CXIX. THE RÔLE OF THE PLACENTA IN THE FAT METABOLISM OF THE RABBIT FOETUS.

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IN recent years, the author has been interested in the study of fat metabolism during the reproductive cycle. The results of previous investigations leading to the present contribution may be briefly indicated. The preliminary step consisted in the elaboration of a method whereby all known lipoids could be simultaneously estimated in micro-amounts, in the same extract and using the same analytical principle—isolation followed by oxidation [Boyd, 1933, 1]. This differential micro-analysis was then applied to a study of lipoids in the blood of pregnant women in whom it was shown [Boyd, 1934, 1] that the concentration of lipoids is increased in plasma but not in the red blood cells and that the increase in plasma is different in regard to time and extent for each lipoid. The white blood cells were noted to contain smaller amounts of fatty substances in pregnant [Boyd, 1934, 2] than in non-pregnant [Boyd, 1933, 2] women but after parturition there occurred a remarkable increase of several hundredfold in the phospholipin, free cholesterol and neutral fat of these cells. Experiments which have not been published to date confirmed the observations of Baumann and Holly [1926] and others that in rabbits the blood lipoids are decreased rather than increased during pregnancy. In the ovary of pregnant and pseudopregnant rabbits, certain changes were encountered in lipoid composition which were interpreted as signifying that the physiological activity of this organ in this species increases up to the mid-point of pregnancy and retrogresses during the latter half [Boyd, 1935, 1].

Two possible effects of the lipaemia of pregnancy in women have so far been investigated. It has been shown that normal lactation following the termination of pregnancy is accompanied by a decline in the value of all plasma lipoids but that if lactation is prevented by drying up the breasts the lipaemia persists or may be augmented [Boyd, 1935, 2]. This was interpreted as signifying that the lipaemia assists in the production of milk fat or that the prevention of lactation causes some further maladjustment in fat metabolism. The second effect investigated was the possibility that the lipaemia may act in the nature of an increased pressure head forcing lipoids into the umbilical circulation through the placenta and on to the foetus where they are utilised in the building up of the developing organs. In this connection, it was found that the human placenta at full term pregnancy adds considerable amounts of lipoids to umbilical blood from which they are absorbed by the growing offspring [Boyd and Wilson, 1935]. The average large, well-nourished infant was recorded to absorb in the neighbourhood of 50 g. of lipoids per day from umbilical blood, 40 g. of these being phospholipin and the remainder equally divided between free cholesterol and cholesterol esters; neutral fat was occasionally absorbed and occasionally given up by the foetus.

From these results it was concluded that the placenta plays an important part in the transfer of lipoids from the mother to the foetus *in utero*. Two

possible explanations may be offered regarding the manner in which this exchange takes place. First, lipoids in the maternal blood-stream may simply be passed across the placental membrane to the umbilical blood. In referring to this process, the term permeability has been purposely avoided since the manner in which lipoids are transferred across animal membranes is at present obscure; it may be through the intermediate formation of semi-soluble phospholipin although even this is disputed. Secondly, the placenta may act in the nature of a secreting gland, taking up lipoids from the maternal blood-stream and passing them on, with or without structural modification, to the foetal circulation. The absence of glandular tissue from the placenta in no way militates against this conception, since apparently all tissues of the body are capable of taking up and discharging lipoids of the blood. If the tissues of the placenta are actively engaged in the production or elaboration of lipoids for the foetus, then it appears that any alteration in the lipid composition of the foetus at the various stages of pregnancy may be accompanied by corresponding changes in the lipid composition of the placenta. The present investigation was concerned with the development of this postulate.

Rabbits were used in this investigation because the placenta in this species is functionally similar to that in man, *i.e.* it is of the haemo-chorial type, and hence results obtained with this animal may be more readily applied to and compared with our previous findings in the human placenta [Boyd and Wilson, 1935]. The procedure adopted was to select female rabbits at different periods during pregnancy, open the abdomen and uterus, take out a foetus with its corresponding placenta and then measure the lipid content of each. When a sufficient number of such experiments had been done, it was possible to plot the lipid composition of the foetus against the lipid composition of the placenta at the various stages of pregnancy, noting whether or not there were any indications of variations in the fat metabolism of the placenta during gestation.

EXPERIMENTAL PROCEDURE.

