nitrogen retained during pregnancy in rats was incorporated into the reproductive tissues and the foetus. During the entire period of gestation products of conception in the cow account for 1,500 g.N (Mitchell *et al.*, 1931) and in the sow 270 g.N (Jakobsen, 1957). However, if the course

of deposition of protein is studied during pregnancy, it is found that protein deposition occurs mainly during the second half of pregnancy and then at an ever increasing rate. This is true both for laboratory animals and humans (Blaxter, 1964).

1(ii) Protein deposition in uterus and uterine contents

The growth of the placenta(e) occurs rapidly at first and in many species the weight of the placenta does not change very much in the last trimester. In the guinea pig, (Flexnor and Pohl, 1941) however, the placenta continues to increase in weight throughout pregnancy. The weight of the placenta undergoes little change after the 70th day in the ewe and goat and after the 21st day in the rabbit (Barcroft, 1948). With most species the placenta accounts for 10-15% of the total products at term, though in women it is as high as 20% of the total N of uterus and contents (Widdowson and Spray, 1951) and, in the sow, as little as 6% (Jakobsen, 1957).

Pregnancy can be maintained in rats fed a protein-free diet provided they receive exogenous oestrone and progesterone. Hazelwood and Nelson (1965) showed that in these circumstances placentae at term were only 60% of the weight of those in rats fed a 24% casein diet without hormone supplementation. Thus deposition of placental

protein can occur in the absence of a dietary supply of amino acids. The smaller placentae of these animals probably accounts for decrease in foetal number and weight which these workers observed in this group.

The massive increase in N content of the uterine contents in the latter half of pregnancy is largely accounted for by the growth of the foetus. The weight of the foetus increases at a less rapid rate than does its protein content and consequently as the foetus grows, its N content per g. increases (Blaxter, 1964).

Measurement of the concentration of nitrogenous compounds in the arterial supply and venous drainage from the uterus has been used to give an indication of the compounds utilised in the synthesis of proteins in the foetus and placenta and for hypertrophy of the uterine wall. In pregnant rats, less amino N was found in the venous blood supply from the pregnant uterus than in the arterial blood suggesting that plasma amino acids are used as building blocks for uterine placental and foetal protein. Jakobsen (1957) found differences in the rates of uptake of individual amino acids. There was no evidence for utilisation of plasma proteins (Setnikar, 1957; Setnikar and Barron, 1958; Jakobsen, 1957). The arterio-venous

differences in amino acids were found by Setnikar and Barron (1958) to have no significant correlation with the stage of pregnancy. Since the N demand increases as pregnancy advances, this implies that the uterine blood flow increases in direct proportion to intra-uterine protein synthesis which is in keeping with earlier work on changes in uterine blood supply in sheep during pregnancy (Barcroft, 1948).

The fact that the concentration of free amino acid N in foetal vessels has been shown to be higher than in maternal vessels (Hagerman and Villee, 1960) suggests that the transport of amino acids across the placentae is an active process.

1(iii) N-metabolism in extra-uterine tissues

Numerous investigations have been made on the increase of extra-uterine body weight in pregnant compared to non-pregnant rats. Most, but not all, show an increase in weight. Morse and Schmidt (1944) showed that N retention in extra-uterine tissues in the rat over 21 days of pregnancy varied from a maximum of + 2.08 g. to a minimum of — 1.6 g. The extra-uterine loss of N was associated with a low consumption of food.

Poo, Lew, Lee and Addis (1940) noted a considerable

maternal storage of N in the carcass of pregnant rats, with a tendency for the storage to increase as the protein content of diet was increased to 27%. A similar type of experiment by Spray (1950) showed a maternal storage of 1.4 gN during pregnancy in the rat. Boyne et al. (1953), however, were unable to show maternal storage. Bourdel and Jacquot (1960) fed pregnant rats at three different levels of protein intake and found that, although the N content of the uterine contents was independent of protein intake, the total N retention during gestation varied from 59% to 354% of the N content of the uterine contents.

Thus the extra-uterine tissues need not necessarily increase in total N content and whether they do so appears to be dependent on dietary factors. The dietary dependency of the weight of the pregnant rat is supported by the work of Morrison (1956).

The critical level of dietary protein required to sustain pregnancy in rats appears to be 6%. At levels below this reabsorption of foetuses in at least 85% of the animals occurs (Nelson, 1959). Hazelwood and Nelson (1965) found that rats fed on a protein free

diet and injected with oestrone and progesterone from the third to the twentieth day of gestation successfully maintained pregnancy without decreased nitrogen excretion or increased food intake. This points to the efficient mobilisation, transport and/or utilisation of maternal protein precursors in absence of dietary protein and they found that the most important maternal source of protein precursors in absence of dietary protein was the skeletal muscle.

During pregnancy in the rat, certain tissues invariably gain in N content. This suggests that the maternal rat has an ability to translocate amino acids from sites other than those at which deposition occurs (Blaxter, 1964). The liver increases markedly in size and N content (Bokelman and Scheringer, 1932; Sonders and Morgan, 1957) and this point will be discussed in more detail later. Smaller increases in weight occur in the gut and kidney (Boyne et al., 1953). Rat mammary glands show a significant increase in weight on the fifth day of pregnancy (Griffiths and Turner, 1961) compared to those of non-pregnant controls. Similar increases in growth of the rat mammary gland in pregnancy have been reported by Greenbaum and Slater (1957) and

McLean (1958). There is an increase in both cell size and cell number in the uterine wall during pregnancy (Needham and Cawkwell, 1957) while the total N per cell increases fourfold (Wakid and Needham, 1960).

A progressive decrease in plasma protein concentration throughout pregnancy, of the order of 7-8% of the non-pregnant values, has been reported in women (Mack, 1955). Other workers have found the fall to occur only in the first part of pregnancy (McGillivray and Tovey, 1957) after which the level stabilised. Lange (1946) claimed that there was a rise in concentration at the sixth month of pregnancy after an initial fall, and Liddelow (1953) described an abrupt terminal rise in concentration prior to delivery. The albumin/globulin ratio falls during pregnancy in women from 1.49 : 1 in early stages, to 1.08 : 1 in later stages (Lai-Wen Wu, 1959). Most authors agree that this is due to both a fall in the concentration of albumin and a rise in all the globulin fractions except γ-globulin which tends to fall (Mack, 1955; Bang and Paaby, 1955; McGillivray and Tovey, 1957; Langman, van Druen and Bouman, 1959). Some authors have reported that albumin and the α and β globulins behave similarly during pregnancy but that

γ- globulins increased (Matiar-Vohar and Geinert, 1961) or remained constant (Mansfield and Sheltar, 1963; Mofty, Zawahri and Megged Maamoun, 1960). Tovey (1959) found an increase in the α_2 globulin concentration of the order of 50% while the β globulin increased by only 30%.

In pregnant animals, too, there are alterations in the various plasma protein fractions. Decreased serum protein concentrations have been reported in pregnant rabbits (Langman and van Druen, 1959) and from the fourth to seventh month in pregnant cows (Kovyndikov and Prostyakov, 1959; Prostyakov, Fortushnyi and Kovyndikov, 1961). In the latter case, the albumin concentration decreased while the concentration of the β and γ globulin fractions increased. Thus, in most species, a change in the ratios of the plasma proteins occurs with a decrease in the concentration of total plasma proteins..

There appears, however, to be an increase in the plasma volume during pregnancy. Malagoli and Donatelli (1962) reported an increase from 44 to 48 ml./kg. body weight in pregnant women. Paaby (1960) has investigated the changes in plasma proteins and plasma volume during pregnancy in women and found the same changes in the

concentrations of plasma proteins as observed by most authors. When he took account of the increase in the plasma volume, however, the total circulating amounts of total plasma protein, globulin and even albumin were found to be increased. Paaby suggested that differences in the increases are a response to increased functional capacities of the various fractions, e.g. as carriers for steroids, metabolic products and raw materials. A study of total serum glycoprotein by Mansfield and Sheltar (1963) showed that they were increased in pregnant women. These workers found an increased percentage of bound carbohydrate in the albumin and γ-globulin but a decreased amount in the α_1, α_2 and β globulin fractions.

Plasma fibrinogen was also decreased in pregnant women (Alvarez, Alfonso and Scherrard, 1961; Malagoli et al., 1962).

2. A comparison of changes in liver composition during pregnancy with those reported for other instances of rapid liver growth.

As already stated, the liver increases in size during pregnancy in rats. This is accompanied by increases in content of various constituents, in particular, the nucleic acids and the aim of the present work is to investigate further some of these changes.

Sonders and Morgen (1957) in their study of the liver of pregnant rats found no change in the water content nor in the N-content per g. solids or per g. non-fat solids at the end of pregnancy. Davidson and Weymouth (1944) found an increase in the total nucleoprotein concentration in the pregnant rat liver. Campbell and Kosterlitz (1949) have shown that the DNA.P content expressed as mg/liver increased slightly while RNA.P mg/liver increased considerably, and these changes were independent of the protein content of the diet. The DNA.P increase expressed as the difference between the observed values and those predicted from the maternal body weights minus weight of uteri and their contents were significant at the 14th day of pregnancy (+ 0.362 $\pm$ 0.077 mg/liver) and

21st day (+ 0.127 $\pm$ 0.044 mg/liver) but not on the 10th day. When RNA.P content of pregnant livers was expressed similarly, an increase was found at all stages of pregnancy examined, i.e. 10th, 14th, 18th and 21st days. This increase was greatest on the 21st day (1.4 mg. per mg. DNA.P) and least on the 14th day (0.2 mg. RNA.P per mg. DNA.P).

The rise in liver RNA in pregnancy is not common to all species. The liver cell RNA of non-pregnant mice, guinea pig, rat and cat varies with the liver cell protein content, and for a given protein content is lower for guinea pig and cat than for mouse and rat (Campbell and Kosterlitz, 1953). In late pregnancy, the liver cell RNA is considerably increased for mouse and rat, less for guinea pig and unaltered in the cat. Campbell and Kosterlitz (1953) also found a rise in the DNA and protein content of liver cells of mice and rats but not for guinea pigs.

Campbell and Kosterlitz (1949) made an intensive study on the RNA content of livers of pregnant and non-pregnant rats. The "excess RNA" (defined as the RNA present above the expected value calculated from body and liver weights) was present on the 10th day of gestation,

rose steeply from the 14th day to the end of pregnancy, and decreased rapidly in the first few days of lactation. In further work, Campbell, Innes and Kosterlitz (1953) showed that provided the placentae were left intact, removal of the foetuses did not cause the "excess RNA" to disappear. These workers concluded that at least two factors play a role in the "excess RNA" phenomenon. Firstly an unknown factor secreted by the placenta, acting independent of the pituitary, and secondly increased amounts of oestrogens apparently requiring the presence of the pituitary.

Most normal plasma proteins with the exception of γ- globulins are synthesised in the liver (McFarlane, 1964). The increase in total plasma proteins demonstrated in pregnancy is another reflection of the increased synthesising capacity of the liver. In vitro studies have shown an increase in the ability of the liver of pregnant rats to synthesise protein. Little and Lincoln (1960) incubated liver slices with L-valine-1-C^{14}. A significant increase in the uptake of radioactivity into liver protein between the 6th and 16th days of gestation in pregnant rats was observed. These workers found that administration of various steroid sex hormones (progesterone, oestradiol and testosterone) had no effect

on the rate of *in vitro* incorporation of L-valine-1-C^{14} into protein by liver slices prepared from non-pregnant rats.

Addis and Gray (1950) found a constant relationship between liver and body weights. In an animal such as the rat, where the body weight increases throughout life, the liver continues to grow slowly with a tendency to decrease a little in senescence. In animals which have a determined growth, the liver growth ceases when the typical adult level is reached. Other evidence supporting this comes from studies on the mitotic rate in liver and its rate of uptake of tritiated thymidine. Brues and Marble (1937) reported only 1 mitosis per 10,000 to 20,000 liver cells and Leblond and Walker (1956) reported a slightly higher value of 0.1% in the rat. This has been confirmed by the more recent work of Guzek (1964) who showed negligible incorporation of tritiated thymidine into DNA in the normal adult rat liver.

All this evidence gives a picture of rigid control of liver growth in the normal adult rat liver. This control is obviously upset in pregnancy. In other conditions, too, disproportionate liver growth is observed. In the foetal and early post-natal liver

as well as in regenerating adult liver, rapid growth is observed. There is often, too, a phase of rapid liver growth in the tumour-bearing animal. The information available on the relevant changes undergone by liver in these circumstances will now be considered.

During gestation the foetal liver increases in weight (Williamson, 1948) although in most animals the liver weight relative to total body weight decreases during this period (Doljanski, 1960). There is an increase in liver weight relative to body weight during the weaning period (Jackson, 1913), coinciding with the liver gaining full functional activity. In both foetal and early post-natal growth, the rate of increase in liver size is high and decreases to zero at different ages depending upon the species (McKellar, 1949; Tier and Lavant, 1953). The cells are diploid in foetal liver but by the time the weaning period is reached in rats (body weight c. 30 g.) there is a decrease in the number of diploid cells and a concomitant appearance of tetraploid cells (Naora, 1957). The number of diploid cells continues to fall with a rise in tetraploid cells as liver growth continues. In the growing rat when a body weight of 100 g. has been

achieved, a small number of cells of greater DNA content than tetraploid occur. These continue to increase in number as the mature rat continues to grow.

The nucleic acids of foetal liver show marked changes during development. The concentration of DNA on a dry weight basis is relatively constant from 16th to 19th pre-natal days and then falls to a third by the 21st day. This decrease is part of the maturation process since it continues at a slower rate after birth. The RNA concentration falls far less rapidly so that the ratio of RNA to DNA changes from a value of 0.9 at the 16th day of foetal life to 2.9 at the 10th day of extra-uterine life (Gershwind and Li, 1949). Wirth and Scheier (1956) confirmed the fall in the DNA concentration of foetal liver but found a steady rise in the RNA concentration.

The concentrations of DNA and RNA in rat liver in the immediate post-partum period were shown to reach the maximum level at 5 and 15 days after birth respectively (Oliver, Bollard, Shield and Bentley, 1962). Palade (1955) studied the distribution of ribosomes in over 40 different cell types, and found that the highest concentrations existed in embryonic cells and in rapidly proliferating cells in the adult. In these cells the endoplasmic

reticulum is poorly developed. Oliver, Blumer and Witham (1963) found very high concentrations of free ribosomes (i.e. not attached to the membranes of the endoplasmic reticulum) in late foetal liver and during the first five days post partum. The concentration thereafter fell to a low level. In young rats the plasma protein concentration remains at a low level until ten days after birth, and thereafter it rises rapidly (Oliver et al., 1962) coinciding with the decrease in free ribosome concentration. It was suggested that the free ribosomes become attached to the membranes of the endoplasmic reticulum at this stage (Oliver et al., 1963). Thus the increase in plasma protein may be a reflection of a change in the protein synthetic mechanisms of the liver.

Another instance where the mitotic rate in liver increases is after partial hepatectomy. As normal adult liver weight is approached, mitosis slows down and eventually normal adult liver size is reached (Harkness, 1957; Weinbren, 1959). Leong, Pessotti and Brauer (1959), however, consider regeneration ceases before normal adult weight is reached. Bucher and Glinos (1950) have suggested that the age of the rat is a factor in determining how far regeneration will proceed.

Examination of regenerating rat liver in the electron

microscope has shown that there is a disaggregation of the endoplasmic reticulum with some loss of membranes and dispersion of ribosomes (Bernhard and Rouiller, 1956; Bernhard, 1958). By sixteen to eighteen hours, the endoplasmic reticulum begins to reform in close contact with groups of mitochondria (Bernhard et al., 1956), starting in cells in the centro-lobular area and progressing rapidly towards the periphery (Glinos, 1958). The normal structure is restored by thirty-six to forty-eight hours (Bernhard et al., 1956).

DNA synthesis, which is negligible in normal liver, exhibits an abrupt rise around fifteen to eighteen hours after partial hepatectomy. There is a sudden active incorporation of labelled precursors which reaches a peak at twenty-two to twenty-six hours, falls off somewhat but continues at elevated levels for some days. The DNA content per nucleus is highest during the phase just preceding the onset of mitosis (Bucher, 1963). The concentration of liver RNA increases after partial hepatectomy.

A net increment of protein synthesis is not easily detectable until nearly twelve hours after partial hepatectomy. The most rapid rate of increase is from twelve to about thirty-six hours (Ferrari and Harkness, 1954). Compared to normal liver, the soluble pH5 enzymes from regenerating

liver augment the activity of either normal or regenerating microsomal fractions by 1.5 to 2 times. It has been suggested that this effect is due to increased amino acid activating enzymes or to enhancement of the transfer mechanism (Hultin and Von der Decken, 1958; Rendi, 1959). Microsomes from regenerating liver have been shown to be approximately twice as active as those from normal liver when incubated with the same soluble fraction (Von der Decken and Hultin, 1958). During regeneration the RNA content of the microsomes is increased, indicating a higher proportion of ribosomal relative to membranous elements (Von der Decken et al., 1958; McCorquodale, Veach and Mueller, 1960). When compared on the basis of equal RNA content, the regenerating microsomes remain at least 1.5 times as active as normal microsomes. However Hoagland and his colleagues (Hoagland, Scornik and Pfefferkorn, 1964) have put forward evidence that bound ribosomes in normal liver contain an inhibitor of protein synthesis, and the concentration of this substance is much reduced in microsomes from regenerating liver. Thus in general, similar types of changes are found in both regenerating liver and foetal and post-natal liver growth when compared to normal adult liver. There are increases

in nucleic acid concentration involving particularly RNA. Disorganization of the endoplasmic reticulum is present and there is a high concentration of free ribosomes.

