OBSERVATIONS ON

THE COMPOSITION OF THE BLOOD

IN THE NEONATAL PERIOD

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by

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INTRODUCTION

It has been realised for many years that the infants of diabetic mothers have notably high foetal and neonatal mortality rates, the reasons for which remain obscure. These infants tend to be large, puffy and hyperkinetic. If they are delivered at term, the mortality rate is very high. Even if pregnancy is terminated two or three weeks earlier, the incidence of respiratory distress and of pulmonary hyaline membrane syndrome is high.

Hypertrophy of the pancreatic islet cells in such infants has been established (Driscoll, Benirschke and Curtis, 1960). Pederson (1952) considers that this is a result of stimulation of the foetus by maternal hyperglycaemia. There is evidence that these infants become hypoglycaemic immediately after birth (Komrower, 1954; Farquhar, 1961). Some evidence of adrenocortical disturbance is present (Farquhar, 1958). There are also some indications of acidosis (Ibid).

This study was begun to investigate the possibility that there might be a disturbance in lipid and lipoprotein metabolism in these infants, since it is already known that such a disturbance exists in the mothers (Albrink and Man, 1958).

Information about normal serum lipid concentrations in blood, at birth and in the neonatal period, is limited so that any study of the infants of diabetic mothers necessitates a parallel study of infants of normal mothers. In the course of obtaining blood specimens from so called "normal infants", a considerable number of samples were obtained from infants whose mothers had various ante-natal complications. These were notably toxaemia and abortive tendencies. The investigation has therefore been extended to cover these groups.

Obtaining blood specimens was naturally a rather erratic process, depending entirely on the availability of hospital staff. In order that the intervening periods might be occupied constructively, certain subsidiary investigations were begun.

To supplement the earlier work in the Department of Child Life and Health in this University by Dr. J. W. Farquhar on adrenocortical disturbances in the infants of diabetic mothers, a very limited investigation of the urinary excretion of 17-ketosteroids and 17-ketogenic steroids has been made in newborn infants. Difficulties in obtaining complete twenty-four hour urine collections are considerable. The amount of blood necessary for studying serum cortico steroid concentrations is prohibitive in infants. Therefore only a small number of urinary steroid estimations were made.

Finally some information has been collected on the amino-acid content of urine and serum in the neonatal period in both normal and abnormal infants.

The scope of the work to be described in this thesis may be summarised as follows:

1. The estimation of serum lipid and lipoprotein lipid concentrations in the cord blood of infants, including those with both normal and abnormal pre- and post natal histories. The term "lipid" includes total lipid, total cholesterol, total esterified fatty acid and phospholipid.
2. The estimation of serum lipid and lipoprotein lipid concentrations in the first week of life in the infants of diabetic mothers.
3. The estimation of the concentrations of 17-ketosteroids and 17-ketogenic steroids excreted in the urine of normal infants on the first day of life and of infants of diabetic mothers during the first three days of life.
4. A chromatographic investigation of amino acid patterns in cord blood and in venous blood and urine during the neonatal period in normal and some abnormal infants.

The methods of chemical analysis used throughout the investigation are given in Appendix I and the methods of statistical analysis in Appendix II. Both appendices will be found at the end of the thesis.

General conclusions are drawn at the end of each section and not at the end of the thesis.

TABLE 1

SUMMARY OF PUBLISHED DATA ON THE LIPID CONTENT OF SERUM AND PLASMA IN THE NEONATAL PERIOD

Values in mg./100 ml.

Age	Author	Date	No. of Cases	Total Lipid	Phospholipid	Total Cholesterol	Total Fatty Acid
Cord	Hermann & Neumann	1912	?	436	207	98	
Cord	Slemons & Curtis	1917	10			115 - 225	
Cord	Rosenthal & Meier *	1921	7			57 42 - 66	
Cord	Slemons & Standar	1923	7	750	210 - 240 104	120 - 200	
Cord	Plass & Tompkins *	1923	9		81 - 168		
Cord	Hornung *	1926				62	
Cord	Gyorgy	1924 1926	6 14		95 103	70 89	
Cord	Hellmuth *	1925	30	660	3 - 4 (Lipid P)	75	80 - 200
Cord	Gordon & Cohn	1928	8		3.6 - 5.0 (Lipid P)	89 75 - 100	
Cord	Boyd & Wilson	1935	15		Umbilical vein 204 162 - 300 Umbilical artery 160 83 - 223		
Cord	Boyd	1936	29	198 97 - 367	61 21 - 166	34 17 - 86	140 59 - 297
Cord	Rosenbloom	1934	65			120 50 - 275	
Cord	Sadowsky et.al.	1947	24			69 - 244	
Cord	Whitelaw	1948	9			71 51 - 96	
Cord	Russ, Eder & Barr	1954	12		124 ± 22	68 ± 15 47 - 98	
Cord	Rafstedt & Swahn	1954	50	347 ± 11 210 - 600	75 ± 3 48 - 133	75 ± 2 50 - 110	
Cord	Rafstedt	1955	15	313 170 - 440	124 76 - 170	74 48 - 98	
Cord	Furuhje lm	1956	?	284 110 - 460			
Cord	Sohar & Bossak et.al.	1956	10			79 ± 15	
Cord	Brown McGurdy et.al.	1959	20	371 220 - 605		82 ± 17 45 - 121	
3 Days	Hornung *	1926	?			80	
5 - 7 Days	Hornung *	1926	?			90	
0 - 7 Days	Gordon & Cohn	1928	16		4.2 (Lipid P)	87	
4 - 25 Days	Sperry	1936	63			133 71 - 190	
Varied	Kugelmass & Greenwald	1931	?		2.5 (Lipid P)		
1 - 13 Hours	Senn & McNamara	1937	24		1.2 ± 0.97 0.0 - 4.0 (Lipid P)	80 ± 14 55 - 120	
6 - 10 Days	Senn & McNamara	1937	25		4.4 ± 2.1 0.8 - 8.1 (Lipid P)	135 ± 19 108 - 179	
1 - 6 Days	Rafstedt & Swahn	1954	50	591 340-890	131 70 - 178	138 98 - 200	
3 - 10 Days	Rafstedt	1955	15	608 430 - 760	207 160 - 260	134 110 - 167	

* Quoted by Rafstedt (1955)

CHAPTER I

EARLIER INVESTIGATIONS INTO THE CONCENTRATION OF SERUM LIPIDS AND LIPOPROTEIN LIPIDS IN THE NEONATAL PERIOD

Electrophoretic separation of lipoproteins became an established practice by 1950. Prior to this, investigations of serum lipids were usually limited to measurements of cholesterol, phospholipid in terms of lecithin or lipid phosphorus, and occasionally total lipids and total fatty acids. The numbers of cases were usually small and methods varied. As a result, the data are often contradictory. Some order was established after the publication of the Schoenheimer-Sperry micro-method for cholesterol determinations (1934). The intervention of the war period ensured that by the time investigations were resumed, reports had become fairly comparable.

SERUM LIPID CONCENTRATIONS

A review of the available data on serum lipids in the neonatal period is given in Table I. Some earlier German reports are quoted from Rafstedt (1955) because the original journals were not readily available. All the investigators agree that the lipid levels in cord blood are lower than

those in the mother or in the non-pregnant adult but the actual concentrations quoted are not necessarily those now generally accepted. Slemons and Stander (1923) found very high cholesterol concentrations unlike the majority of the Germans whose early results are generally quite close to modern ones.

Opinions on the rate of change of the lipid concentrations during the neonatal period vary considerably. Thus Hornung (1926) and Gyorgy (1926) both found increases in serum cholesterol and phospholipid concentrations during the first few days or weeks of life whereas Gordon and Cohn (1928) and Kugelmass and Greenwald (1931) found no alteration. Sperry (1936) found a 76 per cent increase in the concentration of total serum cholesterol in the first three or four days of life and a subsequent slow rise which, in the next few weeks, reached the minimum level for adults. Senn and McNamara (1937) confirmed this although the inclusion in their data of some cases having no serum phospholipid is remarkable.

Two other investigations of cord blood cholesterol levels were published by Sadowsky, Brzezinski, Bromberg and Rosenthal (1947) and Whitelaw (1948) confirming Sperry's figures and this was followed by the more elaborate investigations resulting from the development of electrophoretic and ultra-centrifugal techniques of protein fractionation.

TABLE 2

SUMMARY OF PUBLISHED DATA ON THE LIPOPROTEIN CONTENT OF SERUM AND PLASMA IN THE NEONATAL PERIOD

Values in mg./100 ml.

Age	Author	Date	No. of Cases	γ-Lipoprotein	β-Lipoprotein	α-Lipoprotein	Total Lipid
Cord	Russ, Eder and Barr	1954	12	Cohn Fractionation of serum. Cholesterol and phospholipid measured in Cohn fractions 1 & 3, 2, and 4&5&6			
Cord	Rafstedt and Swahn	1954	50	83 ± 4 46 - 195	105 ± 9 46 - 191	α_1 103 ± 50 48 - 198 α_2 57 ± 5 14 - 174	347 ± 11 210 - 600
Cord	Longsworth et.al.	1945	?	Serum protein electrophoresis. Lipid content of $\alpha_2+\beta$-globulins calculated using data from Boyd (1936)			
Cord	Rafstedt	1955	15	77 ± 4.9 48 - 106	103 ± 7.2 51 - 158	134 ± 9 71 - 176	313 ± 14 170 - 440
Cord	Kempe	1952	51	Ultra centrifuge analysis of Sf 10 - 20 class lipoproteins No difference from those of children up to 15 years old			
Cord	Brown,McGurdy et.al.	1959	20		224 ± 61 125 - 474	147 ± 40 92 - 264	371 ± 75 220 - 605
1 - 6 Days	Rafstedt and Swahn	1954	15	141 ± 6 61 - 260	265 ± 10 148 - 260	α_1 140 ± 6 71 - 264 α_2 44 ± 3 22 - 94	591 ± 18
3 - 10 Days	Rafstedt	1955	15	138 ± 7.4 84 - 190	277 ± 11.5 215 - 320	194 ± 12 116 - 266	608 ± 30 430 - 760
4 Days	Brown,McGurdy et.al.	1959	20		588	217	805

SERUM LIPOPROTEIN CONCENTRATIONS

The data are summarised in Table 2. The early work of Longsworth, Curtis and Pembroke (1945) is of doubtful value. There was obviously a long delay in analysing the serum which was stored at 0° C after travelling from Baltimore to New York packed in solid carbon dioxide. Only serum proteins were analysed. On the grounds of the report by Blix, Tiselius and Svennson (1941) that the combined a_2 - and β - globulins contain all the serum lipid, they assigned values for total lipid and total cholesterol content to this protein fraction. Data for total lipid and cholesterol concentrations were obtained from Boyd (1936) and his values were abnormally low.

The earliest, and the only detailed studies to be made in this field were by Rafstedt and Swahn (1953) and Rafstedt (1955) who measured lipid and lipoprotein lipid concentrations in cord blood and in venous blood from infants and children. Several measurements were made on the same child at different times in many cases. Their lipid concentrations agree with the more reliable of the earlier figures. There appear to be no published data with which their lipoprotein lipid results may be compared. Kempe (1952) made an ultracentrifugal analysis of fifty one cord bloods and found that the pattern of distribution of lipoproteins of Sf value* 10 - 20 was no different from that of children up to fifteen years of age and little different from that of adults.

* Sf: Svedberg. Flotation Unit. 1 unit corresponds to a flotation rate of 1 x 10 - 13 cms/sec/dyne/gm.

Since this fraction is not separable, electrophoretically, from β -lipoprotein, these data are not comparable with those of Rafstedt. Neither are those of Russ, Eder and Barr (1954). These workers used a fractionation method based on differences in solubility and measured cholesterol and phospholipid in the fractions richest in a- and in β -lipoprotein. Further reference will be made to this in Chapter III.

Rafstedt and Swahn (1953) found that the low cord blood lipid levels were not equally reflected in both a- and β -lipoproteins. Whereas a-lipoprotein lipid concentrations were only a little lower in cord blood than in older children, the mean β -lipoprotein lipid concentrations were very much lower: lower in fact than the a-lipoprotein lipid level in the same blood. The β -lipoprotein lipid concentration rose rapidly during the first few days of life, and this rise was paralleled by the increase in serum cholesterol and phospholipid concentrations. The a-lipoprotein lipid concentration increased only slightly.

There are few other pertinent data. Brown, McGurdy, Gillie and Doyle (1959) used Swahn's method (1952) for the determination of serum lipids but did not attempt to separate the β -lipoprotein fraction from that fraction which does not migrate during electrophoretic separation. Their mean value for β -lipoprotein lipid concentration is thus much higher than that of Rafstedt (1955). Sternberg,

Dagenais-Perusse and Dreyfuss (1956) found very much lower cord blood lipoprotein lipid concentrations than Rafstedt and in some cases they found none at all. Sohar, Bossak, Wang and Adlersberg (1956) give percentages of the different lipoproteins only. Their value for the percentage of a-lipoprotein is curiously low but is justified by them on the grounds that all other investigators used faulty methods. They used ten blood specimens only.

For the purpose of comparison of serum concentrations of lipids and lipoprotein lipids obtained in this investigation, only the data of Rafstedt (1955) will be used.

CHAPTER II

A SURVEY OF THE LITERATURE RELATING TO THE METABOLISM OF ADIPOSE TISSUE AND ITS RELATIONSHIP TO THE CONCENTRATIONS OF LIPIDS AND LIPOPROTEINS IN THE BLOOD.

THE LIPOPROTEINS OF THE BLOOD

The main lipoprotein fractions which are found in the blood serum are the chylomicrons, the low density lipoproteins which include a_2-lipoprotein and the high density or a_1-lipoprotein. The lipid fractions of the lipoprotein are composed of varying amounts of triglyceride, cholesterol, phospholipid, and small amounts of other lipids. A detailed description of these different fractions will be given in Chapter III. It will be summarised briefly here.

THE CHYLOMICRONS

The chylomicrons are the carriers of exogenous fat and they contain over 80 per cent triglyceride. Unless neutral fat is transferred in toto across the placenta into the foetal circulation, without prior hydrolysis, the chylomicrons are unlikely to play a large part in the lipid metabolism of the foetus or of the infant at birth. Cord blood has never been observed in this investigation to be

lactescent, and the non-migrating fraction observed during the electrophoresis of cord blood lipoproteins is always small. On the other hand placental transfer of neutral fat is regarded as a possibility in some species such as the rabbit (Popjak, 1952).

THE THREE MAIN LIPOPROTEIN FRACTIONS: a_1-, a_2-, and β-Lipoprotein.

The liver is the site of synthesis of a_1- a_2-, and β-lipoprotein in the adult. It is probable that this is also the case in the foetus since the constituent lipids - triglycerides phospholipids, and cholesterol - are synthesized there to the extent of at least 90 per cent. (Popjak 1952). Almost all the maternal phospholipids and cholesterol entering the placenta circulation are degraded in the placenta and then resynthesized (Ibid).

No specific metabolic role has yet been found for β-lipoprotein. Vaughan (1960) suggests that it might serve as an acceptor for the triglycerides released from the liver in the same way that albumin is the acceptor of free fatty acids. The addition of triglyceride to β-lipoprotein is said to convert it into a_2-lipoprotein (Cornwell and Kruger 1961). The main carrier of endogenous triglyceride is a_2-lipoprotein.

Lipoprotein lipase, an enzyme responsible for liberating free fatty acids from triglycerides in the presence of serum albumin, is known to be activated by

a_1-lipoprotein. The serum albumin acts as an acceptor and carrier of free fatty acids. The stabilization of chylomicrons is also thought to be due to a_1-lipoprotein (Cornwell and Kruger 1961). The protein of the chylomicrons can be shown to equilibrate with the a_1-lipoprotein protein (Ibid).

Whereas the liver is the most active site of synthesis of lipoproteins and their constituent lipids (Byers and Friedman 1960) the triglycerides contained in lipoproteins are both synthesized and stored in adipose tissue. Lipoprotein synthesis appears to be geared to triglyceride synthesis and hence to the metabolism of adipose tissue.

THE METABOLISM OF ADIPOSE TISSUE

The Sources of the Majority of the Available Data.

The majority of the information available on the metabolism of adipose tissue derives from in vitro studies of the rat. The most commonly used material is the epididymal fat pad. However Vaughan (1960) quotes investigations demonstrating the basic similarity of events in other types of white fat. Brown fat cannot be compared with white (Ibid) and at least one other type exists - that in the sucking pad of the cheeks of infants - which differs from both. This pad is remarkable in that it is never utilized, even in severe marasmus. It is reported to have a different fatty acid content from other fat (Feldman, 1920).

THE SITES OF ORIGIN OF THE TRIGLYCERIDES AND THE FREE FATTY ACIDS

Like the liver, adipose tissue can synthesize long chain

fatty acids from a variety of substrates including acetate, pyruvate and glucose. A review of these data is given by Vaughan (1960). After synthesis, the fatty acids are esterified with glycerol of endogenous origin and the triglycerides so formed are stored in the fat droplets in the adipose cells (Ibid).

These triglycerides act as a source of free fatty acids after lipolysis by one or more enzymes, one of which is reported to be activated by adrenaline (Ibid). Lipoprotein lipase, although present in adipose tissue, is not thought to liberate free fatty acid from triglycerides synthesized is situ, but only those derived from chylomicrons (Ibid). It may be noted that adrenaline decreases heparin activity (Donzelot and Kaufman, 1952) and heparin activates lipoprotein lipase (Korn, 1955).

The synthesis and degradation of triglycerides take place simultaneously so that the accumulation and release of free fatty acid could be regulated by the rate of esterification of fatty acids or by the rate of lipolysis of triglycerides, or by both factors (Vaughan 1960).

THE ESTERIFICATION OF FREE FATTY ACIDS

The glycerol liberated by the hydrolysis of triglycerides is apparently not available for re-esterification of free fatty acids. Therefore esterification must depend upon the adequate supply of a-glycerophosphate and hence on the metabolism of glucose by oxidative pathways. In fact, under

optimum conditions, glycerol synthesis is fifty times greater than would be necessary to esterify all the newly synthesized fatty acid. Even during starvation it is ten times greater, although triglyceride synthesis is at a minimum then (Ashmore, Cahill and Hastings, 1960).

The store of triglycerides in adipose tissue is not solely dependent on the synthesis of fatty acids in situ. Free fatty acids, liberated by the action of lipoprotein lipase upon triglycerides which pass into the circulation from the intestine, are taken up by adipose tissue cells and re-esterified there. It is possible that triglycerides can enter both adipose and liver cells without prior lipolysis, possibly by a process of pinocytosis (Rodbell, 1960; Stein and Shapiro, 1960).

THE REGULATION OF FATTY ACID AND TRIGLYCERIDE METABOLISM

The uptake and release of free fatty acids and the synthesis and lipolysis of triglycerides by both the adipose tissues and the liver are regulated by the sympathetic nervous system and by the hormones in a complex manner. They are also influenced by the nutritional status of the animal.

THE DEPENDENCE OF FAT METABOLISM UPON CARBOHYDRATE METABOLISM

The nutritional status and carbohydrate metabolism of an animal are closely related. The effects of insulin are closely connected with both of these.

THE EFFECT OF INSULIN UPON ADIPOSE TISSUE

In vitro, the action of insulin in the epididymal fat pad is to stimulate the uptake of glucose, increase its oxidation, increase the rate of incorporation of glucose, acetate and other precursors into glyceride -glycerol, and decrease the rate of release of free fatty acid (Winegrad and Renold, 1958; Bally, Cahill, Leboeuf and Renold, 1961; Vaughan 1960; Wertheimer and Shafrir, 1960; Ashmore, Cahill and Hastings, 1960). These effects are a reflection of increased triglyceride synthesis and are similar to those observed when a high concentration of glucose is present in the medium (Vaughan 1960). Increased incorporation of acetate into fatty acid in response to insulin and glucose has been found in vitro in mammary gland slices (Ballmain, Folley and Glascock, 1952) and in liver slices, although in the latter, the concentration of insulin necessary was very high (Brady and Gurin, 1950). The rate of inactivation of insulin may well be important in this case.

Carbohydrate metabolism is in fact essential to lipogenesis. It apparently makes available a-glycerophosphate, and possibly reduced triphosphopyrideine nucleotide, both of which are essential to the synthesis of triglycerides from long chain fatty acids and glycerol (Vaughan 1960).

It is notable that the appearance of triglyceride in lipid storage cells is always preceded by a transient deposition of glycogen which almost ceases when fat formation

reaches its peak (Wertheimer and Shafrir, 1960). The degree of hypoglycaemia following insulin administration is paralleled by the magnitude of glycogen synthesis. Rat epididymal fat pads from alloxan diabetic rats do not contain detectable amounts of glycogen and do not convert glucose to lipid. This is corrected within three hours of insulin administration to the intact animal (Ibid).

Wertheimer and Shafrir (1960) formulated the generalization that "whenever glycogen stores are adequate the catabolism of glucose seems to receive priority and the release of fat is at its lowest. When carbohydrate reserves are exhausted or on a sudden upsurge of energy demand, adipose tissue responds swiftly with the release of free fatty acids".

THE EFFECT OF DIABETES AND FASTING ON ADIPOSE TISSUE

Diabetes and fasting produce the same kind of picture. Rat epididymal fat pads from fasting animals release free fatty acid even into an artificial saline medium containing them (Vaughan 1960). In the intact fasting rat the concentration of free fatty acid in the blood rises: the additional amount is derived from tissue not from chyle i.e. from endogenous rather than exogenous sources (Robinson 1960). Millstein and Driscoll (1959) and Shafrir, Sussman and Steinberg, (1959-1960a) report that in rats and in dogs, fasting and alloxan diabetes deplete the fat depots and produce

in them a markedly elevated capacity for fatty acid oxidation. Bally et.al. (1960) showed that triglyceride synthesis in rat epididymal fat pads is markedly decreased by fasting and increased by feeding. Vaughan (1960) states that in the epididymal fat pad from fasting animals, the incorporation of acetate into fatty acid is practically zero and is only partly restored by the addition of glucose to the medium. Glucose uptake, oxidation and convertion to glyceride-glycerol are all depressed.

In relation to this, some work on sugar tolerance may be mentioned. Himsworth (1934) found that sugar tolerance is impaired by fasting and by diets deficient in carbohydrate. It is improved by high carbohydrate diets and particularly by the administration of repeated small amounts of glucose at frequent intervals. Wertheimer and Shafrir (1960) note that the glycogen deposition preceding lipogenesis is particularly prominent in animals which are re-fed after a few days fasting and their respiratory quotient goes above one. Himsworth (1934) also found that re-feeding produced an increased sensitivity to insulin which was apparently mediated by the hypophysis. In hypophysectomized animals, low carbohydrate diets no longer reduced insulin sensitivity, whereas a combination of a high carbohydrate diet and the administration of an anterior pituitary extract had the same effect as a low carbohydrate diet on the intact animal. Himsworth and Scott (1938) suggested that this might be a

result of the production of an insulin antagonist by the hypophysis in response to the low carbohydrate diet. This may well be the case but it is likely that responses to the hypophyseal trophic hormones are also involved.

In addition to the increase in serum free fatty acid concentrations produced by fasting and alloxan diabetes, Shafrir et.al. (1959-1960a) report a rise in the concentrations of serum lipoproteins in dogs. It is accompanied by an increase in the low density lipoprotein fraction (density 1.019-1.063) and in the serum cholesterol and phospholipid concentrations which are known to occur mainly in this low density fraction. The high density lipoproteins are found to contain more cholesterol than usual. Enhanced cholesterol synthesis in the diabetic rat was reported by Hotta and Chaikoff (1952).

THE EFFECTS OF HORMONES OTHER THAN INSULIN UPON THE METABOLISM OF ADIPOSE TISSUE

Adrenaline and Nor-Adrenaline

A release of free fatty acid into the blood stream and a subsequent increase in the concentrations of plasma lipoproteins follows the injection of adrenaline and nor-adrenaline. This is a reflection of an increased release of free fatty acids from adipose tissue (Shafrir, Sussman and Steinberg, 1959-1960a). The increased rate of release of free fatty acid is the result of an increased rate of

lipolysis of the triglycerides in adipose tissue and a reduced synthesis of fatty acids. It is accompanied by an increased rate of glucose uptake, of release and synthesis of glycerol and of oxidation of glucose to carbon dioxide. (Cahill, Lebouef and Flinn, 1960).

At the same time, adrenaline produces an increase in blood glucose concentrations which is not produced by nor-adrenaline. This in its turn, leads to a fall in plasma free fatty acid concentrations (Wertheimer and Shafrir, 1960). The administration of glucose or insulin can prevent the increases in plasma concentrations of free fatty acid caused by adrenaline and nor-adrenaline (Shafrir et.al. 1959-1960a). This may explain why the concentration of plasma free fatty acids falls more abruptly after adrenaline than after nor-adrenaline.

Shafrir et.al. (1959-1960a) found that twenty four hours after the changes in the plasma concentrations of free fatty acids following the administration of adrenaline, there are alterations in the concentrations of lipids and lipo-proteins. Cholesterol, phospholipid and the low density lipoproteins appear in increasing concentrations and the high density lipoproteins not only increase in concentration but also contain more cholesterol than normal. Daily injections of adrenaline into dogs and rats for periods of three, and of

six-to-eight days produced large increases in the serum cholesterol concentrations. The phospholipid concentrations in the serum also increased but the response of the triglyceride fraction was small and variable. There was a three-to-eight-fold increase in the low density or β-lipoprotein and a smaller one in the high density or a_1-lipoprotein, (which contained more cholesterol than usual). There was little change in the concentration, and none in the composition, of the very low density or a_2-lipoprotein of rats and dogs. There is evidence that prolongation of the treatment increases these effects. There are however, species variations. Rabbits respond with raised plasma triglyceride concentrations whereas rats and dogs do not. Wertheimer and Shafrir (1960) consider that the elevation of lipoproteins seen in these experiments does not represent the mobilization of liver fat.

It will be noticed that the response to adrenaline in vivo is very similar to the response to fasting. This may be related to the fact that, as Armin and Grant (1959) have confirmed, lowering the concentration of sugar in the blood will stimulate the secretion of adrenaline.

ADRENALINE AND THE SYMPATHETIC NERVOUS SYSTEM

Adrenaline is also released as a response to stimulation of the sympathetic nervous system. Adrenergic blocking

agents such as hexamethonium produce a prompt and substantial fall in plasma free fatty acid concentrations in dogs (Havel and Goldfien, 1961). Schotz and Page (1960) found that the presence of such agents in the medium surrounding rat epididymal fat pads inhibits the release of free fatty acid in vitro. Thus it seems that the release of free fatty acid, like that of adrenaline, is at least partially under the control of the autonomic nervous system. This system will be absent from in vitro preparations and any deductions made therefrom must take this into consideration.

ADRENALINE, ADRENOCORTICOTROPHIN AND THE CORTICOSTEROIDS

The effects of adrenaline are modified by other hormones. Adrenocorticotrophin (ACTH) affects the release of free fatty acid from adipose tissue and the concentrations of free fatty acids in the plasma in a manner very similar to that of adrenaline but not to quite such a great extent.. (Vaughan 1960). Glucose uptake, its oxidation and its conversion to glycerol are increased and there is a relative decrease in the conversion of glucose to fatty acids. (Ibid). Epididymal fat pads taken from either adrenalectomized or hypophysectomized rats were found by Shafrir et.al.(1959-1960a) to show a very much lower response to adrenaline than those fro normal animals. The immediate response of the plasma free fatty acid concentrations and the delayed response of the

lipoproteins, in vivo, are substantially reduced by adrenalectomy and abolished by hypophysectomy (Ibid).

Shafrir, Sussman and Steinberg (1959-1960b) found that the effects of adrenalectomy are prevented by treatment with three to five milligrams of cortisone per kilo body weight for seven-to-ten days prior to the injection of adrenaline. The same treatment given to normal dogs potentiates the action of adrenaline in a striking manner. Cortisone alone, i.e. without the subsequent administration of adrenaline, does not cause any significant elevation of plasma lipids in normal animals, whereas ACTH treatment acts in a manner very similar to that of adrenaline. Shafrir et.al. (1959-1960b) consider that the effect of cortisone is a permissive one and is not additive in nature. Long (1952) reported that adrenaline induces the secretion of ACTH but Vaughan (1960) suggested that ACTH releases adrenaline in adipose tissue. More recently, Smith, Paoletti and Brodie (1962) treated rats with reserpine to remove all the adrenaline and nor-adrenaline from the normal storage sites in adipose tissue. Epididymal fat pads removed from these animals did not respond to the presence of ACTH in the medium surrounding them whereas fat pads from normal untreated animals liberated free fatty acids into the medium. Results confirming these observations were obtained in vivo.

The prevention, by hypophysectomy, of the delayed increase in serum lipoprotein concentrations in response to adrenaline administration in vivo may be reversed by treatment with ACTH. This does not completely restore the response of the plasma free fatty acids (Shafrir et.al. 1959-1960b). It is interesting that ACTH administered to a human Addisonian diabetic after eleven days without insulin resulted in a marked increase in serum cholesterol concentrations (Conn, Vogel, Louis and Fajans, 1950). No change had been observed when ACTH was given to an uncomplicated case of Addison's disease.

Thyroxine

Shafrir et.al. (1959-1960b.) consider it possible that hypophysectomy deprives the animal not only of ACTH but also of other lipid mobilizing factors which interact with adrenaline. This seems very likely. Debons and Schwartz (1961) found that the lipolytic action of adrenaline in vitro is dependent on the condition of the thyroid gland in the intact animal. Adrenaline had no effect on the release of free fatty acids from epididymal fat pads taken from hypothyroid rats and a greatly exaggerated one on those from hyperthyroid animals (Ibid). Goodman and Knobill (1959) had previously found that pre-treatment with thyroid-stimulating hormone partially restored the free fatty acid response to

adrenaline in hypophysectomized monkeys and that tri-iodothyronine completely restored it.

GROWTH HORMONE

Growth hormone is also active in the regulation of lipid metabolism. Like adrenaline and ACTH, growth hormone preparations will mobilize free fatty acid from adipose tissue both in vivo and in vitro. It will elevate plasma free fatty acid concentrations in adrenalectomized dogs so that unlike adrenaline, it is independent of adrenal function (Wertheimer and Shafrir, 1960). In vitro effects of growth hormone on the rat epididymal fat pad have been described by Ashmore et.al. (1960), but the concentrations required are so large as to be completely unphysiological.

