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# MATERNAL AND FOETAL TISSUE- AND PLASMA-LIPIDS IN NORMAL AND CHOLESTEROL-FED RABBITS

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It was previously reported (Barcroft & Popják, 1945) that the plasma-lipid values in the sheep foetus follow closely those of the maternal plasma, but at a lower level. The relationship was so striking that it was suggested that the foetal plasma-lipids in the sheep are dependent upon and probably derived from the lipids of the maternal plasma. A rapid utilization of these substances by the foetus would explain their lower concentration on the foetal side. Boyd & Wilson (1935) showed that the concentration of lipids in the umbilical arterial blood of the mature human foetus is usually lower than in the umbilical venous blood, indicating a utilization by the foetus.

In the investigation to be described the aim was to induce lipaemia in pregnant rabbits and to examine the extent to which the foetal tissue- and plasma-lipids could be influenced by this means.

## MATERIAL AND METHODS

For the production of lipaemia in the rabbit a convenient method was available. It was found (Popják, 1945) that the feeding of amorphous cholesterol in a watery suspension by the method of Dömösi & Egyed (1939) caused in a short time an increase of all the plasma-lipid fractions in the rabbit.

Pregnant rabbits of about 3 kg. weight received daily, from the 8th until the 28th day of gestation, 1 g. of amorphous cholesterol. In the initial experiments the watery cholesterol suspension was administered through a stomach tube. Five out of six rabbits thus fed miscarried before the 28th day and the foetuses were lost. These early deliveries can be attributed, at least partly, to the struggle some rabbits make against the passing of a stomach tube. After these failures, therefore, the cholesterol suspension was mixed with a small amount of bran and oats and offered to the rabbits first thing in the morning. The rest of their daily ration was given to them after they had consumed the cholesterol-containing food. Since a small portion of the ration containing the cholesterol was usually spilt from the feeding troughs, it is probable that the actual amount of cholesterol consumed was less than 1 g. per day. The control rabbits were maintained on the same bran, oats and cabbage diet as the experimental ones, only the cholesterol being omitted.

Blood was taken from the experimental animals on the 1st, 8th, 15th and 28th days, and from the controls on the 1st and 28th days, and in some cases on the 15th day of gestation also. Heparin was used to prevent clotting.

On the 28th day the rabbits were anaesthetized by the intravenous injection of nembutal (1 ml. of a 10% solution per 5 lb.). The foetuses were delivered one by one, by the intra-abdominal route, after the uterine horns had been opened along the antimesometrial surface. Blood was drawn from the umbilical vein of each foetus with a small syringe and hypodermic needle. The piston of the syringe was moistened with a small drop of heparin solution. About 1–2 ml. of blood could be obtained from each foetus; the samples were pooled separately from each litter and were heparinized. The differential lipid analyses were carried out on 4–6 ml. of foetal plasma. When the mother bore few foetuses, only 1–2 ml. of foetal plasma could be obtained, and in such cases total cholesterol and total fatty acid alone were determined.

*Preparation of tissues for analyses.*

All lipid determinations were carried out on tissues dried *in vacuo* from the frozen state over $P_2O_5$.

*Placenta.* The placentae were freed of the membranes, wiped with a filter paper to remove adherent blood, then the maternal and foetal portions torn apart. Only the foetal placentae were used for the determinations. The tissues of two to three placentae from each case were pooled. For histological examination whole placentae were taken.

*Foetal livers.* The whole livers were dissected out from at least two foetuses from each case and pooled. The gall bladder was not removed.

*Whole foetuses.* The foetuses were minced, dried and ground to a fine powder.

*Maternal livers.* A sample of about 2–3 g. was excised from the middle of the right lobe.

*Lipid analyses.*

The plasma was extracted by boiling with at least 20 vol. of an ethanol-ether mixture (3 : 1, v/v) by the method of Bloor (1928). The finely powdered tissues were extracted by boiling with five portions of the ethanol-ether mixture under a reflux condenser.

The filtered extracts were evaporated to dryness under reduced pressure (20–40 mm. of Hg) at an external temperature of 30–40° C. while a slow current of nitrogen was passing through the extract. The dry residues were extracted with light petroleum (b.p. 40–60° C.) and the lipid fractions isolated from this extract. The phospholipids were precipitated and determined by the method of Bloor (1929). For the determination of non-phospholipid fatty acids (derived from neutral fats and cholesteryl esters) a sample of the supernatant fluid obtained after the phospholipid precipitation was evaporated to dryness and saponified with ethanolic KOH (0·2–0·3 ml. of a 40% aqueous KOH solution added to 5 ml. of ethanol) for 2 hr. at 75° C. in an incubator. After completion of hydrolysis most of the ethanol was evaporated off on a hot plate, then about 2 ml. of water were added. The last traces of the alcohol were removed by further heating. The mixture was then acidified with 5% (w/v) HCl and extracted with light petroleum. The fatty acids, redissolved in absolute ethanol, were determined by titration at 75° C. with a 0·02 N-aqueous NaOH solution. The average molecular weight of the fatty acids was assumed to be 275.

Free and total cholesterol were determined by a digitonin method as described by Popják (1943). Ester cholesterol was calculated by difference.

Neutral fats were calculated by the formula:

$$\text{neutral fat} = (\text{non-phospholipid fatty acids} - 0{\cdot}72 \times \text{ester cholesterol}) \times 1{\cdot}05.$$

## RESULTS

The results obtained afford a comparison between lipids of (*a*) placentae, (*b*) foetal livers, (*c*) whole foetuses, (*d*) maternal and foetal plasmata, and (*e*) maternal livers of normal and cholesterol-fed rabbits on the 28th day of pregnancy.

Rabbits tolerate very well the administration of amorphous cholesterol in a watery suspension: there is no gastro-intestinal upset as there is when cholesterol is fed in a high fat diet. The only disturbance that cholesterol-feeding (even without a stomach tube) produces in pregnant rabbits is that there is a tendency for premature deliveries, although these are fewer when a stomach tube is not used (three out of nine).

Since some of the results obtained have to be considered in the light of the placental changes brought about by the experimental procedure, these will be described first.

*Placentae of cholesterol-fed rabbits*

The mean weight of the placentae (foetal + maternal), without the membranes, was approximately the same in normal and in cholesterol-fed rabbits: $6{\cdot}4 \pm 1{\cdot}4$ and $6{\cdot}3 \pm 1{\cdot}4$ g. respectively. The macroscopical appearance of the organ, however, was very different in the two groups of animals (Pl. 1, fig. 1). In normal rabbits the foetal portion of the placenta is of a dark purplish colour and is sharply demarcated from the creamy white maternal part. In cholesterol-fed rabbits the foetal placenta was pale yellow ochre, and it merged indistinctly into the maternal portion.

Histological examination revealed that the paleness of the foetal placenta in the experimental animals was due to the excessive accumulation of lipids in the form of granules within the cells of the foetal capillaries (Pl. 1, fig. 2). Examination of the sections with a polarizing microscope showed that most of this lipid material is anisotropic, i.e. cholesterol and its esters. This histological finding was confirmed by the chemical analyses. The normal foetal placentae on the 28th day of pregnancy did not contain any stainable fatty material, which is in agreement with the description of Chipman (1901–3). Capillary endothelium elsewhere (e.g. kidney) in the body of the mother has an ability also to take up cholesterol, but not nearly to the same extent as the foetal placenta. In the maternal portion of the normal placenta the decidual cells contain stainable fat in the form of distinct globules (Pl. 1, figs. 3, 4). These cells are most obvious around maternal sinuses, arranged in broad sheaths. In cholesterol-fed animals the decidual cells contained far greater quantities of lipids: the fat globules were tightly packed, almost completely obscuring the structure of the cells (Pl. 2, figs. 1, 2). Examination with polarized light showed that the fat in the decidual cells of both control and experimental animals is isotropic, i.e. neutral fat. It is probable that this excessive accumulation of fatty materials in the placentae is the cause of early deliveries by the cholesterol-fed rabbits. It was observed that it was easier to separate the placentae of cholesterol-fed animals from the uterine wall than those of the controls.