It has been proposed on morphological grounds that ribosomes in close contact with membranes participate in the synthesis of proteins rich in cell products for secretion whereas ribosomes unattached to membranes are involved in protein synthesis for intracellular purposes. This pattern is exhibited by liver when it shifts from production of protein for export to production for cellular growth and proliferation as seen in regenerating liver and in foetal and new born liver. This hypothesis has been put forward by a number of electron microscopists including Porter (1954), Hay (1958), Slautterback and Fawcett (1959), Birbeck and Mercer (1961) and others and discussed by Prescott (1960).

Little information is available on the nature of the changes in nucleic acid content which have been observed in livers of pregnant rats. One of the aims of the present work was therefore to throw some light on this problem, and to compare morphological changes occurring in the liver during pregnancy with those which

have been reported in other instances of liver growth.

Preliminary work (Goodlad, unpublished) had shown that most of the increase in RNA in livers of pregnant rats occurred in the microsomal fraction. This fraction is a heterogeneous mixture consisting mainly of rough and smooth endoplasmic reticulum and free ribosomes. In the present work the changes in the various components of the microsomal fraction were studied by electron microscopy and also by ultracentrifugal techniques.

In regenerating liver the increase in protein synthesising capacity of the ribosomes has been mentioned. It was therefore decided to investigate the amino acid incorporating activity of the various fractions of the microsomes from pregnant rats.

3. Control of Liver Growth

As already discussed, the liver is under rigid control in the normal adult animal, but this control can break down in certain circumstances. The exact mechanism whereby this control is effected is not known but certain hypotheses have been proposed.

Evidence from experiments in tissue culture (Akamutsu, 1923; Glinos and Gey, 1952) and from others using parabiotic animals (Bucher, Scot and Aub, 1951)

pointed to the evidence of factors in the blood being involved in the control mechanism. That these have an inhibiting action was suggested by Glinos (1958) in view of his finding that the mitotic rate after partial hepatectomy was much reduced when water intake was restricted and plasma volume subsequently reduced. Glinos visualised an automatic self-regulating mechanism with liver as the site of synthesis of inhibitory factors. Partial hepatectomy would reduce the concentration of serum constituents and thus mitosis would be stimulated. As liver size increases, the concentration of inhibitory factors would increase and so regeneration would slow down and eventually cease. Glinos (1956) showed that cell division can be reduced in vivo by lowering the concentration of serum constituents and can be inhibited in regenerating liver by increasing the concentration of serum constituents. This and other evidence led him to propose the plasma proteins as the factor controlling liver mitosis.

Hemingway (1960), however, showed that administration of cortisone to hypophysectomised, partial hepatectomised rats caused complete inhibition of mitosis. When the rats were treated with growth hormones and cortisone the inhibition was overcome in proportion to growth

hormone administered. Hemingway thus suggests that the withdrawal of corticosteroid influence is a likely means of instigating growth processes. The work of Guzek (1964) with tritiated thymidine incorporation into DNA of regenerating liver also supports this conclusion. He showed that all cells were rapidly labelled in partially hepatectomised rats, but that after treatment with cortisone there was a marked decrease in uptake of radioactivity compared to the controls. Since an increase in precursor concentration was also shown, Guzek suggests that inhibition is at the level of thymidine incorporation into DNA.

One instance where control of liver growth breaks down is in tumour-bearing animals. The characteristics of liver growth in this condition are an increase in relative liver weight accompanied by cell proliferation and increased DNA turnover rate (Annau, Manganelli and Roth, 1951; Kelly and Jones, 1950) and disproportional increase in RNA. Hepatomegaly in tumour-bearing animals is accompanied by an increase in adrenal size and there is some evidence to suggest they are hyper-functional with respect to both glucocorticoid and aldosterone production (Begg, 1958; Hilf, Burnett and

Borman, 1960; Rechcigl, Grantham and Greenfield, 1961). Yet it is difficult to correlate a hypercortical condition as the above suggests with increase in liver growth in the light of Hemingway's work with cortisone.

In the case of rats bearing the Walker 256 carcinoma, Clark and Goodlad (1961) have shown an increase in Δ^4-steroid hydrogenase activity in the liver. This enzyme system seems to be the rate-limiting step in the inactivation of corticosteroids (Tomkins, 1959). The reduction of ^{4}-3-ketosteroid in rat liver at ring A was studied by Yates, Herbst and Urquhart (1958) under various physiological and experimental conditions and they concluded that the rate of adrenal cortical secretion may be determined by the capacity of the liver to inactivate corticosteroids by reduction at ring A. Δ Further studies (Urquhart, Herbst and Yates, 1959) showed that the inactivation of corticosteroids in liver has a controlling effect on adrenal size rather than the reverse. The result of an increase in the rate of inactivation of adrenal corticosteroids would be a fall in the level of corticosteroids in the blood. Since the rate of secretion of the hormone corticotrophin is under a negative feedback control, [redacted] a

decrease in blood corticosteroids would promote its increased production which in turn would stimulate adrenal enlargement. Hence the increase in hepatic Δ^4-steroid hydrogenase activity in tumour-bearing animals found by Clark and Goodlad may explain both the release of liver from rigid growth control and also adrenal hypertrophy.

The situation in the pregnant rat bears certain similarities to that in the tumour-bearing rat. There is in both cases an increase in liver size and disproportionate increase in RNA. An increase in the weight of the adrenal gland has been shown at the 18th and 19th days of pregnancy in rats by Ricci, Viola and Gori (1963) which is again comparable to the situation found in tumour-bearing rats. An increase in adrenal steroid output in pregnant women has been shown by Cope and Black (1959) by isotope dilution techniques. From this they calculated the daily output of adrenal corticosteroids to be 11 mg. in non-pregnant women and 25 mg. in women during late pregnancy.

Nichol and Rosen (1963) showed that hepatic alanine-transaminase activity was very sensitive to glucocorticoids. Administration of cortisol or

corticosterone to normal rats caused a large increase in alanine transaminase activity, while adrenalectomy caused a decrease (Harding, Rosen and Nichol, 1961). The activity of this enzyme in pregnant rats was, however, found to decrease markedly after the tenth day of gestation. From the fifteenth to the twenty-first days of pregnancy, when growth of foetuses is most rapid and adrenal hypertrophy occurs, the fall in activity was most marked. Furthermore, during this period, the activity was no longer sensitive to exogenous cortisol. From the knowledge of the increase in total circulating plasma proteins and presumed increase of adrenal output in pregnancy, this is a surprising result. One reason for this might be the fact that corticosteroids are present in the blood in both the free state and also bound in a biologically inactive form to a plasma protein of α_1 globulin mobility termed transcortin, and the level of transcortin has been shown by several workers to be increased in pregnancy (Daughaday, Kozak and Biederman, 1959; Doe, Zinneman, Flink and Ullstrom, 1960; Farese and Plager, 1962).

However it was felt that hepatic inactivation of steroids might also be a factor involved, and this was

studied in the present work. The activity can be assayed in tissue slices or homogenates. Urquhart et al. (1959) have discussed the relevance of results obtained by both these methods. Both reduced NADPH and enzyme protein are limiting in the tissue slice, and it is thought, therefore, that this method gives a closer approximation of Δ^4-steroid hydrogenase activity in the conditions found in the cell. For this reason the tissue slice method was selected for the present work.

EXPERIMENTAL

Treatment of Animals

Female adult virgin albino rats of the Wistar strain were used in these experiments. The rats were housed in individual cages. Some of the rats were mated and the day on which spermatazoa and/or copulation plug was noted in the vagina was taken as the start of pregnancy and the male rat removed from the cage. Rats were fasted overnight for initial fasting body weight. As has been discussed in the introduction, the changes in protein content of the extra-uterine tissues in the pregnant rat vary with its nutritional status and since these animals tend to increase their food intake, particularly at the final stages of pregnancy (Blaxter, 1964), an attempt was made to keep food intake of control and pregnant rats constant during the experimental period. Both control and pregnant rats were therefore fed daily 12 g. of a synthetic diet, the composition of which is shown in Table 1. Groups of pregnant rats were killed on the 14th, 16th, 18th and 20th day of pregnancy along with groups of control rats. Rats were killed by stunning and exsanguination. Food was withheld from the rats

from the evening of the day before sacrifice. Body weight, liver weight, adrenal weight and, in the case of the pregnant rats, the weight of the placentae and uteri and contents were noted. The liver was analysed by methods given below.

Isolation and Separation of the Microsomal Fraction of the Livers of Pregnant and Non-pregnant Rats.

The method employed was essentially as described by Hallinan and Munro (1965) and is illustrated diagrammatically in Figure 1. Rats were killed and livers removed, weighed and chilled. About 2.5 g. liver were accurately weighed and homogenised in 15 ml. ice-cold 0.88M sucrose using a Potter-Elvehejm (1936) homogeniser. All homogenisations were carried out using six slow plunges of a close fitting pestle. The volume of the homogenate was adjusted to 25 ml. and two 10 ml. samples taken. Centrifugations were carried out on an M.S.E. "Super-speed 40" refrigerated centrifuge using the 8 x 25 ml. rotor. The samples were first centrifuged for 20 minutes at 16,500 r.p.m. (18,000 g.) to remove nuclei, mitochondria and any unbroken cells. The supernatant containing the microsomal fraction was removed using a Pasteur pipette and centrifuged for 1 hour

TABLE 1

DIET

V.M.R.	.....	100 g.
Casein	.....	240 g.
Margarine	.....	42 g.
Starch	.....	636 g.
Glucose	.....	182 g.

V.M.R. (Vitamin Mineral Roughage)

Sodium Chloride	.....	65 g.
Salt Mixture	.....	260 g.
Vitamin-Starch Mixture		500 g.
Radiostoleum B.D.H.		1.6 ml. (containing 0.114 g. α tocopherol acetate)
Agar	.....	125 g.

Salt Mixture

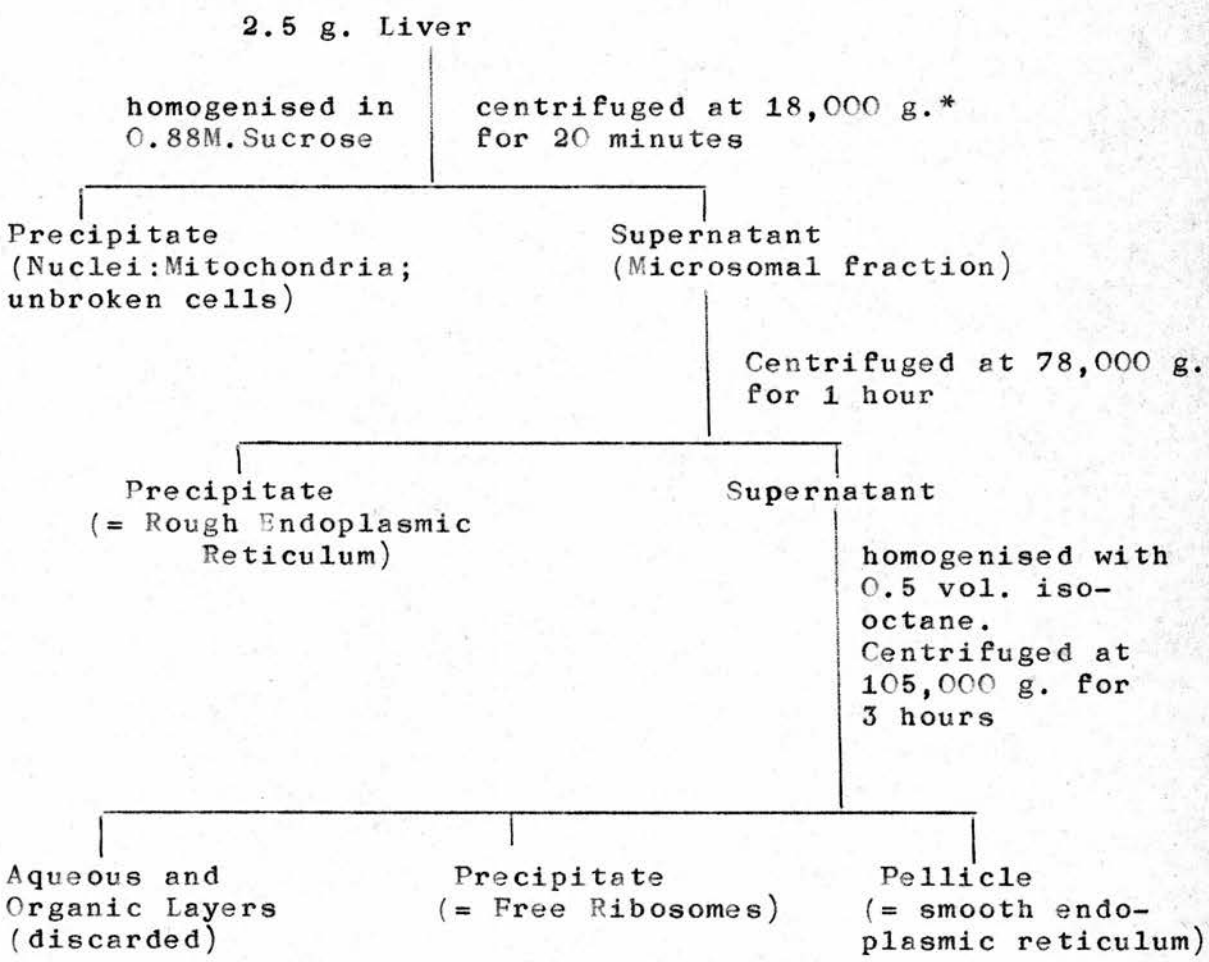
Sodium Chloride	243.2 g.
Potassium Citrate	533.0 g.
Calcium Hydrogen Orthophosphate	800.0 g.
Di-Potassium Phosphate	174.0 g.
Calcium Carbonate	368.0 g.
Ferric Citrate.$3H_2O$	360.0 g.
Copper Sulphate.$5H_2O$	0.4 g.
Potassium Alum.$24H_2O$	0.2 g.
Magnesium Carbonate	92.0 g.
Manganese Sulphate	2.8 g.
Potassium Iodide	0.1 g.
Zinc Carbonate	0.1 g.
Cobalt Chloride.$6H_2O$	0.2 g.
Sodium Fluoride	0.002g.

Vitamin-Starch Mixture

Pyridoxine HCl.	25 mg.
Riboflavin	25 mg.
Thiamine HCl.	25 mg.
Nicotinic Acid	100 mg.
Menaphthone	5 mg.
Biotin	5 mg.
Calcium Pantothenate	200 mg.
p.Amino Benzoic Acid	500 mg.
Inositol	1 mg.
Choline Chloride	10 g.
Folic Acid	trace
Potato Starch	to 500 g.

at 34,000 r.p.m. (78,000 g.) to bring down the rough endoplasmic reticulum. The supernatant was pipetted off and homogenised with 0.5 volumes of iso-octane at 0°C. for 1 minute. The emulsion was allowed to stand in ice for at least 1 hour before it was centrifuged for 3 hours at 40,000 r.p.m. (105,000 g.). The ribosomes now formed a dark red pellet at the bottom of the tube while the smooth membranes formed a thin membrane at the interface of the two layers. Estimations of RNA and phospholipid P were carried out on each of the separated fractions.

Figure 1



* All R.C.F. calculated for centre of tube.

Estimation of Ribonucleic Acid

Estimations of RNA using methods depending on ultra-violet absorption (following alkaline hydrolysis) may yield values which are erroneously high due to the presence of protein breakdown products. These must be eliminated if such methods are to be used. Fleck and Munro (1962), however, have developed a rapid and reliable method for the estimation of RNA in liver, using ultra-violet absorption based on the fact that if the conditions of alkaline incubation (Schmidt and Thannhauser, 1945) are controlled, negligible quantities of protein are broken down while the RNA is completely hydrolysed to a stage where its ultra-violet absorption is maximal (hyperchromicity effect complete). They have shown that these conditions can be fulfilled by a 60 minute incubation at 37°C. in 0.3N potassium hydroxide without prior lipid extraction.

To each sample to be analysed 0.5 vol. ice-cold 2.1N Perchloric Acid (PCA) was added and the mixture allowed to stand for 15 minutes at 0°C. The precipitate was centrifuged for 10 minutes at 2,000 r.p.m. on an M.S.E. "Major" refrigerated centrifuge. The precipitate was washed twice with ice-cold 0.7N PCA. The PCA was

carefully drained off before the addition of alkali for digestion.