Wertheimer and Shafrir (1960) suggest that the sustained elevation of plasma free fatty acids produced by growth hormone preparations, the concomittant loss of body fat, increased fatty acid oxidation and infiltration of fat into the liver may represent part of the protein anabolic reaction of this hormone. The action on fat would supply an additional source of calorigenic substrate, allowing glucose and amino acids to be spared for protein synthesis. There is of course, some doubt as to the nature of the fundamental effect of growth hormone and those effects described above may be ancillary ones mediated by interactions with other hormones.

GLUCAGON

The action of glucagon on adipose tissue, while similar to that of adrenaline and ACTH, also shows some important differences. The increase in plasma concentrations of free fatty acids produced by glucagon administration in vivo is less than that produced by either adrenaline or ACTH. The same is true in vitro for the rate of release of free fatty acid from adipose tissue in media containing glucagon. Like adrenaline, glucagon produces a prompt elevation of blood glucose concentrations in vivo and an increase in the rate of glucose uptake by adipose tissue in vitro, but both of these increases are greater than those induced by adrenaline. (Vaughan 1960; Hagen 1961). The mobilization of free fatty acid by adrenaline is prevented by high glucose concentrations: this is not the case for glucagon action. The elevation of serum lipids and lipoproteins observed twenty four hours after adrenaline administration to dogs by Shafrir et.al. (1959-1960a), has been observed six days after glucagon administration to healthy men by Oliver and Boyd (1956).

THE INTERACTION OF THE VARIOUS MEANS OF REGULATION OF ADIPOSE TISSUE METABOLISM IN THE NORMAL HUMAN SYSTEM

It is highly unlikely that the information summarised above represents more than a small fraction of the ways in which the metabolism of adipose tissue and of the lipids

reaching the blood is regulated by different agents acting separately. It is even more difficult to relate them to what is observed in the normal human and in particular, the new-born infant. Numerous other permutations will be possible as a result of variations in the response of the liver as distinct from the response of adipose tissue.

The situation may be summarised as follows.

1. Adrenaline, ACTH, growth hormone, glucagon and fasting or diets which are deficient in carbohydrate, all accelerate the release of free fatty acid from adipose tissue.
2. This results in an increase in the plasma concentrations of free fatty acids in the intact animal, probably via the liver.
3. This is followed by a delayed increase in the concentrations of lipids and lipoproteins in the blood.
4. Concomittantly with the initial increase in the liberation of free fatty acid from adipose tissue there are increases in the rates of the uptake of glucose by adipose tissue, its oxidation to carbon dioxide and its conversion to glycerol, and in the rate of release of glycerol.
5. The increase in the rate of release of free fatty acid results from an increased rate of lipolysis of triglycerides accompanied by a reduced rate of synthesis of fatty acids.

6. The effect of adrenaline is

   (a) potentiated by ACTH, cortisone and thyroxine.

   (b) blocked by insulin and elevated glucose concentrations.

7. Glucose, or insulin in the presence of glucose, decreases the rate of release of free fatty acid from adipose tissue and the rate of oxidation of glucose to carbon dioxide.

8. Glucose, or insulin in the presence of glucose, increases the rate of synthesis of fatty acids and of glycerol and the rate of uptake of glucose by adipose tissue.

9. The pictures presented by diabetic and fasted animals are very similar to one another. This is because the metabolism of fat is dependent upon the metabolism of carbohydrate.

An attempt may now be made to relate this to the multiplicity of clinical effects of the hormones and of nutritional status on the lipids and lipoproteins of the blood in the human subject.

INSULIN

The loss of body fat, fatty livers and ketonuria of diabetes have been recognised for many years. So have the raised concentrations of triglycerides, cholesterol and the cholesterol-containing lipoproteins in the blood. These are all corrected by the administration of insulin.

THYROXINE

Hypothyroidism is accompanied by elevated serum cholesterol concentrations. Treatment with thyroxine lowers them in both hypothyroid and euthyroid subjects. This is also true of the serum concentrations of the low density β -lipoprotein. As has already been noted, Debons and Schwartz (1961) found that adrenaline had no effect on the release of free fatty acids from epididymal fat pads taken from hypothyroid rats. Therefore the action of adrenaline on fatty acid release and on serum lipid and lipoprotein concentrations may be blocked, not only by high glucose concentrations but also by elevated concentrations of cholesterol and low density lipoproteins.

GROWTH HORMONE

Oliver and Boyd (1956) observed no change in the concentrations of circulating lipids and lipoproteins in healthy men as a result of an intravenous infusion of porcine growth hormone. The same preparation had restored hepatic cholesterol biosynthesis in the rat after inhibition by hypophysectomy. They consider it likely that their negative result may have been caused by too small a dose or by a mechanism of species specificity preventing the action of this particular preparation in man.

ACTH and the Corticosteroid Hormones

The lipid response in man to ACTH and the adrenal hormones and also to the sex hormones, are multiple and often contradictory. Oliver and Boyd (1956) found that the administration of ACTH and of cortisone to hypercholesterolaemic men results in a significant depression of the serum cholesterol and β -lipoprotein concentrations, with an elevation of the α -lipoprotein concentrations.

On the other hand, Adlersberg, Schaefer and Drachman (1951) found that both ACTH and cortisone raised the serum cholesterol concentrations in patients who were severely ill with collagen diseases. Oliver and Boyd (1958) consider that this effect was produced by the symptomatic improvement in the patients such as the reduction of fever and the return of appetite, which contributed to the rise in cholesterol concentrations. This would account for the fact that Adlersberg et.al. (1951) found that ACTH and cortisone had no effect on the serum cholesterol concentrations of hypercholesterolaemic patients without collagen disease. Oliver and Boyd (1958) also quote Mann and White (1953) who had come to similar conclusions about Adlersberg's data on the grounds that both ACTH and cortisone would relieve the acutely stressful phase of the disease and that stress produces a reduction in serum cholesterol concentrations.

THE DEFINITION OF STRESS

A further examination of the data of Mann and White (1953) suggests that a clarification of the definition of "stress" would appear to be necessary. These authors include in this category the effects of fasting for periods varying from one hundred hours to nine days and exposure to a temperature of 38°C for eight hours. They also quote observations made by various people on the effects of acute infection on serum cholesterol concentrations, their own experience of the treatment of collagen diseases with cortisone and ACTH and the effects of pyrogenic vaccines on rabbits.

The effects of long periods of fasting cannot be compared with those of eight hours exposure to heat because the former would have a direct effect on the metabolism of carbohydrate and fat metabolism. Heat stress can be compared with the effects of emotional stress investigated by Wertlake, Wilcox, Haley and Peterson (1958) and by others quoted by Shafrir and Steinberg (1960). Increases in serum cholesterol concentrations were found in these investigations and it is suggested that this is a result of the secretion of adrenaline in response to the stress applied.

Consideration of the data of Mann and White (1953) reveals that heat exposure increased the total cholesterol concentrations in dogs. This increase was found to be a

result of an elevation of free cholesterol concentrations which more than compensated for a simultaneous fall in the concentration of esterified cholesterol. Fasting, on the other hand, had little effect on the serum cholesterol concentrations in dogs until after the third day when there was a marked decrease in concentration. This had corrected itself by the seventh day. A similar pattern emerges for rats in which the concentration of cholesterol was higher on the ninth day than it had been before the fasting period had begun. The effects of cortisone on normal dogs were variable but there appears to have been an increase in the serum total cholesterol concentrations approximately thirty six hours after the last injection. During the actual period of administration, the cholesterol concentrations had in fact fallen slightly but the final increase was more than double the earlier fall. In the clinical cases cited, cortisone also appeared to have elicited increased cholesterol concentrations.

ACTH on the other hand, produced a prompt and reproducible fall in serum cholesterol concentrations in normal fed dogs. After withdrawal of the treatment, the cholesterol concentrations increased again to levels above those obtained initially. In fasting dogs however the withdrawal of treatment did not produce this correction within the time limits of the experiment.

Conn, Vogel, Louis and Fajans (1950) investigated the effect of cortisone on a normal human male subject. The administration of 200 mg. per diem for ten days produced only a "mild and not well maintained depression" of serum total cholesterol concentrations. In the five day period following the cessation of cortisone treatment the serum cholesterol concentrations rose above the original control levels and it was during this period that the most intense "metabolic effects of cortisone were observed". Treatment of the same man with 68 mg. ACTH per diem for six days produced the same result as that obtained by Mann and White (1953): there was a pronounced fall in serum total cholesterol concentrations, the fall in esterified cholesterol being responsible for most of this. The cholesterol concentrations began to rise again immediately treatment was stopped. They had also risen on the first day of the experiment.

The results of Mann and White (1953) and of Conn et.al. (1950) are thus in agreement with those of Shafrir et.al. (1959-1960a) and Shafrir and Steinberg (1960) in so far as the lack of effect on serum cholesterol concentrations of cortisone alone is concerned. It seems possible that the reduction in cholesterol concentrations reported by Oliver and Boyd (1958), quoted earlier, only occurs in the hypercholesterolaemic state and is not, a typical effect of cortisone. The effects of heat stress are similar to those

obtained with emotional stress by Wertlake et.al. (1958).

On the other hand the effects of ACTH upon serum cholesterol concentrations reported by Mann and White and by Conn. et.al. are in direct opposition to those of Shafrir et.al. (1959-1960a). So too are the effects of fasting. It is impossible to do more than report that the disagreement exists and to remark that it serves to illustrate the complexity of the problem of hormonal interactions and of the difficulties inherent in the evaluation of experimental date.

On particular point does emerge from this summary of the literature referring to the metabolism of adipose tissue and its effect upon that of the circulating lipids and lipoproteins: that a remarkable number of different agents have the same end effect upon the circulating lipids and hence on the lipoproteins. Furthermore, even allowing for the fact that cholesterol is one of the more easily measured of the lipids and hence has been studied more frequently, it does appear to be peculiarly prone to alteration.

CHAPTER III

THE LIPID CONSTITUTION OF THE LIPOPROTEINS IN CORD BLOOD

INTRODUCTION

The most important lipids in the blood, from the point of view of the composition of the lipoproteins, are the fatty acids, the phospholipids and cholesterol. There are also small amounts of other lipids present.

Being lipids, these compounds are insoluble in the aqueous serum in the free state. They are mainly carried by protein molecules, thus forming lipoproteins (Blix, Tiselius and Svenson, 1941). Their solubilities and amphoteric properties are those of the protein and not of the free lipid, even though there may be three times as much lipid as thereis protein (Oncley, Gurd and Melin 1950). The main proteins associated with the lipids are the globulins, but most of the small amount of unesterified fatty acid in the blood is associated with albumin.

FATTY ACIDS

In the normal fasting human adult, the concentration of total fatty acid in the serum is approximately 300 mg./100 ml.

(Stern and Shapiro, 1953). Of this, 80 per cent is present in the phospholipids as triglycerides, the latter constituting the majority of the "neutral fat" fraction. Of the remainder, 15 per cent is present as cholesterol esters and 5 per cent is unesterified or "free" although it is still carried by protein. The type of fatty acid in the lymph varies with the diet (Blomstrand and Dahlback, 1960). The fatty acids present as cholesterol esters are relatively more unsaturated than those in phospholipids and triglycerides (Gould 1951).

PHOSPHOLIPIDS

These consist of three main groups. They are as follows:

1. The lecithins, which constitute approximately 80 per cent of the phospholipids (Gould 1951): the phosphoric acid moiety is esterified with choline.
2. The cephalins, which constitute approximately 5 per cent of the total: choline is replaced by ethanolamine, serine or inositol (Ibid).
3. The sphingomyelins, which constitute approximately 15 per cent of the total: glycerol is replaced by sphingosine (Ibid).

Phospholipids are usually estimated as lipid phosphorus, for which the normal adult serum concentrations range from

6 - 11 mg/100 ml. (Stewart and Hendry 1936). To obtain the concentration of phospholipid from that of lipid phosphorus, the average molecular weight of the phospholipids, which is approximately 800, is utilized. Different authors have used slightly different molecular weights giving a multiplying factor varying from 23.6 to 26.0.

CHOLESTEROL

The normal range of concentrations of cholesterol in the adult blood serum is 130 - 250 mg./100 ml. (Rafstedt 1955). Of this approximately 20 - 40 per cent is unesterified under normal conditions (Ibid).

THE INCORPORATION OF THE INDIVIDUAL LIPIDS INTO THE LIPOPROTEIN MOLECULES: METHODS OF ANALYSIS

The mode of incorporation of the lipids into the lipoprotein molecules is being widely investigated. The main methods of analysis are as follows:-

1. Electrophoresis.
2. The Cohn fractionation Method 10, which is based on differential solubilities in a system in which the hydrogen ion concentration, ethanol concentration, protein concentration, ionic strength and temperature are rigidly controlled (Cohn, Gurd, Surgenor, Barnes, Brown, Derouaux, Gillespie, Kahnt, Lever, Liu, Mittleman, Mouton, Schmidt and Uroma, 1950).

TABLE 3

Correlation of the Different Nomenclature Used in the Literature of Lipoprotein Metabolism

Electrophoresis	Density		Sf	Cohn	Hillyard
	Fractionation			Fraction	Flotation
γ-Lipoprotein and Start Fraction	0.94	= Chylomicrons	Ca.40,000	-	D
α_2-Lipoprotein	a)0.958 b)0.990	= Very Low Density Lipoprotein	10-400	-	C
β-Lipoprotein	1.063 Mean = 1.032	= Low Density Lipoprotein	0-10	III-0	A
α_1-Lipoprotein	1.063-1.107	= High Density Lipoprotein	-	IV,VI, V	B
Albumin plus Free Fatty Acid	-	= -	-		

TABLE 4

A Further Subdivision in the Nomenclature of Lipoproteins Devised by Smith (1957)

Electrophoresis	Sf	Cohn Fractionation
Deposition on Origin	-	-
Trail from Origin to β-Lipoprotein	70-400	II
Pre-β-Lipoprotein	18-90	II,III
Fast Migrating β-Lipoprotein	12-20	IV
Normal β-Lipoprotein	0-12	V

3. The Gofman ultracentrifuge method in which the unit is the Svedberg flotation unit (Gofman, Jones, Lindgren, Elliot and Strisower, 1950). One Svedberg flotation unit corresponds to a flotation rate of 1×10^{-13} cms./sec./dyne/gm.
4. Variations on the Gofman ultracentrifuge technique by Hillyard, Entennan, Feinberg and Chaikoff, (1955) and by Havel, Eder and Bragdon (1955).
5. The specific separation of lipoprotein by precipitation with polysaccharides such as dextran sulphate (Oncley, Gurd and Melin, 1950).

NOMENCLATURE

The different terms used in the different methods are extremely confusing. They have been correlated as far as possible in Tables 3 and 4.

THE VARIATION IN THE DATA FROM DIFFERENT SOURCES

Lipoproteins are not very stable compounds. They are readily broken down, particularly by dehydrating agents and by low temperatures. The phospholipid moiety is particularly subject to oxidation. For these reasons and because of the differences in the fractionation techniques, there is considerable variation in the data available on the constitution of the lipoproteins.

TABLE 5

The Composition of the different Lipoprotein fractions in Human Serum which are distinguishable by electrophoresis in mg. per cent of Lipoprotein according to different authors.

Pr = Protein Trig. = Triglyceride F.C. = Free Cholesterol E.C. = Esterified Cholesterol
P = Phospholipid TC = Total Cholesterol

Lipoprotein	Pr	Trig.	P	FC	EC	TC	C/P	Ref.
Chylomicrons	2	81	7	-	-	9	-	Havel, Eder and Bragdon(1955)
α_2-Lipoprotein	7	52	18	-	-	22	-	Havel, Eder and Bragdon(1955)
	7-10	49-52	18-19	6-7	14-16	20-23	0.78-0.90	Cornwell and Kruger(1961)
β-Lipoprotein	21	9	23	-	-	47	-	Havel et.al. (1955)
	21.9	9-12.5	19-32	7.9-9.5	37-39.4	45-49	1.3-1.45	Cornwell and Kruger(1961)
	-	-	29.3	8.31	22.5	30.9	-	Oncley, Gurd and Melin(1950)
	-	-	21.1-	-	-	28.7-	-	Oncley, Walten and Cornwell (1950)
	-	-	25.1	-	-	34.1	-	
α_1-Lipoprotein	46	8	26	-	-	19	-	Havel et.al. (1955)
	46-58	4-8	20-21	2-3	13-15	15-18	0.38-0.58	Cornwell and Kruger(1961)

THE MAIN LIPOPROTEIN FRACTIONS IN THE BLOOD

Serum lipoproteins may be considered as composed of four main fractions. These are:

1. The chylomicrons.
2. The very low density lipoproteins corresponding to a_2-lipoproteins in the electrophoretic separation.
3. The low density lipoproteins corresponding to the β-lipoprotein in the electrophoretic separation.
4. The high density lipoproteins corresponding to a_1 lipoprotein in the electrophoretic separation.
5. To this may be added the albumin bound free fatty acid fraction.

A summary of some of the available data on the constitution of the different lipoprotein fractions is given in Table 5.

1. THE CHYLOMICRONS

The chylomicrons were originally defined as the fat particles in plasma and chyle which are made visible by dark field microscopy. It is preferable to define them as the vehicle for the for the transport of triglyceride of alimentary origin in the passage of the latter from the alimentary tract to the blood via the chyle. They have a density of approximately 0.94 and consist mainly of triglyceride with a small proportion of cholesterol,

phospholipid and protein (Cornwell and Kruger, 1961). They do not migrate during electrophoresis of serum lipoproteins and are included in the γ -lipoprotein fraction.

These are three distinct protein molecules in the chylomicrons. One is identical with that found in the high density or α_1-lipoprotein: aspartic acid is the N-terminal amino acid (Rodbell and Frederickson, 1959). Since high density lipoprotein activates synthetic triglyceride emulsions prior to lipolysis by the enzyme lipoprotein lipase (Korn 1955), Rodbell and Frederickson (1959) consider that high density lipoprotein may be intimately associated with chylomicrons. Rodbell, Frederickson and Ono (1959) found that during the disappearance from plasma of chylomicrons containing labelled protein, there is an immediate appearance of labelling in the plasma high density lipoprotein, suggesting a rapid equilibration of the common protein. This protein may also be added to the chylomicrons on entry into the plasma at the expense of the high density lipoprotein (Cornwell and Kruger, 1961).

The second protein species in the chylomicrons is that which predominates in the low density or β -lipoprotein and has an N-terminal serine unit. (Ibid). The third may be an N-terminal threonine protein. Plasma chylomicrons contain more of this protein than the chylomicrons in lymph, suggesting that it is added to them after entry into the plasma (Ibid).

2. THE VERY LOW DENSITY OR a_2-Lipoprotein

The very low density lipoproteins which correspond to the a_2-lipoprotein in the electrophoretic separation have a density between 0.958 and 0.990. They have an Sf value between 10 and 400. They are considered to be the main vehicle for the transport of endogenous triglyceride which constitutes approximately 50 per cent of their total weight. (Cornwell and Kruger, 1961). They may be converted to low density or β-lipoprotein by the removal of triglyceride because the protein moiety is probably common to both of these lipoproteins. (Ibid).

3. THE LOW DENSITY OR β-Lipoprotein

The low density or β-lipoprotein is the most readily available for analysis. It is so-called because it has a mean density of 1.032 and it migrates at approximately the same rate as β-globulin during electrophoresis. It has an Sf value between 0 and 10. The protein moiety is probably identical with that in the a_2-lipoprotein (Ibid). Oncley, Gurd and Melin (1950) estimated the anhydrous molecular weight at 1,300,000, the hydration being to the extent of 0.6 gm. water/gm. anhydrous lipoprotein. Its physical properties may be explained by assuming a spherical complex in which the lipid fraction is on the inside, the polypeptide moiety forming a broken film on the

surface (Ibid). Only 42-57 per cent of the surface can be covered, depending on the location of the water molecules. If the lipid part of the surface was mainly phospholipid, the protein-like solubility properties of the molecule would be retained (Ibid). The molecule measures approximately 185 A.° (French, Morris and Robinson, 1958).

Low density lipoproteins may arise either as products of very low density or a_2-lipoprotein, or directly from the liver (Cornwell and Kruger, 1961). Although they constitute the largest group of the lipoproteins in human adult blood, their metabolic role is still obscure. Vaughan (1961) suggests that they serve as an acceptor for triglyceride (which would eventually convert them to a_2-lipoprotein) in the same way that albumin is the acceptor of free fatty acid.

4. THE HIGH DENSITY OR a_1-Lipoprotein

The high density or a_1-lipoproteins which migrate with the a_1-globulins during electrophoresis have a density of 1.063 to 1.107. They are synthesized in the liver and are intimately connected with the transport of triglyceride and with chylomicron metabolism. (Cornwell and Kruger, 1961). Since their molecular size is only approximately 50 A.° (French, Morris and Robinson, 1958), they are capable of passing through the capillary membrane. It is notable that the serum of cats, dogs and rats and of human umbilical

cord blood contain a majority of this lipoprotein instead of β-lipoprotein which is the preponderant adult human lipoprotein.

DATA OBTAINED IN THIS INVESTIGATION ON THE CONSTITUTION OF THE LIPOPROTEINS OF THE BLOOD

Methods

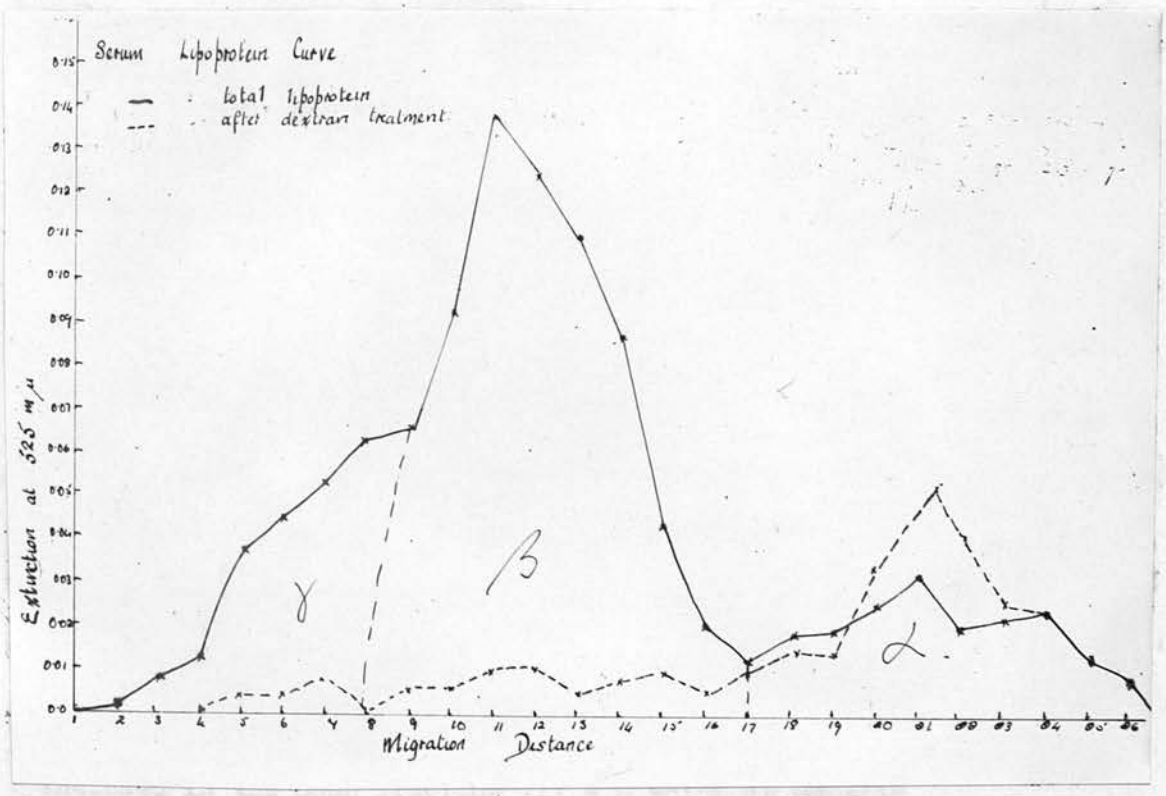
Dextran sulphate has been used to precipitate the β-lipoprotein in a small number of specimens of umbilical cord blood and maternal venous blood. The lipid content was measured in the untreated serum and in the residue left after precipitation. The blood which was analysed in this manner was obtained from the following cases.

1. The umbilical cord blood of 4 infants of diabetic mothers.
2. The umbilical cord blood of 1 twin infant of a toxaemic mother: the infant was badly distressed at birth.
3. The venous blood of 3 diabetic mothers taken after the delivery of the child.
4. The venous blood of one child aged 2 years.

Measurements were made of the concentration of total lipid, cholesterol and phospholipid in each specimen of blood. In each case, simultaneous lipid estimations and electrophoretic separations of the lipoproteins were performed on the untreated serum and on the residue after precipitation

Figure 1.

The Effect on the Serum Lipoprotein Curve Obtained by Electrophoretic Separation of Maternal Serum Caused by the Preliminary Addition to the Serum of Dextran Sulphate Solution.



The solid line represents the total lipoprotein.

The interrupted line represents the lipoproteins remaining after dextran sulphate precipitation.

with dextran sulphate. This indicated that whereas the residue containing no β -lipoprotein gave values for α-lipoprotein lipid which are comparable with those obtained by electrophoresis of the whole serum, subtraction of this figure from the total lipid concentration gave a value corresponding to a combination of β - and γ -lipoprotein lipid. This occurred in both maternal and in cord blood serum. Fig. 1 illustrates the lipoprotein distribution curve obtained from a sample of maternal serum before and after dextran sulphate precipitation. It will be observed that the α -lipoprotein fraction is approximately the same in both curves but the β - and γ-lipoprotein fractions have both been lost after the dextran sulphate treatment. It seems likely that the γ -lipoprotein fraction corresponds to the trail fraction of Smith (1957) which has an Sf value of 70 - 400 and is included in Fraction II of the Cohn separation (See Table 3). If this is the case it will be included in the Cohn fraction III - 0 which is usually referred to as β -lipoprotein. It is impossible to say what proportion of chylomicrons is contained in this fraction but as mentioned in Chapter II Page 9 , it is unlikely that they play a large part in the lipoprotein content of cord blood.

For this reason the fraction precipitated by dextran sulphate is referred to as (β +γ)-lipoprotein. Where

TABLE 6

The Composition of β-Lipoprotein Lipid from different sources. Data in mg./fraction/100 ml. Serum with reference to Cholesterol and Phospholipid.

C = Cholesterol P = Phospholipid LP = Lipoprotein Lipid d = Density

Reference	Method	Source of LP	Serum C	Serum P	Serum C/P	C in β-LP	P in β-LP	C/P in β-LP
Havel et.al. (1955)	d 1.063	Adult Males	172 ±22	218 ±22	0.79 ±0.06	125 ±21	100 ±16	1.25 ±0.08
Havel et.al. (1955)	d 1.063	Adult Females	178 ±27	230 ±33	0.78 ±0.04	116 ±23	92 ±19	1.26 ±0.08
Russ et.al. (1954)	Cohn III-0	Adult Females	189 ±35	372 ±28	0.84 ±0.11	123 ±32	98 ±23	1.25 ±0.16
Hillyard et. al.(1955)	d 1.063	Adults	-	-	-	163	89	-
Russ et.al. (1954)	Cohn III-0	Mothers at Term	282 ±62	372 ±80	0.84 ±0.11	207 ±59	206 ±63	1.07 ±0.14
Russ et.al. (1954)	Cohn III-0	Normal Cord Blood	68 ±15	124 ±22	0.57 ±0.08	36 ±11	39 ±12	0.87 ±0.14

TABLE 7

The Composition of α-Lipoprotein Lipid from different sources. Data in mg./fraction/100 ml. Serum with reference to Cholesterol and Phospholipid.

C = Cholesterol P = Phospholipid LP = Lipoprotein Lipid d = Density

Reference	Method	Source of LP	Serum C	Serum P	Serum C/P	C in α-LP	P in α-LP	C/P in α-LP
Havel et.al. (1955)	d 1.063	Adult Male	172 ±22	218 ±22	0.79 ±0.06	46 ±7	116 ±11	0.39 ±0.04
Havel et.al. (1955)	d 1.063	Adult Female	178 ±27	230 ±33	0.78 ±0.04	57 ±12	134 ±20	0.43 ±0.05
Russ et.al. (1954)	Cohn IV,V,VI	Adult Female	189 ±35	225 ±28	0.84 ±0.11	61 ±13	117 ±20	0.52 ±0.06
Hillyard et. al.(1955)	d 1.063	Adult	-	-	-	60	79	-
Russ et.al. (1954)	Cohn IV,V,VI	Mothers at term	282 ±62	372 ±80	0.84 ±0.11	63 ±18	139 ±34	0.47 ±0.08
Russ et.al. (1954)	Cohn IV,V,VI	Normal Cord Blood	68 ±15	124 ±22	0.57 ±0.08	23 ±8	66 ±10	0.44 ±0.08

normal mean values have been taken from other sources, the sum of the β-lipoprotein lipid and the γ -lipoprotein lipid have been equated with the concentrations of the lipoprotein lipid precipitated by dextran sulphate. Since Russ, Eder and Barr (1954) have used the Cohn Method 10, and their results are in good agreement with those of Havel et.al. (1955) who used the ultracentrifuge density fractionation, both these sets of data may be used as a comparison with data obtained in this investigation. The data of Burstein and Samaille (1958) who also used the dextran sulphate method, are difficult to interpret for comparative purposes because their results are given as the percentage of cases having α - and β -lipoprotein cholesterol concentrations within a number of different ranges.

RESULTS

The data obtained are given in Table 8. In Tables 6 and 7 are similar data obtained from Russ, Eder and Barr (1954) and Havel et.al. (1955). Even allowing for the very small number of cases in this investigation, the agreement between the data obtained and those of Russ et.al. and of Havel et.al. is surprising. This is especially good for the percentages of total cholesterol and total phospholipid which are found in each of the lipoprotein

TABLE 8

The Phospholipid and Cholesterol content of α- and β -lipoprotein Lipid in the Cord Blood of Four Infants of Diabetic Mothers; in the Venous Blood of Three Diabetic Mothers and in that of Two Other Cases.