The results of lipid determinations on the foetal portions of the placentae (Table 1) show that there was a very marked increase of the free and ester cholesterol, and a moderate increase of the neutral fat content in the cholesterol-

fed animals as compared with the controls. The ability of the foetal placenta to accumulate cholesterol and its esters is as great or even greater than that of the maternal liver (cf. Table 9). The mean free and ester cholesterol contents of the experimental placentae were $0{\cdot}617 \pm 0{\cdot}173$ and $2{\cdot}000 \pm 0{\cdot}686\%$ respectively, those of the livers of the same animals $0{\cdot}294 \pm 0{\cdot}074$ and $1{\cdot}290 \pm 0{\cdot}373\%$ respectively. Considering that these values are expressed as percentage of moist tissue weight and that the water content of the placenta is about 80% while that of the maternal liver is only about 70%, the higher cholesterol content of the placentae becomes even more impressive. This behaviour suggests that the placenta is a highly active organ in respect of the fat metabolism of the foetus.

TABLE 1. Lipid content of placentae of normal and cholesterol-fed rabbits on 28th day of pregnancy. Lipid content expressed as g./100 g. fresh tissue

| Experiment No. | Neutral fat | Phospholipids | Cholesterol | | Fatty acids of cholesteryl esters | Total lipid | |
|---|---|---|---|---|---|---|---|
| | | | Free | Ester | | | |
| 61 | 0·95 | 1·19 | 0·226 | 0·598 | 0·431 | 3·395 | Placentae of normal rabbits |
| 62 | 0·62 | 1·21 | 0·177 | 0·195 | 0·140 | 2·342 | |
| 67 | 0·59 | 1·33 | 0·189 | 0·246 | 0·177 | 2·532 | |
| 77 | 0·54 | 1·50 | 0·246 | 0·430 | 0·310 | 3·026 | |
| 80 | 0·44 | 1·33 | 0·186 | 0·141 | 0·101 | 2·198 | |
| Mean | 0·63 | 1·31 | 0·205 | 0·322 | 0·232 | 2·699 | |
| S.D. | ±0·19 | ±0·12 | ±0·030 | ±0·190 | ±0·136 | ±0·499 | |
| 41 | 1·46 | 1·25 | 0·686 | 2·109 | 1·576 | 7·081 | Placentae of cholesterol-fed rabbits |
| 64 | 0·90 | 1·00 | 0·650 | 1·490 | 1·073 | 5·113 | |
| 65 | 1·03 | 1·19 | 0·598 | 2·139 | 1·540 | 6·497 | |
| 71 | 0·91 | 1·53 | 0·491 | 2·171 | 1·564 | 6·666 | |
| 78 | 0·70 | 2·20 | 0·952 | 3·318 | 2·390 | 9·560 | |
| 79 | 0·82 | 1·98 | 0·513 | 1·377 | 0·992 | 5·682 | |
| 81 | 0·59 | 1·66 | 0·431 | 1·389 | 1·001 | 5·071 | |
| Mean | 0·92 | 1·54 | 0·617 | 2·000 | 1·448 | 6·525 | |
| S.D. | ±0·27 | ±0·44 | ±0·173 | ±0·686 | ±0·496 | ±1·557 | |

### *Lipids of whole foetuses and of foetal livers*

The great accumulation of lipids in the placenta apparently interfered with the nutrition of the foetus, if this is judged from foetal body weights. The mean weight of thirty-eight 28-day foetuses from normal mothers was $32 \pm 3{\cdot}6$ g., and that of thirty-eight foetuses of the same age from cholesterol-fed rabbits $22 \pm 2{\cdot}2$ g. It seems probable that the accumulated lipids blocked the placentae for the passage of nutrient materials. There is further evidence from the lipid analyses of foetal livers and of whole foetuses that in the cholesterol-fed rabbits the nutrition of the foetus was impaired.

The percentage lipid contents of whole foetuses and of foetal livers from normal and cholesterol-fed animals are shown in Tables 2 and 3. It can be seen that the lipid composition of whole foetuses and of the foetal livers differed

TABLE 2. Lipid content of 28-day foetuses from normal and cholesterol-fed rabbits. Lipid content expressed as g./100 g. dry matter

| Experiment No. | Neutral fat | Phospholipids | Cholesterol | | Fatty acids of cholesteryl esters | Total lipid | |
|---|---|---|---|---|---|---|---|
| | | | Free | Ester | | | |
| 77–1 | 22·47 | 3·69 | 0·507 | 0·076 | 0·055 | 26·80 | Foetuses of norma rabbits |
| 77–2 | 22·53 | 4·75 | 0·696 | 0·104 | 0·075 | 28·16 | |
| 80–1 | 22·62 | 4·76 | 0·577 | 0·106 | 0·076 | 27·54 | |
| 80–2 | 19·92 | 4·68 | 0·602 | 0·280 | 0·202 | 25·68 | |
| 80–3 | 21·69 | 4·48 | 0·563 | 0·296 | 0·213 | 27·24 | |
| Mean | 21·73 | 4·47 | 0·589 | 0·172 | 0·124 | 27·08 | |
| S.D. | ±1·10 | ±0·45 | ±0·070 | ±0·110 | ±0·077 | ±0·93 | |
| 78–1 | 17·54 | 5·18 | 0·586 | 0·128 | 0·092 | 22·53 | Foetuses of cholesterol-fed rabbits |
| 78–2 | 16·36 | 5·56 | 0·609 | 0·104 | 0·075 | 22·71 | |
| 78–3 | 14·68 | 5·37 | 0·609 | 0·079 | 0·057 | 20·79 | |
| 78–4 | 17·14 | 5·66 | 0·681 | 0·169 | 0·122 | 23·77 | |
| 79 | 23·42 | 4·57 | 0·672 | 0·119 | 0·086 | 28·87 | |
| Mean | 17·83 | 5·27 | 0·631 | 0·120 | 0·086 | 23·73 | |
| S.D. | ±3·3 | ±0·43 | ±0·040 | ±0·030 | ±0·024 | ±3·06 | |

TABLE 3. Lipid content of livers of 28-day foetuses from normal and cholesterol-fed rabbits. Lipid content expressed as g./100 g. fresh tissue

| Experiment No. | Neutral fat | Phospholipids | Cholesterol | | Fatty acids of cholesteryl esters | Total lipid | |
|---|---|---|---|---|---|---|---|
| | | | Free | Ester | | | |
| 61 | 15·40 | 0·95 | 0·107 | 0·123 | 0·089 | 16·669 | Foetuses of normal rabbits |
| 62 | 18·06 | 1·65 | 0·136 | 0·054 | 0·039 | 19·939 | |
| 67 | 13·50 | 1·41 | 0·158 | 0·089 | 0·064 | 15·221 | |
| 77 | 10·07 | 0·92 | 0·100 | 0·126 | 0·091 | 11·307 | |
| 80 | 11·46 | 1·33 | 0·086 | 0·120 | 0·087 | 13·093 | |
| Mean | 13·70 | 1·252 | 0·117 | 0·102 | 0·074 | 15·246 | |
| S.D. | ±3·17 | ±0·313 | ±0·027 | ±0·031 | ±0·023 | ±2·469 | |
| 41 | 10·00 | 1·45 | 0·135 | 0·093 | 0·067 | 11·745 | Foetuses of cholesterol-fed rabbits |
| 64 | 8·13 | 0·97 | 0·095 | 0·082 | 0·059 | 9·336 | |
| 65 | 6·66 | 0·97 | 0·090 | 0·048 | 0·035 | 7·803 | |
| 71 | 11·23 | 1·78 | 0·147 | 0·073 | 0·053 | 13·283 | |
| 78 | 8·86 | 1·68 | 0·189 | 0·122 | 0·088 | 10·939 | |
| 81 | 17·46 | 0·80 | 0·101 | 0·129 | 0·093 | 18·583 | |
| Mean | 10·39 | 1·275 | 0·126 | 0·091 | 0·066 | 11·948 | |
| S.D. | ±3·80 | ±0·415 | ±0·038 | ±0·031 | ±0·022 | ±3·766 | |