The smooth membrane fraction isolated from the microsomal fraction was contaminated with iso-octane and if acid precipitation is proceeded with immediately the membranes tend to float to the surface during centrifugation making quantitative recovery difficult. To remove iso-octane, the fraction is first precipitated in hot 80% (v/v) ethanol (final concentration) and boiled for 5 minutes before the addition of PCA. It has been demonstrated that this use of hot ethanol does not reduce the recovery of RNA (Hallinan and Munro, 1965).

The acid-extracted samples were digested for 1 hour at 37°C. with 0.3N KOH known to be free of ultra-violet absorbing contaminants. The amount of alkali used per sample was 4 ml., 1 ml. of which was allowed for neutralisation of the residual acid in the sample. At the end of the incubation, the samples were chilled then neutralised with 10N PCA and acidified with 1 vol. ice-cold 1N PCA. The precipitate was centrifuged for 10 minutes at 2,000 r.p.m. at 0°C. and washed twice with ice-cold 0.5N PCA. The combined supernatants were generally made up to 100 ml. and the final concentration of PCA was 0.1N. The optical density at 260 mμ was recorded against a blank of 0.1N PCA.

Where the quantity of RNA in the sample was small, as in the "Smooth Membrane fraction", the procedure was adjusted so that the total final volume was 25 ml.

The Extraction Coefficient at 260 mμ for purified rat liver RNA given by Fleck and Munro (1962) as $E^{1\%}_{1\ cm.}=213$ was used for calculation of the RNA content of the samples.

Isolation and Estimation of Phospholipids

(i) Isolation

The method of Folch, Lees and Sloane-Stanley (1957) was used. This involves extraction with a chloroform/methanol mixture followed by washing the extract with NaCl solution to remove any extracted substances of a non-lipid character.

Each fraction was extracted twice with 20 vol. chloroform/methanol 2:1 (v/v), 1 g. tissue being taken as equivalent to 1 ml. The specific gravity of the solvent mixture was occasionally too close to that of the suspended material for adequate separation on centrifugation. In these instances the specific gravity of the solution was lowered by addition of methanol (usually 0.2 vol. methanol suffices). The amount of methanol added was noted and twice as much chloroform was added to the extract before starting the washing procedure.

Chloroform, methanol and water were mixed in the proportion 8:4:3. The upper phase was separated off and made 0.29% with respect to sodium chloride. To the crude phospholipid extract 0.2 vol. of this solution was added, mixed and then 2 phases allowed to separate. As much of the upper phase was pipetted off as possible. The residual sodium chloride solution was removed by raising the interphase twice with small amounts of the upper phase of the chloroform, methanol and water mixture referred to above in such a way as to leave the lower phase undisturbed. Finally, the washed extract was diluted with methanol to a known volume for analysis.

(ii) Estimation

The phospholipid content of the purified extract was obtained by measuring its phosphorous content according to the method of Allen (1940).

A suitable aliquot was taken from the phospholipid extract and solvents removed in a water bath. To this 0.48 ml. 10N.H_2SO_4 was added and the sample digested on a sand bath. Complete digestion was achieved with the aid of hydrogen peroxide (100 vol. M.A.R.). After cooling, 5 ml. of distilled water were added followed by

0.8 ml. amidol reagent (1% (w/v) solution of amidol in 25% (w/v) sodium metabisulphite) and 0.4 ml. 8.3% (w/v) ammonium molybdate (A.R.). The total volume was adjusted to 10 ml. with distilled water. The optical density of the blue colour was read on a Unicam SP 600 spectrophotometer at 640 mμ after 10 minutes and before 30 minutes had elapsed.

The P content of the sample was estimated from a calibration curve.

Isolation and Estimation of DNA

(i) Isolation

The procedure was essentially according to Schmidt and Thannhauser (1945) using a 15-18 hour incubation in N.NaOH at 37°C. which hydrolysed RNA but not DNA. The DNA was precipitated by acidification and analysed by the phosphorous method.

0.5 g. liver were accurately weighed and homogenised in 8 ml. ice-cold distilled water in a Potter-Elvehejm (1936) homogeniser for 2½ minutes. The final volume was adjusted to 12 ml. and 2 x 4 ml. aliquots taken. To each, 2 ml. ice-cold 2.1N PCA was added with stirring. The mixture was allowed to stand at 0°C. for 10 minutes and then centrifuged at 0°C. for 7 minutes at 2,000 r.p.m.

on an M.S.E. "Major" refrigerated centrifuge. The precipitate was washed twice with 5 ml. ice-cold 0.7N PCA, centrifuging after each washing at 2,000 r.p.m. for 5 minutes. The supernatants were discarded.

The precipitate was extracted twice with chloroform/methanol 2:1 (w/v) and twice with ether at room temperature to remove phospholipids.

The resultant acid insoluble non-lipid powder was digested overnight (18 hours) with 2.5 ml. N.NaOH at 37°C. in a water bath. The solution was neutralised with 2.5N.HCl. (0.5 ml. per ml. N.NaOH present), cooled to 0°C. and 0.5 vol. ice-cold 2.1N PCA added. After 10 minutes, the precipitate of DNA and protein was spun down at 2,000 r.p.m. at 0°C. for 10 minutes and washed twice with ice-cold 0.7N.PCA.

(ii) Estimation

The precipitate of DNA was dissolved in 1 ml. N.NaOH, 0.58 ml. 10N.H_2SO_4 added, and digested on a sand bath, complete digestion being achieved using hydrogen peroxide. The P content was then estimated by the method of Allen (1940).

Studies on the *in vitro* Amino Acid Incorporation by Microsomal Fractions.

The method for determining the amino acid incorporating activity of the microsomal preparations was based on those of Campbell, Cooper and Hicks (1964) and Cammarano, Guidice and Lukes (1965).

The various fractions were prepared by the method previously given for the microsomal fraction separation. A liver homogenate in 0.88M sucrose was prepared and centrifuged at 16,500 r.p.m. (18,000 g.) for 20 minutes in the M.S.E. "Super Speed 40" refrigerated centrifuge to remove particles heavier than microsomes.

To obtain a total microsome fraction, the 18,000 g. supernatant was centrifuged for 3 hours at 40,000 r.p.m. (105,000 g.). The cell sap supernatant was retained.

To obtain a rough membrane fraction and a free ribosome plus smooth membrane fraction, the 18,000 g. supernatant was centrifuged for 1 hour at 34,000 r.p.m. (78,000 g.). This gives a pellet of rough membrane. The resultant supernatant was then centrifuged for 3 hours at 40,000 r.p.m. (105,000 g.) to give a pellet of free ribosomes and smooth membranes. Again the supernatant of cell sap was retained.

The cell sap fraction was dialysed against 3,000 vol. Medium A [0.25M sucrose; 0.005M tris HCl. pH 7.6; 0.004M. magnesium acetate; 0.025M potassium chloride] for 3 hours. This procedure removes inhibitors of protein synthesis (Cammarano et al., 1965). During this time the RNA fractions were stored at 0°C.

The fractions were homogenised in Medium A such that they contained approximately 10 mg. protein/ml. (Campbell et al., 1964). Two 0.4 ml. aliquots were taken from each homogenate and incubated, one with and one without an energy source. All experiments were carried out in duplicate.

The 105,000 g. supernatant and microsomal fractions were all in Medium A. To achieve the final concentrations per ml. in the incubation mixture as given by Cammarano et al., 1965, viz:- 250 µmoles sucrose; 25 µmoles tris HCl.pH 7.6; 4 µmoles magnesium acetate; 50 µmoles potassium chloride; 20 µmoles 2-mercapto-ethanol; 12.5 µmoles phosphocreatine; 20 µg. phosphocreatinekinase; 0.5 µmoles ATP (disodium salt); 0.5 µmoles GTP (sodium salt); 0.25 µC valine-1-C^{14}; 700 µg. protein (approximately) 105,000 g. supernatant; 700 µg. (approximately) ribosomal for microsomal fraction

RNA - a supplementary medium (Medium S) had to be used. Medium S contained 0.25M sucrose; 0.004M magnesium acetate; 0.075M potassium chloride.

The incubation mixture consisted of 0.4 ml. RNA fraction in Medium A; 0.1 ml. dialysed cell sap; and the following:- 20 µmoles 2-mercapto-ethanol; 12.5 µmoles phosphocreatine; 20 µg. phosphocreatine-kinase; 0.5 µmoles ATP; 0.5 µmoles GTP, each of 0.1 ml. of Medium S. To each tube 0.25 µC L-valine-1-C^{14} were added. In the incubations carried out without an energy source, ATP, phosphocreatine and phosphocreatine-kinase were omitted, and the incubation mixture adjusted to 1 ml. using Medium S.

Each tube was stoppered and shaken gently for 45 minutes in a water bath at 37°C. The reaction was stopped by precipitation of the protein with an equal volume of ice-cold 1N PCA.

The protein was prepared for counting by the method of Munro, Jackson and Korner (1964). The precipitated protein was centrifuged at 2,500 r.p.m. for 10 minutes at 0°C. The precipitate was washed three times with ice-cold 0.5N PCA containing 1% ^{12}C-valine, to allow exchange with any labelled free

valine in the protein solution.

The protein was next extracted for 15 minutes at 100°C with 0.5N.PCA to remove RNA present and prevent protein loss at later stages. After centrifuging, the supernatant was retained for RNA estimation and the RNA free precipitate was freed from lipid components by washing twice with a mixture of ethyl alcohol, ether and chloroform in proportions 2:2:1.

Finally, the protein was dried by washing twice with ether. A small aluminium planchette was smeared around the edge with silicone grease and weighed.

The protein was dissolved in 1 ml. formic acid and an aliquot of this solution transferred to the weighed planchette. The planchette was dried under an infra-red lamp and weighed. Drying and weighing were continued until constant weight recorded. The samples were counted at infinite thinness using a thin end-window Geiger tube. The observed radioactivity was corrected for background and efficiency of the instrument and expressed as cts/min./mg. protein per mg. RNA.

Electron Microscopy

(i) Liver Cells

Small fragments of liver (less than 1 mm^3) from freshly killed rats were fixed for 1.5 hr. in 1% buffered osmium pH 7.4 (Palade, 1952) at 2°C. A stock buffer solution was made containing 19.4 g. sodium acetate . $3H_2O$; 29.4 g. veronal per litre. The fixative was prepared by mixing 2% osmium tetroxide, veronal buffer, 0.1N HCl and water in the proportions 5:2:2:1. The tissue was then rinsed briefly with distilled water, dehydrated in a graded series of acetone solutions and embedded in araldite.

Thin sections (500-1,000 Å) were cut on an LKB 4800 A Ultramicrotome, and mounted on copper grids with pyroxylin/carbon supporting film.

The material was stained in the section with 2% uranyl acetate for 15 minutes and subsequently with lead citrate (Reynolds, 1963) for 10 minutes. The lead citrate solution was prepared by mixing 1.33 g. lead nitrate and 1.76 g. sodium citrate.$2H_2O$ with 30 ml. distilled water and shaking vigorously for 1 minute. The solution was shaken intermittently for 30 minutes to ensure complete conversion of lead nitrate to lead

citrate. 8.0 ml. N.NaOH (prepared from 10N NaOH carbonate-free by dilution) were now added and the suspension diluted to 50 ml. The lead citrate dissolves and is ready for use. Uranyl acetate is used for general enhancement of the contrast of the sections, while lead citrate specifically stains the cytoplasmic membranes (Reynolds, 1963). Lead citrate solution has the advantage over lead hydroxide stains in that it is more stable and less likely to cause contamination of the sections.

After having been stained with lead citrate, the sections were washed first in 0.02N NaOH for 2 minutes and then in distilled water. Stain sections were examined in a Siemens Elmiscop I using an accelerating voltage of 80 kV.

(ii) Microsomal Fractions

The free ribosome pellet and smooth membrane pellicle of the microsomal fraction separation were cut into small fragments (less than 1 mm^3) and fixed in cold buffered osmium pH 7.8 (Palade, 1952) for 1.5 hours. Subsequently the method given for liver cells was followed.

Estimation of hepatic Δ^4 Steroid-Hydrogenase Activity.

Δ^4 Steroid-hydrogenase inactivates adrenal steroids by hydrogenation of ring A of the steroid. Corticosterone is used as substrate in the assay of the enzyme since it is the principal steroid secreted by rat adrenals (Bush, 1953). Hydrogenation of the 4, 5 double bond of ring A causes a drop in the absorption at 240 mμ which is the basis for the assay.

The slices were prepared using a tissue slicer as described by McIlwain and Buddle (1953) and the method was essentially as described by Urquhart et al. (1959).

The reagents were:-

1. Phosphosaline buffer:-

1 l.	0.9% NaCl.
40 ml.	1.5% KCl.
10 ml.	3.84% $MgSO_4.7H_2O$
300 ml.	Phosphate buffer [17.8% $NaHPO_4.2H_2O$ in 20 ml. N.HCl. and made up to 1 litre]

2. Corticosterone solution: 17.2 mg. corticosterone in 0.72 ml. absolute ethanol.

The reaction mixture was prepared from the above by mixing 100 ml. phosphosaline buffer containing 163.8 mg. glucose with 0.4 ml. corticosterone solution and oxygenating for 30 minutes standing in ice.

2 ml. aliquots of the reaction mixture were placed in 50 ml. ground glass stoppered flasks and oxygenated for 1 minute. Samples of 50 mg. liver slices about 0.3 mm. thick were added and incubated at 37°C with shaking for exactly 15 minutes. At the end of this time, the reaction was stopped by adding 20 ml. purified methylene chloride (b.p. 38°-43°) and the flask was shaken vigorously for 1 minute to extract remaining corticosterone and breakdown products.

Zero time control blanks were prepared using 2 ml. reaction mixture and 20 ml. methylene chloride, then adding 50 mg. liver slices and shaking. The optical densities of the methylene chloride extracts were read on the S.P.500 spectrophotometer.

The results were expressed in terms of μ moles corticosterone inactivated per g. liver weight per 15 minutes. It was found that 0.124 μ moles corticosterone gave an optical density change of 0.100 at 240 mμ under the conditions of the assay.

RESULTS

Alteration in body weight and in weight of uteri, placentae and foetuses during later stages of pregnancy in rat.

Body weights were recorded for all rats at the start of the experiment and before sacrificing. The combined weight of the uteri, placentae and foetuses were also recorded for the pregnant rats. Under the experimental conditions there was no significant change in body weight or in any of the other parameters measured of the non-pregnant rats, even when they were maintained for as long as 20 days on the synthetic diet. The control values have therefore been averaged in Tables 2-4. There was an increase in body weight in the pregnant rats which was still significant ($P < 0.05$) after subtraction of the weight of uteri, placentae and foetuses (Table 2). Thus the body weight change during pregnancy cannot be solely accounted for by changes in the maternal reproductive system and weight of the foetuses. This has been shown by Bond (1958) to be due, in part, to changes in the maternal hydration and blood volume, and also to the fact that the pregnant rat stores N in her own tissues early in pregnancy to meet demands made later in pregnancy by

Table 2

Change in Body Weight during Pregnancy and Weight of Uteri, Placentae and Foetuses

	No. of Rats	Initial Body Weights	Difference between Initial Body Wt. and Final Body Wt. = A.	A less wt. of uteri, placentae, and foetuses.	Combined Wt. Uteri, placentae and foetuses.	% Wt. Uteri, placentae and foetuses of total body wt. increase.
Controls	21	184.0 ± 2.3*	0.43 ± 0.94	0.43 ± 0.94*	-	-
14 days pregnancy	11	165.0 ± 3.1	18.1 ± 2.4	12.3 ± 2.1	5.8 ± 0.36	32.0
16 days pregnancy	4	176.5 ± 13.4	22.5 ± 7.5	12.8 ± 6.8	9.7 ± 0.71	43.1
18 days pregnancy	11	172.0 ± 7.0	39.8 ± 6.2	17.3 ± 3.8	22.5 ± 2.4	56.5
20 days pregnancy	7	177.7 ± 5.8	58.7 ± 6.8	20.0 ± 4.5	38.7 ± 2.3	65.9

* Standard Error of Mean

the foetuses (Campbell et al., 1953). Spray (1950) and Poo et al. (1940) found considerable maternal N storage in rats from carcass experiments.

Effect of Pregnancy on Liver and Adrenal Weights in Rat.

Liver and adrenal weights were recorded for all rats and are summarized in Table 3. The weights of the liver per 100 g. initial or final body weight were significantly increased during the period studied. The adrenal weights per 100 g. initial or final body weight was increased at the 14th day of pregnancy and thereafter began to decrease. On the 20th day of pregnancy the adrenal weights, though lower than at the 14th day of pregnancy, were still significantly higher than those of the controls. The results are shown in Figure 2.