Foetuses and placentas were obtained from the uteri of gravid rabbits at various days during pregnancy from the 14th day to the 32nd day or term. No determinations were made earlier than the 14th day because the foetus before this date is too small for a complete lipid micro-analysis. The structures were kept in pairs so that the lipid composition of the foetus could be compared with that of the placenta to which it had been attached while in the uterus. After removal from the uterus, the foetus and placenta were separately weighed and ground up to a fine mud in a mortar using rough quarry sand which had been de-carbonated with hydrochloric acid, washed with water and extracted with alcohol and was of a uniform size between 18 and 20 mesh. The mixture of sand and tissues was transferred quantitatively to a flask and shaken with at least 50 volumes (*v/w*) of a mixture of alcohol and ether, 3 : 1, both redistilled and the ether peroxide-free. The mortar was washed out several times with small amounts of this solvent and the tissue-sand, which tended to cake when the proteins were precipitated, broken up with a clean spatula. The contents of the flask were then brought to boiling on a steam-bath for a few minutes, thus completing the extraction of lipoids. After cooling to room temperature, the extracted fatty substances were filtered off through alcohol-extracted filter-paper into a volumetric flask, the residue was washed with repeated portions of alcohol-ether, and the combined filtrate made up to volume. Foetuses weighing over 10 g. were cut into fine pieces and continuously extracted with 10 to 15 volumes of alcohol-ether, for 10 to 15 hours in Bloor percolator-extractors. Both of

these methods were found to give complete extraction and the choice was purely for convenience in adjusting the final volume of the extracts, the first method requiring large amounts of solvent and a short extraction time and the Bloor method using smaller amounts of solvent but a longer extraction time.

In each extract the lipoids determined experimentally were total fatty acids, total cholesterol, free cholesterol and phospholipins, using the Bloor oxidative micro-technique as modified by Boyd [1933, 1]. From these values determined experimentally were calculated the concentrations of the various lipoids present. Thus phospholipin and free cholesterol were obtained directly, ester cholesterol calculated by subtracting the value of free cholesterol from total cholesterol, and neutral fat by subtracting from the total fatty acids the fatty acids of phospholipin and cholesterol ester. These residual fatty acids, which were taken to represent neutral fat fatty acids and calculated to comprise 95 % of the neutral fat fraction, are probably present in the tissues mostly in the form of glycerol esters but may have other combinations and some may be present as free fatty acid. In view of this probability and of the absence of definite knowledge concerning their nature, they have been referred to collectively as neutral fat.

EXPERIMENTAL RESULTS.

The concentrations of the four main groups of lipoids, namely phospholipins, free cholesterol, ester cholesterol and neutral fat, in the rabbit foetus and placenta during the latter two-thirds of pregnancy are depicted respectively in Figs. 1, 2, 3 and 4. Curves for total lipid, total fatty acid, total cholesterol and the other lipoids have not been given because they are not essential to the interpretation of the data and because they may be calculated approximately from the information given. The ordinates and abscissae in all figures are in the same units. The ordinate represents mg. of lipid per 100 g. tissue, wet weight. The abscissa represents the weight of the foetus in g. In order to demonstrate clearly the variations in the smaller foetuses, the progression of the abscissa was made geometric rather than arithmetic. This should be remembered when interpreting the slope of the curves. The weight of the foetuses has been used for the abscissa rather than the day of gestation because the lipid composition was found to vary with the weight of the foetus rather than with the duration of pregnancy. This was particularly apparent when three or four foetuses were analysed from a single uterus, especially in the earlier stages of gestation. To obtain the approximate duration of pregnancy in each curve, the following figures may be given as guides: at 14 days, average weight of foetus about 0.25 g.; at 17 days, 1.0 g.; at 24 days, 12 g.; at 31 days, 40 g.

A. *Phospholipins.*

The concentration of phospholipins in the foetus (Fig. 1) increased in the earliest embryos studied and in every embryo throughout pregnancy whenever the weight of the foetus increased, the most rapid increase being around the 14th day of gestation. As the weight of the foetus rose from 0.2 to 0.4 g., the phospholipin content rose from 122 to 513 mg. per 100 g. From then until the termination of pregnancy, phospholipin continued to rise in value but at a much smaller rate. At the termination of pregnancy, phospholipin composed on the average 1 % of the weight of the foetus.

While the phospholipin content of the foetus was steadily increasing, the phospholipin content of the placenta was found to exhibit two phases. As the foetus increased in weight from 0.2 to 2 g., the concentration of phospholipin

in the placenta fell from over 1500 mg. per 100 g. to about 1000 mg. per 100 g. From then on to term, phospholipin rose to and remained at a plateau level of around 1500 mg. per 100 g., with considerable variation both high and low in different placentas. The appearance of this double phase became obvious only after examining a number of placentas, particularly those in the earlier stages of gestation. It is not shown in the results of previous investigators on other

Fig. 1.