Case	(β + γ)-Lipoprotein Lipid. Mg/100 ml. serum	α-Lipoprotein Lipid. Mg/100 ml. serum	Phospholipid in (β + γ)-Lipoprotein Lipid. Mg/100 ml. serum	Phospholipid in α-Lipoprotein Lipid. Mg/100 ml. serum	Cholesterol in (β+γ)-Lipoprotein Lipid. Mg/100 ml. serum	Cholesterol in α-Lipoprotein Lipid. Mg/100 ml. serum	Lipoprotein	Lipid in mg/ 100 ml. serum found by electrophoretic separation			Lipoprotein Lipid in mg/ 100 ml. serum found by Dextran sulphate method		Total serum cholesterol. Mg/100 ml.	Total serum phospholipid. Mg/100 ml.
							α	β	γ	β+γ	α	"β"		
A. The Cord Blood of 4 Infants of Diabetic Mothers														
Re.	96	132	28	68	32	34	102	65	61	126	132	96	66	96
Ph.	138	100	32	66	45	29	133	58	46	104	100	138	74	98
Mo.	120	160	40	50	45	39	140	104	36	140	160	120	84	90
No.	104	232	39	61	38	50	210	88	38	126	232	104	88	100
B. Venous Blood of 3 Diabetic Mothers														
Ph.	948	340	265	148	262	80	225	802	260	1062	340	948	342	413
Mo.	899	222	180	94	229	80	291	566	263	829	222	899	309	274
No.	1132	288	187	162	384	84	264	929	227	1156	288	1132	468	349
C. Venous Blood of Infants of 2 years														
La.	509	222	74	104	133	41	210	320	199	519	222	509	174	178
D. Cord Blood of one of Twin Infants of a Toxaemic Mother														
Co.	270	110	91	44	56	38	115	194	70	264	102	270	94	135

fractions. One is therefore encouraged to make further calculations of the percentage composition of the lipoprotein fractions using the data from all three sources.

Table 9 is a summary of the data obtained by Russ et.al. (1954), Havel et.al. (1955), and in this investigation, and the calculations made therefrom. Figures in brackets are those deduced from the observations from different investigations. The sources of these data will be obvious from their position in the table.

NORMAL INFANTS

It appears from the data of Russ et.al. (1954) and of Havel et.al. (1955) in Tables 6 and 7 that the distribution of cholesterol and phospholipid in α- and in ($\beta+\gamma$) -lipoprotein is different at different stages of development. Thus cholesterol is apparently equally divided between the two lipoprotein fractions in cord blood but in a ratio of one to three in maternal blood at the end of labour. Similarly, phospholipid is reported to be present in a ratio of two to one in cord blood, and approximately two to three in maternal blood α- and ($\beta+\gamma$)-lipoprotein respectively. But these results are given in terms of the percentage of serum cholesterol and phospholipid in the α- and ($\beta+\gamma$)-lipoprotein in 100 ml. of serum. No data are given for the amount of total lipoprotein lipid

TABLE 9

Phospholipid and Cholesterol Content of α - and β-Lipoprotein Lipid in Blood From Various Sources in this Investigation and Data From Similar Investigations Quoted by Russ, Eder and Barr (1954) and by Havel, Eder and Bragdon (1955).

Type of Blood	Number of cases	Source of Data See Key	Serum cholesterol mg./100 ml.	Serum Phospho-lipid mg./100 ml.	Serum ($\beta+\gamma$)-Lipoprotein Lipid mg./100 ml.	Serum α-Lipo-protein Lipid mg./100 ml.	Cholesterol in ($\beta+\gamma$)-Lipo-protein Lipid mg./100 ml.
Normal Cord Blood	50	1	72 ± 14	115 ± 20	177 ± 51	141 ± 34	(40)
	12	2	68 ± 15	124 ± 22	(177)	(141)	36 ± 11
Cord Blood of Infants of Diabetic Mothers	4	1a	78	96	115 ± 19	156 ± 56	40 ± 6
	18	1b	84 ± 22	113 ± 24	159 ± 34	139 ± 28	(43)
Normal Maternal Venous Blood	20	1	281 ± 78	302 ± 49	835	253 ± 71	(211)
	14	2	282 ± 62	372 ± 80	(835)	(253)	207 ± 59
Venous Blood from Diabetic Mothers at Delivery	3	1a	373 ±	345	993	283	292
	12	1b	312 ± 19	335 ± 17	910 ± 20	243 ± 69	(242)
Venous Blood from Non-Pregnant Adults	?	3	178 ± 27	230 ± 33	(600)	(250)	116 ± 23
	24	2	189 ± 35	225 ± 28	(600)	(250)	123 ± 32
Venous Blood from Child Aged 2 years	1	1a	178	174	509	222	133
Cord Blood from Twin Infants of Toxaemic Mother	1	1b	94	135	270	110	56
Column Number	1	2	3	4	5	6	7

Cholesterol in α-Lipoprotein Lipid. Mg./100 ml.	Phospholipid in ($\beta+\gamma$)-Lipoprotein Lipid. Mg./100 ml.	Phospholipid in α-Lipoprotein Lipid. Mg./100 ml.	% Serum cholesterol in ($\beta+\gamma$)-Lipoprotein Lipid	% serum cholesterol in α-Lipoprotein Lipid	% serum Phospho-lipid in ($\beta+\gamma$)-Lipoprotein Lipid	% serum Phospho-lipid in α-Lipoprotein Lipid	% of ($\beta+\gamma$)-Lipoprotein Lipid which is cholesterol	% of α-Lipo-protein Lipid which is cholesterol	% of ($\beta+\gamma$)-Lipoprotein Lipid which is phospho-lipid	% of α-Lipoprotein Lipid which is Phospholipid	Ratio of cholesterol to Phospholipid in ($\beta+\gamma$)-Lipoprotein Lipid	Ratio of cholesterol to Phospholipid in α-Lipoprotein Lipid	Ratio of cholesterol to Phospholipid in serum (C/P)	Ratio of ($\beta+\gamma$)-Lipoprotein Lipid to α-Lipoprotein Lipid in serum ($\beta+\gamma/\alpha$)
(31)	(42)	(73)	(55)	(43)	(37)	(63)	(23)	(22)	(24)	(52)	(0.95)	(0.42)	0.64 ± 0.16	1.36 ± 0.68
28 ± 8	39 ± 12	66 ± 10	55 ± 10	43 ± 9	37 ± 9	63 ± 10	(20)	(19)	(22)	(47)	0.87 ± 0.14	0.44 ± 0.08	0.57 ± 0.08	
38 ± 9	35 ± 6	61 ± 6	52 ± 8	49 ± 8	36 ± 7	64 ± 7	34.9 ± 2	25 ± 3	30 ± 6	45 ± 18	1.18	0.64	0.85	0.83
(41)	(40)	(72)	(52)	(49)	(36)	(64)	(27)	(30)	(25)	(52)	(1.08)	(0.57)	0.77 ± 0.29	1.22 ± 0.46
(67)	(179)	(123)	(76)	(24)	(59)	(41)	(26)	(26)	(21)	(49)	(1.18)	(0.54)	0.93 ± 0.26	2.6
63 ± 18	206 ± 63	139 ± 34	76 ± 8	24 ± 7	59 ± 10	41 ± 10	(25)	(25)	(25)	(59)	1.07 ± 0.14	0.47 ± 0.08	0.84 ± 0.11	
81	231	135	78	22	61	39	29	29	23	48	1.43	0.64	1.10	3.59
(70)	(205)	(130)	(78)	(22)	(61)	(39)	(27)	(29)	(23)	(54)	(1.18)	(0.54)	0.94 ± 0.19	3.75
57 ± 12	92 ± 19	134 ± 20	65	32	40	58	(19)	(23)	(15)	(54)	1.26 ± 0.08	0.43 ± 0.05	0.78 ± 0.04	
61 ± 13	98 ± 23	117 ± 20	65 ± 9	33 ± 9	45 ± 8	55 ± 7	(20)	(24)	(16)	(47)	1.25 ± 0.16	0.52 ± 0.06	0.84 ± 0.11	
41	74	104	76	24	42	58	26	19	14	47	1.79	0.39	0.98	2.29
38	91	44	60	40	67	33	21	35	34	40	0.62	0.86	0.70	2.45
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22

KEY TO SOURCES OF DATA

1 = This investigation: Cord Blood of 50 Normal Infants

1a = This investigation: Dextran sulphate precipitation of (+)-lipoprotein

1b = This investigation: Cord Blood of 18 infants of diabetic mothers

2 = Russ, Eder and Barr (1954) 3 = Havel, Eder and Bragdon (1955)

NOTE. All figures in brackets have been calculated or interpolated from other data in the Table: their source will be obvious from their position in the table.

present so that no deductions of the differences in the composition of the different lipoprotein fractions can in fact be made.

This difference in distribution could be accounted for largely in terms of the difference in the proportion of the two lipoprotein fractions in cord blood and in maternal venous blood. The ratio of (β+ γ) - to α-lipoprotein lipid in cord blood is approximately half that in adult blood. It will be seen from Table 9, Columns 13 - 16 that the proportion of the two lipoprotein lipid fractions made up by cholesterol and phospholipid respectively are in fact very much of the same order in normal cord blood and in normal maternal blood.

Nevertheless, although the differences between the cholesterol and phospholipid contents of the different lipoprotein fractions in maternal and cord blood are small, they may be real differences. They will be summarised in Table 10, and Table 11.

TABLE 10

	(β+γ)-Lipoprotein Lipid		a-Lipoprotein Lipid	
	Cholesterol%	Phospholipid%	Cholesterol%	Phospholipid%
Normal Cord Blood (50)	(22.6)	(23.7)	(22.0)	(51.7)
Normal Cord Blood (12)	20.3	22.0	19.9	46.8
Normal Maternal Blood (30)	(26.0)	(21.4)	(26.4)	(48.6)
Normal Maternal Blood (14)	24.8	24.7	24.9	59.1

(Figures in Brackets are calculated values)

It seems likely that there might be more cholesterol in maternal ($\beta+\gamma$)-lipoprotein lipid than in that in cord blood. It seems unlikely that the proportion of phospholipid differs significantly. The possibility exists that with a sufficient number of cases systematic differences could be established.

At present, knowledge of the incorporation of lipid into lipoprotein is scanty. It would be difficult to distinguish between the existence of one species of molecule capable of carrying variable amounts of lipid, and two or more species of lipoprotein molecule, each with a fixed amount of lipid, occurring in variable proportions. Either possibility could account for the differences observed. It will be remembered that Shafrir et.al. (1959-1960a) also report the possibility of lipoprotein molecules in dog serum carrying more cholesterol than is considered normal, as a result of long term starvation and of diabetes. In their case however, the change was found in the a-lipoprotein lipid fraction.

In view of all these considerations, the use of the ratio of cholesterol to phospholipid concentrations in the serum for diagnostic purposes is of doubtful value. Russ et.al. (1954) have pointed out that, while this ratio tends to be high when the ratio of β- to a-lipoprotein is high and vice versa, there is sufficient variation in the ratio

of cholesterol to phospholipid in the β-lipoprotein fraction alone to render this relationship of little value.

2. INFANTS OF DIABETIC MOTHERS

One should regard the data for the composition of the lipoprotein lipid fractions in the cord blood of the infants of diabetic mothers in the light of the data given above. The mean serum cholesterol concentration is significantly higher than normal while the α- and (β + γ)-lipoprotein lipid concentrations are significantly lower than normal. (See Chapter IV Page 65-66). Calculation of the lipid content of the different lipoprotein lipid fractions in these infants suggests that the cholesterol content of their lipoproteins is more akin to that of the mothers than that of normal cord blood lipoprotein, for there is a higher proportion of cholesterol in their (β +γ)-lipoprotein lipid fraction. There is also a higher proportion of phospholipid in their (β + γ)-lipoprotein lipid fraction and this differs from normal cord blood (β +γ)-lipoprotein lipid in that the latter is more like that found in the maternal serum. The ratio of cholesterol to phospholipid concentrations in the serum in the four cases investigated here and in the 18 cases in the main group of infants of diabetic mothers, is closer to the adult range than to that for normal cord blood. The same applies to the separate cholesterol to phospholipid ratios in

the ($\beta+\gamma$)- lipoprotein lipid found in these four cases. It does not apply to that in the a- lipoprotein lipid which is of the same order as that found in normal cord blood α- lipoprotein lipid. The data will be summarised in Table 11 on the following page.

TABLE 11

	(β+γ)-Lipoprotein Lipid			α-Lipoprotein Lipid			Serum
	C/P	Cholesterol%	Phospholipid%	C/P	Cholesterol%	Phospholipid%	C/P
Normal Cord Blood (50)	(0.95)	(22.6)	(23.7)	(0.42)	(22.0)	(51.7)	(0.64)
Normal Cord Blood (12)	0.87	20.3	22.0	0.44	19.9	46.8	0.57
Cord Blood of Infants of Diabetic Mothers							
(a) 4 cases	1.18	34.9	30.4	0.64	25.2	45.2	0.85
(b) 18 cases	(1.08)	(27.0)	(25.2)	(0.57)	(29.5)	(51.8)	0.77
Normal Maternal Blood (30)	(1.18)	(26.0)	(21.4)	(0.54)	(26.4)	(48.6)	0.93
Normal Maternal Blood (14)	1.07	24.8	24.7	0.47	24.9	59.1	0.84
Diabetic Maternal Blood (3)	1.43	29.4	23.3	0.64	28.6	47.7	1.10
Diabetic Maternal Blood (12)	1.13	(26.6)	(22.5)	(0.54)	(28.8)	(53.5)	0.94

(Figures in brackets are calculated values)

Obviously a larger number of cases must be investigated before any attempt is made to explain this but if it can be regarded as indicating a difference in the ($\beta + \gamma$)-lipoprotein in these infants it would be most interesting.

Unfortunately no blood was obtained in sufficient quantity from infants of mothers who have had a previous abortion to allow this kind of analysis and from only one infant of a toxaemic mother. This was a twin pregnancy and the twin studied was badly distressed at birth. It is therefore by no means representative of infants of toxaemic mothers but the data are included in Tables 8 and 9 purely out of interest, as are those for the child aged 2 years.

CONCLUSIONS

1. The composition of the main lipoprotein lipid fractions in cord blood and in maternal blood are very similar with regard to their content of cholesterol and phospholipid.
2. Small differences exist which may be real ones. It seems likely that there might be more cholesterol in the maternal ($\beta+\gamma$)-lipoprotein lipid fraction that in the same fraction in cord blood.
3. The ($\beta + \gamma$)-lipoprotein lipid in the cord blood of infants of diabetic mothers appears to resemble that in maternal blood in this respect rather than that in normal cord blood.

4. There is also a higher proportion of phospholipid in the ($\beta+\gamma$)-lipoprotein lipid in the cord blood of infants of diabetic mothers and this differs from normal cord blood ($\beta+\gamma$)-lipoprotein lipid which is more like that found in the maternal serum.

5. The ratio of cholesterol to phospholipid in the ($\beta+\gamma$)-lipoprotein lipid and in the whole serum in the infants of diabetic mothers is closer to the adult value for this ratio than that for normal cord blood.

CHAPTER IV

THE CONCENTRATION OF LIPIDS AND LIPOPROTEIN LIPIDS IN THE SERUM OF UMBILICAL CORD BLOOD

THE SOURCES OF THE BLOOD USED IN THIS INVESTIGATION

Serum lipid and lipoprotein lipid concentrations have been measured in the umbilical cord blood of a number of infants, and in the venous blood of a number of mothers at the end of labour. The data are divided into the following groups:

1. Infants who had a normal delivery, who showed no abnormality during the first week of life, whose mothers were not reported to have shown any abnormality during pregnancy: 50 cases.

1a. Mothers of the infants in Group 1 : 20 cases.

2. Infants whose mothers suffered from diabetus mellitus : 18 cases.

2a. The diabetic mothers of the infants in Group 2 : 12 cases.

3. Infants whose mothers had an abortion in a previous pregnancy or a threatened abortion in this pregnancy : 19 cases.

4. Infants whose mothers had suffered from toxaemia of pregnancy or from hypertension or both : 30 cases.
5. Infants who were themselves abnormal at birth or whose mothers showed abnormalities during pregnancy other than those in Groups 2, 3 and 4.

It will be noted that whereas blood from both infants and mothers in Groups 1 and 2 has been studied, data have been given for the infants only in Groups 3,4 and 5. This is not by design, for obviously it would be advantageous to study both umbilical cord blood and the corresponding maternal blood in every case. It is simply a result of the difficulties inherent in an investigation such as this. It is relatively easy to obtain cord blood for it can be drained from the umbilical cord by junior staff. Obtaining maternal blood, on the other hand, requires venepuncture at a time when it is frequently inconvenient. Blood was obtained from the diabetic mothers only because considerable interest is being focussed on diabetic pregnancies. If an investigation of this nature is to be really successful, it would require close liason with the ante-natal clinics so that the labour ward staff could be directed accordingly. In this investigation, blood was collected entirely at random and the clinical history obtained at a later data.

The data which have been accumulated are given in

TABLE 13

Serum Lipid and Lipoprotein Lipid Concentrations in the Venous Blood of 20 Normal Mothers at Delivery.

Case	Total Lipid mg/100 ml.	Total Esterified Fatty Acid mg/100 ml.	Lipid Phosphorus mg/100 ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	C/P	γ-Lipoprotein %	β-Lipoprotein %	α-Lipoprotein %	γ-Lipoprotein Lipid.Mg/100 ml.	β-Lipoprotein Lipid.Mg/100 ml.	α-Lipoprotein Lipid.Mg/100 ml.	β/α	Calculated Total Lipid.Mg/100 ml.
Brl.	931	341	8.4	218	187	0.86	14.6	54.8	30.6	136	510	285	1.80	593
Bro.	1090	651	13.5	351	274	0.78	15.9	59.2	24.8	173	646	271	2.40	1030
Cr.	1081	368	12.0	312	324	1.04	19.2	53.3	27.4	207	576	296	1.90	786
Cl.	701	468	7.1	186	178	0.96	18.7	49.0	32.3	137	358	229	1.52	702
D'A	1351	430	14.0	369	263	0.72	20.9	46.6	32.5	282	630	439	1.43	802
Do.	1381	746	13.2	356	242	0.68	14.6	70.1	15.2	202	968	210	4.61	1095
Gi.	886	490	9.3	240	181	0.75	35.2	44.5	20.3	312	394	180	2.20	743
Gu.	947	562	10.0	260	274	1.05	14.7	64.9	20.2	139	615	191	3.20	914
Ha.	1334	536	11.9	309	347	1.12	17.4	68.6	14.0	232	915	187	4.90	976
He.	1208	745	13.8	359	228	0.63	9.8	63.9	26.3	118	772	318	2.43	1081
Lo.	1337	438	12.3	321	236	0.73	17.3	66.3	16.3	231	886	218	4.10	770
Mu.	931	526	12.0	313	268	0.85	21.1	53.1	25.8	196	494	240	2.06	888
Mo.	1094	652	12.3	320	239	0.75	30.6	49.5	19.8	335	541	217	2.50	987
McN.	1038	475	13.0	337	341	1.01	17.8	61.7	20.4	185	640	212	3.03	917
McK.	1032	467	14.3	372	474	1.27	12.3	58.9	28.6	127	608	295	2.05	1053
Og.	996	306	10.3	268	279	1.04	9.7	56.1	34.2	97	559	341	1.64	665
Ri.	1220	400	10.4	270	372	1.38	17.4	58.9	23.6	212	719	288	2.50	853
Sc.	1172	215	12.5	325	425	1.31	7.9	65.2	26.9	93	764	315	2.43	738
St.	1061	582	10.6	275	260	0.94	17.7	64.4	17.9	188	683	190	3.60	925
Ta.	954	557	11.0	287	224	0.78	16.9	69.6	13.5	161	664	129	5.10	867

TABLE 14

Serum Lipid and Lipoprotein Lipid Concentrations in the Cord Blood of 18 Infants of Mothers who are Diabetics.

Case	Placental Weight lbs. ozs.	Birth Weight lbs. ozs.	Sex	Maternal Age	Parity	Duration of Pregnancy	Total Lipid mg/100 ml.	Total Esterified Fatty Acid.Mg/100 ml.	Lipid Phosphorus mg/100 ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	C/P	γ-Lipoprotein %	β-Lipoprotein %	∝-Lipoprotein %	γ-Lipoprotein Lipid.Mg/100 ml.	β-Lipoprotein Lipid.Mg/100 ml.	∝-Lipoprotein Lipid.Mg/100 ml.	(β+γ)-Lipoprotein %	(β+γ)-Lipoprotein Lipid.Mg/100 ml.	β/∝	(β+γ)/∝	Calculated Total Lipid.Mg/100 ml.	Duration of Diabetes	Notes
Co.	1.3	6.5	M	22	0	36	310	177	5.1	133	90	0.68	27.0	30.0	42.8	84	93	133	57.1	177	0.70	1.33	307	11 years	Badly controlled
Sc.	1.4	6.8	M	32	2+2	36	292	122	4.2	109	75	0.68	19.0	42.8	38.2	55	125	111	61.8	180	1.12	1.62	230	14 years	Pregnancy 2:abortion at 16/52. Pregnancy 4:abortion at 5/12.
Bu.	1.6	5.15	F	27	1	36	270	106	4.2	109	86	0.79	24.0	25.3	50.6	65	68	137	49.3	133	0.50	0.97	225	9 years	Badly controlled
Do.	1.0	7.5	F	34	4+1	37	340	159	5.0	130	68	0.52	23.8	29.6	46.6	81	101	158	53.4	182	0.63	1.15	266	8 years	Rh.-ve.Pregnancy 5:abortion at 18/52. 1 infant with paroxysmal auricular tachycardia.
Sa.	1.11	6.12	F	32	1+1	38	328	185	5.0	129	55	0.43	15.0	34.8	50.2	49	97	165	49.8	146	0.43	0.99	279	7 years	Pregnancy 1:abortion at 24/52.1st infant stillborn.Badly stabilised with hypoglycaemic coma.
Du.	-	8.12	F	21	0	38	328	147	2.7	71	128	1.80	28.6	33.1	38.3	94	109	125	61.7	203	0.86	1.61	296	10 weeks	Grade II cardiac.Obese.Mild essential hypertension.
Wa.	1.9	6.3	M	35	0+2	34	264	122	4.0	105	72	0.68	23.5	21.2	55.3	62	56	146	44.7	118	0.38	0.81	226	12 years	Threatened abortion at 13/52.Previous abortions at 8/52 and 20/52.Rh.-ve.Child died at 5 hours.
In.	)1.14	5.7	F	31	2	36	384	207	5.0	130	128	0.94	10.1	46.4	43.5	39	178	167	56.5	217	1.06	1.30	374	9 years	Anaemia
In.	)	5.1	M	-	-	-	292	178	4.2	110	90	0.82	15.6	36.5	47.9	46	107	140	52.1	153	0.76	1.09	301		
Pa.	-	-	M	-	-	-	274	111	3.4	89	64	0.72	29.8	22.1	48.1	82	60	132	51.9	142	0.45	1.08	202		Infant stillborn.
Gu.	1.7	7.1	F	21	0	36	252	116	3.8	98	57	0.58	16.7	54.9	28.4	42	138	72	71.6	170	1.92	2.52	202	11 years	Infant stillborn - foetal anoxia. Mother Rh.-ve.
Ph.	1.6	7.6	F	25	0	37	238	127	3.8	98	74	0.80	19.3	24.6	56.1	46	58	133	43.9	104	0.44	0.78	230	1 year	Well controlled
Go.	1.3	6.3	F	41	4	36	316	166	5.2	135	79	0.59	14.0	34.6	51.4	44	109	162	48.6	153	0.67	0.95	286	9 years	Mother has palpable thyroid.4th infant diabetic with muscular paralysis.
Re.	1.6	7.6	F	25	0	36	228	116	3.7	96	66	0.69	26.9	28.4	44.6	61	65	102	55.3	126	0.64	1.22	211	1 year	Well controlled
Gl.	0.15	6.4	M	19	0+1	36	270	144	4.6	120	80	0.67	20.0	46.4	33.6	54	125	91	66.4	179	1.37	1.98	260	2-3 years	Previous incomplete abortion at 12/52.
Mo.	1.4	6.12	M	34	0	35	280	93	3.5	90	84	0.83	12.9	37.1	50.0	36	104	140	50.0	140	0.74	1.00	204	15 weeks	Well controlled
No.	1.6	8.4	F	34	4	38	336	-	3.8	100	88	0.88	11.4	26.2	62.4	38	88	210	37.6	126	0.42	0.60	298	4 weeks	Well controlled
Ro.	1.2	7.7	F	36	6	37	340	143	6.8	176	121	0.71	21.0	30.1	48.9	104	112	124	51.1	216	0.62	1.04	321	2 years	Grossly obese.Dietary control of diabetes. 1st infant died at 20 minutes.6th stillborn.

TABLE 15

Serum Lipid and Lipoprotein Lipid Concentrations in the Venous Blood of Twelve Diabetic Mothers at Delivery.

Case	Total Lipid mg/100 ml.	Total Esterified Fatty Acid mg/100 ml.	Lipid Phosphorus mg/100 ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	C/P	γ -Lipoprotein %	β -Lipoprotein %	α -Lipoprotein %	γ -Lipoprotein Lipid. mg/100 ml.	β -Lipoprotein Lipid. mg/100 ml.	α -Lipoprotein Lipid. mg/100 ml.	β/α	Calculated Total Lipid. mg/100 ml.
Gl.	846	448	11.5	300	320	1.07	12.5	56.5	31.0	106	478	262	1.82	858
Sa.	1061	424	11.0	287	226	0.79	19.0	63.5	17.5	202	674	186	3.63	736
Do.	1061	599	11.8	307	216	0.70	16.7	59.5	23.8	177	631	253	2.50	907
Sc.	1562	586	18.7	486	334	0.69	19.1	61.8	19.1	298	965	298	3.23	1066
Co.	1143	441	13.5	351	342	0.97	22.9	59.2	17.9	262	677	204	3.31	888
Bu.	1049	364	13.4	348	298	0.86	22.4	39.8	37.8	235	417	396	1.05	766
Ro.	1263	571	13.7	356	310	0.87	17.5	63.7	18.7	222	804	236	3.41	988
No.	1420	627	13.4	349	468	1.34	16.0	65.4	18.6	227	929	264	3.52	1200
Mo.	1121	428	10.5	274	309	1.13	23.5	50.5	26.0	263	566	291	1.94	819
Go.	963	515	11.1	289	272	0.94	24.0	62.4	13.6	231	601	131	4.59	874
Gu.	882	389	10.8	280	292	1.04	14.3	66.0	19.7	126	582	174	3.35	765
Pa.	1473	613	15.1	393	353	0.90	14.2	70.6	15.1	209	1040	224	4.64	1084

Serum Lipid and Lipoprotein Lipid Concentrations in the Cord Blood of Nineteen Infants of Mothers who have had Previous Abortions or stillbirths or a threatened Abortion in this Pregnancy.

Case	Placental Weight lbs. ozs.	Birth Weight lbs. ozs.	Sex	Maternal Age	Parity	Duration of Pregnancy. Weeks.	Total Lipid mg/100 ml.	Total Esterified Fatty Acid mg/100 ml.	Lipid Phosphorus mg/100 ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	C/P	γ-Lipoprotein %	β-Lipoprotein %		γ-Lipoprotein Lipid.Mg/100 ml.	β-Lipoprotein Lipid.Mg/100 ml.	α-Lipoprotein Lipid.Mg/100 ml.	(β+γ)-Lipoprotein %	(β+γ)-Lipoprotein Lipid.Mg/100 ml.	β/α	(β+γ)/α	Calculated Total Lipid.Mg/100 ml.	Notes
Di.	1.4	4.9	F	19	0	32	508	168	5.0	130	77	0.59	28.2	29.3	4	143	149	216	57.5	292	0.69	1.35	284	Threatened Abortion
Chr.	2.2	8.9	F	32	2+1	40	493	229	5.8	150	109	0.73	24.6	45.3	3	121	223	148	69.9	344	1.50	2.32	383	Complete abortion at 8/52. This infant cyanosed
Cr.	1.10	8.0	F	22	1+1	38	610	267	8.0	207	111	0.54	-	-		-	-	-	-	-	-	-	440	Complete abortion
Ros.	1.4	7.10	M	23	1	39	432	155	5.7	148	95	0.64	12.6	35.2	5	54	152	226	47.8	206	0.67	0.91	294	Threatened abortion. Placenta Praevia
Co.	1.10	7.9	M	25	1+1	40	298	163	3.0	79	77	0.98	12.4	50.7	3	36	147	107	63.1	183	1.37	1.71	264	Complete abortion
Dun.	0.10	7.15	F	40	4+1	47	419	178	5.2	135	89	0.66	18.9	26.5	5	79	111	228	45.4	190	0.49	0.83	308	Stillbirth. Fourth infant has speech defect
Em.	1.9	8.15	F	32	2+2	33	360	168	5.2	136	82	0.60	19.7	34.5	4	71	124	165	54.2	195	0.75	1.18	291	Pregnancies 1 and 3 complete abortion at 16/52
Gr.	2.0	6.13	M	28	0+1	40	468	134	5.2	135	112	0.83	24.3	38.9	3	114	182	172	63.2	296	1.06	1.72	287	Complete abortion at 24/52. Threatened abortion at 22/52 this pregnancy.
Lau.	1.5	6.12	M	25	0+1	42	238	128	3.4	89	71	0.78	27.0	17.5	5	64	42	132	44.5	106	0.32	0.80	226	Complete abortion at 8/52
Mun.	1.12	7.11	F	33	2+1	37	428	203	5.9	156	78	0.50	-	-		-	-	-	-	-	-	-	328	Complete abortion at 16/52
Mor.	1.8	9.9	F	30	4+3	40	298	133	4.9	127	89	0.70	18.7	46.2	3	57	141	107	64.9	198	1.32	1.85	260	Three complete abortions at 9/52. Achondroplastic dwarf twin born living.
Sw.	1.5	7.3	M	31	2+1	38	302	-	3.8	99	81	0.82	16.7	26.7	5	50	81	171	43.4	131	0.47	0.77	241	Complete abortion at 10/52
McM.	1.8	6.14	M	33	9+1	40	242	161	3.7	96	68	0.70	23.5	42.6	1	57	103	82	66.1	160	1.26	1.95	258	Third infant spina bifida. Sixth pregnancy complete abortion at 12/52. Ninth stillborn
Nic.	2.0	7.15	F	38	6+1	37	468	186	6.3	164	108	0.66	13.5	42.5	4	63	199	206	56.0	262	0.97	1.27	343	One stillborn. Sixth infant with congenital heart disease
Pat.	1.6	7.13	F	31	3+1	39	280	91	4.6	119	71	0.59	25.4	36.4	1	71	102	107	61.8	173	0.95	1.62	198	First pregnancy complete abortion at 10/52. Second threatened abortion. Third mongol infant
Fy.	1.8	7.7	F	28	1+1	40	432	202	4.7	124	102	0.82	20.0	45.6	1	86	197	149	65.6	283	1.32	1.90	336	Complete abortion at 16/52
McW.)		6.5	M	27	1+1	39	316	132	3.6	94	74	0.79	29.0	45.8	2	92	145	86	74.8	237	1.69	2.76	234	(Second pregnancy, complete abortion at 10/52
)																								(
McW.)		7.5	M	27	1+1	39	508	173	6.1	159	153	0.96	5.2	46.7	4	26	237	244	51.9	263	0.97	1.08	374	(Twin pregnancy
McE.	1.11	8.6	M	26	2+1	41	410	148	4.9	126	76	0.60	19.2	39.0	4	79	160	171	58.2	239	0.94	1.40	262	Complete abortion at 15/52

TABLE 17

Serum Lipid and Lipoprotein Lipid Concentrations in the Cord Blood of 30 Infants of Mothers who had Toxaemia or Hypertension or both during Pregnancy.