Table 3

(i) Liver Weights

	No. of Rats	g/100g. Initial body weight.	P+	g/100g. Final body wt. minus Uteri plus contents.	P+
Controls	21	2.93 ± 0.07*		2.92 ± 0.06	
14 days Pregnancy	11	3.98 ± 0.21	< 0.01	3.69 ± 0.18	< 0.01
16 days Pregnancy	4	3.66 ± 0.18	< 0.01	3.44 ± 0.17	< 0.01
18 days Pregnancy	11	4.35 ± 0.20	< 0.01	3.92 ± 0.17	< 0.01
20 days Pregnancy	7	4.74 ± 0.25	< 0.01	4.24 ± 0.19	< 0.01

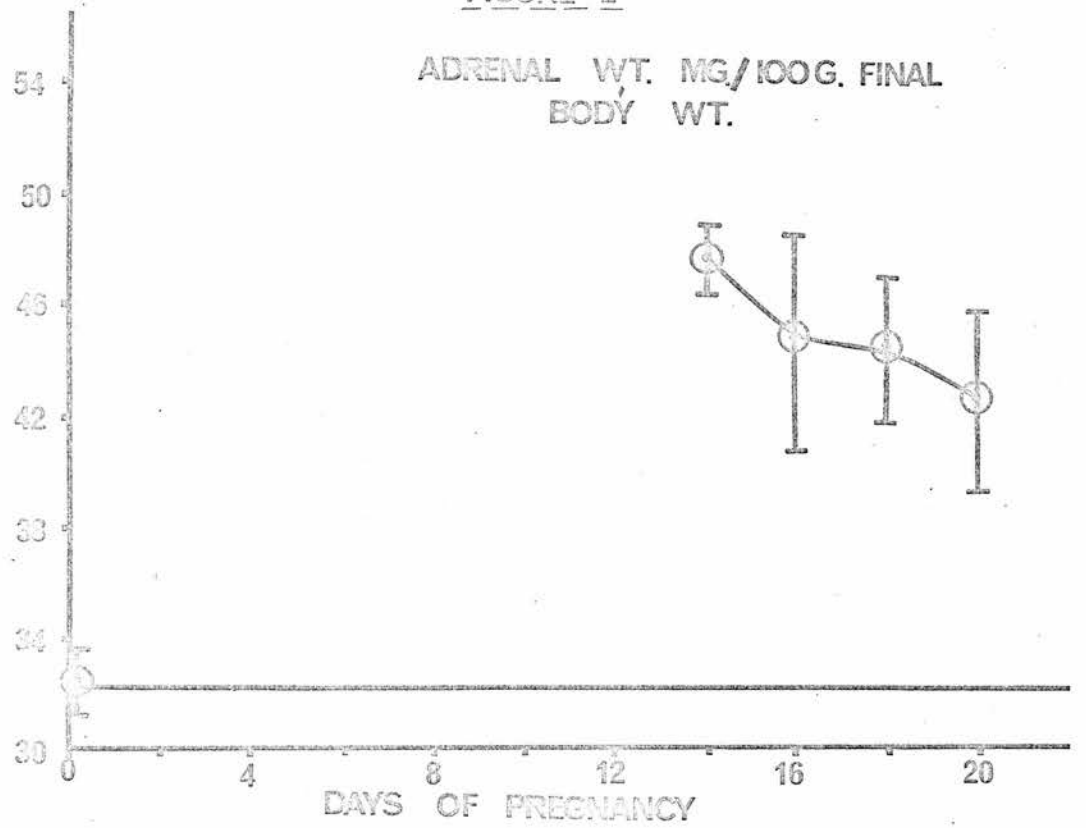
(ii) Adrenal Weights

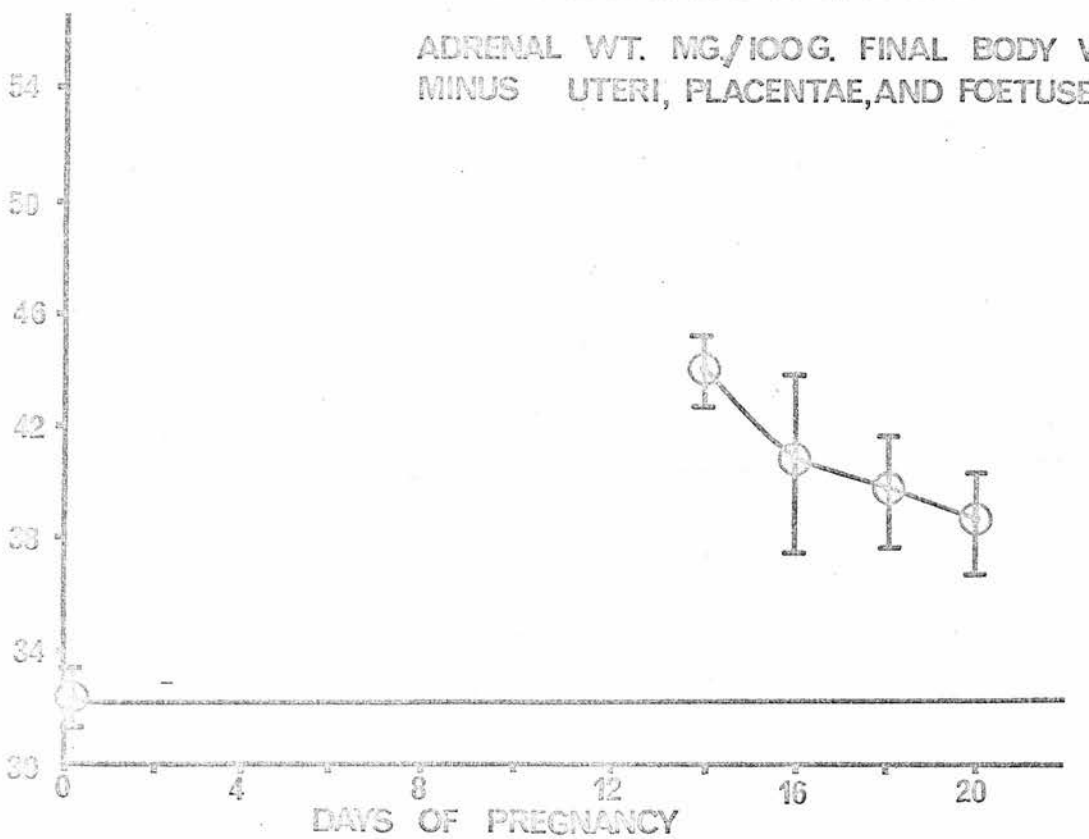
	No. of Rats	mg/100g. Initial body weight.	P+	mg/100g. Final body wt. less Uteri plus contents.	P+
Controls	21	32.7 ± 0.9		32.6 ± 0.7	
14 days Pregnancy	10	47.9 ± 1.6	< 0.001	43.8 ± 1.1	< 0.001
16 days Pregnancy	3	45.2 ± 3.9	< 0.001	41.4 ± 4.1	< 0.002
18 days Pregnancy	10	44.8 ± 2.4	< 0.001	39.8 ± 1.9	< 0.001
20 days Pregnancy	8	42.9 ± 2.8	< 0.001	38.8 ± 1.6	< 0.001

* Standard Error of the Mean

\+ Significance of difference between control and pregnant animals.

FIGURE 2


ADRENAL WT. MG./100 G. FINAL BODY WT.
54
50
46
42
38
34
30
0
4
8
12
16
20
DAYS OF PREGNANCY


ADRENAL WT. MG./100 G. FINAL BODY WT.
MINUS UTERI, PLACENTAE, AND FOETUSES
54
50
46
42
38
34
30
0
4
8
12
16
20
DAYS OF PREGNANCY

Effect of Pregnancy on Nucleic Acids in Rat Liver

The concentrations of RNA and DNA expressed in mg. per g. liver and in terms of total liver per 100 g. body weight were estimated in some of the rats and the values are shown in Table 4. There was no significant difference in the DNA values per g. liver during pregnancy. The DNA per total liver per 100 g. initial body weight was significantly increased on the 14th and 18th days of pregnancy, just failed to reach significance at the 3% level on the 20th day but was not significant at the 16th day. Campbell and Kosterlitz (1949) found a similar variation of DNA.P in total liver with each stage of pregnancy. They found an increase in DNA.P at 14 days and a smaller increase at 20 days, but made no estimations between 14th to 21st days of pregnancy.

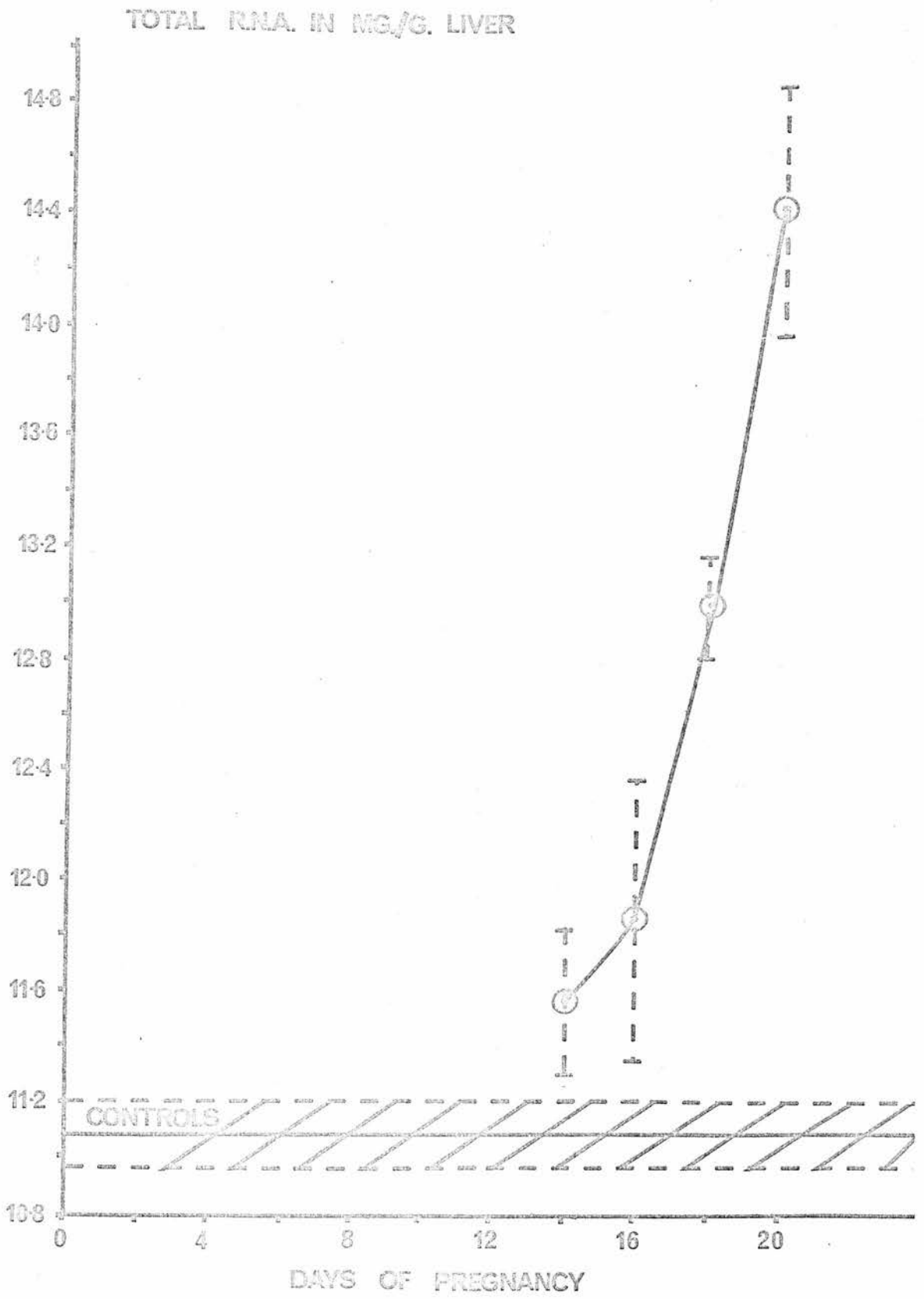
The concentrations of RNA during the stage of pregnancy studied are graphed in Figure 3. There was a tendency for RNA per g. liver to increase on the 14th and 16th days of pregnancy. On the 18th and 20th days of pregnancy the increases in RNA concentration were highly significant. The total amount of RNA per liver per 100 g. body weight was increased over the period of pregnancy studied. This is in accordance with the increase in liver weight per 100g. body weight which occurs during pregnancy.

Table 4

	Controls	Number of Days Pregnant			
		14	16	18	20
DNA: mg/g liver	3.01 ± 0.08*	3.29 ± 0.10	2.80 ± 0.05	2.99 ± 0.14	2.60 ± 0.07
P		NS	NS	NS	NS
DNA:mg/total liver per 100g Initial body wt.	9.99 ± 0.74	14.15 ± 0.76	10.49 ± 0.47	14.00 ± 1.24	11.90 ± 1.19
P		P < 0.01	NS	P < 0.01	P < 0.10
No. of Rats	3	3	3	3	3
RNA:mg/g liver	11.09 ± 0.11	11.56 ± 0.30	11.86 ± 0.51	13.00 ± 0.16	14.43 ± 0.44
P	-	NS	< 0.10	< 0.001	< 0.001
RNA: mg.in total liver/100g. initial body wt.	33.35 ± 1.80	49.99 ± 4.96	44.42 ± 2.05	60.62 ± 2.01	65.91 ± 5.19
P		< 0.01	< 0.01	< 0.01	< 0.01
No. of Rats	8	3	3	3	4

* Standard Error of the Mean

FIGURE 3


TOTAL R.N.A. IN MG./G. LIVER
14·8
14·4
14·0
13·6
13·2
12·8
12·4
12·0
11·6
11·2
10·8
CONTROLS
0
4
8
12
16
20
DAYS OF PREGNANCY

Electron Microscopy of Liver Parenchymal Cells of Pregnant Rats.

Electron microscopy studies were carried out on samples of liver obtained from virgin non-pregnant rats and also from rats on the 14th and 16th days of pregnancy. The double staining technique, selected to enhance the contrast of the sections, involved the use of lead citrate which is a specific stain for membranes. Lead ions also show up glycogen areas as clusters of intensely electron dense particles (Mukherjee, Gustafsson, Afzelius and Arrhenius, 1963).

The distribution of membranes throughout the cell is not even. For example, smooth membranes tend to be concentrated at the cell periphery whilst the rough endoplasmic reticulum is more central (Palade, 1956). Thus since the area of the cell is likely to be important, a comparison of electronmicrographs taken at similar regions of the cell was aimed at.

Plates 1 and 2 show typical areas of the non-pregnant and 14 days pregnant rat liver cell taken in the vicinity of the nucleus. There did not appear to be any difference in the concentration of ribosomes and the majority were bound in both instances.

Plate 3 shows an area near the nucleus of a liver parenchymal cell of a rat on the 18th day of pregnancy. No difference in the quantity of membranes or amount of ribosomes can be detected.

Plate 4 shows a typical area near a liver cell boundary of a non-pregnant rat. More smooth membranes than rough membranes were seen in this region compared to the nuclear region (Plate 1). A similar distribution was found in 14 day pregnant rats.

Plate 5 shows an area near the cell boundary in 18 day pregnant rat liver. The electron micrographs of this area suggested that there was an increase in the amount of membranes on the 18th day of pregnancy. There also appeared to be more rough membranes than were found in this region of the cell in non-pregnant rats.

Plates 6 and 7 are of non-pregnant and 18 days pregnant rats respectively, showing typical general areas of the cell. Again there was an indication of an increase in the total quantity of membranes present in the cells of 18 days pregnant rats. There appeared to be more ribosomes present which might account for the known "excess RNA" in rats during pregnancy (Campbell and Kosterlitz, 1949). It is, however, impossible to judge on a strict quantitative basis

from electron micrographs whether or not there are more bound or free ribosomes. It was, therefore, decided to investigate this question further by fractionating the microsomal fraction of the non-pregnant and pregnant rats.

Relative distribution of RNA and phospholipid P in sub-fractions of microsomal fractions.

The distribution of phospholipid P and RNA in the microsomal fraction is presented in Table 6. The results are expressed as percentage of the total phospholipid P or RNA of the microsomal fraction.

The distribution pattern for RNA and phospholipid P observed in rats on the 14th day of pregnancy was similar to that observed in non-pregnant rats. On the 16th day of pregnancy there was a slight increase in the percentage of RNA in the rough membranes and a slight decrease in the percentage of RNA in the free ribosomes. These changes were not statistically significant.

At 18 days of pregnancy the percentage RNA associated with the rough membranes was significantly lower than the controls ($P < 0.01$). It was also significantly lower than that of the 16 days and 20 days pregnant groups ($P < 0.001$;

P $\leq$ 0.05 respectively). Similarly, the RNA of the free ribosomes at the 18th day of pregnancy was significantly higher than those of the non-pregnant, 16 days and 20 days pregnant groups, the values of P obtained being $\leq$0.01, $\leq$ 0.002, and $\leq$0.01 respectively.

The total RNA in the rat liver is significantly increased at the 18th day of pregnancy. Thus the results expressed in Table 6 suggest that the increase of RNA appears first in the free ribosomes which then is transferred to the rough membranes.

Table 6

RNA and Phospholipid P distribution in the microsomal fraction during pregnancy.