Fig. 2.

Fig. 1. Changes in the percentage of phospholipin in the foetus and in the placenta of the rabbit during intra-uterine growth. $\circ-\circ$ placenta; $\bullet-\bullet$ foetus.

Fig. 2. Changes in the percentage of free cholesterol in the foetus and in the placenta of the rabbit during intra-uterine growth. $\circ-\circ$ placenta; $\bullet-\bullet$ foetus.

types of placentas; most of this previous work reports only one or two stages, but Watanabe [1923] obtained a "step down" type of curve for the human placenta, that is a high plateau in the first trimester, a drop to a lower plateau in the second trimester and then the lowest level of all in the last trimester. The only previous report on the placenta of the rabbit gives 600 mg. per 100 g. as the value for phospholipin on the 29th day of gestation [Baumann and Holly, 1926].

The results suggest that the initial fall in the level of placental phospholipin is due to removal of phospholipin either to certain maternal organs such as the mammary glands, which were found by Baumann and Holly [1926] to have an infiltration of phospholipin during pregnancy, or to the foetus in which there is a very rapid increase in phospholipin as shown above. Since the weight of the placenta increases during pregnancy more rapidly than the concentration of phospholipin falls, there is actually a slight increase in the total amount of phospholipin segregated in this organ. Hence the increase in the phospholipin of the foetus cannot be due to a simple transfer of a certain number of mg. of

phospholipin from the placenta. The phospholipin content of a tissue is a dynamic value, being increased by absorption of lipid from the blood and decreased by oxidation, hydrolysis, and removal by the blood. Hence the placenta of early pregnancy, or rather the early part of the second half of pregnancy, may be giving up to the foetus or to some other organ more phospholipin than it actually requires to satisfy its own needs.

B. *Free cholesterol.*

Changes in the concentration of free cholesterol (Fig. 2) in the foetus during pregnancy closely paralleled the changes in phospholipin. This was not unexpected since the two lipoids appear to be closely related in vital economy, the one being antagonistic to the other in many physiological reactions, for example, haemolysis of the red blood cells. In the foetus, free cholesterol rose from a value of 58 mg. per 100 g. in a 0.2 g. embryo to 167 mg. per 100 g. in a 34 g. embryo almost at term. Again the increase in the foetus was more marked in the earlier cases studied.

The placenta exhibited considerable variation in regard to its free cholesterol with a tendency toward increasing amounts of the lipid as gestation proceeded. The curve as represented in Fig. 2 was considered best adapted to the various points. The relationship to phospholipin may be shown more clearly if the first eight points for free cholesterol in the placenta are united by a straight line progressing downward followed by a rise in the latter part of pregnancy as in the case of phospholipin. In such a case, most of the discussion which was applied to phospholipin may also be applied to free cholesterol. Be this as it may, the results clearly indicate that cholesterol metabolism of the placenta varies according to the duration of pregnancy.

C. *Ester cholesterol.*

This lipid underwent a remarkable change in the rabbit placenta during pregnancy. As shown in Fig. 3, it rose from a value of 139 mg. per 100 g. on the 14th day of gestation to almost 500 mg. per 100 g. between the 16th and 22nd days of pregnancy. After that, the concentration of placental ester cholesterol fell and at the end of pregnancy the value was similar to that found in the 14-day animals. During this time, small and comparatively insignificant amounts of ester cholesterol were present in the foetus, the values varying between 0 and 98 mg. per 100 g. but showing no definite trends.

Variations in the concentration of placental ester cholesterol are in many respects the opposite of those for phospholipin and free cholesterol, particularly phospholipin. Thus, while the values for phospholipin fell in the placenta between 0.2 and 10 g. foetuses, the ester cholesterol rose. Ester cholesterol in tissues is bound with fatty acids and the results suggest that the decrease in phospholipin may have been due to a liberation of fatty acids which were then combined with cholesterol to produce cholesterol esters. The concentration of total cholesterol also increased in this period, so that there must have been a considerable infiltration or synthesis of cholesterol.

It is worthy of note that the placenta of the rabbit was found to contain more cholesterol esters than are usually found in tissues. On the average, about one half of the total cholesterol was present as ester; in adult tissues, ester cholesterol rarely comprises more than 10 % of the total cholesterol. Since cholesterol esters appear to act as transporters of fatty acids [Bloor, 1924;

Schaible, 1932; Ling, 1931], there may be some significance in the high cholesterol ester content of the placenta in relation to the postulated transport of fatty acids to the foetus.

Fig. 3.