TABLE 4. Average lipid content, in milligrams, of 28-day foetuses from normal and cholesterol-fed rabbits

| | Foetuses of | |
|---|---|---|
| | Normal mothers (mg.) | Cholesterol-fed mothers (mg.) |
| Neutral fat | 1180 | 630 |
| Phospholipids | 240 | 185 |
| Free cholesterol | 32 | 22 |
| Ester cholesterol | 9 | 4 |
| Total lipid | 1467 | 844 |

only in respect of the neutral fat content, this being lower in the cholesterol-fed rabbits. It is of some interest that the foetal livers contained considerably more neutral fat per 100 g. moist tissue than did the maternal livers. Histological examination of the foetal livers (Pl. 2, fig. 3) confirmed the intensive fatty infiltration which, however, was less marked in the foetuses from cholesterol-fed mothers.

If the absolute amounts of lipids contained in the foetuses of the two groups are calculated (Table 4), it is found that the foetuses of cholesterol-fed mothers contained approximately 33% less phospholipid and cholesterol, and approximately 50% less neutral fat, than the controls. The weight of the experimental foetuses was 33% less than that of the controls; therefore it is evident that the phospholipid and cholesterol contents were less by the same proportion as their weights, while the neutral fat content was disproportionately less. When the figures in Tables 1, 2 and 3 are compared, it appears that the neutral fat content of the experimental foetal livers and of whole foetuses is approximately inversely related to the cholesterol content of the placentae. In rabbits Nos. 79 and 81 the placentae differed the least from the normal, as judged by their morphological appearances and cholesterol content. In these two cases the percentage neutral fat content of the whole foetus and of the foetal liver was similar to those of controls, although the foetuses were underweight (22–26 g.). It appears, therefore, that the foetuses of cholesterol-fed mothers are able to build up their tissues with the same phospholipid and cholesterol composition as the controls; when the cholesterol content of the placenta, however, increases beyond a certain point (approximately 1·8% total cholesterol) the foetuses are unable to accumulate the same amounts of neutral fat as the controls either in the liver or in the body as a whole.

The experiments throw very little light on the source of foetal cholesterol. From Tables 2 and 3 it is evident that the percentage cholesterol content of the experimental foetuses and of foetal livers was the same as that of the controls: the absolute amounts (cf. Table 4) were actually less because of the lighter weight of the experimental foetuses. If the foetus obtains its cholesterol from the mother, the controlling mechanism of the placenta must be very exact in this respect, being able to prevent the flooding of the foetus by this compound when the latter is available in excess.

### *Comparison of maternal and foetal plasma lipids in normal and cholesterol-fed rabbits*

Table 5 gives the maternal and foetal plasma-lipid values in the control and experimental animals. These data are represented graphically in Text-fig. 1. It can be seen that normally the concentration of all the lipids is higher in the foetal than in the maternal plasma. Baumann & Holly (1925–6) found that the concentration of lipoid phosphorus and of cholesterol is higher in the blood of

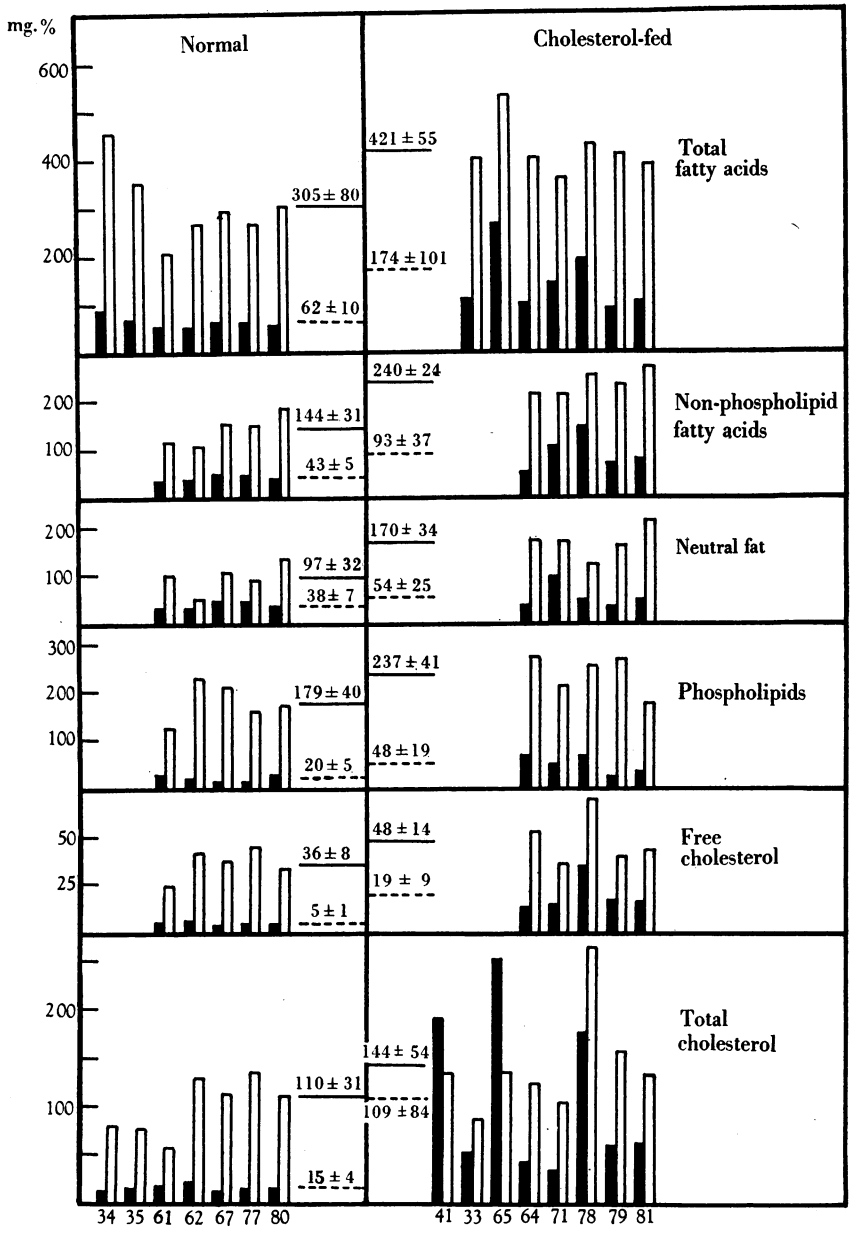
TABLE 5. Maternal and foetal plasma lipids of normal and cholesterol-fed rabbits on 28th day of pregnancy. Lipid content expressed as mg./100 ml. of plasma