	No. of Rats	RNA			PL		
		Rough Membranes	Free Ribosomes	Smooth Membranes	Rough Membranes	Free Ribosomes	Smooth Membranes
Controls	8	74.3 ± 1.25	21.2 ± 1.18	4.9 ± 0.44	79.1 ± 1.55	1.82 ± 0.25	18.9 ± 1.63
14 days Pregnancy	3	74.6 ± 2.51	21.6 ± 2.92	3.8 ± 0.43	80.9 ± 1.35	1.60 ± 0.45	17.5 ± 0.94
16 days Pregnancy	3	77.8 ± 0.61	17.8 ± 1.11	4.8 ± 0.52	79.1 ± 2.77	1.70 ± 0.88	19.2 ± 2.11
18 days Pregnancy	3	66.0 ± 1.1	29.1 ± 1.1	4.9 ± 0.35	76.4 ± 0.41	2.4 ± 0.52	21.0 ± 0.93
20 days Pregnancy	4	76.3 ± 2.66	18.1 ± 1.31	5.6 ± 1.65	77.7 ± 3.16	1.3 ± 0.31	21.0 ± 2.93

Electron microscopy of the Free Ribosome Pellet and Smooth Membrane Pellicle separated from liver by Ultracentrifugation.

This study was carried out to determine the effectiveness of the iso-octane separation of smooth membranes from free ribosomes. Plate 8 shows a portion of the free ribosome pellet. This was a typical example of the electron micrographs obtained which showed that very few membranes were present in the pellet and can be regarded as negligible.

Plate 9 shows a portion of the smooth membrane pellicle. The membranes were not as clearly defined as expected. The blurring effect was thought to be due to the iso-octane treatment. A few ribosomes were present and occasionally small clusters of ribosomes attached to a membrane were seen. The contamination of the smooth by free ribosomes appeared to be quite low.

Amino acid incorporation in vitro by microsomal fractions during pregnancy.

The 18th and 20th days of pregnancy were selected for study because it is here that a significant change in RNA concentration was found. The results are tabulated in Table 7 as counts/min./mg. protein/mg. ribosomal RNA.

Hallinan and Munro (1964) studied the uptake of ^{14}C-leucine into the fractions of the microsomal separation. They found that the uptake by the rough membranes and free ribosomes was largely energy dependent whereas much of the uptake by the smooth membranes was independent of an energy source and thus not due to protein synthesis. They suggested that transpeptidation or incorporation into the abundant lipids of the fraction may account for this. The results for uptake of L-valine-^{14}C without an energy source present were deducted from the results obtained with an energy source before presentation in Table 7. These results were finally corrected for the efficiency of the counting system.

The activity of the whole microsome fraction was increased on the 18th and 20th days of pregnancy. The increase was significant at the 20th day of pregnancy ($P < 0.05$). The rough membranes showed a tendency towards increased activity but did not reach statistical significance. The activity of the free ribosomes and smooth membrane fraction was significantly lowered at the 20th day of pregnancy ($P < 0.05$).

Table 7

Amino Acid Incorporating Activity in vitro of microsomal fractions during later stages of pregnancy.

	No. of Rats	Whole Microsomes	Rough Membranes	Free Ribosomes & Smooth Membranes.
Non-pregnant	3	54.8 ± 7.1	73.0 ± 18.3	75.7 ± 8.1
18 days Pregnancy	3	85.2 ± 24.3	87.2 ± 3.4	76.0 ± 25.8
20 days Pregnancy	2	160.2 ± 38.3	89.2 ± 15.5	26.4 ± 7.4

Effect of Pregnancy on Hepatic Δ^4-steroid Hydrogenase Activity.

Hepatic Δ^4-steroid hydrogenase activity was estimated on the 14th day of pregnancy and compared to the activity obtained for non-pregnant rats.

The results given in Table 8 are expressed as μ moles corticosterone reduced/15 min./g. liver, and those given in Table 9 are expressed as μ moles corticosterone reduced per total liver/15 min./100g. initial body weight.

The activity of Δ^4-steroid hydrogenase increased per g. liver and also in the total liver adjusted for body

weight. The results of a paired t test showed that both increases are significant ($P < 0.02$; $P \leq 0.01$ respectively). The values obtained for control rats were of a similar order to the results obtained by Clark and Goodlad (1961).

Table 8

Δ^4-Steroid hydrogenase activity per g. liver.

	Non-pregnant Rats	14 days Pregnant Rats
Number of Rats	3	3
μ moles Corticosterone reduced/15 min./g.liver	2.26 ± 0.29	3.30 ± 0.41

Table 9

Δ^4-Steroid hydrogenase activity per total liver.

	Non-pregnant Rats	14 days Pregnant Rats
Number of Rats	3	3
μ moles corticosterone reduced per total liver/ 15 min./100g. initial body weight.	5.89 ± 0.99	11.24 ± 1.02

L E G E N D

br	bound ribosomes
cb	cell boundary
fr	free ribosomes
gl	glycogen
m	mitochondrion
r	ribosomes
ser	smooth endoplasmic reticulum
sm	smooth membrane

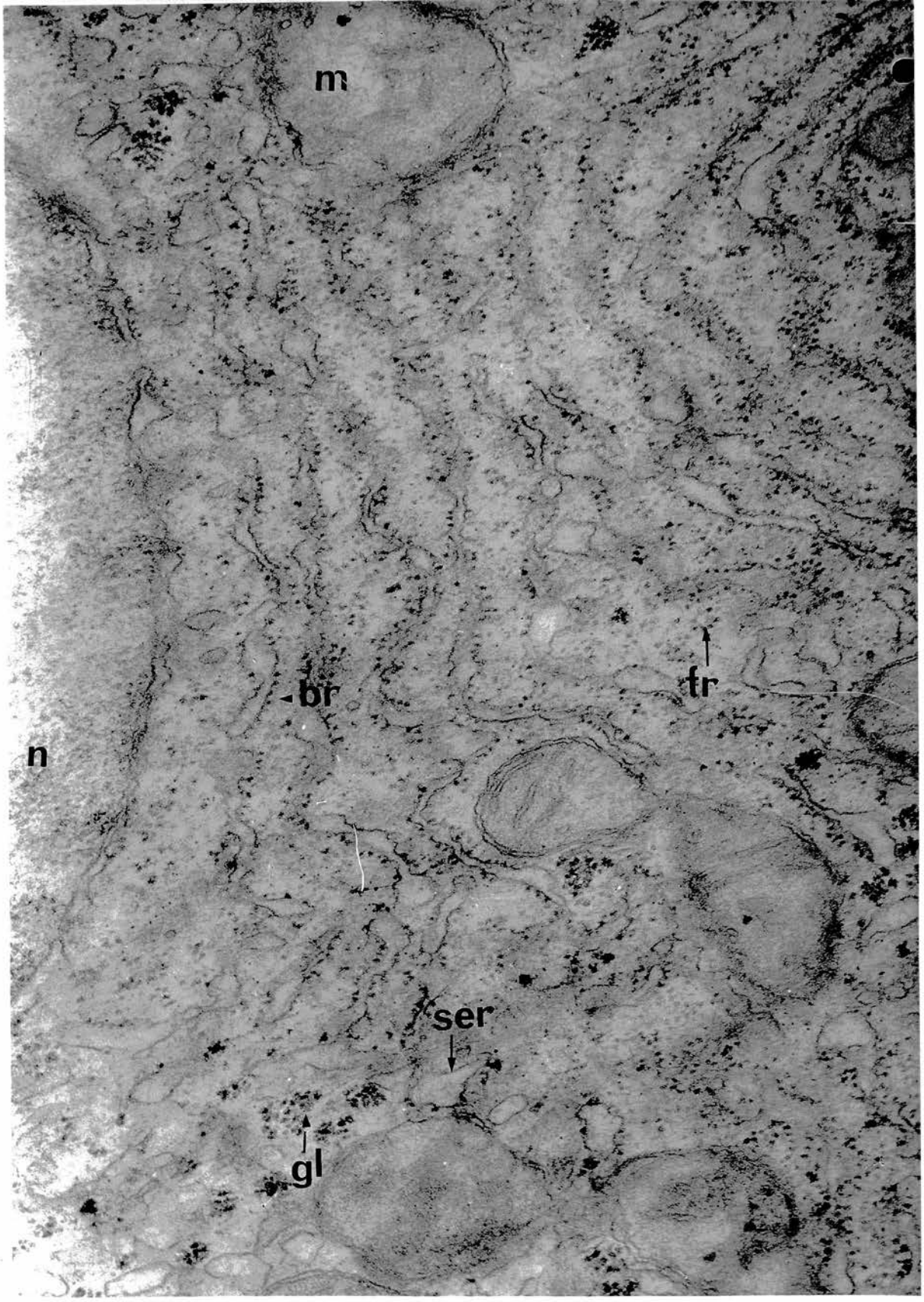


PLATE 1

Portion of liver parenchymal cell, in vicinity nucleus, . from non-pregnant rat (x 60,000)

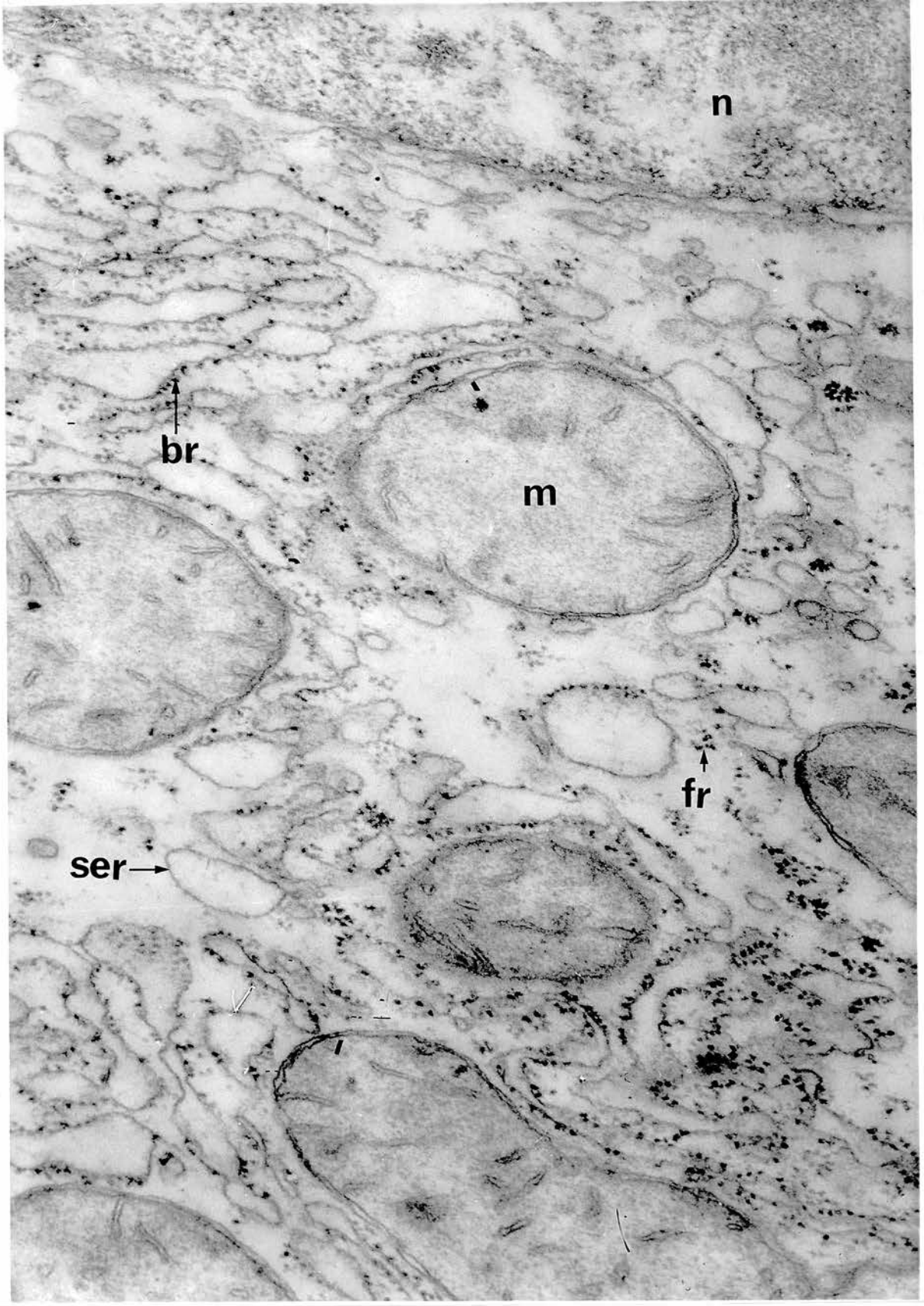


Plate 2

Portion of liver parenchymal cell, in vicinity nucleus, from pregnant (14 days) rat. (x 60,000)

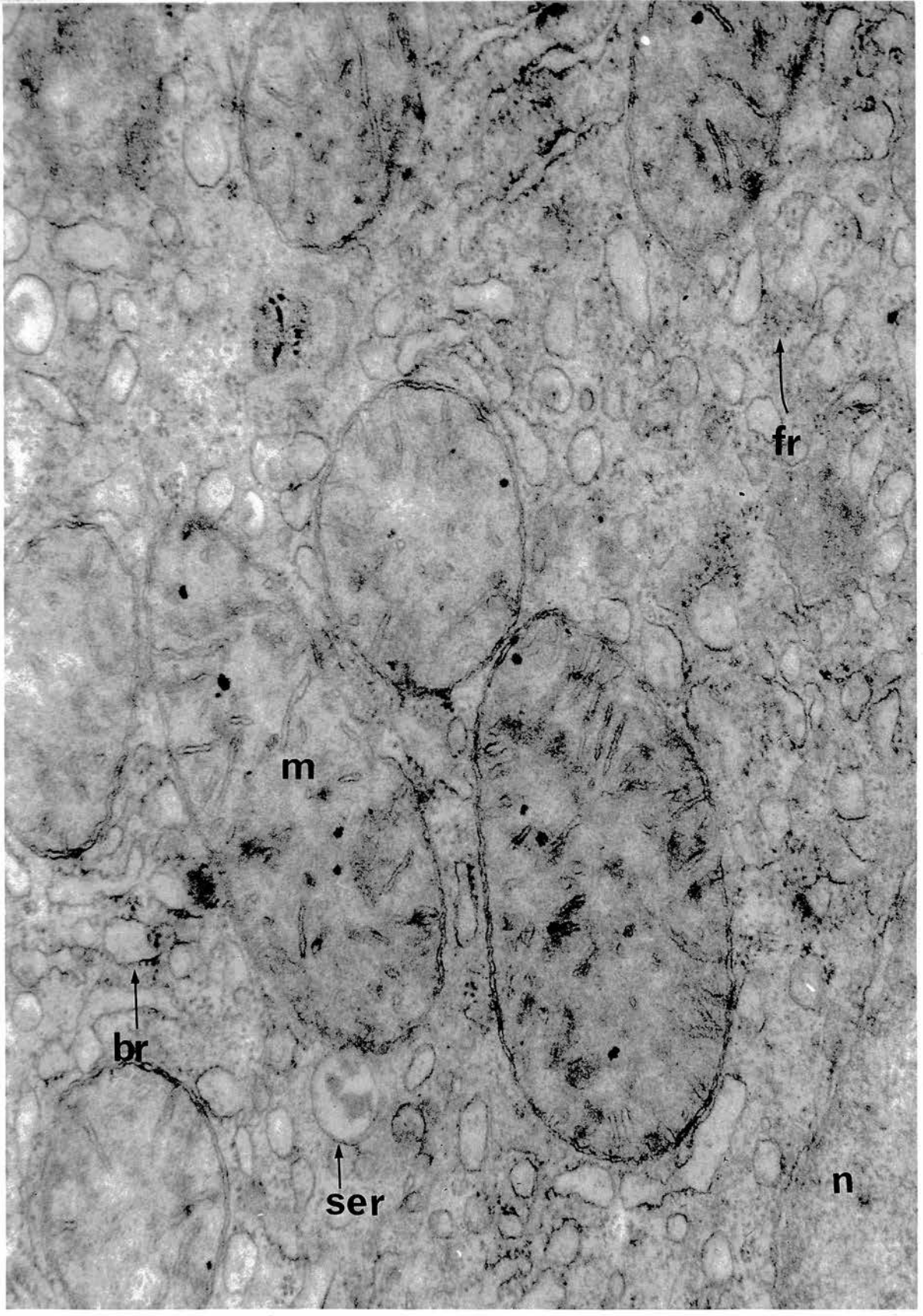


Plate 3

Portion of liver parenchymal cell, in vicinity nucleus, from pregnant (19 days) rat. (x 30,000)