Case	Placental Weight lbs. ozs.	Birth Weight lbs. ozs.	Sex	Maternal Age	Parity	Duration of Pregnancy. Weeks.	Total Lipid Mg/100 ml.	Total Esterified Fatty Acid. Mg/100 ml.	Lipid Phosphorus Mg/100 ml.	Phospholipid Mg/100 ml.	Cholesterol Mg/100 ml.	C/P	γ-Lipoprotein %	β-Lipoprotein %	α-Lipoprotein %	γ-Lipoprotein Lipid. Mg/100 ml.	β-Lipoprotein Lipid. Mg/100 ml.	α-Lipoprotein Lipid. Mg/100 ml.	($\beta+\gamma$)-Lipoprotein %	($\beta+\gamma$)-Lipoprotein Lipid. Mg/100 ml.	β/α	$\frac{(\beta+\gamma)}{\alpha}$	Calculated Total Lipid. Mg/100 ml.	Notes
McD	-	8.8	M	16	0	40	306	152	4.7	122	66	0.54	18.4	27.9	53.6	56	85	164	46.3	141	0.52	0.86	255	Moderate Pre-eclamptic Tetany (PET).
Ki	-	7.2	M	18	0	40	288	96	3.9	101	65	0.64	14.0	32.2	53.8	40	93	155	46.2	133	0.60	0.85	191	Mild PET
He	1.0	7.7	F	37	0	40	310	102	6.1	159	70	0.44	21.7	32.2	46.0	67	100	143	53.9	167	0.70	1.17	220	Mild PET
Ma	1.0	6.5	F	21	0	40	196	113	4.2	109	47	0.43	29.1	15.6	55.2	57	31	108	44.7	88	0.29	0.81	193	Mild PET
Gl	1.10	8.12	M	27	1+1	40	356	111	3.3	86	50	0.58	21.4	38.4	40.2	76	137	143	59.8	213	0.96	1.49	187	Moderate PET
McL	1.5	7.7	M	28	0	40	336	236	1.9	49	58	1.18	20.6	14.1	65.3	69	47	220	34.7	116	0.21	0.53	309	Mild PET. On Saluric.
Gle	0.15	7.5	F	30	0	37	366	96	4.1	107	65	0.61	26.5	40.3	33.2	97	147	121	66.8	244	1.21	2.02	193	Moderate PET
Ke	-	8.2	M	31	1	39	222	90	2.8	72	54	0.74	24.3	28.1	47.6	54	62	106	52.4	116	0.58	1.09	166	Mild PET. On Saluric.
Me	-	8.1	M	20	0	40	398	92	4.2	109	78	0.72	28.2	23.6	48.2	112	94	192	51.8	206	0.49	1.07	203	Moderate PET
Go	-	7.14	F	37	0	38	312	139	4.7	122	68	0.56	-	-	-	-	-	-	-	-	-	-	244	Mild PET + Essential Hypertension(EHT). On Saluric.
Ta	-	4.8	F	25	1+1	40	232	72	3.5	91	50	0.55	23.0	36.4	38.6	58	84	90	61.4	142	0.93	1.58	149	Mild PET + EHT. On Saluric.
Co	1.4	7.1	M	28	0	40	292	166	4.7	122	57	0.47	33.9	28.1	37.9	99	82	119	62.0	181	0.69	1.52	260	Mild Hypertension (HT).
Fo	0.15	6.1	M	19	0	37	288	124	4.2	109	70	0.64	16.7	36.5	46.8	48	105	135	53.2	153	0.78	1.13	227	Mild PET
Gr	-	6.15	F	29	0	41	288	146	4.3	112	73	0.65	28.5	30.2	41.3	82	87	119	58.7	169	0.73	1.42	253	Mild PET
Cl	1.3	5.15	F	29	1	38	340	129	3.8	98	55	0.56	31.4	41.9	26.7	107	142	91	73.3	249	1.56	2.74	213	Mild PET + HT
Sm	-	8.8	F	30	2	42	388	131	5.2	135	76	0.56	16.4	34.7	48.9	64	135	190	51.1	199	0.71	1.05	248	Mild PET. On Saluric.
Po	-	7.15	F	28	1	35	356	148	4.5	116	61	0.53	15.5	37.4	47.1	55	133	168	52.9	188	0.79	1.12	244	Mild PET. On Saluric.
Pl	-	7.5	M	23	1	39	244	135	4.4	114	49	0.43	-	-	-	-	-	-	-	-	-	-	218	Mild PET. On Saluric.
McI	-	8.0	F	26	1	31	274	131	4.4	114	55	0.48	-	-	-	-	-	-	-	-	-	-	220	Mild PET. On Saluric.
McK	-	8.10	F	23	1	39	264	89	4.0	103	58	0.56	20.5	40.4	39.1	54	107	103	60.9	161	1.04	1.56	178	Very mild PET. On Saluric.
Ha	1.1	7.3	F	27	0	40	248	100	4.2	109	66	0.60	24.2	20.0	53.8	60	50	133	44.0	110	0.38	0.83	199	Mild EHT
Gr	1.2	6.11	M	29	0	39	190	122	3.0	79	43	0.54	26.8	24.3	48.8	51	46	93	51.1	97	0.49	1.04	189	Mild EHT
Ba	1.7	8.4	M	19	1+1	42	288	102	3.9	101	57	0.56	14.1	44.9	41.0	41	129	118	59.0	170	1.09	1.44	189	EHT
McH	1.4	6.3	M	30	1+1	40	284	133	3.6	95	43	0.45	23.9	26.5	49.5	68	75	140	50.4	143	0.54	1.02	205	Mild EHT
Ol	1.8	7.14	F	32	2	37	264	96	3.7	96	45	0.47	26.7	46.0	27.3	70	121	72	72.1	191	1.68	2.65	170	Mild EHT
Wa	1.9	9.1	M	27	1	41	212	111	3.7	96	59	0.61	39.4	27.5	33.1	83	58	70	66.9	141	0.83	2.01	199	Mild EHT
Be	1.2	8.3	F	22	0	40	384	152	4.5	117	66	0.56	19.9	23.5	56.6	76	90	217	43.4	166	0.41	0.76	253	Mild EHT
Gi	-	7.13	M	27	1	39	316	170	3.4	89	95	1.16	29.7	22.4	47.9	94	71	151	52.1	165	0.47	1.09	292	Mild EHT
Os	1.1	6.13	F	28	1	40	360	133	5.2	135	92	0.68	25.5	31.3	43.2	92	113	156	56.8	205	0.72	1.31	266	Mild EHT
Hu	1.6	7.10	F	27	1	39	310	81	3.9	101	53	0.51	32.2	21.3	46.4	100	66	144	53.5	166	0.46	1.15	164	Mild EHT

TABLE 18

Serum Lipid and Lipoprotein Lipid Concentrations in the Cord Blood of a number of Infants whose Mothers or who themselves showed abnormalities other than those of the main groups of Infants. (Mg./100 ml. serum).

Case	Placental Weight lbs. ozs.	Birth Weight lbs. ozs.	Sex	Maternal Age	Parity	Duration of Pregnancy.Weeks	Total Lipid Mg/100 ml.	Esterified Fatty Acid. Mg/100 ml.	Lipid Phosphorus mg/100 ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	C/P	γ -Lipoprotein %	β -Lipoprotein %	α -Lipoprotein %	γ -Lipoprotein Lipid Mg/100 ml.	β-Lipoprotein Lipid mg/100 ml.	α -Lipoprotein Lipid mg/100 ml.	(β + γ)-Lipoprotein %	(β + γ)-Lipoprotein Lipid. Mg/100 ml.	β / α	Calculated Total Lipid. Mg/100 ml.	Notes
A. Mothers Rh.-ve.																							
Li.	1.5	7.5	M	23	0	37	366	179	4.8	126	111	0.88	23.4	30.1	46.5	86	110	170	53.5	196	0.65	328	
An.	1.6	7.4	F	27	3	40	486	161	4.7	122	85	0.70	22.4	35.7	41.8	109	174	203	58.1	283	0.86	283	
Br.	1.2	6.12	F	-	1	40	275	119	3.4	88	66	0.73	35.6	17.4	47.0	98	47	129	53.0	145	0.36	211	
La.	1.3	6.11	F	32	-	38	366	109	4.3	112	57	0.51	27.9	50.0	22.2	85	153	65	77.9	238	2.35	200	Iso-immunized,Exchange transfusion of infan
McL.	-	7.11	M	25	1	-	340	134	5.1	132	81	0.68	28.5	26.1	45.4	97	89	154	54.6	186	0.58	255	
Pe.	1.2	4.12	F	26	1	34	310	200	4.4	114	45	0.40	19.8	23.0	57.1	61	71	177	42.8	132	0.40	279	Premature.Long labour.Bilirubin 15.7mg.day
Tr.	1.4	6.9	F	24	1	39	284	120	2.9	75	81	1.08	20.1	40.1	39.8	57	114	113	60.2	171	1.01	224	
Mac.	1.6	5.8	F	28	0	40	310	149	3.4	88	79	0.90	39.3	29.6	31.1	122	92	96	68.9	214	0.96	254	
B. Infants Abnormal																							
Bl.	2.1	7.10	M	28	1	40	619	-	3.9	102	132	1.34	18.9	55.2	25.8	117	342	160	74.1	459	2.14	-	Hydrocephalic.Sib.died at 5 days.Mother alkaptonuric
Na.	1.5	7.5	M	20	0	40	415	156	5.5	143	140	0.98	19.4	28.7	51.9	81	119	215	48.1	200	0.55	341	Muscular twitching
Ni.	1.10	6.10	F	23	0	41	448	243	3.4	90	96	1.10	12.5	43.9	43.6	56	197	195	56.4	253	1.01	366	Foetal distress.Meconium staining
McK.	-	8.5	M	-	-	40	428	-	5.0	131	125	0.94	23.7	38.7	37.6	101	166	161	62.4	267	1.03	-	Cerebral irritation.Convulsions
Ba.	1.4	7.0	M	21	0	41	288	89	3.7	96	63	0.66	38.5	50.7	10.7	111	146	31	89.2	257	4.71	181	Died at 5 days.Cerebral anoxia
McH.	-	2.14	F	21	0	32	1164	484	11.2	291	237	0.81	17.6	53.8	28.6	205	626	333	71.4	831	1.87	798	Stillborn.Spina bifida.Foetus macerated
C. Mothers Severely Anaemic																							
Sm.	3.4	9.11	M	22	0	41	350	114	3.6	94	54	0.57	33.3	31.4	35.2	116	110	123	64.7	226	0.89	196	
We.	1.5	7.12	M	23	1	40	212	114	3.8	99	37	0.37	18.6	28.1	53.3	39	60	113	46.7	99	0.53	181	
Ru.	1.1	6.11	F	20	0	40	444	183	5.3	138	61	0.44	17.6	36.3	46.2	78	161	205	53.9	239	0.79	284	Hyperemesis as well as anaemia
Lu.	1.7	7.5	F	35	5	40	274	155	4.3	112	76	0.68	18.0	34.2	47.8	49	94	131	42.2	143	0.72	264	
Ar.	1.12	6.12	M	19	0	42	415	-	3.9	103	79	0.79	41.8	27.6	30.5	113	114	126	69.4	227	0.90	-	
McV.	-	2.12	M	23	1	29?	232	122	3.8	100	87	0.87	30.9	13.7	55.3	72	32	128	44.6	104	0.25	242	Twin pregnancy
D. Isolated Cases																							
La.	1.10	7.6	M	33	1+1	42	476	142	4.1	107	50	0.47	20.9	39.3	39.8	95	187	189	60.2	282	0.99	224	Progesterone deficient.Primolute first 3/1
Ru.	1.6	7.5	F	31	1	40	496	-	5.9	153	124	0.80	26.8	29.4	43.8	131	144	215	56.2	275	0.67	-	Enlarged thyroid with hypertension
Cr.	1.9	8.4	M	41	5	38	486	170	6.0	156	105	0.66	30.2	24.0	45.8	147	117	223	54.2	264	0.52	322	Cardiac grade 11
Ki.	1.8	6.13	F	20	0	37	322	146	4.1	108	85	0.78	24.6	33.1	42.3	79	106	136	57.7	185	0.78	263	Cardiac grade 11

Tables 12 - 18. Examples of the lipoprotein distribution curves obtained by paper electrophoresis are given in Figs. 2. The mean values for the concentrations of the different serum lipids and lipoprotein lipids and their standard errors in Groups 1-4 are given in Tables 19 and 20. The significance of any deviation from normality in the data for Groups 2,3 and 4 is also indicated. Statistical methods have been used to locate any relationship between the concentration of pairs of different lipids or lipoprotein lipids in umbilical cord blood or between concentrations of the same lipid or lipoprotein lipid in the maternal venous blood and the infant's umbilical cord blood. Scatter diagrams showing the distribution of values of the different lipids and lipoprotein lipids in the different groups of infants are given in Figs. 6-8.

MEAN SERUM LIPID AND LIPOPROTEIN LIPID CONCENTRATIONS.

1. NORMAL INFANTS OF NORMAL MOTHERS : (50)

AND NORMAL MOTHERS : (20)

The data obtained for these infants are given in Table 12. The mean serum concentrations of the different lipids and lipoprotein lipids of the umbilical cord blood, with their standard errors are given in Table 19. Similar data for maternal blood are given in Tables 13 and 20. Examples of the lipoprotein distribution curves obtained by

TABLE 19

Concentrations of Lipids and Lipoprotein Lipids in Mg./100 ml. Serum in the Cord Blood of Normal and Abnormal Infants.

	Normal (50)	Diabetic (18)	Abortions (19)	Toxaemics (30)
Observed Total Lipid	317.66 ± 8.78	296.70 ± 9.73	395.26 ± 23.74 (0.01 p 0.001)	297.10 ± 10.36
Calculated Total Lipid	253.08 ± 6.26	262.10 ± 11.54	300.53 ± 17.54 (0.01 p 0.001)	216.57 ± 7.11 (p 0.001)
Difference	64.6 ± 6.3	34.6 ± 6.1	94.8 ± 10.4	80.8 ± 9.3
Total Esterified Fatty Acid	148.26 ± 4.80	142.29 ± 7.57	167.70 ± 9.34	123.27 ± 6.06 (0.01 p 0.001)
Phospholipid	115.20 ± 2.90	112.78 ± 5.61	130.20 ± 7.11 (0.05 p 0.02)	105.57 ± 3.75 (0.05 p 0.02)
Cholesterol	71.82 ± 2.05	83.83 ± 5.29 (0.02 p 0.01)	90.68 ± 4.84 (p 0.001)	61.47 ± 2.35 (0.01 p 0.001)
C/P	0.641± 0.022	0.770 ± 0.068 (0.05 p 0.02)	0.710 ± 0.031	0.600 ± 0.175
α -Lipoprotein Lipid	140.98 ± 4.90	139.68 ± 6.57	159.82 ± 11.85	135.59 ± 7.25
β -Lipoprotein Lipid	95.50 ± 5.18	97.35 ± 7.30	146.76 ± 11.71 (p 0.001)	92.22 ± 5.94
γ -Lipoprotein Lipid	81.78 ± 3.90	61.18 ± 4.91 (0.01 p 0.001)	74.29 ± 6.93	75.63 ± 3.75
(β+ γ)-Lipoprotein Lipid	177.08 ± 7.28	158.53 ± 8.06 (p 0.001)	221.06 ± 14.61 (0.01 p 0.001)	163.70 ± 7.45 (p 0.001)
β-/α-Lipoprotein	0.757 ± 0.060	0.760 ± 0.092	0.985 ± 0.089	0.735 ± 0.064
(β+ γ)/α-Lipoprotein	1.36 ± 0.10	1.224 ± 0.109	1.495 ± 0.129	1.30 ± 0.10

electrophoresis are given in Figs.

Total Lipid

The mean concentration of total lipid in umbilical cord blood is 318 ± 9(50)mg./100 ml., That in maternal blood is 1087 ± 33(20) mg./100 ml.

Total Esterified Fatty Acid

The mean concentration of total esterified fatty acid in the two bloods are 148 ± 5(50) mg./100 ml. and 503 ± 25 (20) mg./100 ml. in the infants and mothers respectively. The ratios of the mean concentration of total lipid to that of total esterified fatty acid are thus almost identical: 2.15 in umbilical cord blood and 2.16 in maternal venous blood.

Phospholipid

The mean concentration of phospholipid in umbilical cord blood is 115 ± 3 (50) mg./100 ml. and in maternal venous blood it is 302 ± 9 (20) mg./100 ml. The ratio of the mean total lipid concentration to the mean phospholipid concentration is therefore 2.8 in umbilical cord blood and 3.6 in maternal venous blood. These values are not significantly different.

Cholesterol

The mean concentration of the cholesterol in umbilical cord blood is 72 ± 2 (50) mg./100 ml. and in maternal venous

TABLE 20

Concentrations of Lipids and Lipoproteins Lipids in Mg./100 ml. serum in Maternal Venous Blood.

	Normal Mothers(20)	Diabetic Mothers(12)
Observed Total Lipid	1087.25 ± 32.69	1153.67 ± 66.22
Calculated Total Lipid	874.25 ± 27.65	912.58 ± 41.72
Difference	213	241
Total Esterified Fatty Acid	502.75 ± 25.44	500.42 ± 27.43
Phospholipid	302.40 ± 8.88	335.00 ± 17.50
Cholesterol	280.80 ± 14.38	311.67 ± 18.73
C/P	0.933 ± 0.040	0.942 ± 0.054
α -Lipoprotein Lipid	252.55 ± 13.00	243.25 ± 19.87
β -Lipoprotein Lipid	647.10 ± 29.16	697.00 ± 44.82
β/α -Lipoprotein	2.769 ± 0.206	3.08 ± 0.31
$(\beta+\gamma)-/\alpha$ -Lipoprotein	2.6	3.75
γ- Lipoprotein Lipid	188.15 ± 12.25	215.17 ± 15.97

blood it is 281 ± 14 (20) mg./100 ml. The ratio of the mean total lipid concentration to the mean cholesterol concentration is therefore 4.4 in the cord blood and 3.9 in the maternal venous blood. These values also, are not significantly different.

Ratio of Cholesterol to Phospholipid Concentrations

Although there is no significant difference between the ratios of the mean serum concentration of total lipid to that of phospholipid or cholesterol in cord blood and in maternal blood, a study of the ratios of the mean serum cholesterol concentration to that of phospholipid (C/P) suggests that there is a difference in the pattern of lipid distribution. The C/P value for umbilical cord blood is 0.64 ± 0.02 (50) mg./100 ml. and that for maternal venous blood is 0.93 ± 0.04 (20) mg./100 ml. which is significantly higher. ($p < 0.001$).

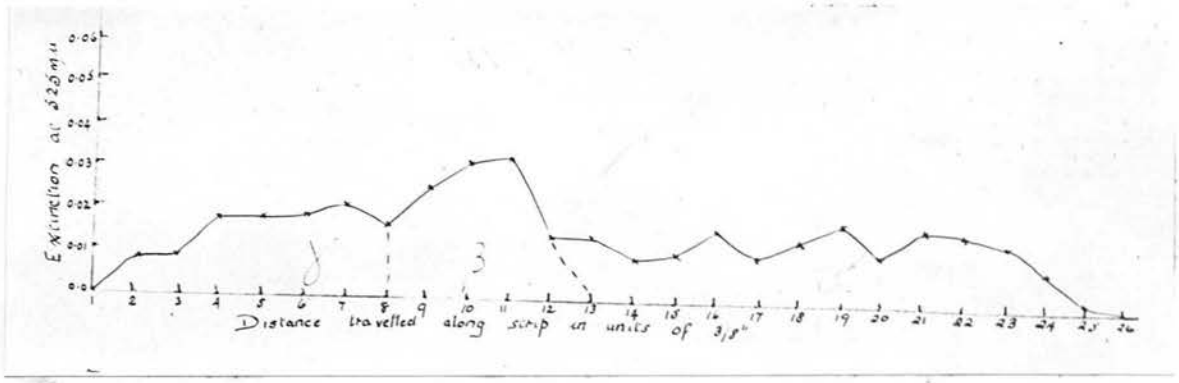
Thus it seems that the total lipid of umbilical cord blood contained a lower proportion of cholesterol and a higher proportion of phospholipid than maternal venous blood. This difference fits in with the relative proportions of the different lipoprotein fractions in the two bloods.

The Lipid Content of the Main Lipoprotein Fractions of the Blood

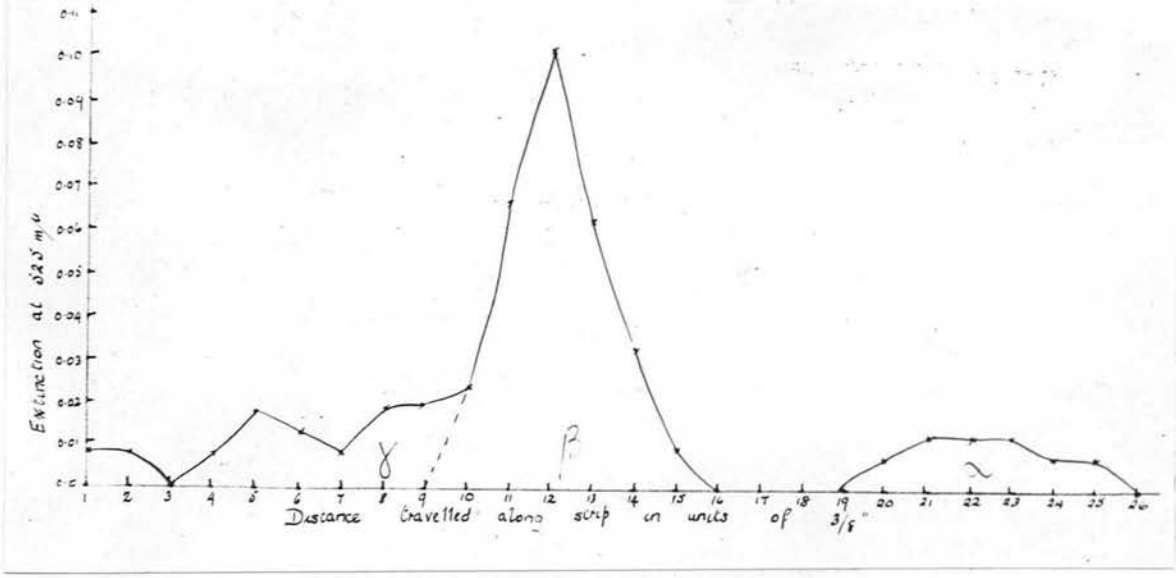
The two main lipoprotein fractions distinguishable by

Figure 2.

Serum Lipoprotein Curves Obtained by Electrophoretic Separation of the Lipoproteins in a) the Cord Blood of Normal Infants and b) the Venous Blood of Normal Mothers at the Time of Delivery.

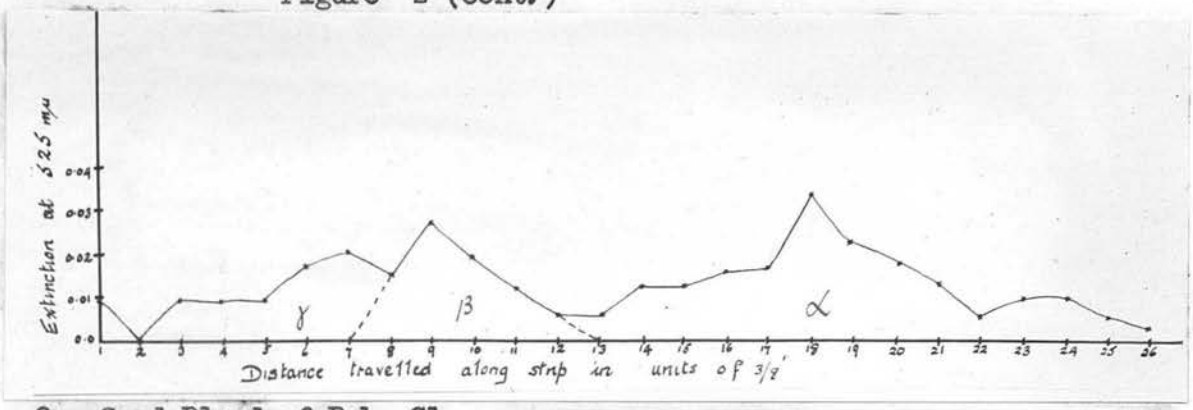


1a. Cord Blood of Baby Ta.

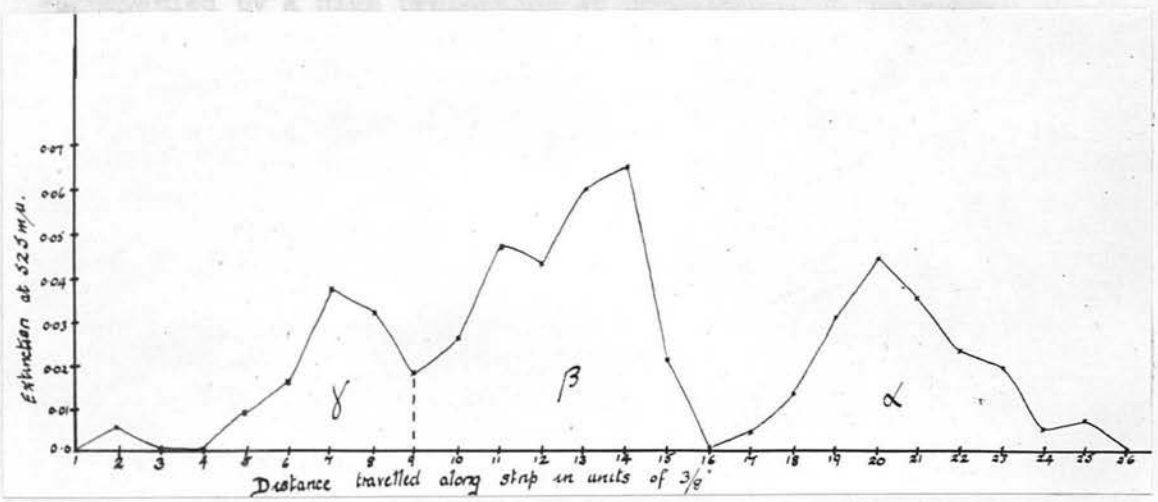


1b. Venous Blood of Mother Ta.

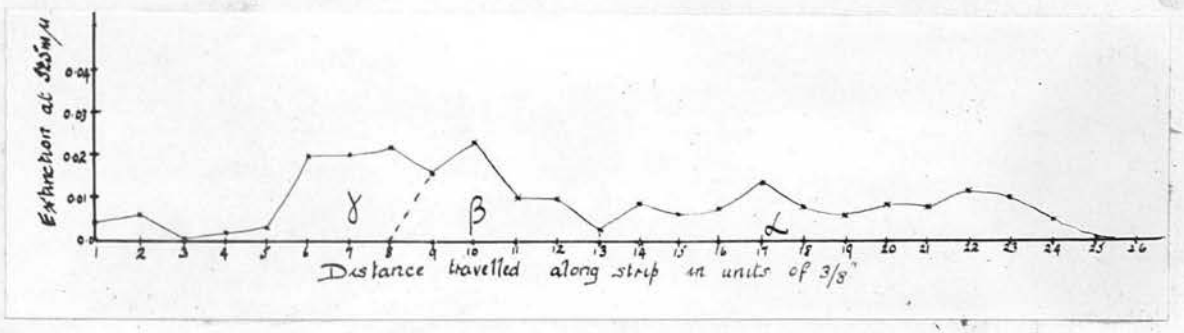
Figure 2 (cont.)



2a. Cord Blood of Baby Cl.



2b. Venous Blood of Mother Cl.



5a. Cord Blood of Baby He.

electrophoresis are α -lipoprotein and β -lipoprotein. Cholesterol constitutes approximately 25 per cent of the total lipid carried by α -lipoprotein, and 30 per cent of that carried by β-lipoprotein. Phospholipid on the other hand constitutes 50 per cent of the α -lipoprotein lipid, and 30 per cent of the β-lipoprotein lipid. Therefore a high proportion of β - to α-lipoprotein will be accompanied by a high proportion of cholesterol to phospholipid and vice versa.

As has been said in Chapter 111, page 42, the γ-lipoprotein lipid fraction is considered to correspond to the trail fraction of Smith (1957) and therefore to be part of the β-lipoprotein fraction separated as fraction 111-0 in the Cohn fractionation. It is likely to contain more triglyceride and less cholesterol and phospholipid than true β-lipoprotein lipid.