| Rabbit No. | Total fatty acids | | Non-phospholipid fatty acids | | Neutral fat | | Phospholipids | | Free cholesterol | | Total cholesterol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mother | Foetus | Mother | Foetus | Mother | Foetus | Mother | Foetus | Mother | Foetus | Mother | Foetus |
| C34 | 83 | 457 | — | — | — | — | — | — | — | — | 11 | 80 |
| C35 | 64 | 358 | — | — | — | — | — | — | — | — | 15 | 78 |
| C61 | 55 | 206 | 38 | 119 | 31 | 100 | 25 | 126 | 5 | 24 | 17 | 57 |
| C62 | 54 | 266 | 40 | 109 | 32 | 49 | 20 | 227 | 6 | 42 | 21 | 130 |
| C67 | 60 | 297 | 50 | 155 | 47 | 105 | 14 | 206 | 4 | 37 | 12 | 113 |
| C77 | 60 | 266 | 48 | 152 | 43 | 90 | 16 | 160 | 5 | 45 | 15 | 136 |
| C80 | 59 | 305 | 41 | 185 | 36 | 139 | 26 | 174 | 5 | 33 | 14 | 111 |
| Mean control | 62 | 305 | 43 | 144 | 38 | 97 | 20 | 179 | 5 | 36 | 15 | 110 |
| S.D. | ±10 | ±80 | ±5 | ±31 | ±7 | ±32 | ±5 | ±40 | ±1 | ±8 | ±4 | ±31 |
| 33E | 110 | 403 | — | — | — | — | — | — | — | — | 52 | 88 |
| 41E | 375 | — | — | — | — | — | — | — | — | — | 192 | 136 |
| 65E | 267 | 537 | — | — | — | — | — | — | — | — | 252 | 138 |
| 64E | 101 | 405 | 55 | 218 | 36 | 175 | 68 | 272 | 13 | 53 | 42 | 124 |
| 71E | 146 | 363 | 108 | 217 | 98 | 173 | 48 | 212 | 14 | 36 | 36 | 106 |
| 78E | 195 | 434 | 149 | 257 | 49 | 123 | 66 | 257 | 35 | 70 | 177 | 265 |
| 79E | 90 | 414 | 72 | 238 | 38 | 161 | 26 | 265 | 16 | 40 | 60 | 158 |
| 81E | 106 | 394 | 81 | 272 | 49 | 218 | 35 | 177 | 16 | 43 | 63 | 133 |
| Mean experimental | 174 | 421 | 93 | 240 | 54 | 170 | 48 | 237 | 19 | 48 | 109 | 144 |
| S.D. | ±101 | ±55 | ±37 | ±24 | ±25 | ±34 | ±19 | ±41 | ±9 | ±14 | ±84 | ±54 |

rabbit foetuses than in that of the mother. The present investigation confirms the findings of Baumann & Holly and extends their observations to all the lipid fractions of the plasma. The real magnitude of the difference between foetal and maternal values, however, is not so obvious from the work of Baumann & Holly, because they analysed whole blood only. In the guinea-pig also the foetal serum contains more lipids than the maternal (Kreidl & Donath, 1910), while in the human (Slemons & Stander, 1923; Boyd, 1936) and in the sheep (Barcroft & Popják, 1945) the reverse relationship is found. The foetal plasma-lipid values in the rabbit are not only higher than in the mother but, except for the neutral fats, they are even higher than the values in normal adult rabbits (Table 6). The maternal plasma, on the other hand, shows a great

TABLE 6. Comparison between plasma lipid values of 28-day rabbit foetuses and of normal non-pregnant adult rabbits. Lipid content expressed as mg./100 ml. of plasma

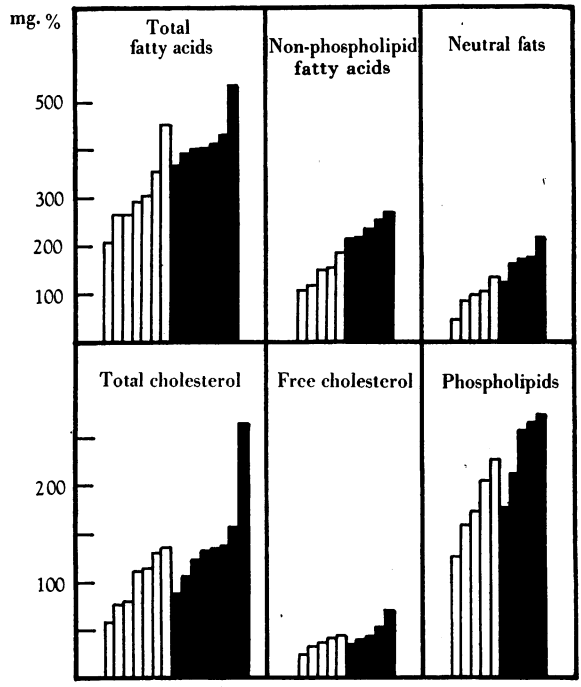
| | Total fatty acids | Non-phospholipid fatty acids | Neutral fat | Phospho-lipids | Cholesterol | |
|---|---|---|---|---|---|---|
| | | | | | Free | Tota |
| Mean of 38 normal non-pregnant adult rabbits | 223 ±76 | 131 ±53 | 93 ±54 | 138 ±40 | 25 ±9 | 74 ±32 |
| Mean normal foetal values on 28th day of pregnancy | 305 ±80 | 144 ±31 | 97 ±32 | 179 ±40 | 36 ±8 | 110 ±31 |




Text-fig. 1. Maternal (black columns) and foetal (white columns) plasma-lipids (mg./100 ml.) in normal and cholesterol-fed rabbits on 28th day of pregnancy. Each pair of columns in the same vertical line represents values for one case whose protocol numbers are given under the columns illustrating total cholesterol. ———mean foetal, - - - - - - mean maternal values with standard deviation.

poverty in lipids on the 28th day of pregnancy. The changes of maternal plasma-lipids in rabbits during pregnancy will be discussed in another section of this paper.

An inspection of the plasma-lipid values of the cholesterol-fed mothers shows that these were higher than in the controls and that there was a considerable individual variation in the response to cholesterol. For this reason mean values for the maternal plasma of the cholesterol-fed rabbits are given




Text-fig. 2. Plasma-lipids of foetuses from normal (white columns) and cholesterol-fed mothers (black columns).

only tentatively in Table 5 and Text-fig. 1; it is better to assess each case on its own merits. The foetal plasma-lipids in this group of animals are at a somewhat higher level than in the controls. A close inspection of the data in Table 5 and Text-fig. 1 reveals that these foetal plasma-lipid values follow a pattern similar to those of the maternal ones (but less sharply). This seems particularly so in the case of total fatty acids, and free and total cholesterol. The usual relationship between maternal and foetal plasma total cholesterol was reversed in experimental rabbits 41 and 65. While it seems that the foetal plasma-lipid values may be influenced to a slight extent by drastic changes in the maternal plasma, the findings in these two rabbits further emphasize the very strict role