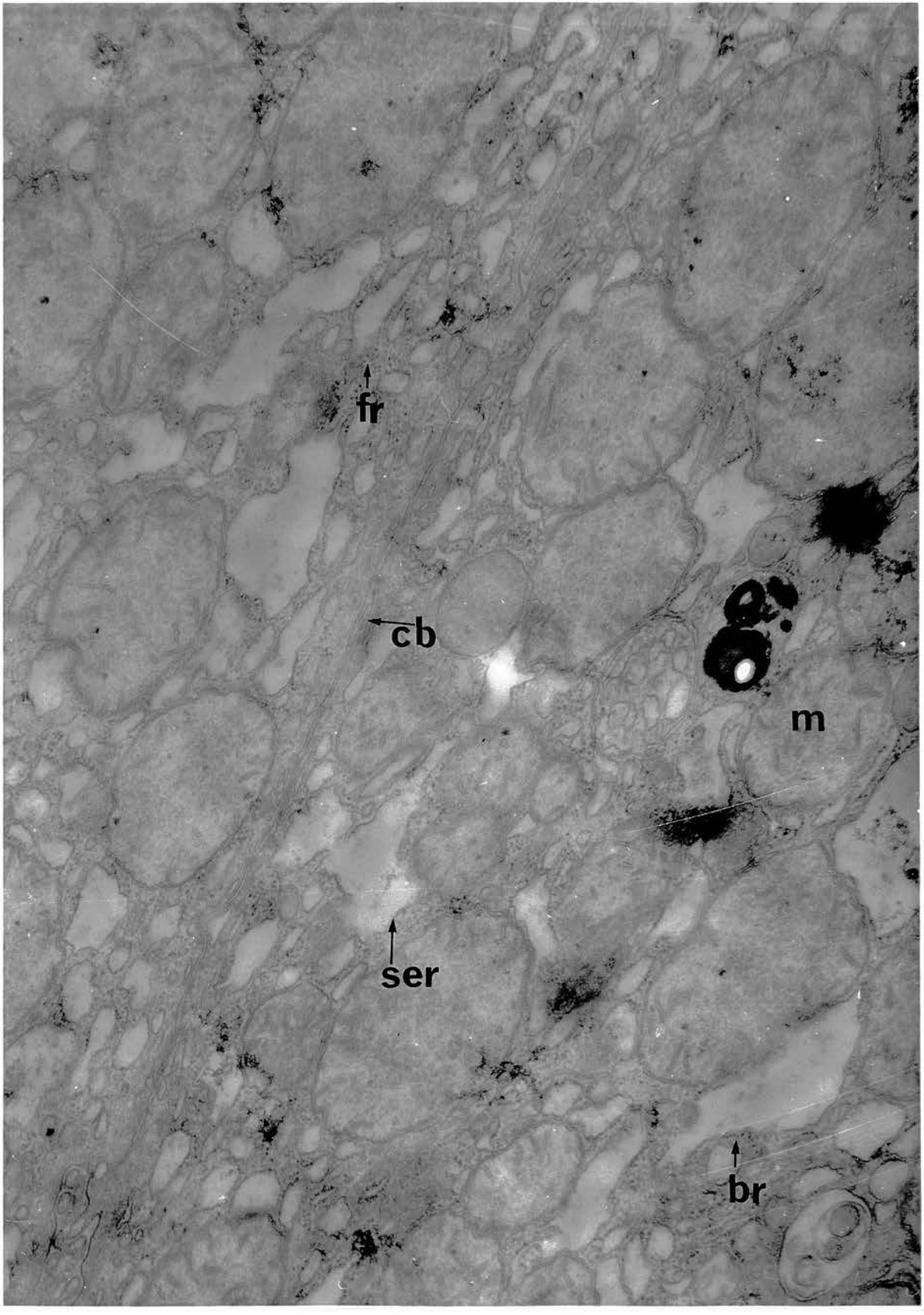


Plate 4

Portion of liver parenchymal cell, in vicinity cell membrane, from non-pregnant rat. (x 30,000)

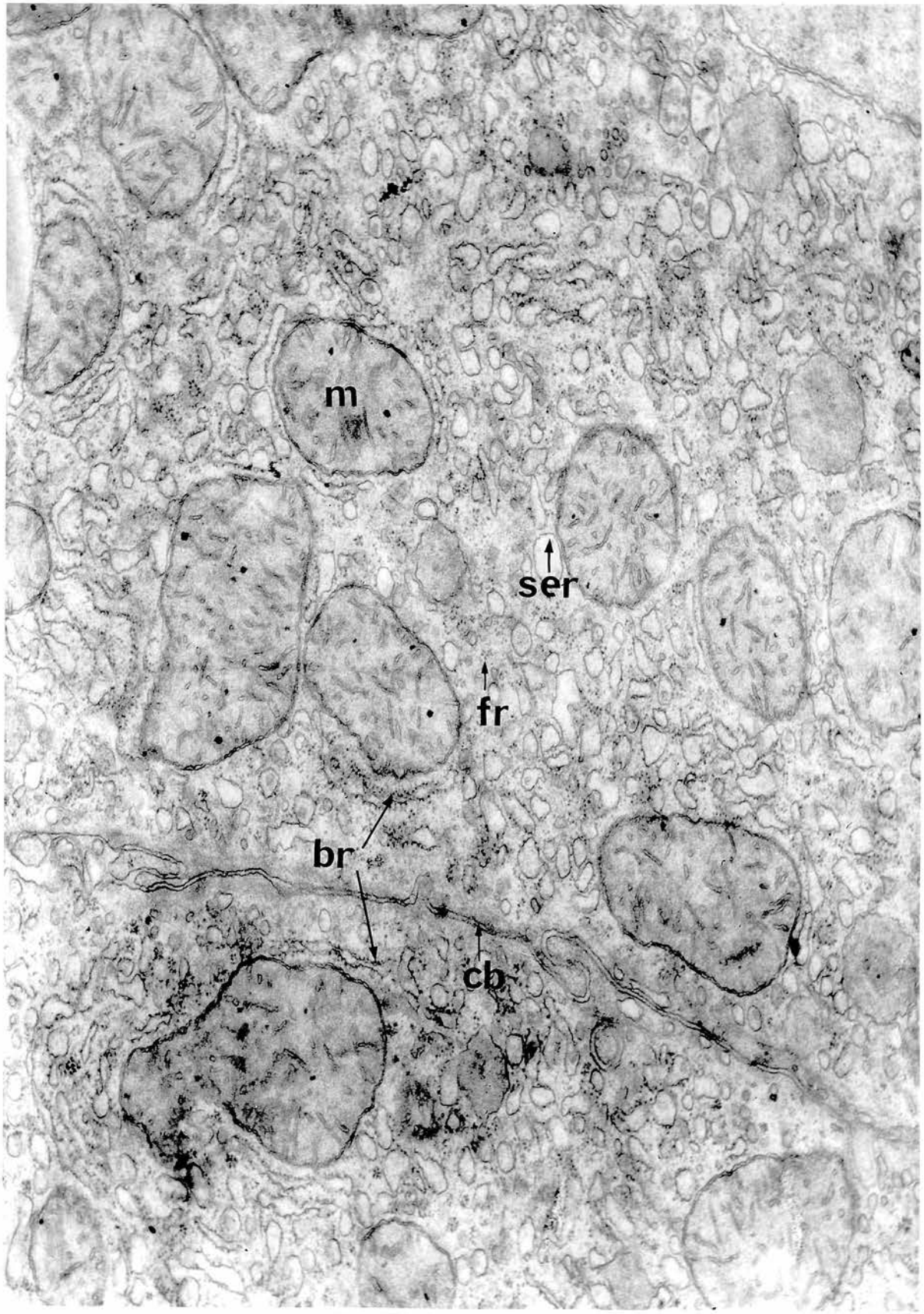


Plate 5

Portion of liver parenchymal cell, in vicinity cell membrane, from pregnant (18 days) rat. (x 30,000)

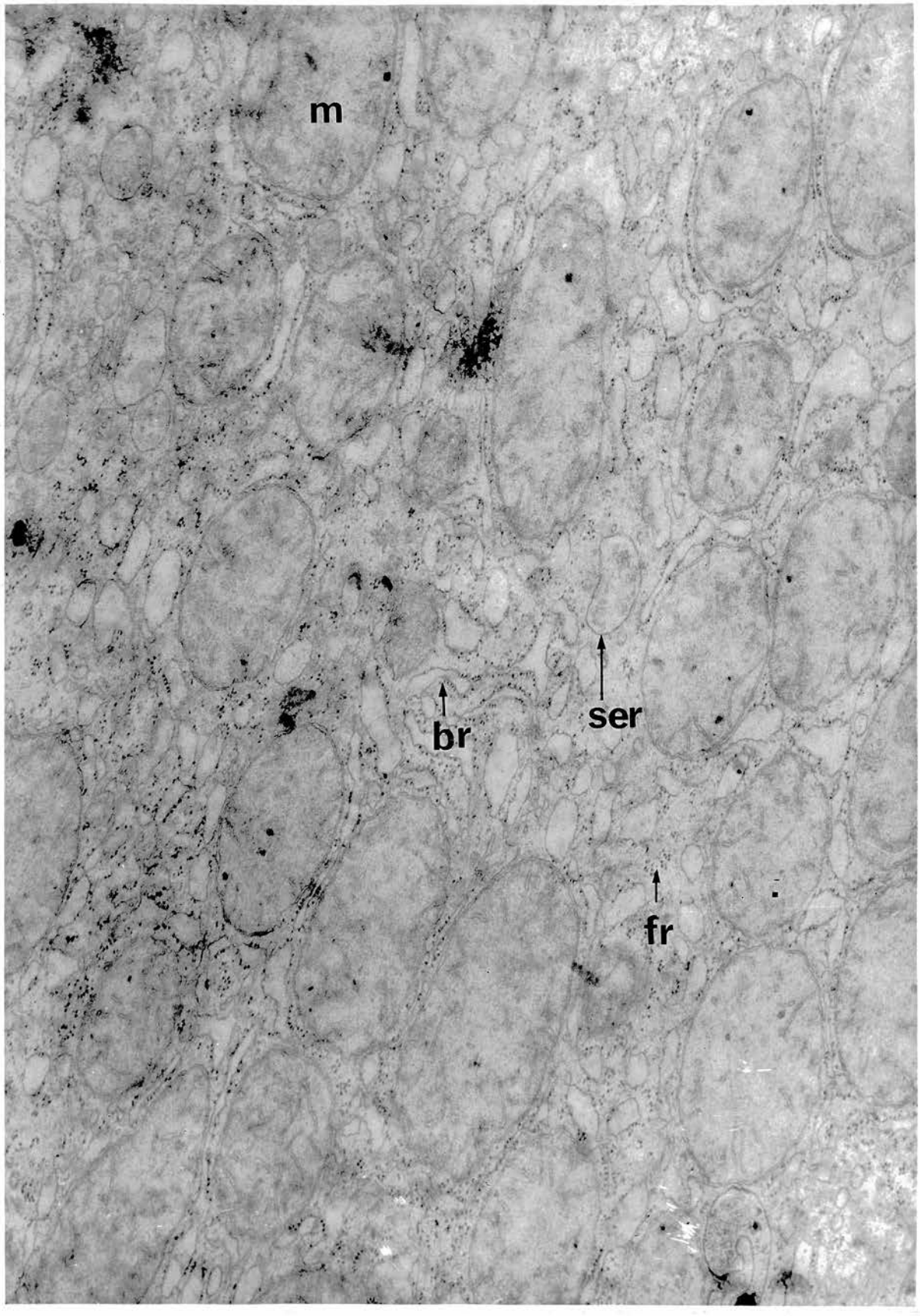


Plate 6

Portion of liver parenchymal cell from non-pregnant rat. (x 30,000)

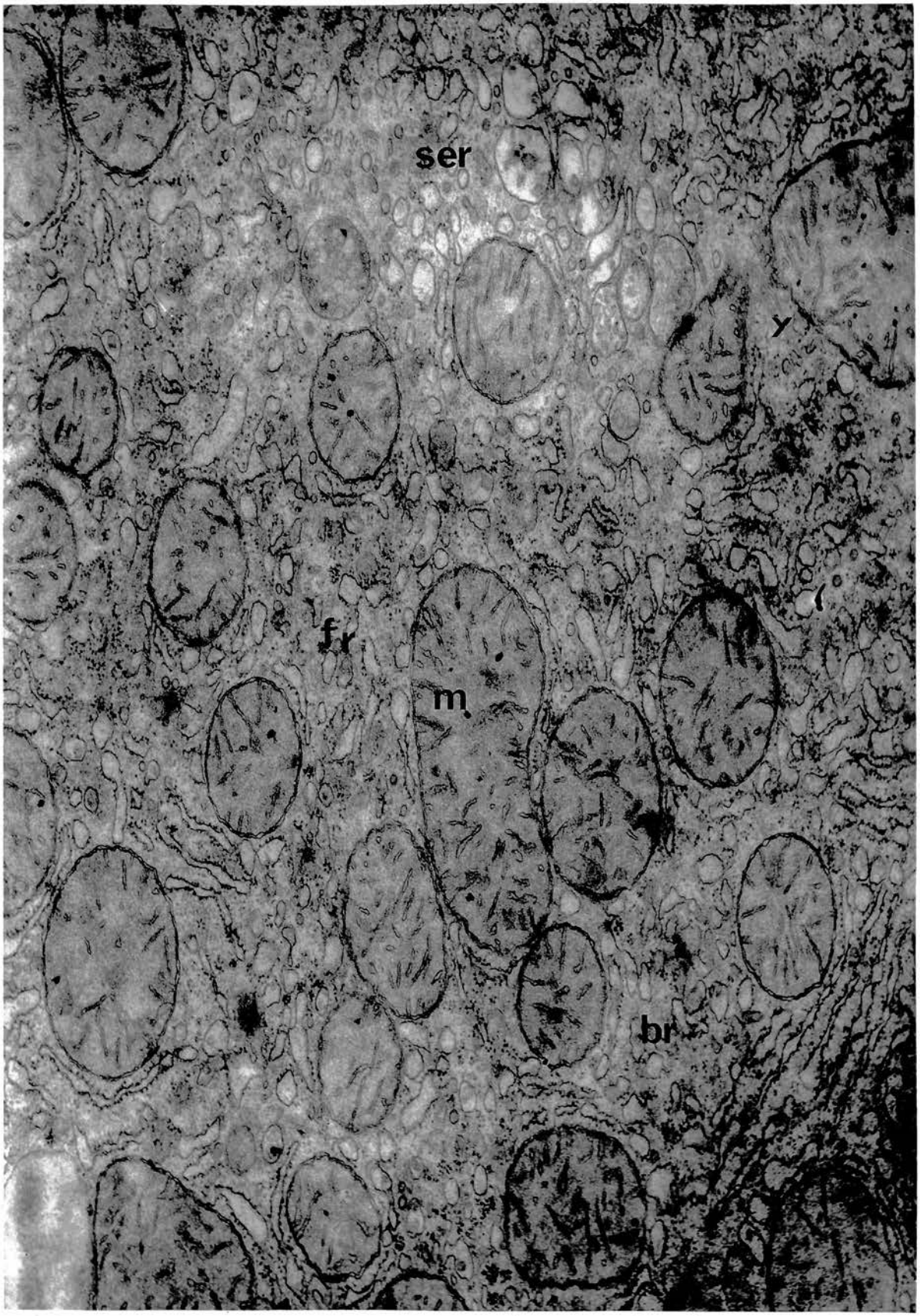


Plate 7

Portion of liver parenchymal cell from pregnant (18 days) rat. (x 30,000)

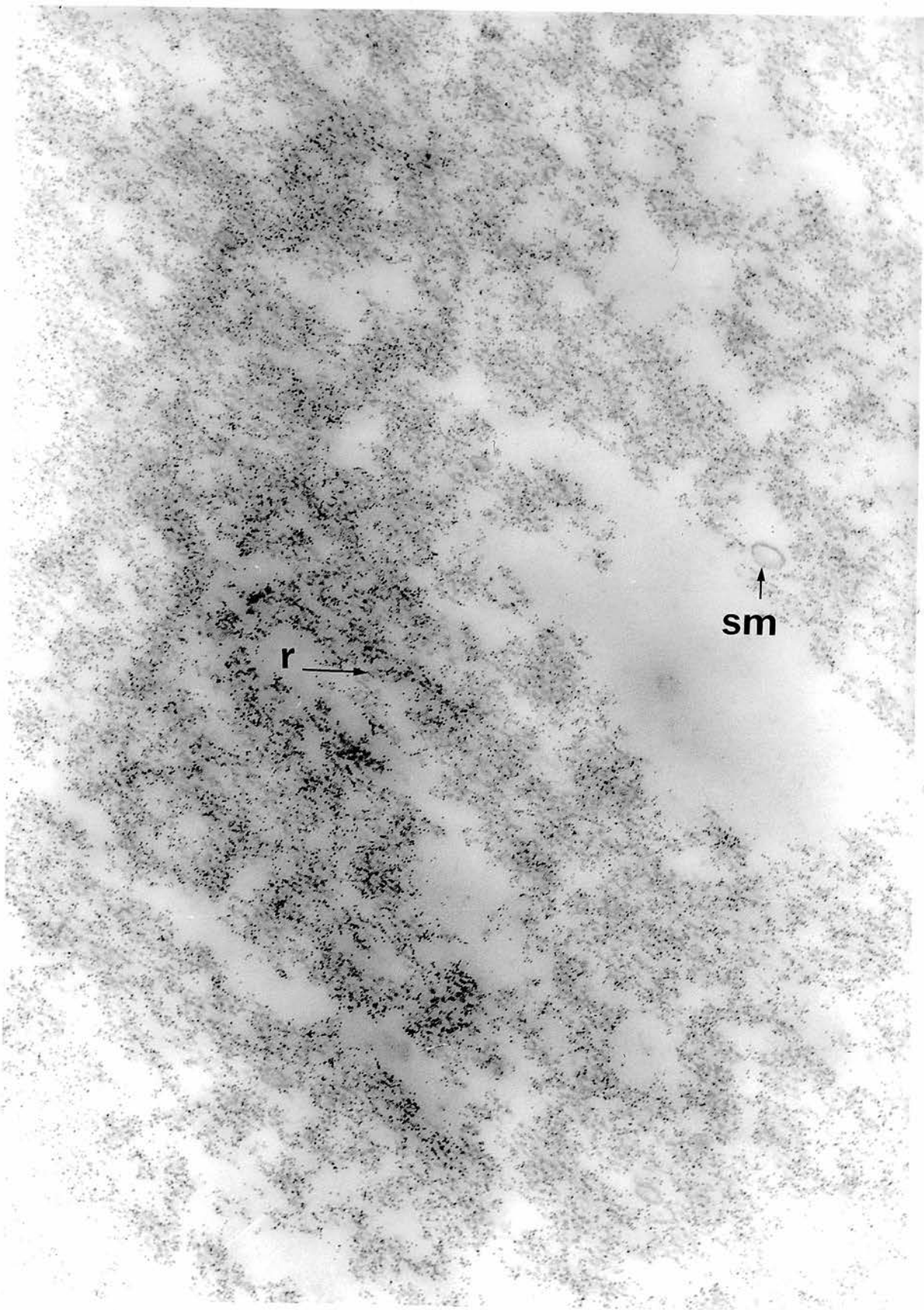


Plate 8

Portion of free ribosome pellet from microsomal separation (x 60,000)