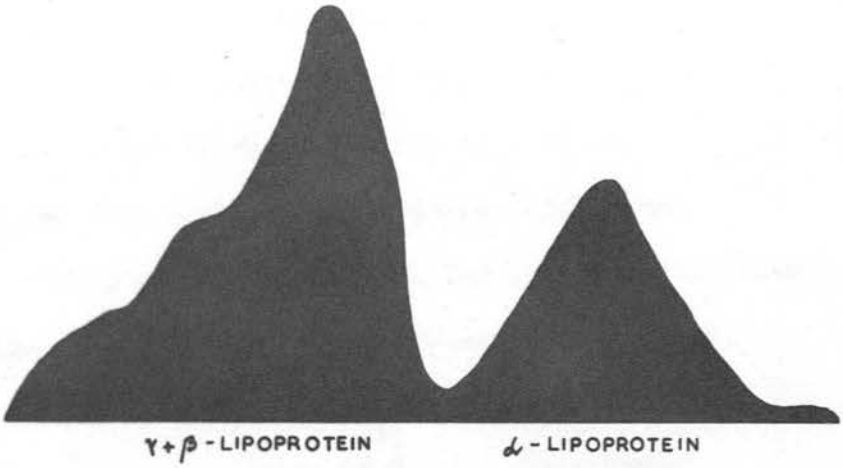
α-Lipoprotein Lipid and β-Lipoprotein Lipid.

The mean serum concentration of α-lipoprotein lipid in the umbilical cord blood is 141 $\pm$ 5 (50) mg./100 ml. and in maternal venous blood it is 253 $\pm$ 13 (20) mg./100 ml. The mean serum concentration of β-lipoprotein lipid in the two bloods are 96 $\pm$ 5 (50) mg./100ml. and 647 $\pm$ 29 (20) mg./100 ml. respectively. These concentrations correspond to44 per cent of α-lipoprotein lipid and 30 per cent of β-lipoprotein lipid in the cord blood and 24 per cent of α - lipoprotein

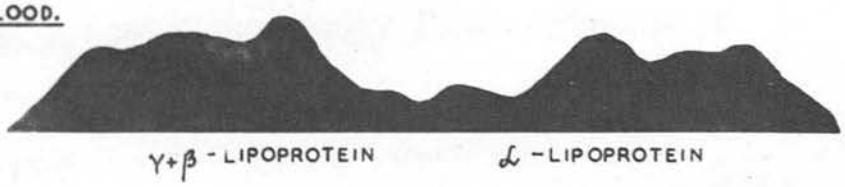
FIGURE 3

LIPOPROTEIN DISTRIBUTION CURVES FROM VARIOUS SOURCES.

HUMAN ADULT BLOOD.



HUMAN CORD BLOOD.



RAT BLOOD [TAKEN FROM MORRIS AND COURTICE, 1955.]

lipid and 59 per cent of β-lipoprotein lipid in the maternal blood.

The Ratio of β- to α-Lipoprotein Lipid.

The mean ratio of the concentration of β- to α-lipoprotein lipid is 0.76 $\pm$ 0.06 (50) in umbilical cord blood and 2.77 $\pm$ 0.21 (21) in maternal venous blood. The difference is highly significant ($p < 0.001$). The individual ratios do not in fact, conform to a normal distribution curve when expressed as arithmetical values but do so when converted to logarithms. The arithmetical value of the logarithmic mean in cord blood is0.659 with a range, corresponding to one standard deviation, of 0.392 to 1.106. For maternal blood the mean value on the same basis is 2.575 with a range of 1.753 to 3.783.

Distribution of α- and β-Lipoprotein Lipid in Species Other than Man.

It is interesting that several animal species also have more α- than β-lipoprotein even when the animal is fully grown. Morris and Courtice (1955) found this in the dog, cat and rat. The lipoprotein curve they obtained by electrophoresis of rat plasma is remarkably similar to that obtained from human cord blood. Several examples of lipoprotein curves obtained from normal umbilical cord blood

in the present investigation are given in Figs. 2. They may be comared with similar curves obtained from maternal serum given in Figs. 2. A representative example of each and the curve obtained by Morris and Courtice (1955) from rat plasma are shown in Fig. 3. As will be seen, the same elision of β - and γ -lipoprotein occurs during electrophoresis of rat plasma and of human cord blood serum.

γ -Lipoprotein Lipid

The mean concentrations of γ -lipoprotein lipid in umbilical cord blood and in maternal venous blood are 82 ± 4 (50) mg./100 ml. and 188 ± 12 (20) mg./100 ml. respectively. The ratios of the aggregate value of ($\beta+\gamma$) lipoprotein lipid concentration to α -lipoprotein lipid concentration is 1.36 ± 0.1 (50) in cord blood and 3.0 ± 0.2 (20) in maternal venous blood. It should be noted that the γ -lipoprotein lipid fraction in maternal blood constitutes a very small proportion of the total lipid and no difficulty exists in distinguishing it from the β -lipoprotein fraction on the lipoprotein distribution curve.

Calculation of the Ratio of Cholesterol to Phospholipid from the Concentrations of the Three Lipoprotein Fractions

The composition of α -lipoprotein lipid is known. As has been said cholesterol contributes approximately

25 per cent of the total lipid and phospholipid approximately 50 per cent of the total. The data of Russ, Eder and Barr (1954) for the lipid content of fractions III-0 in the Cohn separation (See Chapter III) may be regarded as giving the composition of the (+)-lipoprotein lipid fraction. It contains approximately 20 per cent of cholesterol and phospholipid.

The following calculation may be made:

	Cord Blood	Maternal Blood
α-Lipoprotein lipid mg./100 ml.	141	253
($\beta+\gamma$)-Lipoprotein lipid mg./100 ml.	177	835
C/P	0.64	0.93

	Cholesterol mg%	Phospholipid mg%
α-Lipoprotein lipid	25	50
($\beta+\gamma$)-Lipoprotein lipid	20	20

Therefore in cord blood the ratio of cholesterol to phospholipid may be calculated as follows:

$$\frac{141 \times 25 + 177 \times 20}{141 \times 50 + 177 \times 20} = \frac{1417}{2122} = 0.67 \quad \text{(Observed value = 0.64)}$$

and in maternal blood

$$\frac{253 \times 25 + 835 \times 20}{235 \times 50 + 835 \times 20} = \frac{23025}{29350} = 0.78 \quad \text{(Observed value = 0.93)}$$

The calculated ratio of cholesterol to phospholipid concentrations in cord blood agrees well with the observed value. That calculated for maternal blood is somewhat

lower than expected. In view of the variability of the data this is not necessarily significant, but the possibility cannot be discounted altogether.

One may conclude from these calculations that the difference between the ratio of cholesterol to phospholipid concentrations in cord blood and in maternal blood is accounted for by the difference in the ratios of the concentrations of the lipoproteins. It may also be suggested that since the calculated ratio of cholesterol to phospholipid concentrations in maternal blood is lower than the observed value by an appreciable amount, maternal blood may contain more cholesterol than would be expected from a consideration of the relative concentrations of the lipoprotein fractions. Therefore the lipid composition of one or more of the lipoprotein fractions in maternal blood may not be the same as in cord blood. It has been suggested earlier in Chapter III that the $(\beta + \gamma)$-lipoprotein lipid fraction in maternal blood contains more cholesterol than the same fraction in cord blood.

Calculation of the Total Lipid Concentration from the Concentrations of the Individual Lipids

If one adds together the serum concentrations of cholesterol, phospholipid and that part of the total esterified fatty acid not associated with phospholipid,

(phospholipid is approximately 70 per cent esterified fatty acid), a value is obtained which should be similar to the observed total lipid concentration. In many cases the difference between the two values is far too great to be accounted for by experimental error and the larger observed total lipid concentrations are not necessarily associated with larger discrepancies in the calculated total lipid value. The calculated total lipid concentration for normal umbilical cord blood is 253 $\pm$6(50) mg./100 ml. compared with an observed value of 318 $\pm$ 9 (50) mg./100 ml. The difference between the two values is 64 $\pm$ 6 (50) mg./100 ml. Similar descrepancies exist in the data for maternal blood and have also been observed in data published by other investigators in this field. It was found impossible to begin an investigation into the source of the discrepancies but it would be of great interest to do so, especially in view of the variations in the mean differences between the two values in the groups of abnormal infants which will be referred to later.

2. THE INFANTS OF DIABETIC MOTHERS (18):
AND THEIR MOTHERS (12)

The data obtained for these infants are given in Table 14. The mean serum lipid and lipoprotein lipid concentrations in the umbilical cord blood, and the standard

errors of the means are given in Table 19. The significance of any deviation of the mean value from normality is also given in Table 19. Similar data for the diabetic mothers are given in Tables 15 and 20. Some of these data are also given in Table 27 in Chapter V which consists of serum lipid and lipoprotein lipid concentrations, not only in the cord blood of the infants of diabetic mothers, but also in their venous blood during the first week of life. Examples of the lipoprotein distribution curves obtained by electrophoresis of the cord blood serum are also given in Chapter V, Figs.

Total Lipid

The mean serum total lipid concentration in the umbilical cord blood of these infants is 297 ± 10 (18) mg./100 ml. Although this is below the mean value of 318 ± 9 (50) mg./100 ml. found in normal infants, the difference is not significant. The mean serum total lipid concentration in the venous blood of the diabetic mothers is 1154 ± 66 (12) mg./100 ml. This is higher that the concentration found in normal mothers but this difference is also not significant. An increase in serum lipid concentrations is characteristic of diabetes so a higher value is to be expected.

Total Esterified Fatty Acid

The mean total esterified fatty acid concentration in the umbilical cord blood of the infants of diabetic mothers is 142 ± 8 (18) mg./100 ml. In the maternal venous blood it is 500 ± 27 (12) mg./100 ml. These values are almost identical with the normal values which are 148 ± 5 (50) mg./100 ml. and 503 ± 33 (20) mg./100 ml. in the infants and mothers respectively. The ratios of total lipid concentrations to total esterified fatty acid concentrations are also normal.

Phospholipid

The mean concentration of phospholipid in the umbilical cord blood of the infants of diabetic mothers is 113 ± 6 (18) mg./100 ml. This is almost identical with the normal value of 115 ± 3 (50) mg./100 ml. and the ratio of total lipid to phospholipid concentrations is 2.6 in the infants of diabetic mothers and 2.8 in normal infants. The mean concentration of phospholipid in the venous blood of the diabetic mothers is 335 ± 18 (12) mg./100 ml. which is significantly higher than the value of 302 ± 9 (20) mg./100 ml. found in normal mothers. The ratio of the total lipid to phospholipid concentrations in the diabetic mothers, which is 3.4 is, however, of the same order as the ratio in normal mothers which is 3.7.

Cholesterol

The mean total cholesterol concentration in the umbilical cord blood of the infants of diabetic mothers is 84 ± 5 (18) mg./100 ml. This is significantly higher than the normal value of 72 ± 2 (50) mg./100 ml. ($0.02 > p > 0.01$). The mean total cholesterol concentration in the venous blood of the diabetic mothers is 312 ± 19 (12) mg./100 ml. This is very significantly higher than the concentration of 281 ± 14 (20) mg./100 ml. ($p < 0.001$). The ratio of total lipid to cholesterol concentrations in the infants of diabetic mothers is thus 3.5 compared with 4.4 in normal infants and 3.7 in diabetic mothers. The latter value is hardly different from the normal maternal value of 3.6. It will be noted that the value for this ratio in the infants of diabetic mothers is much closer to the two maternal values than it is to the one found in normal infants.

The Ratio of Cholesterol to Phospholipid Concentrations

The mean ratio of cholesterol to phospholipid concentrations in the umbilical cord blood of the infants of diabetic mothers is 0.77 ± 0.07 (18). This is significantly higher than the normal value of 0.64 ± 0.02 (50); ($0.05 > p > 0.02$). In the diabetic mothers on the other hand, the value for this ratio is 0.94 ± 0.03 (20). This is to be expected because the mean concentrations of both these lipids

are increased above normal values by approximately the same amount.

α-Lipoprotein Lipid, β-Lipoprotein Lipid and γ-Lipoprotein Lipid

1. In the Infants

The mean concentrations of the two main lipoprotein lipid fractions in the umbilical cord blood of the infants of diabetic mothers are almost identical with those found in normal infants. The concentration of α-lipoprotein lipid is 140 $\pm$ 7 (19) mg./100 ml. compared with a normal value of 141 $\pm$ 5 (50) mg./100 ml. That of β-lipoprotein lipid is 97 $\pm$ 7 (18) mg./100 ml. compared with 96 $\pm$ 6 (50) mg./100 ml. in the normal infants. The third fraction, γ-lipoprotein lipid, is significantly lower than normal. Its concentration is 61 $\pm$ 5 (18) mg./100 ml. in the infants of diabetic mothers and 82 $\pm$ 4 (50) mg./100 ml. in the normal infants; (0.01 p 0.001). It is this which accounts for the fact that although the total lipid concentration is below normal in the infants of diabetic mothers, the concentrations of α- and β-lipoprotein lipid remain normal. The mean aggregate concentration of (β+γ)-lipoprotein lipid which is 159 $\pm$ 8 (18) mg./100 ml. is significantly lower than the normal value of 177 $\pm$ 7 (50) mg./100 ml.

2. In the Mothers

In the diabetic mothers, the picture is quite different. The mean serum concentration of α-lipoprotein lipid is 243 $\pm$ 20 (12) mg./100 ml. This is slightly but not significantly lower than the normal maternal value of 253 $\pm$ 13 (20) mg./100 ml. But the concentrations of β- and γ-lipoprotein lipid which are 697 $\pm$ 45 (12) mg./100 ml. and 213 $\pm$ 16 (12) mg./100 ml. respectively are both significantly higher than the normal values of 67 $\pm$ 29 (20) mg./100 ml. and 188 $\pm$ 12 (20) mg./100 ml. In both cases $p < 0.001$.

The Ratio of β- to α-Lipoprotein Lipid

In the infants of diabetic mothers the ratio of β- to α-lipoprotein lipid concentrations in the cord blood is not different from normal. The ratio of the concentrations of $(\beta + \gamma)$- to α-lipoprotein lipid is slightly but not significantly lower than normal. In the diabetic mothers, the mean ratio of β- to α-lipoprotein lipid concentrations is 3.1 $\pm$ 0.3 (12) compared with a normal value of 2.8 $\pm$ 0.2 (20). This difference is not significant because the variation of the individual ratios is enormous.

Calculation of the Ratio of Cholesterol to Phospholipid from the Concentrations of the Three Lipoprotein Fractions

A theoretical value for the ratio of cholesterol to phospholipid concentrations in the infants of diabetic

mothers and in the mothers themselves may be calculated from the concentrations of the lipoprotein lipid fractions in the same way as has been done for the normal group if one assumes that the relative proportions of cholesterol and phospholipid have not altered. The results are given in Table 21 below with those for the normal group for comparison.

TABLE 21

	Calculated C/P	Observed C/P	(Observed - Calculated)
Infants of diabetic mothers	0.66	0.77±0.07(18)	-0.11
Normal Infants	0.67	0.64±0.02(50)	+0.03
Diabetic Mothers	0.80	0.94±0.08(12)	-0.14
Normal Mothers	0.78	0.93±0.04(20)	-0.15

It will be observed that there is a greater discrepancy between the calculated and observed values for the infants of diabetic mothers than for normal infants. It is of the same order as the discrepancy observed for the diabetic and normal mothers. One may conclude from this that there is more similarity between the lipid composition of the lipoprotein lipid fractions in the normal mothers, the diabetic mothers, and the infants of diabetic mothers, than between the latter and normal infants. Since the calculated ratio is lower than the observed ratio in both

groups of mothers and in the infants of diabetic mothers, the lipoprotein may be carrying more cholesterol than would be expected if the normal lipid composition of the lipoproteins was maintained. This will be referred to again in the discussion.

Calculation of the Total Lipid Concentration from the Concentrations of the Individual Lipids

Whereas the calculated ratio of the mean cholesterol to phospholipid concentrations for the infants of diabetic mothers is further from the experimental value than it is in normal infants, the mean calculated total lipid concentration is closer to the observed value than in any other group of infants. The mean calculated total lipid concentrations are 262 $\pm$ 12 (17) mg./100 ml. and 253 $\pm$ 6 (50) mg./100 ml. for the infants of diabetic mothers and for normal infants respectively. The observed values are 297 $\pm$ 10 (18) mg./100 ml. and 318 $\pm$ 9 (50) mg./100 ml. in the same order. The mean differences are thus 35 $\pm$ 6 (17) mg./100 ml. for the infants of diabetic mothers and 65 $\pm$ 6 (50) mg./100ml. for the normal infants. The difference between these two values is highly significant so that the discrepancies between the calculated and observed total lipid concentrations are not a result of cumulative experimental errors alone. It would be of the greatest interest to find an explanation for this.

3. INFANTS WHOSE MOTHERS HAD HAD AN ABORTION IN A PREVIOUS PREGNANCY OR A THREATENED ABORTION IN THIS PREGNANCY: (19)

The data obtained for these infants are given in Table 16. The mean serum concentrations of the lipids and lipoprotein lipids in their umbilical cord blood are given in Table 19, with the standard errors and the significance of any deviation from normality. Examples of the lipoprotein distribution curves obtained by electrophoresis are given in Figs.4.

Total Lipid

The mean serum concentration of total lipid in the umbilical cord blood of these infants is 395 ± 24 (19) mg./100 ml. This is above the value of 318 ± 9 (50) mg./100 ml. found in normal infants and the difference is highly significant; $(0.01 > p > 0.001)$.

Total Esterified Fatty Acid

The mean concentration of total esterified fatty acid in the umbilical cord blood of these infants is 168 ± 9 (19) mg./100 ml. Although this is above the normal value of 148 ± 5 (50) mg./100 ml. the difference is not significant. The ratio of total lipid to total esterified fatty acid concentrations is 2.4 which is of the same order as the normal value of 2.2.

Phospholipid

The mean phospholipid concentration in the umbilical cord blood of these infants is 130 ± 7 (19) mg./100 ml. The difference between this and the normal value of 115 ± 3 (50) mg./100 ml. is not highly significant; ($0.05 > p > 0.02$). The ratio of the mean concentration of total lipid to that of phospholipid is 3.1 which is of the same order as the normal value of 2.8.

Cholesterol

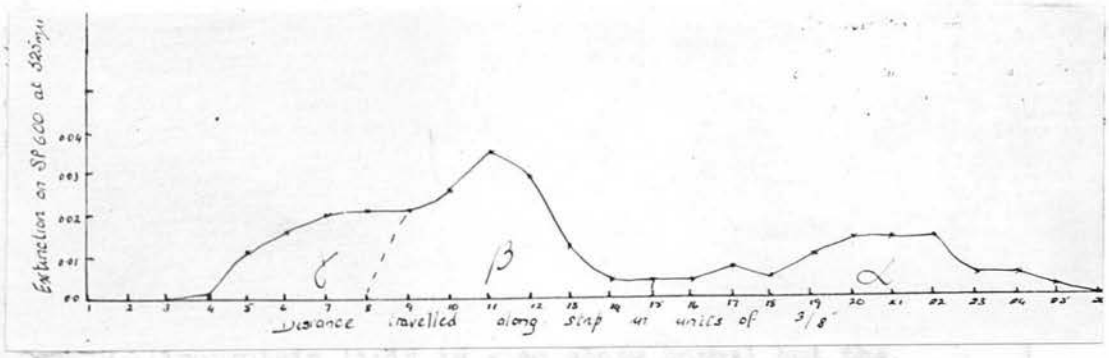
The mean concentration of cholesterol in the umbilical cord blood of these infants is 91 ± 5 (19) mg./100 ml. This is approximately 35 per cent greater than the normal value of 72 ± 2 (50) mg./100 ml. and the difference is highly significant; ($p < 0.001$). The ratio of the mean total lipid concentrations to that of cholesterol is 4.3 which is almost identical with the normal value of 4.4.

Ratio of Cholesterol to Phospholipid Concentrations

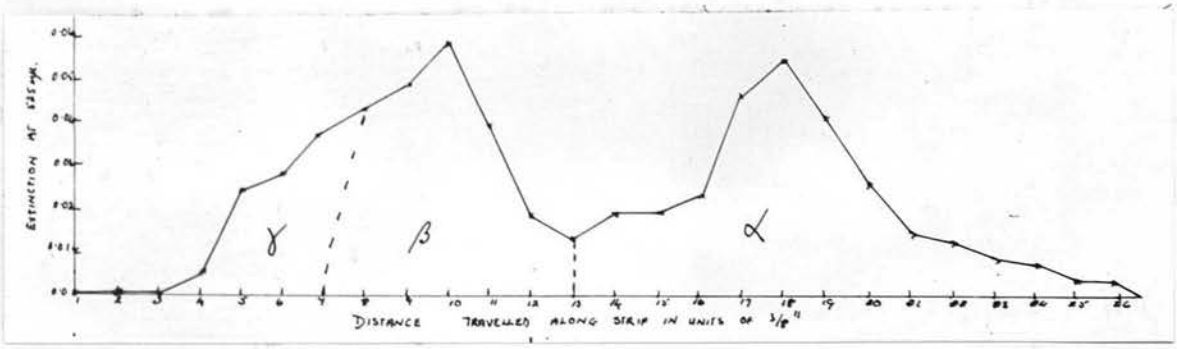
The mean ratio of the concentrations of cholesterol to phospholipid in the umbilical cord blood of these infants is 0.71 ± 0.03 (19) which is not significantly different from the normal value of 0.64 ± 0.02 (50). This is due to the fact that both lipid concentrations are increased to approximately the same extent above normal although the increase in cholesterol concentration is slightly greater

Figure 4

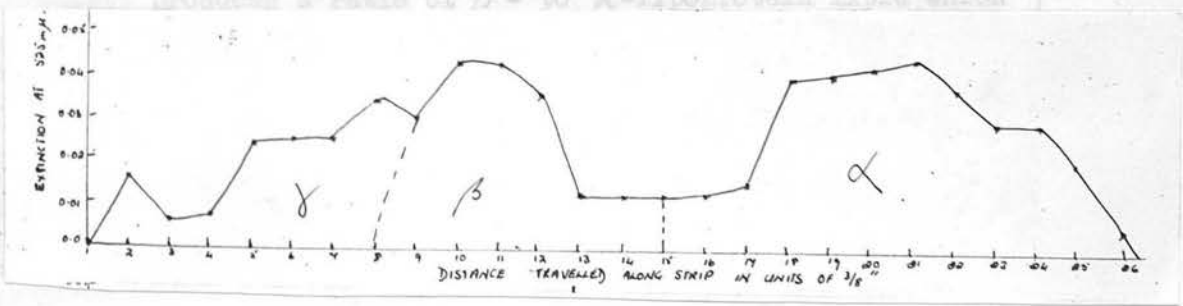
Serum Lipoprotein Curves Obtained by the Electrophoretic Separation of the Lipoproteins in the Cord Blood of Infants Whose Mothers Had Had a Previous Abortion or Stillbirth or a Threatened Abortion in This Pregnancy.



a. Baby Cr.



b. Baby McW.



c. Baby Du.

than that of phospholipid.

α -Lipoprotein Lipid, β -Lipoprotein Lipid and γ-Lipoprotein Lipid

The significant increase in both cholesterol and phospholipid concentrations in these infants is accompanied by a significant increase in the concentrations of β-lipoprotein lipid. The mean concentration of this fraction in the umbilical cord blood of these infants is 147 ± 12 (17) mg./100 ml. compared with a normal value of 96 ± 5 (50) mg./100 ml. ($p < 0.001$). The mean concentration of α-lipoprotein lipid is also above normal but the difference is not significant. It is 160 ± 12 (17) mg./100 ml. compared with the normal value of 141 ± 5 (50)mg./100 ml. The mean concentration of γ-lipoprotein lipid is 74 ± 7 (17) mg./100 ml. which is very slightly lower than the normal value of 82 ± 4 (50) mg./100 ml. The difference is obviously not significant.

The Ratio of β - to α -Lipoprotein Lipid

The far greater increase in the concentration of β-lipoprotein lipid compared with that of α-lipoprotein lipid produces a ratio of β - to α-lipoprotein lipid which is significantly higher than normal. The mean value for the ratio in the infants of mothers who have had a previous abortion is 0.99 ± 0.09 (17) compared with the normal value of 0.76 ± 0.06 (50); (0.05 p 0.02).

The increase in the mean concentration of β-lipoprotein lipid is great enough to produce a highly significant increase in the mean $(\beta+\gamma)$-lipoprotein lipid concentration compared with the normal value, despite the decrease in the mean γ-lipoprotein lipid concentration. The aggregate value is 221 $\pm$ 15 (17) mg./100 ml. compared with a normal value of 177 $\pm$ 7 (50) mg./100 ml.; $(0.01 > p > 0.001)$. The ratio of $(\beta+\gamma)$-lipoprotein lipid to α-lipoprotein lipid concentrations is not, however, significantly different from normal.

Calculation of the Ratio of Cholesterol to Phospholipid from the Concentration of the Three Lipoprotein Fractions

If the ratio of cholesterol to phospholipid is calculated as before, from the relative proportions of the lipoprotein lipid fractions, a value of 0.68 is obtained. The observed value is 0.71. These are of the same order and do not suggest any abnormality in the lipid constitution of the lipoproteins.

Calculation of the Total Lipid Concentration from the Concentrations of the Individual Lipids

Like the observed total lipid concentration in these infants, the calculated concentration, which is 301 $\pm$ 18 (18) mg./100 ml., is significantly higher than the normal value of 253 $\pm$ 6 (50) mg./100 ml.; $(0.01 > p > 0.001)$. The difference between the observed and calculated values is

95 ± 10 (18) mg./100 ml. which is greater than in any other group of infants.

4. INFANTS WHOSE MOTHERS HAD SUFFERED FROM TOXAEMIA OF PREGNANCY OR HYPERTENSION OR FROM BOTH DISEASES (30)

The data obtained from these infants are given in Table 17. The mean serum concentrations of the lipids and lipoprotein lipids in their umbilical cord blood are given in Table 19, with the standard errors of the means and the significance of any deviation from normality. No illustration of the lipoprotein distribution curves obtained by electrophoresis have been given for these infants because they are indistinguishable from normal ones.

Total Lipid

The mean concentration of total lipid in the umbilical cord blood of these infants is 297 ± 10 (30) mg./100 ml. This is slightly lower than the normal value of 318 ± 9 (50) mg./100 ml. but it is not significantly different from it.

Total Esterified Fatty Acid

The mean concentration of total esterified fatty acid in the umbilical cord blood of the infants of toxaemic mothers is 123 ± 6 (30) mg./100 ml. This is highly significantly lower than the normal value of 148 ± 5 (50) mg./100 ml. ($0.01 > p > 0.001$). However, the ratio of total lipid

concentration to that of total esterified fatty acid remains normal.

Phospholipid

The mean concentration of phospholipid in the umbilical cord blood of these infants is 106 ± 4 (30) mg./100 ml. This is significantly below the normal value of 115 ± 3 (50) mg./100 ml., ($0.05 > p > 0.02$). The ratio of total lipid concentration to that of phospholipid is, however, perfectly normal because both values are decreased.

Cholesterol

The mean concentration of cholesterol in the umbilical cord blood of these infants is 62 ± 2 (30) mg./100 ml. compared with the normal value of 72 ± 2 (50) mg./100 ml. The difference is highly significant; ($0.01 > p > 0.001$). The ratio of the total lipid concentrations to that of cholesterol is 4.9 compared with the normal value of 4.4. That is, the decrease in cholesterol concentrations compared with normal values is slightly greater than might normally be expected to occur as a result of such small reduction in the total lipid concentration.

The Ratio of Cholesterol to Phospholipid Concentrations

Although the reduction in the cholesterol concentration is greater, compared with normal, than that in the

phospholipid concentration, the ratio of the two concentrations which is 0.60 ± 0.03 (30), is not significantly below the normal value of 0.64 ± 0.02 (50).

α-Lipoprotein Lipid, β-Lipoprotein Lipid and γ-Lipoprotein Lipid

The reductions in the concentrations of esterified fatty acid, cholesterol and phospholipid compared with normal values are accompanied by small decreases in the concentrations in the three lipoprotein lipid fractions. The mean concentrations of α-, β- and γ-lipoprotein lipid in the umbilical cord blood of these infants are 136 ± 7, 92 ± 6, and 76 ± 4 (27) mg./100 ml. respectively. These values may be compared with the normal values of 141 ± 5, 96 ± 5, and 92 ± 4 (50) mg./100 ml. in the same order. None of these differences is significant but the small reductions in the concentrations of β- and γ-lipoprotein lipid are sufficient to reduce the aggregate (β+γ)-lipoprotein lipid concentrations to 163 ± 7 (27) mg./100 ml. which is highly significantly below the normal value of 177 ± 7 (50) mg./100 ml. ($p < 0.001$).

The Ratio of - to -lipoprotein Lipid

Neither the ratio of β- to α-lipoprotein lipid nor that of (β+γ)- to α-lipoprotein lipid, which are 0.74 ± 0.06 and 1.30 ± 0.1 (27) respectively, are significantly different from the normal values of 0.76 ± 0.06 and 1.36 ± 0.1 (50).

Calculation of the Ratio of Cholesterol to Phospholipid from the Concentrations of the Three Lipoprotein Lipid Fractions

If the ratio of cholesterol to phospholipid concentrations is calculated as before, from the relative proportions of α-, β-, and γ-lipoprotein lipid, a value of 0.66 is obtained. The observed value is 0.60 $\pm$ 0.03(30). The difference between the two values is of the same order as, but slightly greater than that between the two normal values for this ratio. The latter are 0.67 for the observed value. The difference between the two values is insufficiently great to allow one to conclude that there is any difference between the lipid composition of the lipoprotein lipid fractions in the normal infants and in the infants of toxaemic mothers.

Calculation of the Total Lipid Concentration from the Concentration of the Individual Lipids

The calculated total lipid concentration in the infants of toxaemic mothers is 217 $\pm$ 7 (30)mg./100 ml. The observed value is 297 $\pm$ 10 (30) mg./100 ml. The mean difference between the two values if 81 $\pm$ 7 (30) mg./100 ml. Although the observed total lipid concentration is the same as that found in the infants of diabetic mothers, the difference between the observed and calculated values is more than twice as great.