Fig. 3. Changes in the percentage of ester cholesterol in the foetus and in the placenta of the rabbit during intra-uterine growth. ○—○ placenta; ●—● foetus.

Fig. 4.

Fig. 4. Changes in the percentage of neutral fat in the foetus and in the placenta of the rabbit during intra-uterine growth. ○—○ placenta; ●—● foetus.

D. Neutral fat.

Comparatively little is known about the physiology of neutral fat apart from the fact that it is the chief storage form of fat in the body. Analyses for neutral fat in the rabbit foetuses, Fig. 4, confirmed the well-known clinical observation that premature and abortive embryos have a thin, emaciated appearance in contrast to the plump, well-rounded out appearance of full-term foetuses. These latter embryos owe their appearance to the presence of subcutaneous fat which is mostly neutral fat. In the foetuses of rabbits studied, neutral fat increased over 600 % between the 14th and last days of pregnancy, the rise taking place about the 20th day.

The neutral fat of the placenta varied within a wide range of values, especially in the earlier cases studied wherein figures were obtained between 560 and 1470 mg. per 100 g. Although the differences between placentas were also wide at subsequent periods of pregnancy, in general the values tended to fall somewhat. The total loss of neutral fat to the placenta was less than the total gain in neutral fat of the foetus.

DISCUSSION.

The present investigation offers certain further information concerning the relationship between the lipid composition of the foetus and the activity of the placenta. It has been shown that the embryo of the rabbit acquires increased concentrations of phospholipin and free cholesterol as it grows in the uterus. There was no relation found between growth and the cholesterol ester content of the foetus, but about 10 days before the end of intra-uterine development the neutral or glyceride fat of the foetus increased sixfold. At birth the foetus of the rabbit contained a much higher percentage of all lipoids except cholesterol esters than in the early stages of growth within the mother.

The rates of increase in the concentrations of phospholipin and free cholesterol of the foetus were greatest up to about the 16th day or middle of pregnancy. Then ensued slower rates of increase until the last week of gestation when again the percentage of these lipoids rapidly increased. Phospholipin and free cholesterol, especially phospholipin, have been shown in recent years to increase in concentration in a tissue or organ whenever that tissue or organ becomes physiologically active [Bloor and Snider, 1934; Boyd, 1935, 1]. If it may be assumed that the rate of increase in physiological activity varies as the rate of increase in phospholipin, then the embryo of the rabbit may be said to exhibit two stages of marked activity, the first up to the 16th day and the second in the last week of intra-uterine life. The activity of the embryo in the first half of pregnancy is probably referable chiefly to growth. In the last week of gestation, however, the growth rate is falling off and it seems from the data obtained that activity of the embryo at this time is due to increased activity of fat metabolism, resulting in the deposition of large amounts of neutral or storage fat.

This stimulus to foetal fat metabolism in the last days of intra-uterine development may be shown to be accompanied simultaneously by an increase in the physiological activity of the placenta. As previously stated, physiological activity is characterised by increasing percentages of phospholipin and free cholesterol. On the other hand, degeneration, retrogression or inactivity results in a decrease in phospholipin and an increase in cholesterol esters [Bloor and Snider, 1934; Boyd, 1935, 1]. Up to about the middle of pregnancy, the placenta has been shown to exhibit a decreased concentration of phospholipin and an increased concentration of cholesterol esters. This indicates that the placenta declines in activity during the first half of gestation.

Lipoid analyses reveal that about the middle of gestation, the placenta begins to take on increased function, *i.e.* the percentage of cholesterol ester falls while that of phospholipin and free cholesterol rises. At the same time, fat metabolism in the foetus becomes augmented as evidenced by the laying down of great quantities of storage fat and increased concentrations of phospholipin and free cholesterol.

It would appear that these two phenomena may be related. From the data given in Figs. 1 to 4, the foetus of 16 days may be calculated to contain a total of about 6 mg. of phospholipin, 1 mg. of free cholesterol, 4 mg. of ester cholesterol and 2 mg. of neutral fat. The foetus at term may be similarly shown to contain 500 mg. of phospholipin, 75 mg. of free cholesterol, 75 mg. of ester cholesterol and 600 mg. of neutral fat. Thus it is only in the latter part of pregnancy that appreciable amounts of lipoids are segregated in the foetus. Previous studies [Boyd and Wilson, 1935] suggested that in the haemo-chorial type of placentation, the major portion of foetal lipoids is passed through the placenta. Present studies indicate that the bulk of foetal lipoids, excluding a possible portion

which may be synthesised from carbohydrate, must pass through the placenta in the latter part of pregnancy. If this passage were simply one of physical transfer across the placental membranes, it is difficult to see why there should be any change in the lipid metabolism of the placenta, especially a change which indicates increased physiological activity. Rather do the results suggest that the actual tissue substance of the placenta plays an important rôle in the transfer of lipoids from the mother to the growing offspring.