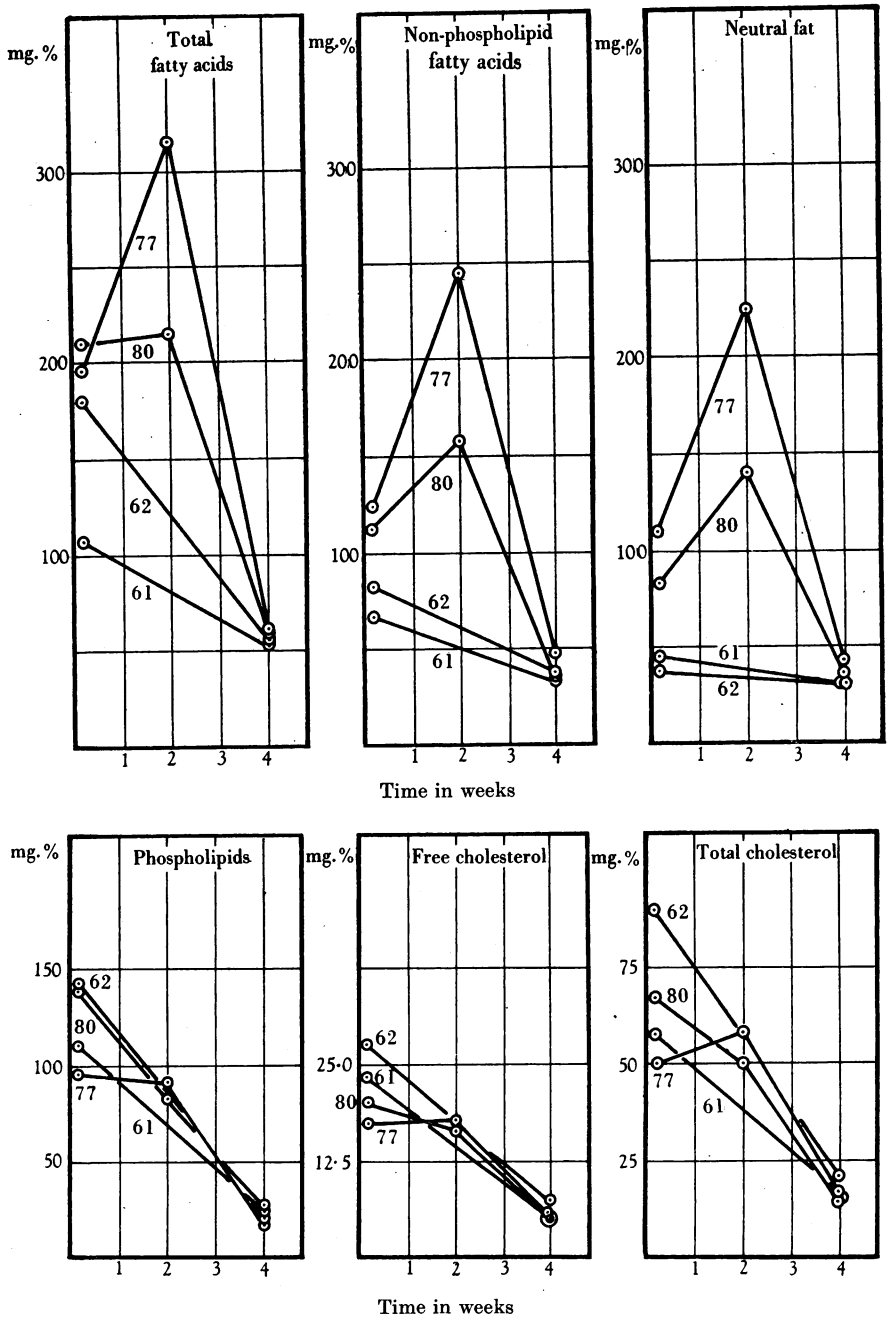
of the placenta in regulating the foetal fat metabolism, providing a certain amount of autonomy to the foetus. Data of such small series do not lend themselves readily to a statistical analysis and it is believed that a side-by-side inspection of the control and experimental foetal values should give a better comparison of the differences in the two groups. Such a comparison is shown graphically in Text-fig. 2. It can be seen that, while the difference between the lowest and highest plasma-lipid values in the experimental animals is about the same as in the controls, the whole range has been shifted to a slightly higher level. If the difference between the two small series is accepted as a real one, it can be inferred that lipids do pass through the placenta of the rabbit from the mother. The placenta, however, by its powerful controlling mechanism allows the passage of only minute quantities in excess of that which is required to build up foetal tissues of a normal composition. If the absolute amounts of circulating plasma-lipids in the two groups of foetuses were calculated, these would not differ by more than small fractions of a milligram, if it be assumed that the total plasma volume of the smaller experimental foetuses is proportionately less than that of the controls. Certainly the amounts that might have passed through the placenta in excess of normal requirements during a period of 3 weeks of intra-uterine growth were not sufficient to upset the normal concentration of cholesterol in the tissues. It should be pointed out that the percentage cholesterol content of the foetuses of experimental rabbit 78 was no more than that of the controls, although the foetal plasma in this instance showed the highest cholesterol concentration among the cases investigated.

### *Changes of plasma-lipids in normal and cholesterol-fed rabbits during pregnancy*

Normal rabbits develop a marked lipopenia by the 28th day of pregnancy. This change was first observed by Baumann & Holly (1925–6) in whole blood. The decrease occurred in all the normal pregnant rabbits and in each case the change was of the same type. For this reason only four cases are presented in Text-fig. 3. The response of the plasma-lipids in pregnant rabbits to the administration of cholesterol was markedly different from that of normal non-pregnant animals. In Text-fig. 4 two cases representing the maximum and two cases representing the minimum response to cholesterol during pregnancy are shown.

It can be seen from Text-fig. 4 that by the end of the first week there was usually a slight fall in the plasma phospholipid values and neutral fat, although free and total cholesterol may not show any changes. During the first week of gestation the animals did not receive cholesterol.

Text-fig. 3 shows that in the normal rabbits by the end of the second week of pregnancy the plasma-lipids generally show a further slight fall, although the




Text-fig. 3. Behaviour of plasma-lipids of normal rabbits during pregnancy. The figures beside the graphs are the protocol numbers of the rabbits.

neutral fats (and consequently total fatty acids and non-phospholipid fatty acids also) may increase. By the end of the 4th week (cf. Text-fig. 3) all the plasma-lipids are at a very low level as compared with the initial values. From Text-fig. 3 it can be seen also that, whatever is the initial concentration of the plasma-lipids in the various rabbits, by the 28th day of pregnancy the concentrations have fallen to very nearly the same level in each animal (cf. also Table 5). The lipopenia in the rabbit during pregnancy is the opposite to the lipaemia of pregnancy in the human (Boyd, 1934). That this change is definitely associated with the presence of the foetus and its appendages *in utero* was clearly demonstrated by Baumann & Holly, who found that after either normal parturition or an early delivery the blood lipids of the rabbit quickly returned to normal. Baumann & Holly suggested that the lipopenia of rabbits at term could be readily due to an extensive utilization of the lipids during the period of very active growth and differentiation of the embryos. That this explanation is not adequate will be evident from the consideration of the behaviour of plasma-lipids in cholesterol-fed pregnant rabbits.