5. INFANTS WHO WERE ABNORMAL AT BIRTH OR WHOSE MOTHERS SHOWED ABNORMALITIES DURING PREGNANCY OTHER THAN THOSE IN GROUPS 2, 3 AND 4

The number of cases in each section of this group is frequently so small that a statistical analysis of the data would be meaningless. Some are included because they illustrate combinations of the abnormalities detailed in Groups 2,3 and 4, others because they are of individual interest; others again because they illustrate abnormalities existing in some of the cases in Groups 2, 3 and 4 but subsidiarily to the group abnormality. The data are given in Table 18 and some of the lipoprotein curves obtained from these cases are given in Figs. 5.

a) Infants of Mother with Rhesus Incompatibility

The majority of these infants showed no differences from normal in their umbilical cord blood lipid and lipoprotein lipid concentrations. The exceptions are one infant who was iso-immunized and required exchange transfusion, and one premature infant who was delivered after a prolonged labour.

The infant who required exchange transfusion had entirely normal cord blood concentrations of total lipid, total esterified fatty acid and phospholipid but a low cholesterol concentration and such abnormal lipoprotein

lipid distribution that the ratio of β- to α-lipoprotein lipid was 2.35 compared with the normal value of 0.76. This can only be compared with the value for the ratio found in maternal venous blood, which is 2.77 $\pm$ 0.2. The concentration of β-lipoprotein lipid was 154 mg./100 ml. and that of α-lipoprotein lipid was 65 mg./100 ml. compared with normal values of 96 $\pm$ 5 and 141 $\pm$ 5 mg./100 ml. respectively. The concentration of γ-lipoprotein lipid was normal.

The premature infant is distinguished only by having a very low cord blood cholesterol concentration, and a normal phospholipid concentration. This produces a very low ratio of cholesterol to phospholipid concentration - 0.40 compared with a normal value of 0.64 $\pm$ 0.02 (50).

b) Infants of Severely Anaemic Mothers (6 cases)

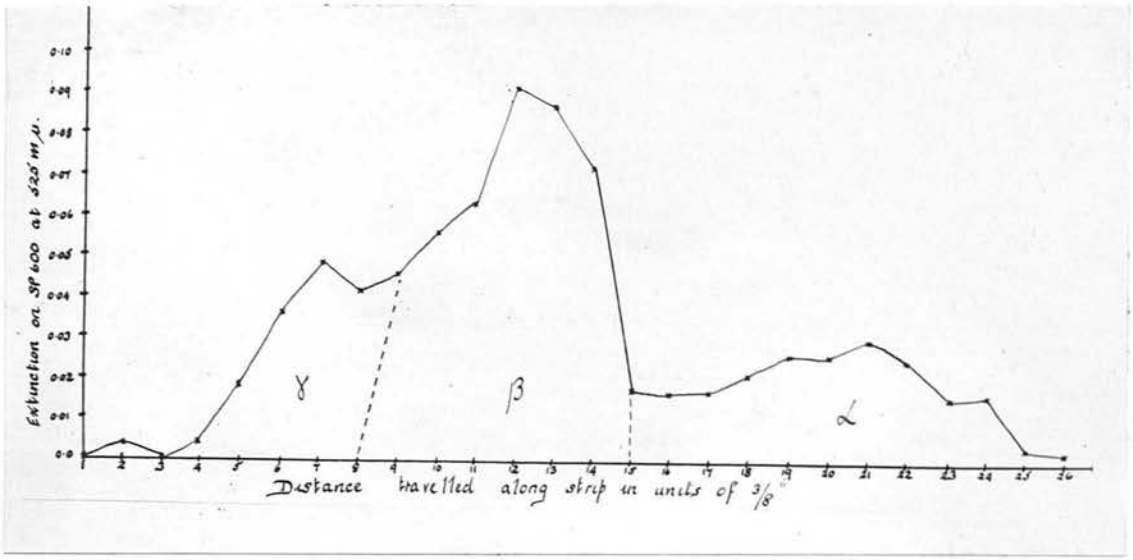
The cord blood lipid and lipoprotein lipid concentrations showed no difference from normality.

c) Infants of Mothers who have Cardiac Disease of Grade II Severity (2 cases)

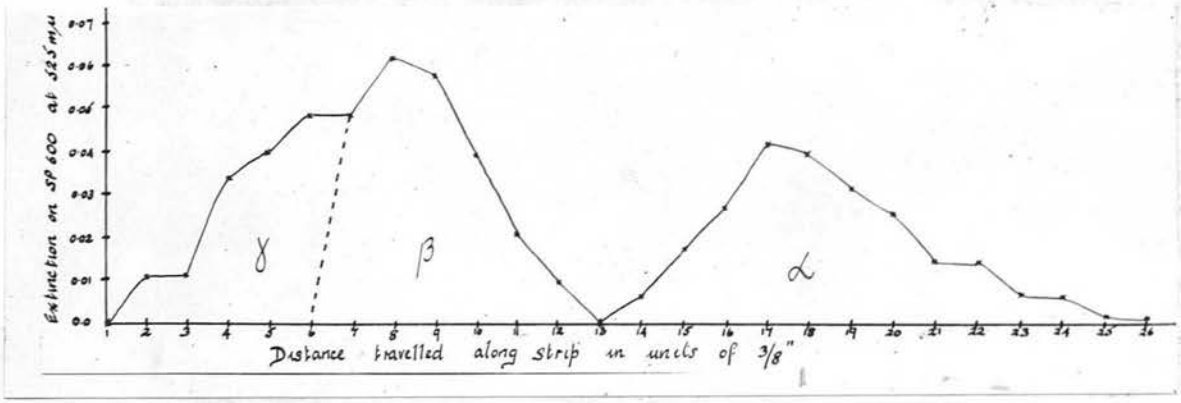
Of these two cases one showed no difference from normality. The other had raised concentrations of all the cord blood lipids and lipoprotein lipids but a normal ratio of β- to α-lipoprotein lipid. That is, the lipoprotein distribution was normal.

Figure 5

Serum Lipoprotein Curves Obtained by the Electrophoretic Separation of the Lipoproteins in the Cord Blood of Infants Suffering From Abnormalities Too Diverse to be Classified.

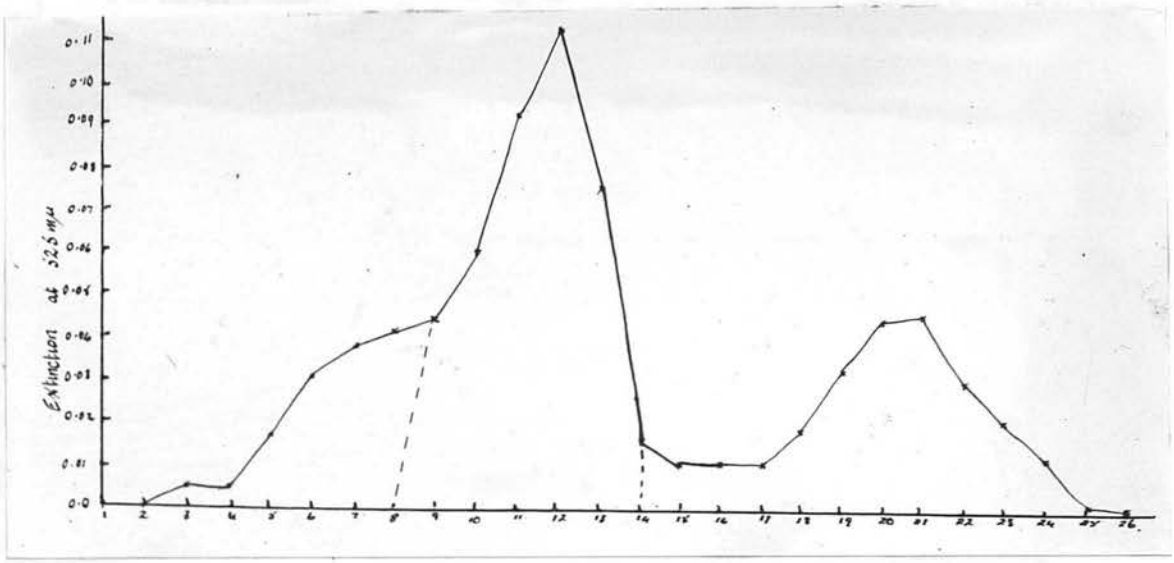


a. Baby Bl. : Hydrocephalus; Mother Alkaptonuric.

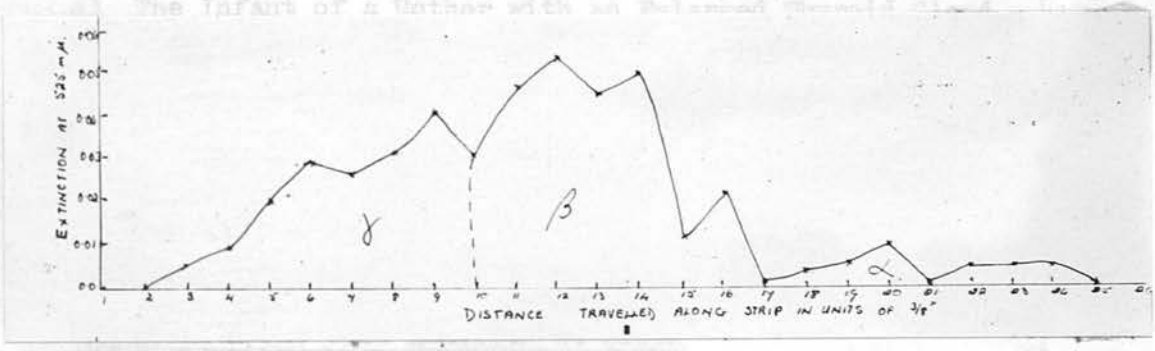


b. Baby McK. : Cerebral Irritation with Convulsions.

Figure 5 (cont.)

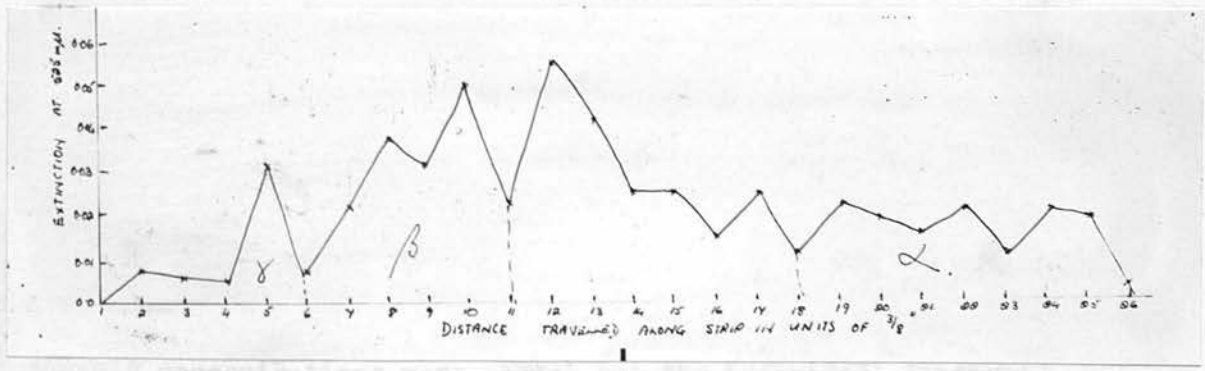


c. Baby McH. : Stillborn; Spina Bifida.



d (i) Baby Ba. Cord Blood. Post-mature. Died at 5 Days.

Cerebral Anoxia Diagnosed.



d (ii) Baby Ba. Post - mortem Heart Blood.

d) One Infant of a Progesterone Deficient Mother who had been treated with an Oral Progestin for the First Three Months of Gestation

This infant had a high cord blood total lipid concentration but normal concentrations of phospholipid and total esterified fatty acid and a low cholesterol concentration. The discrepancy between the experimental and calculated total lipid concentration was very high. The lipoprotein distribution was normal.

e) The Infant of a Mother with an Enlarged Thyroid Gland and Hypertension

In this infant's cord blood all the lipid concentrations were high but the lipoprotein distribution remained normal.

f) Infants who were Abnormal at Birth

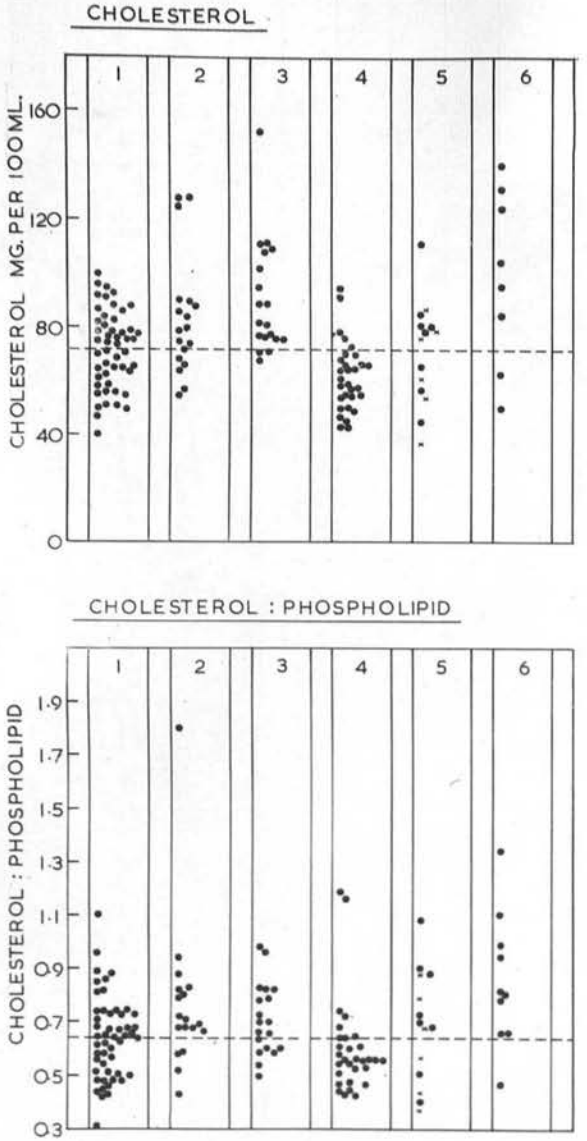
One of these cases (Baby McH) was a stillborn spina bifida. The foetus was macerated and it is assumed that the placental barrier no longer existed because all the cord blood lipid and lipoprotein lipid concentrations are those expected in an adult.

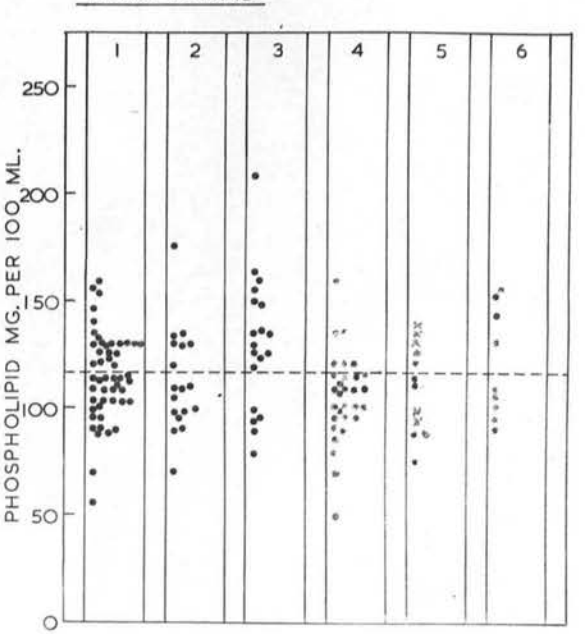
One of these cases (Baby Ba) died when five days old. The post mortem examination found no definite lesion but supported a diagnosis of cerebral anoxia. Both umbilical cord blood and post mortem heart blood were analysed. The lipid concentrations were normal but the individual lipoprotein lipid concentrations were not. The concentration

of α-lipoprotein lipid was very low and the ratio of β- to α-lipoprotein lipid concentrations was 4.7 compared with the normal value of 0.76. The ratio of the aggregate ($\beta+\gamma$)-lipoprotein lipid to α-lipoprotein lipid was 8.3 compared with the normal value of 1.36. The actual lipoprotein distribution curve was abnormal on inspection. A double peak appearing in the γ- to β-lipoprotein area in the cord blood distribution curve was intensified in the curve obtained from the post mortem heart blood. This is illustrated in Fig. 5d:i&ii. The double peak could be produced by the existence of the so-called "pre-beta lipoprotein" (Smith 1957). This lipoprotein is considered to be equivalent to the Sf 20 to 30 group of lipoproteins and has been found to occur in the blood of adults after a myocardial infarction. (Ibid).

The infant was born to a healthy prima gravid woman aftwe a normal pregnancy but it was slightly post mature by date. Post maturity accompanied by placental insufficiency was confirmed in the opinion of the paediatrician, by the very dry cracked skin and the infrequent cogwheel respiration which developed on the second day.

Of the remaining four abnormal infants, all are now perfectly normal. They were all found to have elevated cord blood total lipid and cholesterol concentrations and





1 – NORMAL NORMAL MEAN ---
2 – DIABETES
3 – ABORTIONS
4 – TOXAEMIA
5 – RHESUS INCOMPATIBILITY AND ANAEMIA
6 – OTHER ABNORMALITIES

CONCENTRATIONS OF
CHOLESTEROL AND PHOSPHOLIPID
IN UMBILICAL CORD BLOOD

FIGURE 9

CONCENTRATIONS OF
TOTAL LIPID AND TOTAL ESTERIFIED FATTY ACID IN UMBILICAL CORD BLOOD

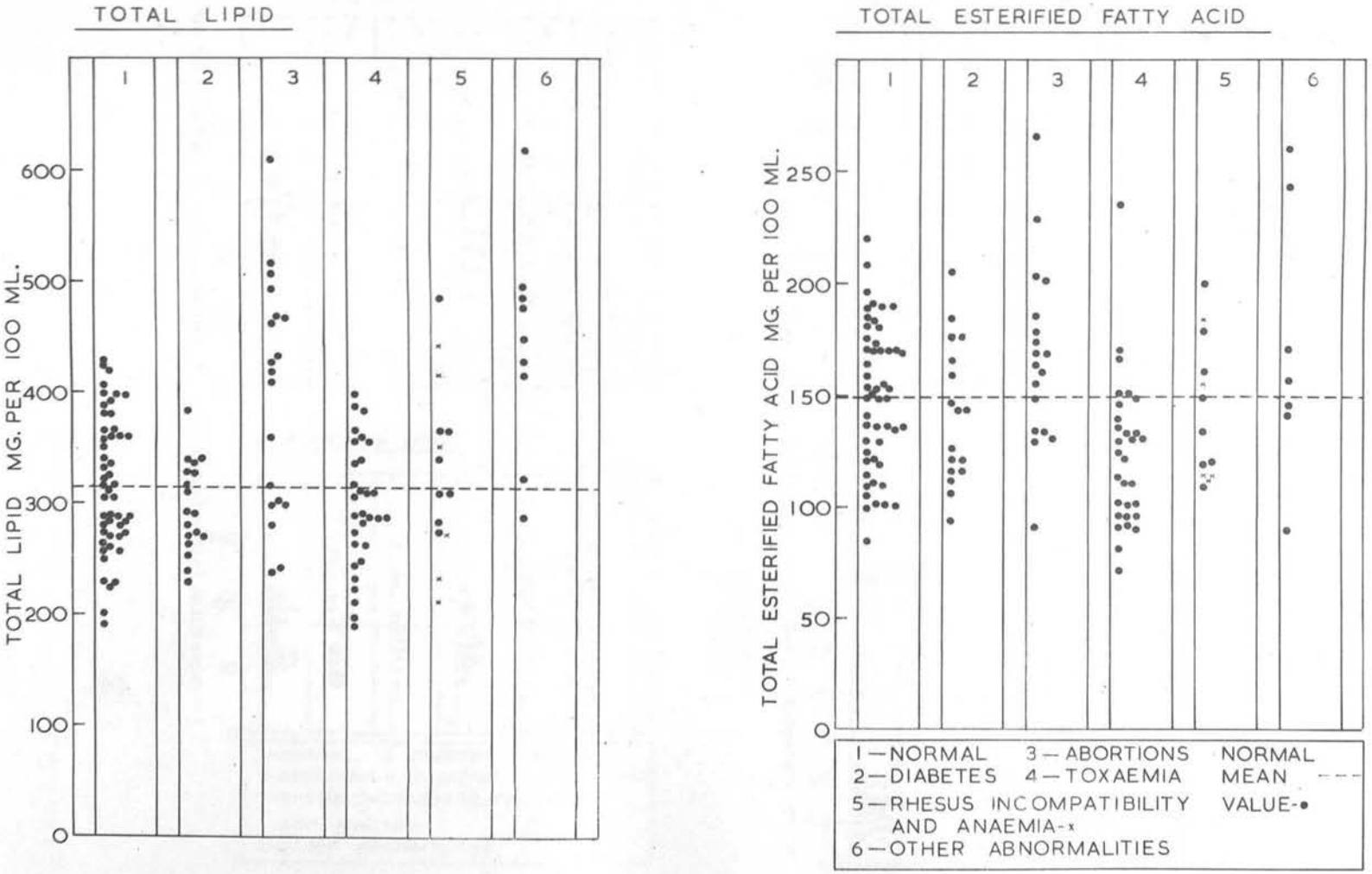


FIGURE 7

CONCENTRATIONS OF α– LIPOPROTEIN LIPID,
β-LIPOPROTEIN LIPID AND γ-LIPOPROTEIN LIPID IN UMBILICAL CORD BLOOD.

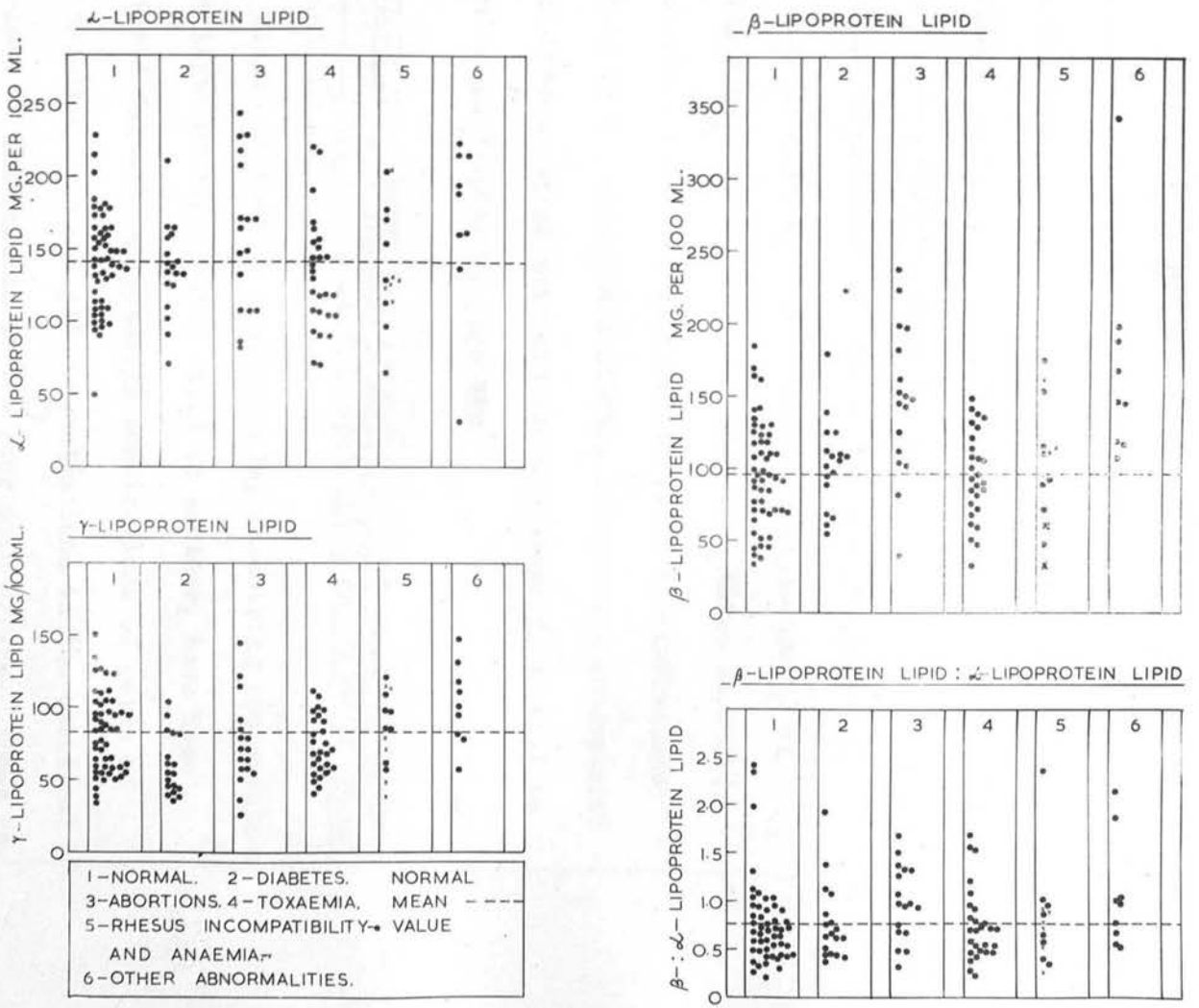


FIGURE 8

relatively normal phospholipid concentrations. Only one had a normal lipoprotein distribution curve, the other three having higher β-lipoprotein lipid concentrations than normal and ratios of β- to α-lipoprotein lipid concentrations which were greater than 1.0 compared with the normal value of 0.76. The least abnormal of the four (Baby Na) was reported only to be slightly jaundiced and to twitch occasionally. The most abnormal (Baby Bl) was hydrocephalic and his mother is alkaptonuric. A sibling died from "cerebral anoxia" when three days old. The abnormal lipid and lipoprotein lipid concentrations existing in the cord blood of this infant were accompanied by an abnormal amino acid excretion pattern which will be described in Chapter 7, page 183.

RELATIONSHIPS BETWEEN THE CONCENTRATIONS OF PAIRS OF LIPIDS AND LIPOPROTEIN LIPIDS IN THE UMBILICAL CORD BLOOD OF NORMAL AND ABNORMAL INFANTS

Simple regression coefficients, describing the relation of one lipid or lipoprotein lipid to another, have been calculated for all the possible combinations of pairs of lipid and lipoprotein lipid concentrations in the umbilical cord blood of the infants in the four main groups. That is, for

1. Normal infants;
2. Infants of diabetic mothers;

3. Infants of mothers who have had a previous abortion;

4. Infants of toxaemic mothers.

The significance of the regression coefficient was calculated in each case.

Since it is impossible to distinguish between the dependent and the independent variable, both possible regression coefficients have been calculated. In no case did these give the same regression line when graphs were constructed of the concentrations of one component against another. The geometrical means of the regression coefficients i.e. the correlation coefficients, were also calculated but did not contribute to a successful analysis of the data. In the following discussion, only one regression coefficient is used in each case.

Table 22 gives the regression coefficients, their standard errors and the significance of their difference from zero, for each pair of lipid or lipoprotein lipid concentrations. Table 23 is abstracted from Table 22 and includes only those regression coefficients which are of interest.

Examples of scatter diagrams showing the relations between pairs of variables are given in Figs. 9-12. They are composite diagrams, showing the relationships in all four groups of infants. The normal regression lines are

TABLE 22

Data Obtained by Statistical Analysis: The Coefficients of Regression of the Concentrations of One Serum Lipid or Lipoprotein Lipid upon those of Another in the Cord Blood of Various Groups of Infants.