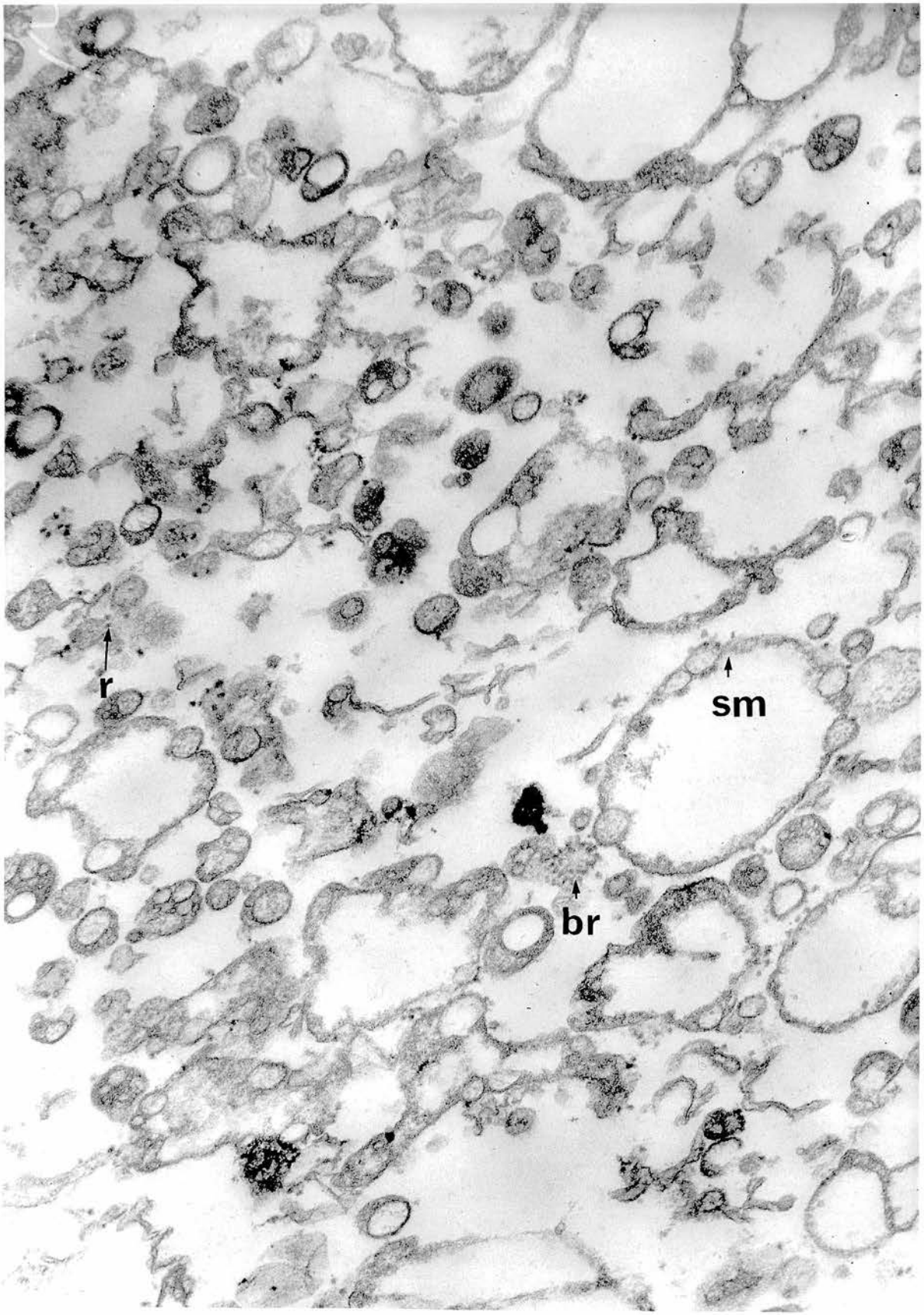


Plate 9

Portion of smooth membrane pellicle from microsomal separation (x 60,000)

DISCUSSION

In the present series of experiments, the increases in body weight observed during pregnancy were greater than could be accounted for by the weight of the uteri, placentae and foetuses, as shown in Table 2. However as pregnancy proceeded, a greater proportion of the increase in body weights was accounted for by the combined weight of the uteri, placentae and foetuses viz:- only 32.0% after 14 days of pregnancy to 65.9% at the 20th day of pregnancy. The maternal weight (i.e. total body weight less weight of uteri, placentae and foetuses) increased throughout pregnancy, though at a decreasing rate as pregnancy proceeded (Table 2). Part of this maternal body weight increase may be accounted for by the mammary glands which have been reported as comprising 7% of the body weight at term in the case of mice (Linzell, 1959). The results of Campbell, Innes and Kosterlitz (1953a) showed a slightly different picture from above. They found that rats given unlimited access to an adequate diet gained about 50-55 g. during the first 13-15 days of pregnancy, most of this gain being maternal. During the last 15-21 days of pregnancy, there was a further increase of about 50 g.,

which was practically entirely due to the growth of the placentae and foetuses. The total increase in body weight of about 100 g. obtained in this study is greater than that obtained in the present work. Also there was little evidence of maternal weight increase during the latter half of pregnancy in the study of Campbell et al., whereas the maternal weight continued to increase during this period in the present work. These differences could be explained by the fact that the food intake of the rats in the present work was restricted and it is well-known that pregnant rats voluntarily keep on increasing their food intake as pregnancy progresses (Cole and Hart, 1938).

An increase in liver weight relative to total body weight has been shown in rats during the period from 14th to 20th days of pregnancy. This is in agreement with the findings of Bokelman and Sheringer (1932).

The total liver DNA was also found to be increased during the period from the 14th to 20th days of pregnancy. The concentration of DNA, on the other hand, was not altered during the period studied. An interpretation of the significance of this increase in DNA is not possible in the absence of data on the amount of DNA per nucleus

in the liver of rats at these stages of pregnancy.

Campbell and Kosterlitz (1949) studied the alterations in liver RNA in the rat during the course of pregnancy. They found a disproportionate amount on the 10th day of pregnancy but by the 14th day the RNA/DNA ratio had returned almost to the control levels. From the 14th day onwards, Campbell and Kosterlitz found another phase of intense hepatic RNA synthesis. In the present work confirmation of this pattern of changes in hepatic RNA during the second half of pregnancy in the rat was obtained. There was no significant difference in the ratio of liver RNA/DNA between control rats and 14-day pregnant rats. After the 14th day of pregnancy, the concentration of RNA in the liver began to rise at a time when DNA concentration remained constant. It has been shown in this laboratory that the microsomal fraction is the site of this increased deposition of liver RNA at least at the 18th day of pregnancy (Goodlad, unpublished results). In the present work, the general appearance of the livers of the pregnant animals under the electron microscope also suggested an increase in the amount of microsomal components (Plates 1 - 7). The distribution of RNA and phospholipids between the

rough endoplasmic reticulum, the smooth endoplasmic reticulum and free ribosomes was similar in control rats and in rats on the 14th and 16th days of pregnancy. On the 18th day of pregnancy, however, these studies suggested that there was an increased amount of free relative to bound ribosomes. The smooth membrane fraction at this stage was similar to control levels. On the 20th day of pregnancy the distribution pattern between the various microsomal compounds was similar to that in non-pregnant animals.

Since the RNA of the microsomal fraction is markedly increased at the 18th and 20th days of pregnancy the change in the distribution pattern of the components might be interpreted in terms of an initial synthesis of free ribosomes which subsequently become attached to the membranes of the endoplasmic reticulum.

In view of the changes observed in the relative amounts of bound and free ribosomes in the present work during the period from the 16th to the 20th days of pregnancy and also in view of the changes which occur in plasma proteins in pregnancy a more critical study of the absolute amounts of the bound ribosome fractions and the rate of synthesis of plasma proteins would appear

desirable in these circumstances.

Peters (1962) has studied albumin formation and secretion in rat liver and has shown that it is first formed by the bound ribosomes, migrates in some way to the smooth surfaced membranes before it is released from the liver in soluble form. Little soluble labelled albumin was detected intracellularly. There are two possibilities of how the migration could occur. Either the protein could actually migrate from one type of membrane to the other, or the protein could remain bound in a fixed position on a membrane which itself undergoes transformation from rough to smooth membranes by a process involving the detachment of the ribosomes. This could mean a flow concept of the membranes, rather than a static concept. The evidence obtained by Manganiello and Phillips (1965) is more in keeping with the latter theory. These workers studied the chemical composition and enzymic activity of the membranes of rough and smooth surfaced microsomes and found them to be qualitatively very similar in both respects.

The ability of the unfractionated microsome fraction from pregnant rats on the 18th and 20th days of pregnancy

to incorporate ^{14}C-valine into protein under *in vitro* conditions tended to be increased compared with the activity observed in microsomes studied from livers of non-pregnant rats. This situation is similar to that in regenerating liver where the microsomal fraction is more active in incorporating labelled amino acid into protein *in vitro* (Hultin *et al.*, 1957; McCorquodale *et al.*, 1962).

Cammarano *et al.* (1965) have shown that the regenerating liver differs from normal rat liver not only in that the polysome population is increased, but also in extent of association of the ribosomes with the endoplasmic reticulum. They also found that the amino acid incorporating ability of regenerating rat liver ribosomes plus 105,000 g. supernatant was about twice as great as that of ribosomes plus 105,000 g. supernatant from normal rat liver. However, the activity of normal rat liver ribosomes was found to be increased if supernatant from regenerating instead of normal rat liver was used, although not to the level obtained with both ribosomes and supernatant of regenerating rat liver, and *vice versa*. How far the increased activity found in the present work is due

to the ribosomes themselves or to factors present in the cell sap must await further investigation. However, Campbell et al., 1964, have studied the activating effect of polyuridylic acid at various concentrations of Mg^{2+} ions on the incorporation of ^{14}C-phenylalanine into protein by preparations of whole rat microsomes. They found that ribosomes from both regenerating and normal rat livers were equally active.

The adrenals were enlarged on the 14th day of pregnancy and were still significantly increased at the 20th day, although to a less degree than on the 14th day. This is in accordance with Cope (1961) and Ricci et al. (1963) who found that the adrenals tend to enlarge in pregnancy. Blaxter (1964), however, records that the adrenals may atrophy in the rat.

An increase in the Δ^4-steroid hydrogenase activity has been shown on the 14th day of pregnancy. One can consider two results that the action of this enzyme could have - a local effect on the liver (Lin, Rivlin and Knox, 1959) and a wider effect on the body as a whole.

The increase in Δ^4-steroid hydrogenase activity would cause a decrease in the concentration of corticosteroids at least within the liver. This might be a factor in the release of liver from rigid growth control since Hemingway (1960) showed that corticosteroids inhibit mitosis in regenerating rat liver. ACTH and all compounds known to have corticosteroid activity have been shown to increase the activity of hepatic alanine-glutamine transaminase in rats (Rosen, Roberts, Budnick and Nichol, 1963). Adrenalectomy on the other hand causes a fall in the level of the enzyme (Harding, Rosen and Nichol, 1961; Miyabo, 1959). Thus the fact that alanine-glutamine transaminase activity is decreased in liver during pregnancy (Beaton, Beare, Ryu and McHenry, 1964; Nichol and Rosen, 1963) supports the suggestion that the concentration of corticosteroids in the liver is lowered by the increased activity of Δ^4-steroid hydrogenase. The transaminase is only decreased in activity after the 10th day of pregnancy in rats (Nichol and Rosen, 1963) and further work in this laboratory has shown that it is at this time that the activity of Δ^4-steroid hydrogenase begins to increase (Goodlad, unpublished results).

The activity of Δ^4-steroid hydrogenase is the rate controlling step in the inactivation of corticosteroids. This enzyme has been postulated as controlling the level of adrenal cortical activity (Yates et al., 1958). Increased activity of the enzyme would cause a drop in the corticosteroid level which would stimulate increased production of ACTH from the pituitary since this hormone is under a negative feedback control mechanism. This in turn would act on the adrenal to stimulate adrenal output and cause an increase in adrenal size, as recorded in the present work.

It is interesting to compare changes in adrenal composition reported in pregnant animals with those found by Farese and Reddy (1963) in non-pregnant rats treated with corticotrophin. These workers showed that the administration of 20 units of corticotrophin-gel twice daily to rats caused a steady increase in adrenal size. Analysis of the nucleoproteins showed a two-phase response. Firstly RNA but not DNA increased. Then after seven days, DNA increased along with continued RNA increase. An investigation of adrenal weight increase on the 18th to 19th day of pregnancy in rats (Ricci et al., 1963) showed no increase in cell number and an increase in

cell volume, associated with an increase in concentration of nucleoproteins. Thus the adrenal growth in pregnancy could be similar to the first phase of response to ACTH administration, which would be in accord with the idea that hepatic Δ^4-steroid hydrogenase activity influences adrenal size by means of a negative feedback control mechanism. Blood corticotrophin has actually been shown to be increased in all months of pregnancy in women (Cassano and Terantino, 1957), although its elevation could be by mechanisms other than the one suggested.

Information of the levels of plasma free corticosteroids and their turnover times would obviously be of interest in this connection. No data for the pregnant rat are available. However, increased secretion of adrenal corticosteroids have been reported in pregnant women (Cope and Black, 1959; Bayliss, Browne, Round and Steinbeck, 1955). The half-life of plasma cortisol has been shown to be increased in human pregnancy from a normal value of 2 hours to 4 hours indicating a decreased rate of breakdown (Migeon, Betrand, Walt, Stempfel and Prystowsky, 1957; Cohen and Gordon, 1958). If a similar picture obtains in the rat, then it could only mean hepatic Δ^4-steroid hydrogenase activity in pregnancy does not have such a fundamental controlling

effect with regard to adrenal function as Yates and his colleagues have claimed for non-pregnant rat under various conditions (Yates et al., 1958).

Another factor, however, assumes importance in pregnancy with respect to the level of circulating corticosteroids. Slaunwhite and Sandberg (1959) showed that cortisol circulates partially in free form and partially bound to protein(s) of the α_1 globulin fraction of plasma proteins. According to these workers only the free form can exert an active biological effect. They also showed that oestrogens cause an increase in the cortisol binding protein(s) which have been named transcortin. This protein fraction has a high affinity for corticosterone as well as for cortisol (Daughaday et al., 1959). Thus the total cortisol concentration of blood can be raised without a simultaneous increase in the non-protein bound fraction (Sandberg and Slaunwhite, 1959). As a result, the half life of the corticosteroids would be increased (Marks, Friedman and Duncan, 1961; Christy, Wallace, Wel and Jailer, 1959; Wallace and Carter, 1960). Despite an increase in the total concentration of corticosteroids, the free fraction of the hormone does

not increase in the first two trimesters and only slightly in the third trimester in pregnant women (Doe et al., 1960). Plasma oestrogens are elevated during pregnancy (Svendsen, 1960) and this could explain the increased binding of corticosteroids by virtue of increased transcortin synthesis.

Oestrogens also cause elevation of hepatic Δ^4-steroid hydrogenase activity (Yates et al., 1958) and may therefore be a factor involved in the increase of this activity found in the present work.

Ring A reduction of corticosteroids requires NADP.H as a cofactor. The greater Δ^4-steroid hydrogenase activity in the livers of female than male rats may be related to the fact that there are also increased levels of glucose-6-phosphate hydrogenase and 6-phospho-gluconic acid dehydrogenase in the livers of female rats and these enzymes catalyse reactions generating reduced NADP (Glenister and Yates, 1961). In pregnant rat liver the ratio of NADP.H to NADP is increased (Colagocomo, Vallerino, Chieffi and Colombo, 1963) which may be interpreted in terms of increased activity of the pentose shunt pathway of glucose metabolism. The extent to which the pentose shunt pathway operates is indicated by

the glucose-6-phosphate dehydrogenase level which is high in lactating mammary and adrenal glands and low in tissues such as brain and skeletal muscle (Glock and McLean, 1953, 1954). It would be interesting to know if the level and/or activity of glucose-6-phosphate dehydrogenase is raised in the pregnant liver. Such an increase would supply not only reduced NADP necessary for Δ^4-steroid hydrogenase action, but also ribose necessary for RNA synthesis (Beaconsfield and Reading, 1964).