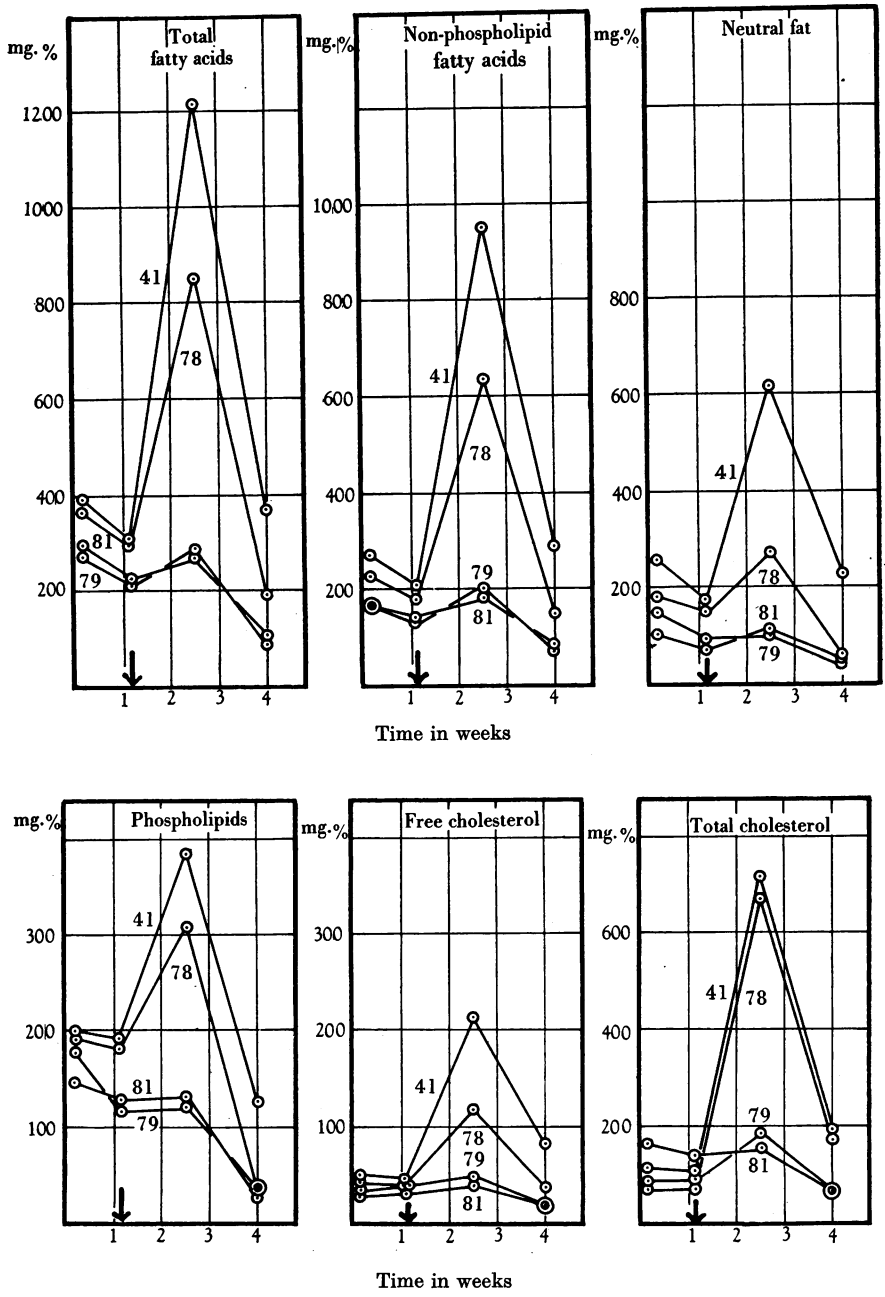
In Table 7 are recorded at weekly intervals the plasma-lipid values of a non-pregnant rabbit which received 1 g. of cholesterol per day. Such observations have been made on about twenty-five rabbits and without exception similar changes occurred in all of them, often of an even greater magnitude. It

TABLE 7. Plasma lipids (mg./100 ml.) of non-pregnant rabbit before and during administration of 1 g. of cholesterol daily. The interval between successive determinations was 1 week

| | Non-phospholipid fatty acids | Neutral fats | Phospho-lipids | Cholesterol | |
|---|---|---|---|---|---|
| | | | | Free | Ester |
| Before cholesterol | 98 | 66 | 134 | 20 | 35 |
| During cholesterol administration | 450 | 97 | 361 | 134 | 495 |
| | 552 | 109 | 521 | 195 | 615 |
| | 1150 | 266 | 860 | 438 | 1228 |
| | 1146 | 282 | 835 | 427 | 1200 |
| | 1261 | 207 | 1010 | 636 | 1464 |

can be seen that all the plasma-lipids show a steady rise from week to week, and this may continue for as long as 2–3 months.

From Text-fig. 4 it can be seen that, when administration of cholesterol was started at the beginning of the second week of gestation, the plasma-lipid fractions usually increased to a high level in a week's time. The two rabbits (Nos. 79 and 81) which reacted with only a slight rise in plasma-lipids to the administration of cholesterol were the only such cases observed; in all other experimental rabbits the response was either as high as the maximum or was intermediate between the maximum and minimum changes recorded in Text-fig. 4. By the 28th day, however, a very pronounced fall in plasma-lipids occurred in spite of the continuous administration of cholesterol, but the values




Text-fig. 4. Behaviour of plasma-lipids of cholesterol-fed rabbits during pregnancy. Cholesterol feeding was started at ↓. The figures beside the graphs are the protocol numbers of the rabbits.

had not fallen to such a low level as in the control rabbits. These cholesterol-fed pregnant rabbits showed on the 28th day plasma-lipid values closer to those found initially but, as may be seen from Text-fig. 4, they might be above or below these. That this fall was not due to an inadequate absorption of cholesterol, but to other factors, can be inferred from the fact that it was observed regularly and that the analyses of maternal livers and foetal placentae showed a very marked increase in cholesterol content in every case, proving that cholesterol was adequately absorbed.

That this fall in plasma-lipids was not due to a lack of absorption is further proven by observations on rabbits which miscarried during the administration of cholesterol. The data of a typical case are shown in Table 8. It can be seen that administration of cholesterol for 1 week in the middle of pregnancy produced the usual rise in the plasma-lipids. The animal miscarried on the

TABLE 8. Plasma lipids (mg./100 ml.) of rabbit No. 39 which miscarried on 26th day of pregnancy

| Date | Total fatty acids | Non-phospholipid fatty acids | Neutral fat | Phospho-lipids | Cholesterol | |
|---|---|---|---|---|---|---|
| | | | | | Free | Total |
| 23. v. 44, mated | 200 | 100 | 56 | 154 | 28 | 93 |
| 1. vi. 44 | 236 | — | — | — | — | 98 |
| 2. vi. 44 | Cholesterol feeding started | | | | | |
| 10. vi. 44 | 766 | 589 | 242 | 314 | 210 | 695 |
| 19. vi. 44 | Miscarried | | | | | |
| 21. vi. 44 | 914 | 642 | 284 | 394 | 236 | 753 |

26th day; unfortunately no observation was made on the plasma-lipids at that time. Two days later, while the administration of cholesterol was continued, the plasma-lipid values were at an even higher level than in the middle of pregnancy.

It seems that the tendency to lipopenia in rabbits during the second half of pregnancy occurs even when lipaemia is induced experimentally. However attractive the hypothesis of Baumann & Holly, that a high rate of utilization of lipids by the foetus is the explanation for this lipopenia, it seems inadequate. In cholesterol-fed rabbits the amounts of circulating plasma-lipids are far in excess of the requirements of the foetus or of the amounts that are available to it from the maternal plasma during a normal pregnancy. Further, the fall in plasma-lipids was independent of the number of foetuses the mother bore; the plasma-lipids decreased to the same extent whether there were three or ten foetuses present. It seems more likely that a hormonal or some other factor associated with pregnancy is responsible for the suppression of plasma-lipids. An increased fixation by tissues, like that observed in placentae of the cholesterol-fed rabbits, might be more important.

*Liver-lipids of normal and cholesterol-fed pregnant rabbits*

The relevant data are presented in Table 9. The lipid composition of the livers of control pregnant rabbits on the 28th day of gestation differed from that of normal non-pregnant animals only in respect of the neutral fat content: this is usually not more than 1·5% of the moist weight in non-pregnant rabbits.