Correlation		50 Normal Infants				19 Infants of Mothers who have had a Previous Abortion				18 Infants of Diabetic Mothers				30 Infants of Mothers with Toxaemia of Pregnancy			
x	y	A	b	*	S.E.of b	A	b	*	S.E.of b	A	b	*	S.E. of b	A	b	*	S.E. of b
Chol	TL	231.20	1.2037	S	0.5800	87.76	3.3909	S	0.8344	203.96	1.1072	S	0.3668	150.20	2.3898	S	-
TL	Chol	50.09	0.0684	S	0.0316	34.91	0.1411	S	0.0346	-13.52	6.3280	S	0.1086	24.88	0.1232	S	0.0359
Chol	TEFA	67.95	0.1182	S	0.2978	94.29	0.8050	-	0.4265	101.25	0.4905	-	0.3360	-			-
TEFA	Chol	41.72	0.2031	S	0.0540	53.28	0.2262	-	0.1190	47.51	0.2536	-	0.1731	-	0.0877	-	-
Chol	PL	83.53	0.4412	S	0.0931	45.31	0.9357	S	0.2665	97.44	0.2480	-	0.2576	64.99	0.2601	S	0.1080
PL	Chol	46.44	0.2203	S	0.0969	34.16	0.4343	S	0.1232	58.96	0.2208	-	0.2300	34.02	0.2600	S	-
TL	TEFA	51.22	0.3055	S	0.0671	55.48	0.2803	S	0.0641	-27.47	0.5765	-	-	-	0.1620	-	0.1063
TEFA	TL	172.90	0.9764	S	0.2161	92.24	1.8376	S	0.4458	158.98	0.9522	-	-	-		-	-
TL	PL	70.05	0.1422	S	0.0436	28.42	0.2574	S	0.0361	28.76	0.2827	S	0.1256	77.02	0.1072	-	0.0653
PL	TL	173.74	1.2491	S	0.3879	21.40	2.8724	S	0.4134	200.98	0.8503	S	0.3779	210.90	0.8193	-	-
PL	TEFA	66.28	0.7115	S	0.2162	50.78	0.8868	S	0.2434	65.71	0.6752	S	0.2685	116.30	-0.0877	-	-
TEFA	PL	76.85	0.2582	S	0.0786	46.12	0.5114	S	0.1400	58.48	0.3861	S	0.1679	99.00	-0.2295	-	-
Chol	$\beta+\gamma$-LP	69.89	1.4800	S	0.4620	68.26	1.6933	S	0.6230	65.76	1.0862	S	0.2849	96.10	0.9191	-	-
$\beta+\gamma$-LP	Chol	50.77	0.1191	S	0.0374	47.17	0.1948	S	0.0492	13.57	0.4532	S	0.1190	41.43	0.0971	-	-
PL	$\beta+\gamma$-LP	44.78	1.1482	S	0.3240	24.77	1.5814	S	0.5107	77.12	0.7171	S	0.3276	91.64	0.6332	-	0.3620
$\beta+\gamma$-LP	PL	83.24	0.1806	S	0.0424	69.61	0.2466	S	0.0804	60.40	0.3376	S	0.1542	76.14	0.1723	-	0.0984
TEFA	$\beta+\gamma$-LP	72.10	0.7081	S	0.1943	52.42	1.0939	S	0.4076	83.66	0.5343	S	0.2430	-	-0.0836	-	-
$\beta+\gamma$-LP	TEFA	94.04	0.3062	S	0.0837	88.90	0.3106	S	0.1157	67.20	0.4779	S	0.2183	-		-	-
Chol	α-LP	108.26	0.4556	-	0.3390	33.28	1.4024	S	0.4560	136.64	0.0366	-	0.3260	44.20	1.3973	S	0.5300
α-LP	Chol	55.91	0.0796	-	0.0590	51.66	0.2444	S	0.0874	82.20	0.0230	-	0.2044	47.89	0.1556	S	-
PL	α-LP	87.37	0.4451	-	0.2435	68.20	1.4808	S	0.3687	103.38	0.1026	-	0.3060	95.98	0.2774	-	-
α-LP	PL	93.65	0.1500	-	0.0820	23.96	0.3498	S	0.0860	44.78	0.0726	-	0.2165	84.18	0.0796	-	-
TEFA	α-LP	112.54	0.1920	-	0.1445	61.89	0.6103	-	0.4049	87.21	0.3346	-	0.2321	62.93	0.5958	S	0.1946
α-LP	TEFA	122.24	0.1846	-	0.1380	122.90	0.2288	-	0.1510	44.52	0.7343	-	0.3439	59.89	0.4577	S	-
Chol	TL-Chol	59.06	1.1693	-	-	87.76	2.2391	S	-	198.70	0.1072	-	-	172.79	0.1172	-	-
TL-Chol	Chol	53.30	0.0762	-	0.0352	-29.72	0.3953	S	0.0231	73.28	0.0496	-	0.1695	45.49	0.0662	-	0.0443
PL	TL-PL	175.73	0.2491	-	-	21.40	1.8724	S	0.0[illegible]	200.97	-0.1496	-	-	266.49	-0.6008	-	-
TL-PL	PL	108.32	0.0341	-	0.0533	52.74	0.2921	S	0.0645	124.60	-0.0649	-	0.1640	121.13	-0.0791	-	0.0637
TEFA	TL-TEFA	125.65	0.3032	-	-	118.38	0.6819	-	-	158.97	-0.0478	-	-	239.21	- 0.5307	-	-
TL-TEFA	TEFA	126.98	0.1247	-	0.0908	126.65	0.1765	-	0.1191	152.02	-0.0639	-	0.2980	154.24	-0.1782	-	0.1798

KEY:

- Chol - Cholesterol
- TL - Total Lipid
- TEFA - Total Esterified Fatty Acid
- PL - Phospholipid
- $\beta+\gamma$-LP - $\beta+\gamma$-Lipoprotein Lipid
- α-LP - α-Lipoprotein Lipid
- TL-Chol. - Total Lipid mimus cholesterol
- TL-TEFA - Total lipid minus TEFA
- TL-PL - Total Lipid mimus Phospholipid
- * - S represents positive significance of correlation

given in each diagram. The regression lines for each group of abnormal infants are superimposed on the diagram if the regression coefficient is significant. No correlation is described as significant unless the p value is less than 0.05. Fig.13. is an example of the kind of diagram obtained when there is no relationship between the two variables. In Fig.9&11, the normal 50 per cent fiducial limits have been superimposed upon the diagrams, i.e. the boundaries within which 50 per cent of the normal values are expected to lie. This is to illustrate that although the values are very variable, they still conform to the normal statistical distributions.

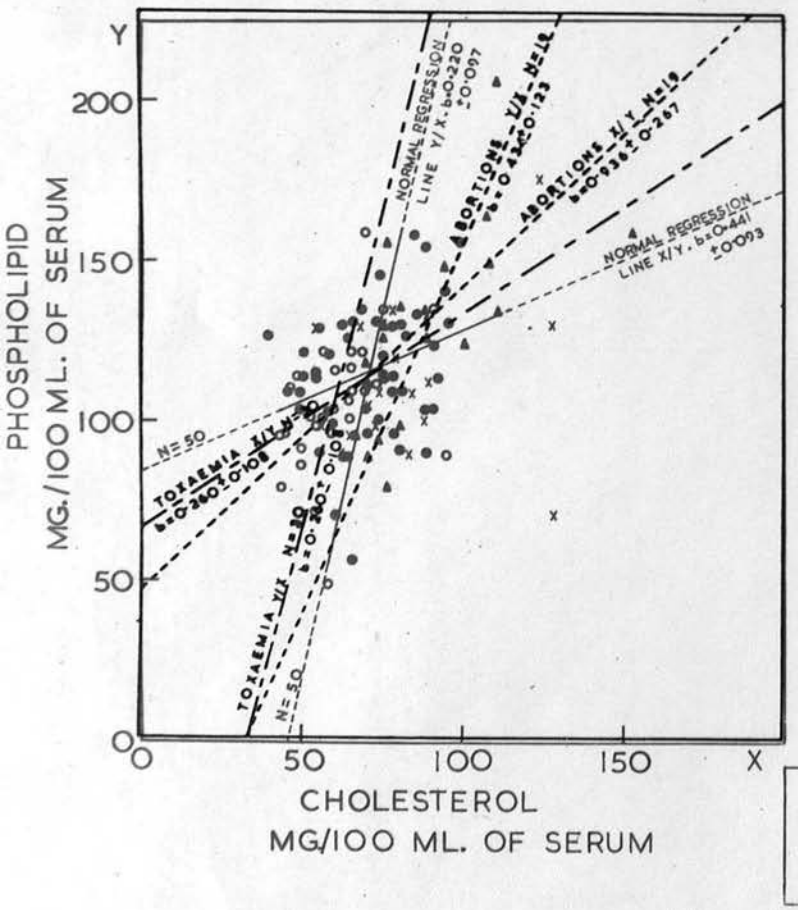
Of the various possible permutations of pairs of lipid and lipoprotein lipid concentrations, there must be a significant correlation between those of total lipid and the three lipoprotein lipids. This is because the latter are calculated from the total lipid concentration on the percentage basis indicated by the electrophoretic separation of the lipoproteins. For each of these pairs, the regression coefficient is very highly significant ($p < 0.001$).

It has been remarked earlier that the difficulties attendant upon distinguishing between β - and γ-lipoprotein on the electrophoretic strip cast considerable doubt upon the individual values obtained for these two lipoprotein lipid fractions. Although significant differences from normal

REGRESSION DIAGRAMS OF:

REGRESSION LINES.

1. CHOLESTEROL / PHOSPHOLIPID



2. TOTAL ESTERIFIED FATTY ACID/CHOLESTEROL

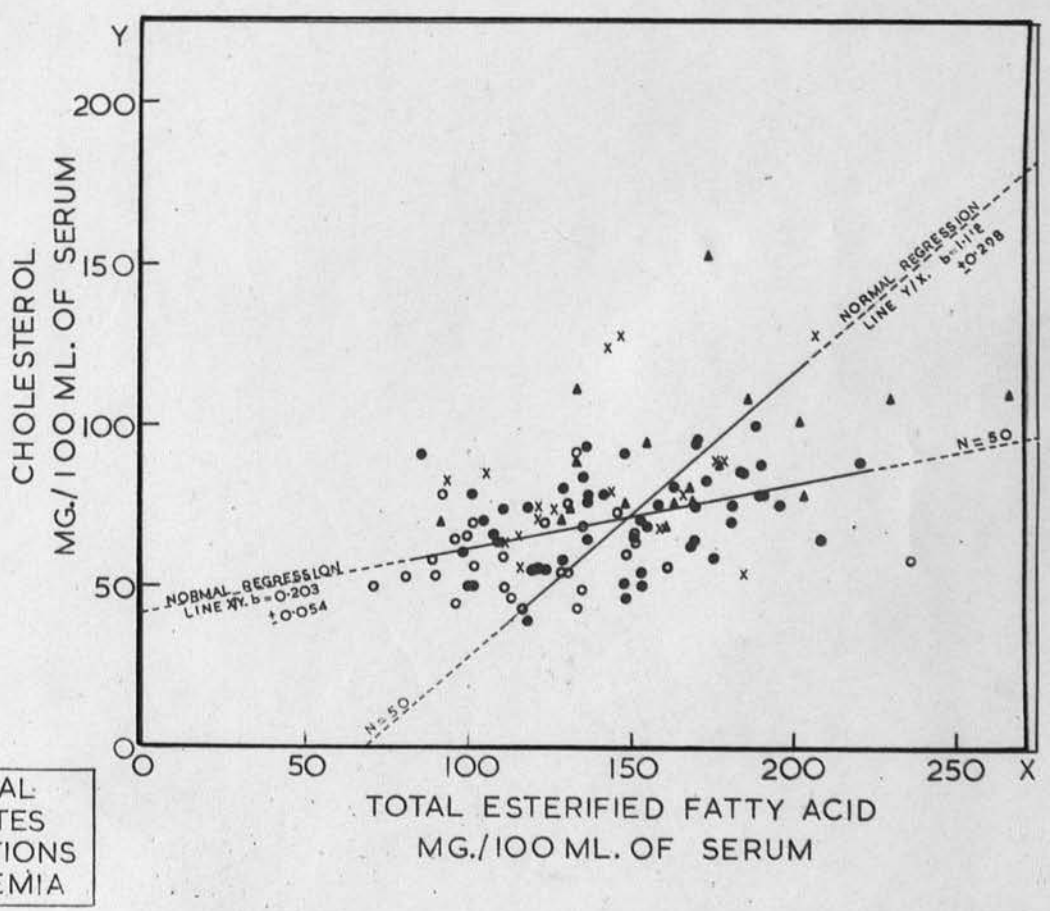
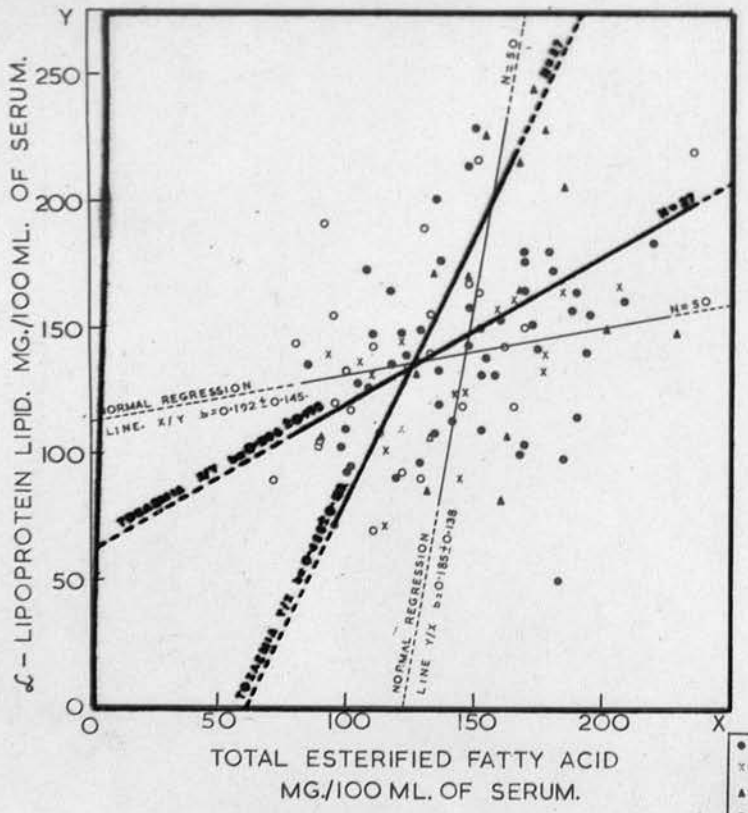


FIGURE 9

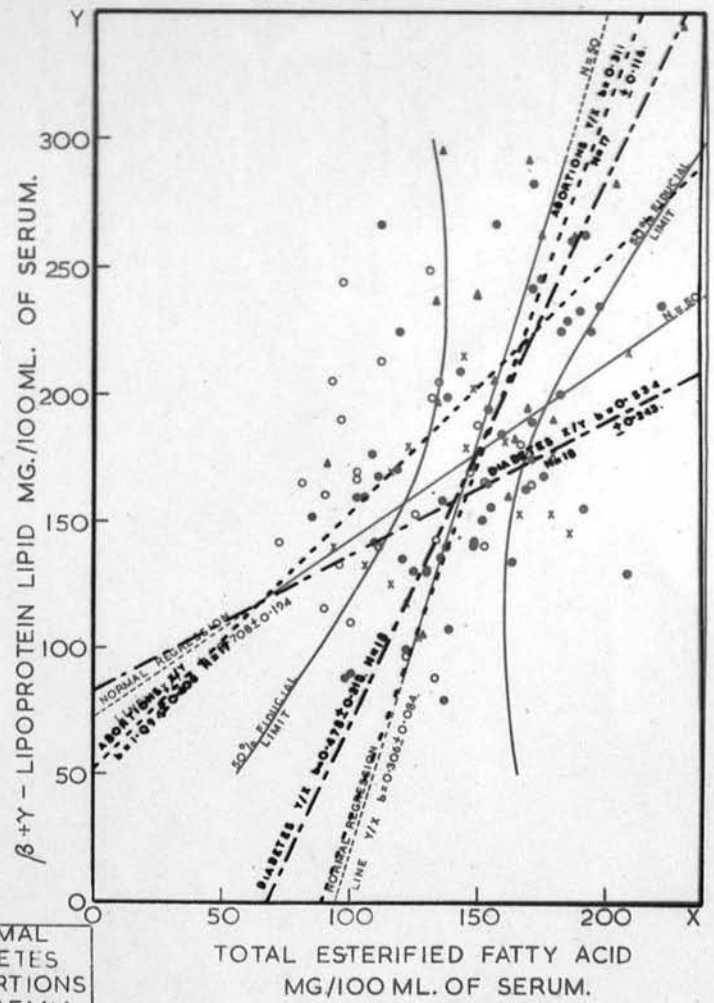
REGRESSION DIAGRAMS OF

REGRESSION LINES.

1. TOTAL ESTERIFIED FATTY ACID / α-LIPOPROTEIN LIPID.



2. TOTAL ESTERIFIED FATTY ACID / $\beta+\gamma$-LIPOPROTEIN LIPID.



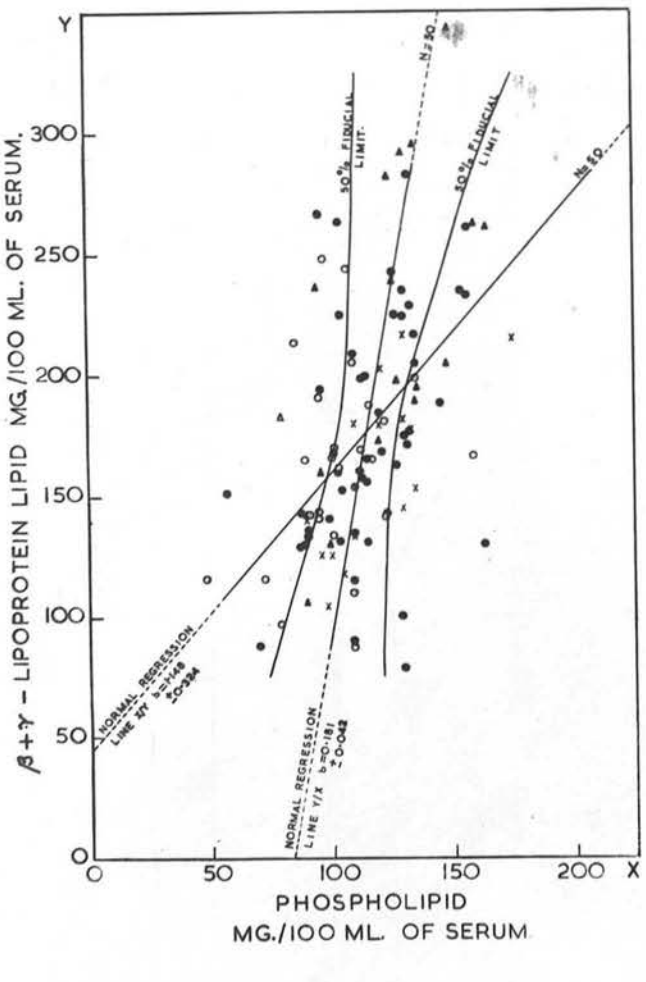
● = NORMAL
× = DIABETES
▲ = ABORTIONS
○ = TOXAEMIA

FIGURE 10

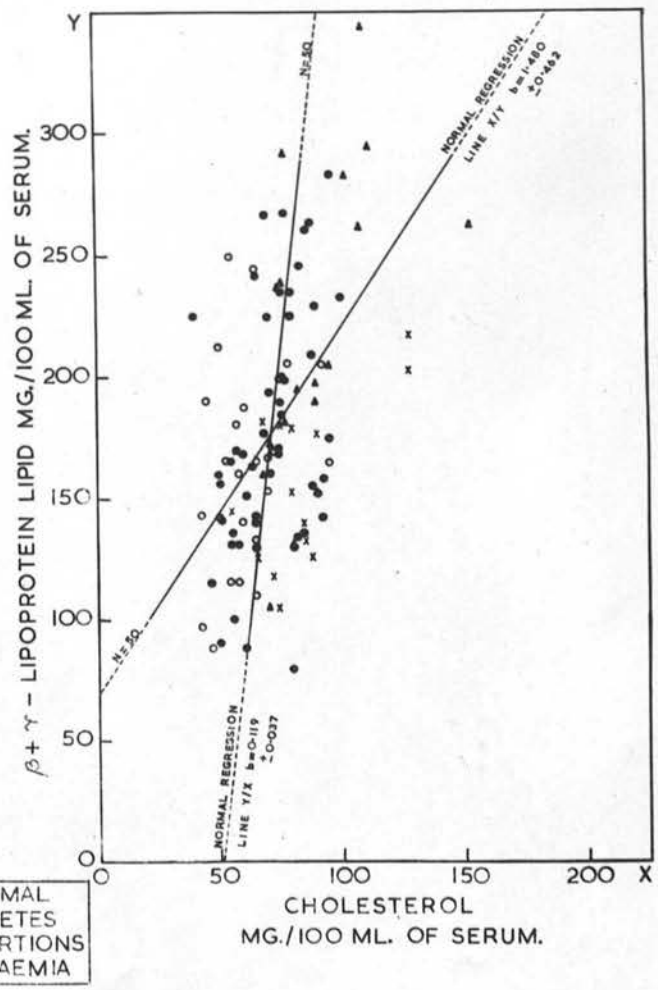
FIGURE 11

REGRESSION DIAGRAMS OF

1. PHOSPHOLIPID/ β+γ-LIPOPROTEIN LIPID.



2. CHOLESTEROL/ β+γ-LIPOPROTEIN LIPID.



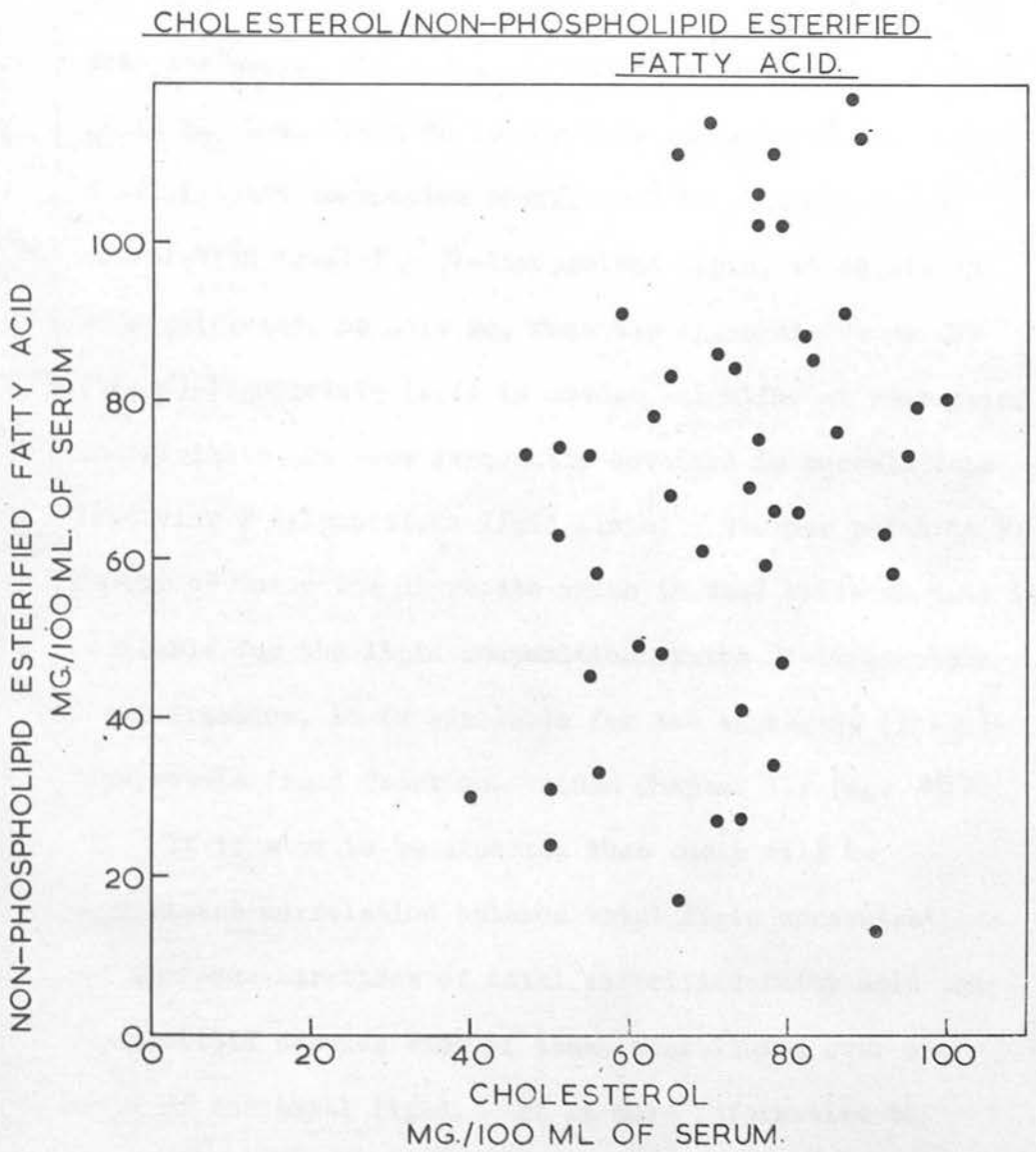
NORMAL
DIABETES
ABORTIONS
TOXAEMIA

FIGURE 12

REGRESSION LINES

FIGURE 13

A NON-SIGNIFICANT REGRESSION



have been observed in the individual values of these two fractions in the groups of abnormal infants, and significant correlation exists between their concentrations and those of one or more of the separate serum lipids in some instances, the aggregate value of ($\beta+\gamma$)-lipoprotein lipid has been found to be the more reliable value. When a significant regression coefficient is obtained for a correlation involving β-lipoprotein lipid, it is always as significant, or more so, when the aggregate value for ($\beta+\gamma$)-lipoprotein lipid is used. Significant regression coefficients are less frequently obtained in correlations involving γ-lipoprotein lipid alone. Another point in f favour of using the aggregate value is that while no data is available for the lipid composition of the γ-lipoprotein lipid fraction, it is available for the aggregate ($\beta+\gamma$)-lipoprotein lipid fraction. (See Chapter III Page 42).

It is also to be expected that there will be significant correlation between total lipid concentrations and the concentrations of total esterified fatty acid and phospholipid because each of these constitutes over one third of the total lipid. It is more informative to calculate the coefficients of regression of the remaining total lipid on the separate lipid concentrations i.e. the coefficients of regression of (total lipid less the total

TABLE 23

Coefficients of Regression of one Lipid or Lipoprotein Lipid on Another, their Standard Errors and their Significance if any.

	b	S.E. of b	p
Normal Infants			
TEFA/ α -LP	0.19	0.14	NSD
TEFA/($\beta+\gamma$)-LP	0.71	0.19	0.001
Chol/ α-LP	0.46	0.34	NSD
Chol/($\beta+\gamma$)-LP	1.48	0.46	0.01 - 0.001
Phospholipid/ α -LP	0.44	0.24	NSD
Phospholipid/($\beta+\gamma$)-LP	1.14	0.32	0.001
Infants of Diabetic Mothers			
TEFA/ α -LP	0.33	0.23	NSD
TEFA/($\beta+\gamma$)-LP	0.53	0.24	0.05 - 0.02
Chol/ α -LP	0.04	0.32	NSD
Chol/($\beta+\gamma$)-LP	1.09	0.28	0.01 - 0.001
Phospholipid/ α -LP	0.10	0.31	NSD
Phospholipid/($\beta+\gamma$)-LP	0.72	0.33	0.05 - 0.02
Infants of Mothers who have had a Previous Abortion			
TEFA/ α -LP	0.60	0.40	NSD
TEFA/($\beta+\gamma$)-LP	1.09	0.41	0.02 - 0.01
Chol/ α -LP	1.40	0.49	0.02 - 0.01
Chol/($\beta+\gamma$)-LP	1.69	0.62	0.01 - 0.001
Phospholipid/ α -LP	1.50	0.37	0.001
Phospholipid/($\beta+\gamma$)-LP	1.58	0.51	0.01 - 0.001
Infants of Toxaemic Mothers			
TEFA/ α -LP	0.60	0.19	0.01 - 0.001
TEFA/($\beta+\gamma$)-LP	-0.08	0.23	NSD
Chol/ α -LP	1.40	0.53	0.02 - 0.01
Chol/($\beta+\gamma$)-LP	0.92	0.59	NSD
Phospholipid/ α -LP	0.28	0.33	NSD
Phospholipid/($\beta+\gamma$)-LP	0.63	0.36	NSD

NSD = not significantly different from zero

esterified fatty acid concentration) on total esterified fatty acid concentration and similarly (total lipid less phospholipid) on phospholipid. Although cholesterol constitutes a smaller proportion of the total lipid, the same treatment has been afforded to this pair of variables.

Likewise, phospholipid is approximately 70 per cent esterified fatty acid so that it is of more interest to see if any relationship exists between phsopholipid concentrations and those of non-phospholipid esterified fatty acid.

Throughout the discussion which follows, the four groups of infants will be referred to by initial letters only, viz:

Normal Infants	-Group N
Infants of Diabetic Mothers	-Group D
Infants of Mothers who have had a previous Abortion	-Group A
Infants of Toxaemic Mothers	-Group T

The Significant Coefficients of Regression

It will be observed in Table 23, that, of the more interesting relationships, the following are significant:

		Coefficients of Regression Significantly Different from Zero in the Following Groups:
TEFA	/α-LP	Group T only.
Cholesterol	/α-LP	Group T; Group A.

Phospholipid	/ α -LP	Group A only.
TEFA	/ (β+γ)-LP	Group N; Group D; Group A
Cholesterol	/ (β + γ)-LP	Group N; Group D; Group A
Phospholipid	/ (β + γ)-LP	Group N; Group D; Group A
Cholesterol	/ Phospholipid	Group N; Group A; Group T
TEFA	/ Cholesterol	Group N only.

It must be remembered that these regression coefficients are a measure of the increase in the concentration of one of a pair of serum constituents when the concentration of the other increases by one unit. The regression lines are illustrations of this. The coefficients are only valid over the range of values found in the investigation. Extrapolation to a zero concentration of one component would be meaningless when dealing with concentrations of serum constituents. Furthermore, although it may be impossible to prove a correlation to be significant, this does not mean that it is necessarily non-significant. A lack of proven significance may be the result of too great a variation in a relatively small number of cases. On the other hand, very small, non-significant regression coefficients are considered to show non-correlation between the two variables concerned.

THE INCREASES IN LIPOPROTEIN LIPID CONCENTRATIONS RESULTANT UPON INCREASES IN INDIVIDUAL LIPID CONCENTRATIONS

A discussion of the increase in the concentration of one serum constituent which accompanies a unit increase in

that of another would be completely unphysiological. This is especially so with such closely inter-related entities as serum lipids and lipoproteins. It may be assumed that if there is an increase in the concentrations of any one serum lipid, there must be a corresponding increase in the concentrations of one or all of the lipoprotein lipid concentrations unless a fraction of that particular lipid is not carried by lipoprotein. Therefore an increase in any one lipid concentration must be accompanied by an increase in the other two lipid concentrations if the normal lipoprotein lipid constitution is to be maintained. If, on the other hand, the lipid in question is being incorporated into lipoprotein molecules in such a way that their lipid constitution becomes abnormal, there would not necessarily have to be the corresponding increase in the other lipid concentrations.

The increases in the lipoprotein lipid fractions expected to result from 100 mg. increases in cholesterol or phospholipid concentrations may be calculated as follows:-

	Cholesterol mg.%	Phospholipid mg.%
($\beta+\gamma$)-Lipoprotein Lipid	20	20
α -Lipoprotein Lipid	25	50

Ratio of ($\beta+\gamma$)- to α -lipoprotein lipid in normal cord blood = 1.36 : 1.0

Per cent cholesterol content of ($\alpha+\beta+\gamma$)-lipoprotein lipid

$$= \frac{1.36 \times 20 + 1 \times 25}{2.36} = \frac{52.2}{2.36} \quad \text{_____(1)}$$

Per cent phospholipid content of ($\alpha+\beta+\gamma$)-lipoprotein lipid

$$= \frac{1.36 \times 20 + 1 \times 50}{2.36} = \frac{77.2}{2.36} \quad \text{_____(2)}$$

From (1), Increases in ($\alpha+\beta+\gamma$)-lipoprotein lipid per 100mg. rise in cholesterol

$$= \frac{236}{52.2} \times 100 = 452$$

From (2), Increases in ($\alpha+\beta+\gamma$)-lipoprotein lipid per 100mg. rise in phospholipid

$$= \frac{236}{77.2} \times 100 = 306$$

The rise in α -lipoprotein lipid $= \frac{1}{2.36}$ of total rise in lipoprotein lipid.

From this, in round figures,

	per 100mg. increase in cholesterol	per 100mg. increase in phospholipid
Increase in ($\beta+\gamma$)- lipoprotein lipid in mg.	260	180
Increase in α -lipoprotein lipid in mg.	190	130

The application of these relationships is referred to in the next section.