S U M M A R Y

1) Increases in liver weight, adrenal weight and total hepatic RNA and DNA in pregnant rats have been reported by various workers. Data for these are presented in the present work and compared to those of other workers.

2) The increase in hepatic RNA concentration in pregnant rat reported by Campbell et al. (1949) has been confirmed. Electron microscopy indicated an increase in the membranes of the liver of the 18 day pregnant rat. Separation of the microsomal fraction showed a change in the ratio of bound to free ribosomes at the 18th day of pregnancy.

3) The incorporation of ^{14}C-leucine into protein by the whole microsome fraction and of the rough membranes and free ribosome plus smooth membrane fractions was determined. A significant increase in the former and decrease in the latter was found on the 20th day of pregnancy.

4) The activity of Δ^4-steroid hydrogenase in the liver on the 14th day of pregnancy was increased. The possible significance of this is discussed.

BIBLIOGRAPHY

Addis, T. and Gray, H. (1950)

Growth 14, 49.

Akamutsu, N. (1953)

Virchow's Arch. Path. Anat. u Physiol., 240, 308.

Allen, R.J.L. (1940)

Biochem. J. 34, 858.

Alvarez, R. di, Alfonso, J.F. and Scherrard, D.J. (1961)

Am. J. Obstet. Gynecol. 82, 1096.

Annau, E., Manganelli, A. and Roth, A. (1951)

Cancer Research 11, 304.

Bang, H.O. and Paaby, P. (1955)

Nord. Med. 59, 1693.

Barcroft, J. (1948) "Researches on Prenatal Life"

Thomas, Springfield, Illinois.

Bayliss, R.I.S., Browne, J.C.McC., Round, B.P. and Steinbeck, A.W. (1955)

Lancet i, 62.

Beaconsfield, P. and Reading, H.W. (1964)

Nature 202, 464.

Beaton, G.H., Beare, J., Ryu, M.H., and McHenry, E.W. (1954)

J. Nutrition 54, 291.

Begg, R.W. (1958) Adv. in Cancer Research, 5, 1.

Bernhard, W. (1958) Exptl. Cell Res. Suppl. 6, 17.

Bernhard, W., Rouiller, C. (1956)
J. Biophys. Biochem. Cytol. 2,
Suppl. 73.

Birbeck, M.S.C., Mercer, E.H. (1961)
Nature 189, 558.

Blaxter, K.L. (1964) Mammalian Protein Metabolism II
Chapter 15.

Bokelman, O. and Scheringer, W. (1932)
Arch. Gynaek. 150, 247.

Bond, C.F. (1958) Endocrinology 63, 258.

Bourdel, G. and Jacquot, R. (1960) in "Amino Acids,
Peptides and Proteins" Vol.4, AEC.
Soc. Chim. et Biol. Paris.

Boyne, A.W., Chalmers, M.I. and Cuthbertson, D.P. (1953)
Z. Physiol. Chem. 295, 424.

Brues, A.M. & Marble, B.B. (1937)
J. Exptl. Med. 65, 15.

Bucher, N.L.R. (1963) Int. Rev. Cytol. 15, 245.

Bucher, N.L.R. and Glinos, D.D. (1950)
Cancer Res. 10, 324.

Bucher, N.L.R., Scot, J.P., and Aub, J.C. (1951)
Cancer Res. 11, 457.

Bush, I.E. (1953) J. Endocrinol. 9, 95.

Cammarano, P., Guidice, G., and Lukes, B. (1965)
Biochem. Biophys. Res. Comm. 19(4),
487.

Campbell, P.N., Cooper, C. and Hicks, M. (1964)
Biochem. J. 92, 225.

Campbell, R.M., Innes, I.R. and Kosterlitz, H.M. (1953)
J. Endocrinol. 9, 52.

Campbell, R.M., Innes, I.R. and Kosterlitz, H.M. (1953a)
J. Endocrinol. 9, 68.

Campbell, R.M. and Kosterlitz, H.M. (1949)
J. Endocrinol. 6, 171.

Campbell, R.M. and Kosterlitz, H.M. (1953)
J. Endocrinol. 9, 45.

Cassano, F. and Terantino, C. (1957)
Rio Obstet. e ginecol. 12, 471.
[Chem. Abstr. 55(23) 23727f, 1961]

Christy, N.P., Wallace, E.Z., Wel, G. and Jailer, J.W. (1959)
J. Clin. Invest. 38, 299.

Clark, C.M. and Goodlad, G.A.J. (1961)
Brit. J. Cancer 15, 833.

Cohen, S. and Gordon, A.H. (1958)
Biochem. J. 70, 544.

Colagocomo, A., Vallerino, V., Chieffi, O. and Colombo, L. (1963)

Boll. Soc. Ital. Sper. 39(7), 428

[Chem. Abstr. 59(11), 13167Of.(1963)]

Cole, H.H. and Hart, G.H. (1938)

Am. J. Physiol. 123, 589.

Cope, C.L. (1961) Proc. Roy. Soc. Med. 54, 743.

Cope, C.L. and Black, E. (1959)

J. Obstet. Gynaecol. Brit. Emp.

66, 404.

Daughaday, W.H., Kozak, I. and Biederman, O. (1959)

J. Clin. Invest. 39, 998.

Davidson, J.N. and Weymouth, C. (1944)

Biochem. J. 78, 379.

Doe, R.P., Zinnerman, H.H., Flink, E.B. and Ultrom, R.A. (1960)

J. Clin. Endocrinol. 20, 1484.

Doljanski, F. (1960) Int. Rev. Cytol. 10, 217.

Farese, R.V. and Player, J.E. (1962)

J. Clin. Invest. 41, 53.

Farese, R.V. and Reddy, W.J. (1963)

Biochim. Biophys. Acta, 76, 145.

Ferrari, V. and Harkness, R.D. (1954)

J. Physiol. (London) 124, 443.

Fleck, A. and Munro, H.N. (1962)

Biochem . Biophys. Acta 55, 571.

Flexner, and Pohl, (1941)
Am. J. Physiol. 132, 594.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957)
J. Biol. Chem. 226, 497.

Gershwind, I. and Li, C.H. (1949)
J. Biol. Chem. 180, 467.

Glenister, D.W. and Yates, F.E. (1961)
Endocrinol. 68, 747.

Glinos, A.D. (1956) Science 123, 673.

Glinos, A.D. (1958) in a Symposium on "The Chemical Basis of Development" Ed. W.D. McElroy and B. Glass. John Hopkins Press.

Glinos, A.D. and Gey, G.O. (1952)
Proc. Soc. Exptl. Biol. Med. 80, 421.

Glock, G.E. and McLean, P. (1953)
Biochim. Biophys. Acta 12, 590.

Glock, G.E. and McLean, P. (1954)
Biochem. J. 56, 171.

Greenbaum, A.L. and Slater, T.F. (1957)
Biochem. J. 66, 155.

Griffiths, R.D. and Turner, C.W. (1961)
Proc. Soc. Exptl. Biol. Med. 106, 448.

Guzek, J.W. (1964) Nature 201, 430.

Hagerman, D.D. and Villee, C.A. (1960)

Physiol. Rev. 40, 313.

Hallinan, T.H. and Munro, H.N. (1964)

Biochim. Biophys. Acta 80, 166.

Hallinan, T.H. and Munro, H.N. (1965)

Quart. J. Exptl. Physiol. 50, 93.

Harding, H,R., Rosen, F. and Nichol, C.A. (1961)

Am. J. Physiol. 301, 271.

Harkness, R.D. (1957)

Brit. Med. Bull. 13, 87.

Hay, E.D. (1958) J. Biophys. Biochem. Cytol. 4, 583.

Hazelwood, R.L. and Nelson, M.M. (1965)

Endocrinol. 77, 999.

Hemingway, J.T. (1960) Nature 185, 106.

Hilf, R., Burnett, F.F. and Borman, A. (1960)

Cancer Research 20, 1389.

Hoagland, M.B., Scornik, O.A. and Pfefferkorn, L.C. (1964)

Proc. Nat. Acad. Sci. 51, 1184.

Hultin, T. and von der Decken, A. (1958)

Exptl. Cell Res. 15, 581.

Hunsher, H.A., Donelson, E., Nims, B., Kenyon, F. and May, I.G. (1933)

J. Biol. Chem. 99, 507.

Jackson, C.M. (1913) Am. J. Anat. 15, 1.

Jakobsen, P.E. (1957) 229th Beretng. Forsøgslab. København.

Kelly, L.S. and Jones, H.B. (1950) Science 3, 333.

Kovyndikov, M. and Prostyakov, A. (1959) Sbonik. Rabot. Leningrad. Vet. Inst. 22, 373 [Chem. Abstr. 55, 16733b (1961)].

Lai Wên Wu (1959) Chung Hua Fu. Chan. Kʻo Tsa. Chik. 7, 280. [Chem. Abstr. 54, 12297h (1960)].

Lange, H.F. (1946) Acta Med. Scand. Suppl. 176.

Langman, J. and van Druen, H. (1959) Anat. Record. 133, 513.

Langman, J., van Druen, H., and Bouman, F. (1959) Am. J. Obstet. Gynecol. 77, 546.

Leblond, C.P. and Walker, B.E. (1956) Physiol. Reviews 36, 255.

Leong, G.F., Pessotti, R.L. and Brauer, R.W. (1959) Am. J. Physiol. 197, 880.

Liddelow, B. (1953) Med. J. Aust. 2, 232.

Lin, E.C.C., Revlin, R.S. and Knox, W.E. (1959) Am. J. Physiol. 196, 303.

Linzell, J.L. (1959) Physiol. Reviews 39, 534.

Little, B. and Lincoln, E. (1964)
Endocrinol. 74, 1.

McCorquodale, D.J., Veach, E.G. and Mueller, G.C. (1960)
Biochim. Biophys. Acta 46, 335.

McFarlane, A.S. (1964) in "Mammalian Protein Metabolism"
Vol. 1, 267. Ed. Munro, H.N. and
Allison, J.B. Academic Press.

McGillivray, I. and Tovey, J.E. (1957).
J. Obstet. Gynaecol. Brit. Emp. 64, 369.

McIllwain, H. and Buddle, H.L. (1953)
Biochem. J. 53, 747.

Mack, H.C. (1955) "Plasma Proteins in Pregnancy"
Thomas, Springfield, Illinois.

McKellar, M. (1949) Am. J. Anat. 85, 263.

McLean, P. (1958) Biochim. Biophys. Acta 30, 303.

Malagoli, F. and Donatelli, G.F. (1962)
Ann. Obstet. Ginecol. 84, 60
[Chem. Abstr. 57, 2776h (1962)]

Manganiello, V.C. and Phillips, A.H. (1965)
J. Biol. Chem. 240, 3951.

Mansfield, R.E. and Sheltar, M.R. (1963)
Proc. Soc. Exptl. Biol. Med. 112, 891.

Marks, J.F., Friedman, G.R. and Duncan, F.J. (1961)

J. Lab. Clin. Med. 57, 47.

Matiar-Vohar, H. and Geinert, F. (1961)

Arch. Gynecol. 194, 558.

Migeon, C.J., Betrand, J., Wall, P.E., Stempfel, R.S. and Prystowsky, H. (1957)

Ciba Foundation Colloquia on Endocrinology

II, 338.

Mitchell, H.H., Carroll, W.E., Hamilton, T.S. and Hunt, G.E. (1931)

Univ. Illinois Agr. Expt. Sta. Bull., 375.

Miyabo, S. (1959) Endocrinol. 6, 113.

Mofty, A. El, Zawahri, L.El, and Megged Maamoun, A.E.El, (1960)

Pharm. Bull. 42(4), 117.

[Chem. Abstr. 58, 2699e (1963)]

Morrison, S.D. (1956)

J. Physiol. (London) 133, 167.

Morse, L.M. and Schmidt, S.L.A. (1944)

Proc. Soc. Exptl. Biol. Med. 46, 472.

Mukherjee, T., Gustafsson, R.G., Afzelius, B.A. and Arrhenius, E. (1963)

Cancer Res. 23, 944.

Munro, A.J., Jackson, J. and Korner, A. (1964)

Biochem. J. 92, 289.

Naora, H. (1957) J. Biophys. Biochem. Cytol. 3, 949.

Needham, D.M. and Cawkwell, J.M. (1957)
Biochem. J. 65, 540.

Nelson, M.M. (1959) in "Reprod. Physiol. and Protein
Metabolism". Ed: Leatham, J.H.
Rutgers University Press.

Nichol, C.A. and Rosen, F. (1963) in "Advances in
Enzyme Regulation" 1, 341.
Ed. G. Weber, Pergamon Press.

Oliver, I.T., Blummer, W.F.C. and Witham, I.J. (1963)
Comp. Biochem. Physiol. 10, 33.

Oliver, I.T., Bollard, F.J., Shield, J. and Bentley, P.J. (1962)
Devel. Biol. 4, 108.

Paaby, P. (1960) J. Obstet. Gynaecol. Brit. Emp. 67, 43.

Palade, G.E. (1952) J. Exptl. Med. 95, 285.

Palade, G.E. (1955) J. Biophys. Biochem. Cytol. 1, 59.

Palade, G.E. (1956) J. Biophys. Biochem. Cytol. 2,
Suppl. 85.

Penzes, L. (1959) Biol. Közlemények 7, 73.
[Chem. Abstr. 55, 27577c (1961)]

Peters, T. Jr (1962) J. Biol. Chem. 237, 1186.

Poo, Z.J., Lew, W., Lee, D.D. and Addis, T. (1940)
~~Proc. Soc. Exptl. Biol. Med.~~ J. Nut. 19, 505.

Porter, K.R. (1954) J. Histochem. Cytochem. 2, 346.

Potter, V.R. and Elvehejm, C.A. (1936)

J. Biol. Chem. 11A, 495.

Prescott, D.M. (1960) Am. Rev. Physiol. 22, 17.

Prostyakov, A.P., Fortushnyi, V.A. and Kovyndikoy M.S. (1961)

Ukrain Biochim. Zhur. 33, 505.

[Chem. Abstr. 56, 1869d (1962)]

Rechcigl, M. Jr., Grantham, F. and Greenfield, R. (1961)

Cancer Res. 21, 238.

Rendi, S. (1959) Biochim. Biophys. Acta 31, 266.

Reynolds, E.S. (1963)

J. Cell Biol. 17(1), 208.

Ricci, P.D., Viola, M.P. and Gori, M. (1963)

Rass. Fisiopatol. Clin. Terop.(Pisa)

35, 388.

[Chem. Abstr. 59(12), 14366d (1963)]

Rosen, F., Roberts, N.R., Budnick, L.E. and Nichol, C.A. (1954)

Endocrinol. 65(2), 256.

Sandberg, A.A. and Slaunwhite, R.W. (1959)

J. Clin. Invest. 38, 1290.

Schmidt, G. and Thannhauser, S.J. (1945)

J. Biol. Chem. 161, 83.

Setnikar, I. (1957) Boll. Soc. Ital. Biol. Sper 33, 1636.

Setnikar, I. and Barron, D.H. (1958) Quart. J. Exptl. Physiol. 43, 284.

Slautterback, D.B. and Fawcett, D.W. (1959) J. Biophys. Biochem. Cytol. 5, 441.

Slaunwhite, R.W. and Sandberg, A.A. (1959) J. Clin. Invest. 38, 384.

Sonders, H.J. and Morgan, A.F. (1957) Am. J. Physiol. 191, 11.

Spray, C.M. (1950) Brit. J. Nutrition 4, 354.

Svendsen, R. (1960) Acta Endocrinol. (Kbh) 35, 161.

Tier, H. and Lavant, K. (1953) Exptl. Cell Res. 7, 367.

Tomkins, G.M. (1959) Ann. N.Y. Acad. Sci., 82, 836.

Tovey, J.E. (1959) J. Obstet. Gynaecol. Brit. Emp. 66, 981.

Urquhart, J., Herbst, A.L. and Yates, F.E. (1959) Endocrinol. 64, 816.

Von der Decken, A. and Hultin, T. (1958) Exptl. Cell Res. 14, 88.

Wakid, N.W. and Needham, D.M. (1960)
Biochem. J. 76, 95.

Wallace, E.Z. and Carter, A.C. (1960)
J. Clin. Invest. 39, 601.

Weinbren, K. (1959) Gastroenterol. 37, 637.

Widdowson, E.M. and Spray, C.M. (1951)
Arch. Disease Childhood 26, 205.

Williamson, M.B. (1948) Growth 12, 145.

Wirth, K. and Schreier, K.(1956)
Z. Physiol. Chem. 309, 182.

Yates, F.E., Herbst, A.L. and Uruqhart, J. (1958)
Endocrinol. 63, 887.