TABLE 9. Lipid content of livers of normal and cholesterol-fed rabbits on 28th day of pregnancy. Lipid content expressed as g./100 g. of fresh tissue

| Experiment No. | Neutral fat | Phospholipids | Cholesterol | | Fatty acids of cholesteryl esters | Total lipid | |
|---|---|---|---|---|---|---|---|
| | | | Free | Ester | | | |
| 61 | 2·35 | 2·73 | 0·143 | 0·224 | 0·161 | 5·608 | Normal pregnant rabbits |
| 62 | 4·38 | 2·79 | 0·151 | 0·101 | 0·072 | 7·494 | |
| 67 | 2·95 | 3·04 | 0·141 | 0·089 | 0·064 | 6·284 | |
| 77 | 5·17 | 2·67 | 0·141 | 0·336 | 0·242 | 8·559 | |
| 80 | 8·24 | 2·51 | 0·176 | 0·150 | 0·108 | 11·184 | |
| Mean | 4·62 | 2·75 | 0·150 | 0·180 | 0·129 | 7·826 | |
| S.D. | ±2·313 | ±0·19 | ±0·015 | ±0·102 | ±0·074 | ±2·192 | |
| 41 | 2·26 | 3·06 | 0·345 | 1·221 | 0·880 | 7·766 | Cholesterol-fed pregnant rabbits |
| 64 | 2·51 | 2·41 | 0·211 | 1·816 | 1·308 | 8·255 | |
| 65 | 3·49 | 2·33 | 0·263 | 1·317 | 0·949 | 8·349 | |
| 71 | 4·09 | 2·90 | 0·281 | 0·643 | 0·463 | 8·377 | |
| 78 | 2·94 | 3·00 | 0·428 | 1·630 | 1·174 | 9·172 | |
| 79 | 9·80 | 2·63 | 0·296 | 1·238 | 0·893 | 14·857 | |
| 81 | 10·04 | 3·02 | 0·231 | 1·165 | 0·840 | 15·296 | |
| Mean | 5·02 | 2·76 | 0·294 | 1·290 | 0·929 | 10·296 | |
| S.D. | ±3·40 | ±0·31 | ±0·074 | ±0·373 | ±0·277 | ±3·291 | |

A moderate degree of infiltration by neutral fat was observed in the control pregnant rabbits. In the cholesterol-fed rabbits, in addition to the infiltration by neutral fat observed in the controls, there was a moderate increase in the free cholesterol, and a marked increase in the cholesteryl ester content. Although rabbits 79 and 81 showed only a slight increase in the plasma-lipids during cholesterol administration, the increased cholesterol content of their livers proves that cholesterol was absorbed. Reference has already been made to this phenomenon.

## DISCUSSION AND CONCLUSIONS

The conclusions that can be drawn from the work described are but few and of a tentative character. It seems impossible to influence the foetal plasma-lipids except to a very limited extent by the production of experimental lipaemia in pregnant rabbits. The function of the foetal placenta appears to be of paramount importance in foetal fat metabolism. It is able to prevent the flooding of the foetus by cholesterol and to provide an almost constant milieu for the foetus even under drastic experimental conditions. Boyd (1935) showed that the lipid composition of the placenta varies with the time of gestation: during the last 10 days there is a marked increase in phospholipid content, which

suggests an increased physiological activity. He concluded that the passage of lipids through the placenta is not a simple physical transfer but required the active participation of placental tissues. The present experiments substantiate this statement. Although the range of plasma-lipid concentrations in the experimental foetuses was somewhat higher than in the controls, the amounts in excess of the normal were so small that there was no deposition of abnormal amounts of lipids in the foetus. If any appreciable amounts of cholesterol in excess of the normal had passed the placenta, one might have expected to find an increased cholesterol content of the experimental foetuses, since the mechanism to eliminate excess amounts of this compound is particularly sluggish in herbivorous animals. Unless the rabbit foetus has a great ability to destroy excess cholesterol, it can be concluded that only very small, almost negligible, amounts of cholesterol in excess of the normal pass through the placenta. This conclusion of course does not exclude the possibility that the normal cholesterol requirements of the foetus are derived from the preformed maternal compound.

While there is evidence from the works of Sinclair (1933), Chaikoff & Robinson (1933), McConnell & Sinclair (1937) on the rat, and of Bickenbach & Rupp (1931) and Rupp & Bickenbach (1931–2) on the rabbit, that fatty acids (or their simple compounds) do pass through the placenta from the mother to the foetus, there has been very little done to investigate with modern methods the possibility of foetal synthesis of fats. The recent investigations of Nielson (1941–2) on the rat with radioactive phosphorus show that probably the major portion, if not all, of the foetal phospholipids are synthesized within the foetus and are not derived from the mother. The higher plasma-phospholipid content of the experimental foetuses in the present experiments may not be due to an excess transfer of this compound through the placenta but may have been synthesized in larger amounts within the foetus or placenta, owing to the abnormal condition of the latter. It is well, however, to bear in mind the species differences. There are two alternative explanations for the higher lipid content of the foetal plasma in the rabbit as compared with the maternal: (*a*) that the placenta in this species has a power to concentrate lipids, assuming that the foetal lipids are derived from the mother; or (*b*) that there is a synthesis of lipids within the foetus in addition to those obtained from the mother. The second alternative is favoured, as further discussion will show.

The placentae of the cholesterol-fed rabbits contained large quantities of free cholesterol and cholesteryl esters and also some excess neutral fat. This abnormal lipoid storage apparently produced a pathological condition resulting in an impaired foetal nutrition, if this is judged from body weight. Not only were the experimental foetuses 33% lighter than the controls, but they contained also less neutral fat, although the concentration of the other lipids was similar to that in the controls. If all the foetal fat were derived from preformed

maternal fat, it is difficult to understand why the fatty acids should not reach the foetus, at least in normal amounts, when they are available in excess within the placenta. The results are most readily interpreted if it be assumed that preformed maternal fat is not the only source of foetal fat but that some of it may be synthesized within the foetus. If this hypothesis be accepted, until further evidence on the question is obtained, the lower neutral fat content of the experimental foetuses may be explained on the basis that the placentae were partially blocked by the accumulated cholesterol and thus limited the free passage of fat precursors from which the foetus could synthesize fat. Boyd (1935) showed that during the last 8–10 days of gestation there is a sudden marked increase in the neutral fat content of the rabbit foetus. If all this neutral fat is derived from the mother, there must be a sudden increase in placental permeability to it. It was pointed out by Huggett (1941), in his review on foetal nutrition, that, owing to degenerative changes, the permeability of the placenta may increase towards the end of gestation. One cannot exclude the possibility, however, that by the 20th–22nd day of the rabbit's gestation the embryonic tissues reach such a degree of maturation that the enzyme systems required for fat-synthesis from carbohydrates may operate. Further investigation of this problem with the aid of isotopes should answer many as yet unsolved questions of foetal fat metabolism.

The lipopenia observed in pregnant rabbits towards the end of pregnancy may be due to hormonal or other factors associated with gestation. The explanation of Baumann & Holly that an increased utilization of maternal lipids by the foetus is the cause of the lipopenia seems inadequate, because (*a*) the same phenomenon was also observed in all the cholesterol-fed rabbits in which the foetuses actually utilized less lipids than did the controls, and (*b*) because the degree of lipopenia was independent of the number of foetuses the mother bore.

SUMMARY

1. Lipid analyses were carried out on the placentae, foetal livers, whole foetuses, maternal livers, and maternal and foetal plasmata of normal and of cholesterol-fed rabbits, on the 28th day of pregnancy.

2. Feeding of amorphous cholesterol to pregnant rabbits during the last 3 weeks of gestation caused a heavy storage of free and esterified cholesterol and an increase of neutral fat content in the foetal placenta. From histological investigations it is inferred that there was also an increase of neutral fats in the decidual cells of the maternal portion of the placenta.