While in this series of investigations attention has been directed chiefly to the placenta as the medium through which lipoids are transferred from the mother to the embryo, it is possible that other mechanisms may exist. In animals possessing an epithelio-chorial or syndesmo-chorial type of placenta, with several layers of cells separating the maternal and foetal blood streams, *e.g.* pigs, horses and ruminants, it is difficult for the foetus to obtain sufficient nourishment directly from the maternal blood *via* the placenta and return to it waste products of metabolism. In such animals, accessory mechanisms such as "uterine milk" are provided for the nourishment of the foetus and accessory organs like the allantois for the disposal of waste products. The decidua and amniotic fluid constitute another possible mechanism for foetal nourishment.

Little is known about the relations of these several possible mechanisms to foetal fat metabolism except in the case of amniotic fluid. This fluid was found to contain but traces of fatty acids in human beings [Konstantinovitch, 1906] but appreciable amounts in the cow [Eckles, 1916]. Traces of cholesterol have been reported in human amniotic fluid [Chauffard *et al.*, 1911]. A sample of pure amniotic fluid, obtained by the author from a human ovum about two months old, gave the following lipid analysis: neutral fat, 0; free cholesterol, 4 mg. per 100 g.; ester cholesterol, 27 mg. per 100 g.; phospholipin, 67 mg. per 100 g. It is of interest that the lipoids concerned with fat transport, *i.e.* phospholipin and cholesterol ester, were present in greatest amounts. A possible manner by which lipoids may be absorbed from amniotic fluid by the foetus can be deduced from the work of Keiffer [1926]. He found that "vernix caseosa," which is a cheesy deposit from amniotic fluid on the skin of the mammalian embryo and contains some 10 % fat, will be absorbed, if left on the skin, within eight hours of birth. These various facts suggest that lipoids may be added to amniotic fluid through the decidua and amnion, deposited on the skin of the embryo and from there absorbed by the foetus. Further work is required to substantiate this postulate.

SUMMARY.

The lipid composition of the foetus and of the placenta was determined in the rabbit at various stages during intra-uterine development with a view to demonstrating any relation between the fat metabolism of placental tissue and fat metabolism of the foetus.

It was found that the lipid composition both of the foetus and of the placenta varied according to the duration of growth of the foetus. In the foetus, the concentrations of phospholipin and free cholesterol increased rapidly up to the middle of gestation, then more slowly until the last week or ten days when again the rate increased. During the last ten days there was a marked increase of 600 % in the neutral or storage fat content of the foetus. The embryo contained variable but similar amounts of cholesterol ester at all stages of growth within the uterus.

Up to the mid-point of pregnancy, the placenta exhibited a fall in the concentration of phospholipin and an increased percentage of cholesterol esters.

In the latter half of gestation, the reverse changes occurred, *i.e.* the value of phospholipin rose while the value of cholesterol esters fell. Throughout pregnancy there was a slight increase in placental free cholesterol and a slight increase in placental neutral fat.

On the basis of current theory regarding the relation between lipid composition and physiological activity, these changes were interpreted as follows. Growth maintains a high degree of physiological activity in the foetus until about the middle of pregnancy. In the last few days of gestation, the foetus again becomes active owing to an increased turnover of fat metabolism resulting in the laying down of large amounts of storage fat in the embryo. The deposition of this fat occurs along with an increased concentration of phospholipin and free cholesterol and is associated with increased activity on the part of the placenta. Such placental activity appears inconsistent with the conception that lipoids are passed through the placenta merely as through an animal membrane and suggests that the actual tissue substance of the placenta is actively engaged in transferring at least a part of the lipoids from the mother to the embryo in the latter part of gestation.

Other possible pathways for the exchange of lipoids between the mother and the foetus were discussed, particularly the decidua and amniotic fluid.

The author takes pleasure in acknowledging that he obtained the idea that the placenta may be more than a permeable membrane in relation to the acquisition of lipoids by the foetus from reading the stimulating description of this subject by Dr Joseph Needham in his book, *Chemical embryology*.

This investigation was begun while the author was associated with the Department of Obstetrics and Gynaecology, The University of Rochester, School of Medicine and Dentistry, Rochester, New York. The author desires to thank Dr A. W. Makepeace of that department for providing much of the experimental material.

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