3. The lipoid-storage in the placenta interfered with the nutrition of the foetus, resulting in foetuses one-third lighter than normal. These foetuses and their livers contained less neutral fat than the controls.

4. It is suggested that not all the foetal fat is derived from preformed maternal fat but that at least a part of it may be synthesized within the foetus.

It is inferred, on the basis of this assumption, that the lower neutral fat content of the experimental foetuses was brought about by the partial blockage of the placentae to the transfer of fat-precursors.

5. The concentration of lipids was considerably higher in the foetal than in the maternal plasma, and was even higher than in normal non-pregnant adults of this species. It was impossible to influence the foetal plasma-lipid values except to a very limited extent by the production of lipaemia in the mothers.

6. The rabbit develops a very marked lipopenia by the end of gestation, in contrast to the lipaemia of pregnancy in the human. Although rabbits usually responded to cholesterol-feeding with a marked lipaemia in the middle of gestation, by the 28th day a tendency towards lipopenia manifested itself. When a cholesterol-fed animal miscarried, there was an immediate rise in plasma-lipids. It is suggested that a hormonal factor, or an increased fixation of lipids by the tissues, may be the cause of the lipopenia.

I gratefully acknowledge my indebtedness to Sir Joseph Barcroft, with whom the first operations were performed and who has given valuable advice throughout these experiments. My thanks are also due to Miss M. Kendall for her technical assistance and to Mr A. E. Clark for the preparation of the histological sections, which, particularly in the case of frozen sections from placentae, was no mean task.

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## EXPLANATION OF PLATES

### Plate 1

Fig. 1. Appearance of placentae from normal (A) and cholesterol-fed rabbits (B) on 28th day of pregnancy. The cross-section of each is also shown. Approximately $\frac{1}{3}$ original size.

Fig. 2. Photomicrograph from the foetal portion of placenta of cholesterol-fed rabbit No. 64. Section cut on freezing microtome and stained with Scharlach R alone; all the black material represents lipids within cells of foetal capillaries. Most of these lipids are anisotropic. 93 ×.

Fig. 3. Photomicrograph from the maternal portion of placenta of control rabbit No. 80. Section stained with Scharlach R and haematoxylin. Picture shows decidual cells containing fat granules and arranged around maternal blood sinuses. All the fat in these cells is isotropic. 80 ×.

Fig. 4. Photomicrograph from the same field as fig. 3; it shows isolated decidual cells containing distinct fat granules. 480 ×.

### Plate 2

Fig. 1. Photomicrograph from maternal portion of placenta from cholesterol-fed rabbit No. 41. The area represented here corresponds to that shown from a control rabbit in Pl. 1, fig. 4. The decidual cells contain large amounts of isotropic fat. Section stained with Scharlach R and haematoxylin. 42 ×.

Fig. 2. Photomicrograph from the maternal portion of placenta from cholesterol-fed rabbit No. 33. It shows decidual cells loaded with isotropic fat and arranged around maternal sinuses. The individual structure of the cells has been completely obscured by fat. 80 ×.

Fig. 3. Photomicrograph from normal foetal liver (28-day foetus of rabbit No. 80). Section stained with Scharlach R and haematoxylin. The liver cells contain large globules of fat. 480 ×.

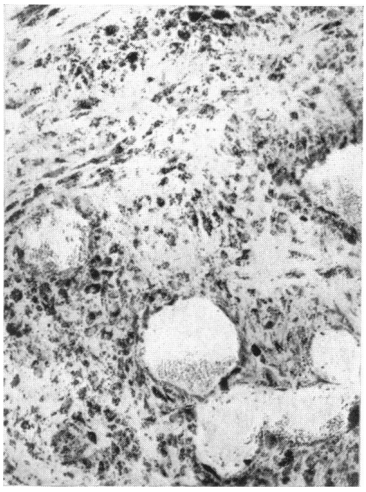
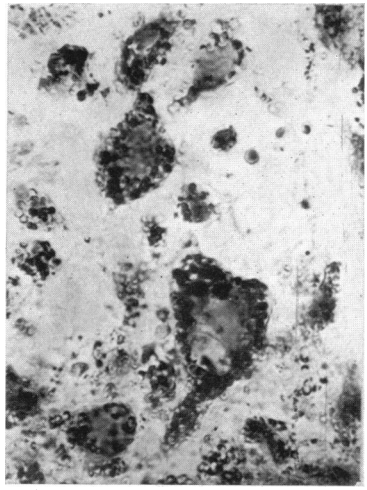


Fig. 3.



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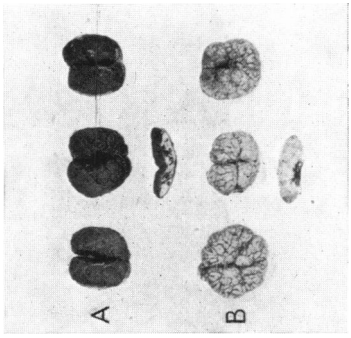


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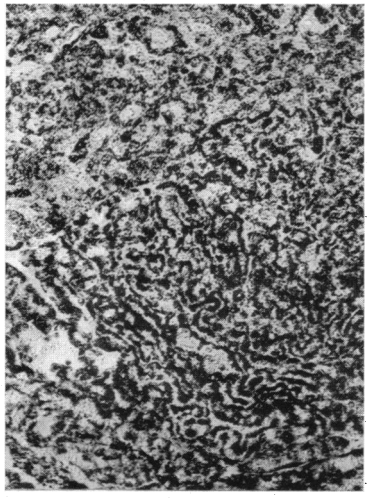

Fig. 2.

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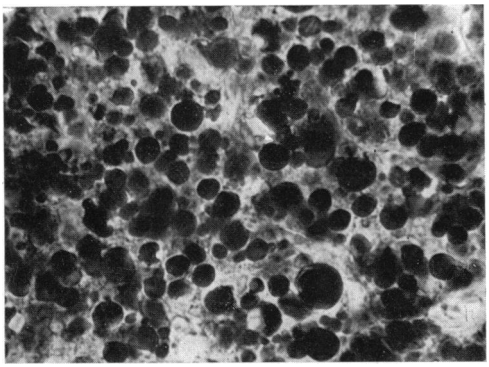
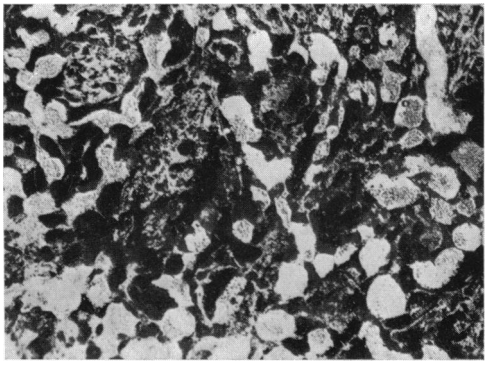


Fig. 3.



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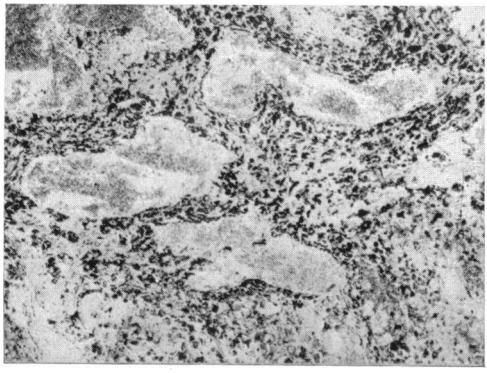

Fig. 1.