THE INDIVIDUAL COEFFICIENTS OF REGRESSION

AI. Correlation Between Concentrations of Total Esterified Fatty Acid and α-Lipoprotein Lipid

Total esterified fatty acid constitutes at least 35 per cent of the α-lipoprotein in the form of phospholipid, in addition to a proportion of triglyceride and the small amount incorporated into cholesterol. Despite this, a significant correlation has only been found in the group of infants of toxaemic mothers (Group T). The coefficient of regression of α-lipoprotein lipid on total esterified fatty acid concentrations in these infants is 0.60 $\pm$ 0.19 (30). An identical value of 0.60 is obtained for the infants of mothers who have had a previous abortion (Group A), but the standard error of the regression coefficient is $\pm$ 0.40 (17) so that the correlation cannot be proved to be significant. This, and the fact that no significant correlation can be proved in the case of the normal infants (Group N) and in the infants of diabetic mothers (Group D), is probably a result of the very wide variation in the data.

A2. Correlation Between the Concentrations of Total Esterified Fatty Acid and (β + γ)-Lipoprotein Lipid

Whereas a significant correlation between total esterified fatty acid and α-lipoprotein lipid can only be proved for Group T, total esterified fatty acid concentrations are significantly correlated with those of (β + γ)-lipoprotein in the three remaining groups. The regression

coefficients are:-

Group N : 0.71 ± 0.19 (50) ($p < 0.001$)

Group D : 0.55 ± 0.24 (18) ($0.05 > p > 0.02$)

Group A : 1.09 ± 0.41 (17)($0.02 > p > 0.01$)

The aggregate of ($\beta + \gamma$)-Lipoprotein lipid will, in fact, have a very high total esterified fatty acid content because triglycerides alone will contribute approximately 50 per cent to the total β-lipoprotein lipid and probably much more to the γ-lipoprotein lipid, in addition to the esterified fatty acid content of cholesterol and phospholipid.

B. CORRELATIONS BETWEEN THE CONCENTRATIONS OF CHOLESTEROL AND THOSE OF THE TWO LIPOPROTEIN LIPID FRACTIONS

1. In Normal Infants

The coefficient of regression of α-lipoprotein lipid on cholesterol concentration in the cord blood of Group N is 0.46 ± 0.34 (50). This is not statistically significant. When ($\beta + \gamma$)-lipoprotein lipid concentrations are substituted for those of α-lipoprotein lipid the regression coefficient is 1.48 ± 0.46 (50) and this is highly significant ($0.01 > p > 0.001$). The coefficients of regression of α- and ($\beta + \gamma$)-lipoprotein lipid on phospholipid concentration are 0.44 ± 0.24 (50) and 1.14 ± 0.32 (50) respectively. Again, only the latter value is significant ($p < 0.001$).

If the values for the regression coefficients are compared with the increases calculated to result from increases of 100 mg. cholesterol or phospholipid given in the previous section, the inaccuracy of the non-significant regression coefficients becomes obvious. These data are summarised in Table 24 below.

TABLE 24

	(a) Calc.inc. in LP for 100 mg. inc. in lipid.	(b) Regression Coefficient x 100	(c) 100 x (b)/(a)
Cholesterol/α-Lipoprotein Lipid	190	46 ± 34	24
Cholesterol/(β+γ)-Lipoprotein Lipid	260	148 ± 46	57
Phospholipid/α-Lipoprotein Lipid	130	44 ± 24	34
Phospholipid/(β+γ)-Lipoprotein Lipid	180	114 ± 32	63

The observed coefficients of regression of α-lipoprotein lipid on both the individual lipid concentrations are not significant. Only if the lipoprotein lipid concentration was invariant would a regression coefficient as high as 1.90 be expected. Conversely, where the variation in the concentration of cholesterol is due to variation, in some measure, in both of the lipoprotein lipid fractions, the coefficients of regression of the two lipoprotein lipid

concentrations on that of cholesterol will be low. The implication here is that most of the changes in cholesterol are linked with those of ($\beta + \gamma$)-lipoprotein lipid.

2. In Infants of Diabetic Mothers

The coefficients of regression of α-lipoprotein lipid concentration on those of cholesterol and phospholipid in Group D are 0.04 and 0.10 respectively. Obviously no significant correlation exists in either case. The coefficients of regression of ($\beta + \gamma$)-lipoprotein lipid concentration on those of cholesterol and phospholipid are 1.09 + 0.28 (18); $(0.01 > p > 0.001)$, and 0.72 $\pm$ 0.33 (18); $(0.05 > p > 0.02)$ respectively. Both of these are significant.

The ratio of ($\beta + \gamma$)- to α-lipoprotein lipid in Group D is 1.22. If the lipid content of the lipoprotein lipid fractions is assumed to be normal, the expected increases in α-lipoprotein lipid in response to an increase of 100mg. in cholesterol and phospholipid would be 200 mg. and 134 mg. respectively. These values bear no relationship to the regression coefficients at all.

The expected increases in ($\beta + \gamma$)-lipoprotein lipid in response to the same increases in cholesterol and phospholipid would be 247 mg. and 164 mg. respectively. Therefore the corresponding regression coefficients are less

than one half of the calculated values despite their being significant.

These data are summarised in Table 25 below.

TABLE 25

	(a) Calc.inc. in LP for 100mg. inc. in lipid	(b) Regression Coefficient x 100	(c) 100 x(b)/(a)
Cholesterol/α-Lipoprotein Lipid	200	4 ± 32	2
Cholesterol/($\beta+\gamma$)-Lipoprotein Lipid	247	109 ± 28	44
Phospholipid/α-Lipoprotein Lipid	134	10 ± 31	7
Phospholipid/($\beta+\gamma$)-Lipoprotein Lipid	164	72 ± 33	44

Since the discrepancies between the calculated and observed coefficients of regression of α-lipoprotein lipid on cholesterol and phospholipid are far greater than the standard errors of the regression coefficients would lead one to expect, this suggests that the lipid composition of the α-lipoprotein lipid fraction is not identical with that of normal infants. These data do not confirm the suggestion made earlier, that there is an abnormality of the ($\beta+\gamma$)-lipoprotein fraction.

3. In Infants of Mothers who have had a Previous Abortion

The coefficients of regression of α- and ($\beta+\gamma$)-

lipoprotein lipid concentrations on those of cholesterol in Group A are 1.4 ± 0.49 (17); $(0.02 > p > 0.01)$, and 1.69 ± 0.62 (17); $(0.01 > p > 0.001)$ respectively. The coefficients of regression of α - and $(\beta + \gamma)$-lipoprotein lipid concentrations on those of phospholipid are 1.5 ± 0.37 (17); $(p < 0.001)$ and 1.58 ± 0.51 (17); $(0.01 > p > 0.001)$ respectively. All of these regression coefficients are highly significant.

The ratio of $(\beta + \gamma)$- to α-lipoprotein lipid in Group A is 1.5. If the normal lipid composition is assumed for the lipoprotein lipid fractions, an increase of 100 mg. cholesterol should be accompanied by increases of 182 mg. α-lipoprotein lipid and 273 mg. $(\beta + \gamma)$-lipoprotein lipid.

These calculated values may be compared with the regression coefficients obtained in each case in Table 26 below.

T A B L E 26

	(a) Calc.inc. in LP for 100 mg. inc. in lipid	(b) Regression Coefficient x 100	(c) 100 x (b)/(a)
Cholesterol/α-Lipoprotein Lipid	182	140 ± 49	77
Cholesterol/$(\beta+\gamma)$-lipoprotein lipid	273	169 ± 62	62
Phospholipid/α-Lipoprotein Lipid	125	150 ± 37	120
Phospholipid/$(\beta+\gamma)$-Lipoprotein Lipid	187	158 ± 51	84

In each case the discrepancies are of the order to be expected from the standard errors of the regression coefficients. Therefore no difference can be deduced in the lipid composition of the lipoprotein lipid fractions from that which has been calculated theoretically. The increased slope of the regression lines compared with normal seems likely to be a result of the differences in the ratio of the individual lipoprotein lipid fractions to each other which has been noted earlier. (See Chapter IV, page 72).

4. In Infants of Toxaemic Mothers

Of all the coefficients of regression which may be calculated from the data for Group T, only that for α -lipoprotein lipid concentrations on cholesterol concentrations is significant. The value for the regression coefficient is 1.4 ± 0.53 (27); $(0.02 > p > 0.01)$. This is surprising because the mean serum cholesterol concentration is significantly below normal while that of α -lipoprotein lipid is almost exactly normal. The coefficient of regression of $(\beta + \gamma)$-lipoprotein lipid on cholesterol concentrations is 0.92 ± 0.59 (27) and those for α - and $(\beta+\gamma)$-lipoprotein lipid on phospholipid concentrations are 0.28 ± 0.33 (27) and 0.63 ± 36 (27) respectively. None of these is significant.

In the data for Group N, the existence of significant

correlation between cholesterol and ($\beta+\gamma$)- but not α-lipoprotein lipid led one to the conclusion that most of the changes in cholesterol are linked with those in ($\beta+\gamma$)-lipoprotein lipid. The converse may be deduced for Group T; i.e. most of the changes in cholesterol are linked with changes in α-lipoprotein lipid. No other evidence points to any difference in the lipid composition of the lipoprotein lipid fractions in these infants so that it is impossible to come to any definite conclusion on this point.

DISCUSSION

The Low Concentrations of Lipids and Lipoprotein Lipids in Cord Blood

The immediate result of this investigation is to confirm the earlier findings that the concentrations of serum lipids and lipoprotein lipids in the umbilical cord blood of infants are lower than those in the venous blood of adults. They are between one half and one third of the concentrations found in maternal blood.

It seems unnecessary to invoke the presence in the foetal circulation of high concentrations of oestrogens of maternal origin to explain this, as was done by Whitelaw (1948). The ovaries of female infants undoubtedly show signs of considerable stimulation by oestrogenic hormones of exogenous origin. However, the presence of concentrations of oestrogens in the infant which would be great

enough to affect the lipid and lipoprotein lipid concentrations to such an extent would be expected to have a similar effect upon the mother at term in whom, in fact, lipid concentrations are raised. It may be that the lipids and lipoproteins respond differently in the mother and in the immature infant, but suggestive evidence will be put forward in Chapter V page 133 , that, to a certain extent, the concentrations of lipids and lipoprotein lipids during the first week of life are dependent upon feeding. Unless this involves a further complication in the lipid response to the hormonal environment, it is hardly compatible with the suggestion that large concentrations of oestrogens of maternal origin are responsible for the low lipid and lipoprotein lipid concentrations in the cord blood.

It is more reasonable to suppose that the enzyme systems involved in the synthesis and the release into the circulation of the lipids and lipoproteins are not functioning maximally until after birth. As will be seen in Chapter V, the concentrations of all the serum lipids and lipoprotein lipids in normal infants increase to almost adult values during the first week of life and the rate of increase is particularly great on the first day. Several specific enzymes are reported to be absent or decreased in the foetal and new-born period. These include glucose-6-phosphatase in human infants (Komrower, Schwarz, Holzel,

and Goldberg, 1956), and adenosine triphosphatase in chicks (Moog and Steinbach, 1945; Moog, 1947), and in rats (Hermann and Nicholas, 1948). Since fat metabolism is geared to carbohydrate metabolism, such a lack would be expected to have some effect upon serum lipid concentrations.

Oestrogens of maternal origin may well play some part in the reduction of the serum lipid and lipoprotein concentrations to such low levels, but even a combination of high, but still physiological, concentrations of all the possible steroid hormones and thyroxine and adrenaline would be unlikely to reduce them to the extent which is observed.

The Difference in the Ratios of β - to α -Lipoprotein in Cord and Adult Blood

The significance of the higher proportion of α-lipoprotein in the umbilical cord blood of human infants and in the blood of the dog, cat and rat, investigated by Morris and Courtice (1955) compared with human adult blood which contains more β-lipoprotein is unknown. It is possible that it is connected is some way with the small molecular diameter of α - compared with β-lipoprotein. In human infants, there is a rapid increase in the concentrations of β-lipoprotein during the first week of life so that adult values are reached in which β-lipoprotein is predominant instead of α-lipoprotein.

The Difference Observed in the Cord Blood Lipid and Lipoprotein Lipid Concentrations in the Different Groups of Infants

Of more interest than a discussion of the differences between umbilical cord blood and maternal blood in terms of lipid and lipoprotein lipid concentrations, is one of the differences between their concentrations in the cord blood of normal infants and that of infants who are themselves abnormal at birth or whose mothers showed some abnormality during pregnancy.

The Frequency of Abnormalities in Cholesterol Concentrations

One conclusion in particular emerges from this investigation: that serum cholesterol concentrations are more subject to change than the concentrations of any other serum lipid.

It will have been noticed that only the infants of toxaemic mothers have a mean serum cholesterol concentration which is lower than normal. Any other abnormality, either of the mother or of the infant, which is accompanied by an alteration in the serum cholesterol concentration, tends to result in an increase rather than a decrease. Infants of mothers who had had a previous abortion and also suffered from toxaemia in this pregnancy tended to have a raised, rather than a lowered, cholesterol concentration.

It must be stressed that all cases in a particular group do not necessarily behave in the way indicated by the mean concentration of any lipid. For instance, the mean cord blood cholesterol concentration of the infants of diabetic mothers is higher than normal. So is that of the infants of mothers who have had a previous abortion. But one infant of a diabetic mother who had had a previous abortion, and a stillborn child, had a cholesterol concentration which is lower than the mean value for infants of toxaemic mothers. This may be connected with the fact that the mother frequently went into hypoglycaemic coma during the few weeks prior to delivery. On the other hand, one of the highest cholesterol concentrations found amongst the infants of diabetic mothers occurred in one whose mother had had a pregnancy which was only abnormal in that it was a twin pregnancy (apart from the diabetes). Twinning may itself affect serum cholesterol concentrations for other cases have been found where one infant of a pair of twins had a high cholesterol concentration. This serves to underline the difficulties in working with clinical material. It is impossible, unless a very large number of cases is studied, to subdivide them in such a way that only one abnormality is exhibited by each group.

This does not detract however from the fact that, in certain infants, the umbilical cord blood cholesterol

TABLE 27

THE MEAN CONCENTRATIONS IN MG./100 ML. AND THE RATIOS OF CONCENTRATIONS OF THE LIPIDS AND LIPOPROTEIN LIPIDS, BOTH OBSERVED AND CALCULATED, AND THE DISCREPANCIES BETWEEN THE TWO VALUES, TO THE NEAREST WHOLE NUMBER IN THE CORD BLOOD OF THE FOUR GROUPS OF INFANTS.

	Normal	Diabetic	Abortion	Toxaemic
Observed total lipid	317	297	395*	297
Calculated total lipid	253	262	301	217
Discrepancy	-64	-35*	-94	-80
α -lipoprotein lipid	141	140	160	136
β -lipoprotein lipid	96	97	147*	92
γ -lipoprotein lipid	82	61*	74	76
Total esterified fatty acid	148	142	168	123*
Observed cholesterol	72	84*	91*	61*
Calculated cholesterol	70	67	84	68
Discrepancy	-1	-17	-7	+7
Observed Phospholipid	115	113	130*	106
Calculated Phospholipid	106	102	124	102
Discrepancy	-9	-11	-6	-4
Observed C/P	0.64	0.77*	0.71	0.60
Calculated C/P	0.67	0.66	0.69	0.64
Discrepancy	+0.03	-0.11	-0.02	+0.04

* Values marked with an asterisk are significantly different from normal.

concentrations are abnormal. Such abnormalities may or may not be accompanied by alterations in the concentrations of the other serum lipids or in the lipoprotein lipid fractions. The data in Table 27 summarises the more significant of these alterations.

The Differences Characteristic of Each Group of Infants

It will be observed in Table 27 that all the "abnormal" groups of infants are distinguished by having cholesterol concentrations which are different from normal. Both the infants of diabetic mothers (Group D) and the infants of mothers who have had a previous abortion (Group A) have cholesterol concentrations which are above those in normal infants. In the infants of toxaemic mothers (Group T), the cholesterol concentrations are below normal. Group D may be further distinguished from Group A because only the latter have a raised phospholipid concentration and correspondingly, a raised total lipid concentration.

In fact the data are consistent with a rise in cholesterol, phospholipid and total esterified fatty acid in Group A and a fall in all three in Group T. That the change in Group D is almost exclusively in cholesterol concentration is confirmed by the fact that only in this group is the ratio of cholesterol to phospholipid concentrations significantly increased.

The distribution of lipoprotein lipid is also different in the three groups. There is a significant fall in γ-lipoprotein lipid in Group D and a significant rise in β-lipoprotein lipid in Group A. Although there are no significant changes in Group T, the mean values for all the lipoprotein lipid fractions are somewhat lower than normal, a finding consistent with the diminution in the concentrations of cholesterol, phospholipid and total esterified fatty acid.

The rest of the data in Table 27 are included to illustrate the problem of the discrepancy between the observed and calculated total lipid concentrations. It will be seen that there is good agreement between the observed and calculated cholesterol and phospholipid concentrations in all groups except Group D, in which there are large differences for both. However, in this group, the discrepancy between the observed and calculated total lipid concentration is smaller than in any other group. Judging by the sign of the discrepancies, that between the observed and calculated total lipid concentrations is not a result of discrepancies between the observed and calculated cholesterol and phospholipid concentrations. Further work is obviously required to clear up this problem and to define the further abnormality in Group D which seems to be implied by this discussion.

One further comment may be made upon the lipoprotein lipid pattern in Group A: the fact that only the β -lipoprotein lipid and not the α -lipoprotein lipid is increased in Group A is suggestive of a changeover from the foetal lipoprotein pattern with preponderant α -lipoprotein, to the adult pattern with preponderant β -lipoprotein.

The Possible Causes of the Differences Observed Between the Four Groups of Infants

Assuming, therefore, that these four groups of infants are quite distinct, the question arises as to why they are distinct and what factors in the mother or in themselves could produce these differences. It must be noted that until there is a greater understanding of the function of the lipoproteins, the reasons for, and the results of any abnormalities in them will remain obscure.

The Response of Serum Lipids and Lipoproteins to Metabolic Controls

A review of the data available on the responses of the serum lipids and lipoproteins to metabolic controls of various natures has been given in Chapter II. It may be summarised as follows.

The Inter-Relation of Fat Metabolism and Carbohydrate Metabolism Insulin

Insulin and a normally functioning carbohydrate metabolism, particularly that of glucose, are required for the

normal synthesis of fatty acids in adipose tissue and in the liver. In their absence there is a release of free fatty acid from the fat depots and a consequent rise in plasma free fatty acid concentrations. This is followed by a rise in blood glucose concentrations and a delayed rise in serum lipid and lipoprotein lipid concentrations.

Adrenaline, Nor-adrenaline, ACTH and the Corticosteroids, Thyroxine and Growth Hormone.

Low blood sugar concentrations promote the secretion of adrenaline and nor-adrenaline which, in their turn, promote the secretion of corticosteroids. Adrenaline, nor-adrenaline and ACTH produce, separately, a mobilization of free fatty acid within a few minutes and an increase in the metabolic rate. Adrenaline produces a hyperglycaemia almost simultaneously with the elevation of plasma free fatty acid concentrations. It also produces a delayed increase in the concentrations of serum lipids and lipoprotein lipids. Nor-adrenaline does not produce a hyperglycaemia but the elevation of free fatty acids which it produces is more long lasting. Both effects of adrenaline are potentiated by cortisone which apparently does not affect normal serum lipid concentrations on its own. It will reduce them in a hypercholesterolaemic state. Thyroxine also potentiates the mobilization of free fatty acid by adrenaline and the subsequent increase in serum lipid and lipoprotein lipid concentrations. Like cortisone, it will

reduce cholesterol concentrations in a hypercholesterolaemic state such as that found in hypothyroidism. That is, cortisone and thyroxine seem to have a dual role. They potentiate an increase in serum lipid concentrations produced by adrenaline but reduce them in hypercholesterolaemic conditions.

Serum Lipids and Lipoproteins, the Autonomic Nervous System and Stress

Stimulation of the autonomic nervous system has the same effects as adrenaline on the serum lipids and lipoprotein lipids and so, therefore, do some types of psychological stress. Stress in the form of complete starvation will obviously interfere with carbohydrate metabolism. It has been variously reported as increasing, or decreasing the serum lipid concentrations.

The Hormonal Environment in the Four Groups of Infants

Insulin

There is no experimental basis for assuming that there is any abnormality in the insulin activity of any of the infants in the abnormal groups except the infants of diabetic mothers. The reasons for believing that these infants have a raised insulin activity are given in Chapter V page 141. However, the infants of toxaemic mothers are remarkable for developing an abnormally low blood glucose

concentration during the first few hours of life. A "physiological" hypoglycaemia develops normally during this period but in the infants of toxaemic mothers, blood glucose concentrations have been observed to fall to values under 10.0 mg./100 ml. compared with a normal fall to between 20 mg. and 30 mg./100 ml. (Farquhar, 1862; Cornblath, Odell and Levine, 1959). In the cases observed by Cornblath et.al. (1959) the very low glucose concentrations in the infants of toxaemic mothers were associated with convulsions, apnoea, tremor and limpness. These symptoms also occur in the infants of diabetic mothers in whom blood glucose concentrations also fall to very low levels as will be remarked in Chapter V page 143.

The very low blood glucose concentrations developed in the infants of diabetic mothers may well be a result of the high insulin activity to which a considerable antagonism is later developed. But it is accompanied by raised cholesterol concentrations as opposed to the low cholesterol concentrations observed in the infants of toxaemic mothers. In fact, low cholesteroland lipoprotein lipid concentrations should accompany high insulin activity and it is possible that the infants of diabetic mothers would have even higher cholesterol concentrations if it were not for the high insulin activity.

Adrenaline and Nor-Adrenaline

There are apparently few data for the cord blood content of hormones other than insulin. It might be thought possible that adrenaline and nor-adrenaline would be produced in large amounts by mothers during labour and cross the placenta; the evidence to data suggests that this is not so (Stone, Pilliero, Hammer, and Portnoy, 1960). This does not disallow that an abnormal pregnancy could result in the production of excessive amounts of these hormones or that the infant could produce them in response to the stress of delivery.

Thyroxine

Thyroxine in known to be present in the venous blood of normal infants during the first week of life in concentrations greater than those normally found in adult blood (Pickering, Kontaxis, Benson and Meecham 1958). There is however, no reason to suppose that there should be even higher concentrations in the cord blood of infants having abnormal serum lipid concentrations. It is notable however, that the infant of a mother whose only abnormality was a palpably enlarged thyroid gland had very high cord blood lipid concentrations.

Corticosteroids

Evidence is presented in Chapter VI page 165

suggesting that the infants of diabetic mothers possess increased serum corticosteroid concentrations. Nothing is known in this respect about the infants of mothers who have had a previous abortion or who are toxaemic. Combinations of increased concentrations of adrenaline, thyroxine and corticosteroids could be responsible for the increased concentrations of lipids and lipoprotein lipids in the infants of mothers who have had a previous abortion. The reverse situation, with the addition of raised insulin concentrations, might result in the low serum lipid concentrations found in the cord blood of infants of toxaemic mothers.

Growth Hormone

The data on growth hormone is contradictory in the extreme. If it does play any part in the development of the large infants of diabetic mothers (Erlich and Randle 1961) its fat mobilising properties must have been suppressed (Osler and Pedersen 1960; Erlich and Randle 1961).

The Supply of Food and Oxygen to the Foetus

Any discussion of the nutritional status of the abnormal infants or of the adequacy of their oxygen supply must, like that of the hormonal environment, be purely theoretical. The investigation of oxygen and carbon dioxide

transport across the placenta is difficult but there are indications that the foetus exists in anoxaemic conditions by post-partum standards (Dancis, 1959).

Hypoxia is, in fact, one of the known teratogenic agents (Ingalls, 1956). According to Villee, Hagerman, Holmberg, Lind and Villee (1958), the new-born mammal can withstand severe hypoxia much better than the adult. They suggest three possible mechanisms for this.

1. A lowered rate of cellular metabolism.
2. The existence of special anaerobic pathways.
3. Quantitative differences in the rates of those enzymatic reactions which are common to both the foetus and the adult.

Villee (1953) had found earlier that in liver slices from a twenty week old human foetus, the rate of lipogenesis under anaerobic conditions is only half that found in aerobic conditions, although anaerobic glycolysis in the same preparation was four times as efficient as aerobic glycolysis.

As has been said earlier in this chapter, several specific enzyme systems involved in the oxidative pathways are reported to be deficient or even absent in the foetal and new-born period. These include glucose-6-phosphatase and adenosine triphosphatase. Should these deficiencies become more pronounced, or the mechanisms for circumventing

them be interfered with, the foetus would be expected to show some abnormality as a result.

Transport Across the Placenta

The placenta exerts a selective control on the passage of all the molecules from the maternal to the foetal circulation and active transport mechanisms function in many cases (Dancis 1959). Glucose will cross the placenta in either direction at rates dependent on the concentration (Huggett, 1954), and in doing so it affects the foetal fructose concentrations. There is active transport of amino acids and this is stereo-specific, the natural L-isomer being favoured. (Page, Glendinning, Margolis and Harper, 1957). Electrolytes such as sodium, potassium, calcium and phosphate ions have no difficulty in crossing the placenta in either direction. The same is true for iron although the latter is only transferred from the mother to the foetus except in cases of placental breakdown (Dancis, 1959). Vitamins, particularly those which are water-soluble, cross the placenta readily and there is active transport of some of them including the vitamins of the B complex. Congenital deficiencies have been reported. (Ibid).

Transport of Hormones and Other Large Molecules

The foetus must relay on maternal thyroxine or thyrotrophic hormone at some stage during gestation because

hypothyroid mothers have been known to have infants with congenital hypothyroidism. Insulin was shown to cross the placenta in 1930 when Rupp showed that it would pass in both directions once the foetal pancreas began to function during the second half of pregnancy. Soule (1938) found equal concentrations of oestrogenic hormones on both sides of the placenta and Lucey (1961) quotes data on the ill-effects on the foetus of treating the mother with excessive amounts of oestrogens, androgens and oral progestins. The adreno-cortical steroids can also be transferred from the mother to the foetus, and in excess, will result in foetal virilization, the development of the adreno-genital syndrome and even abortion (Asling 1961; and Lanman, 1961).

Even such large molecules as viruses (Kaye Rasner and Stein 1953; and Tondery, 1952), and both antigens and antibodies will pass to the foetus. Maternal immunization against polio myelitis will protect the infant until it is approximately six months old. Conversely, the infection of the mother with an infectious disease at the end of pregnancy can cause the infant to develop the disease after the normal incubation period, part of which would be spent in utero. Maternal γ-globulins constitute the whole of the infant's γ-globulin because the infant is incapable of synthesizing its own until it is three months old.

Significant amounts of albumin and possibly other plasma proteins are also interchanged across the placenta and even red blood corpuscles under certain conditions (Dancis, 1959).

Source of Foetal Lipids and Lipoproteins

Despite the fact that so many molecules, both large and small, can cross the placenta either passively or by active transport, only a very small proportion of the foetal lipids and lipoproteins are derived in toto from the mother. After an investigation extending over many years, Popjak (1946, 1952, 1954) and Popjak and Beekmans, (1950) reported that only about 1.5 per cent of the foetal fatty acids had crossed the placenta intact and not more than 11 per cent of the foetal cholesterol. No phospholipid appeared to cross the placenta at all.

Therefore if the condition of the placenta is to affect the foetal synthesis of lipids and lipoproteins, it must be because the transfer of the main raw materials, such as amino acids, one- and two-carbon fragments and phosphate, has been interfered with, or alternatively the mechanisms of their resynthesis into more complex molecules in the placenta.

The Degeneration of the Placenta

The condition of the placenta in part determines the length of gestation. The exact date of onset of labour is

considered to be partly hereditary and partly caused by a diminution in the secretion of progesterone by the mother. This results in the beginning of the degeneration of the placenta (Clifford, 1957).

There is evidence that such degeneration begins early in the infants of diabetic mothers (Farquhar 1958), and reduced availability of oxygen to the foetus was demonstrated in diabetic pregnancy by Mackay (1957). How far the premature induction of labour, which is practised in these cases, prevents this from having any effect is not known. It seems highly probable however that the vascular lesions typical of diabetes would affect the efficiency of the placenta. Burstein, Soule and Blumenthal (1957) have found that approximately one third of diabetic women show marked endarteritic lesions of the placenta. The condition of the placenta also appears to be a primary factor in the stillbirth and neo-natal death rate in toxaemic pregnancies (Dancis, 1959, and Brash, 1949). McGaughey, Jones, Talbert and Anslow (1958) consider that there is a diminished rate of placental transfer in toxaemia. Desmond, Franklin, Blattner and Hill (1961) report that the adverse effects of toxaemia on the foetus include both interference with foetal oxygenation as a result of placental insufficiency, and retardation of intra-uterine growth.

But these reports refer to severe toxaemia and there was no case of more than moderate severity in the present investigation, and no reports of any overt abnormalities of the placenta. The extent to which degeneration of the placenta must progress before it is clinically apparent will undoubtedly be greater than that required to have some adverse effect on the foetus. But the problem still remains as to the nature of the effect of such a degeneration on the lipids and lipoproteins of the cord blood, particularly since in adults, overt starvation is reported to produce increased serum lipid concentrations by Shafrir et.al. (1959) and alternatively, decreased serum lipid concentrations by Mann and White (1953). In view of this disagreement it is impossible to say whether any reduction in the supply of maternal nutrients to the infants of toxaemic mothers caused by placental degeneration could lead to the reduction in cord blood lipid concentrations which were observed or not.

The present investigation did yield one example of known postmaturity and placental insufficiency. This was Baby Ba. (see page 80). The cord blood lipid concentrations were low down in the normal ranges; the α -lipoprotein lipid concentration was very low; the high β -lipoprotein lipid concentration might have been a result of its containing a

"pre-beta lipoprotein" fraction. This bears no resemblance to the picture in the infants of toxaemic mothers and since the infant died, it seems likely that the diagnosis of "cerebral anoxia" covered a combination of several biochemical anomalies. It may be noted that "pre-beta lipoprotein" has been found in the sera of diabetic mothers by approximately the 25th week of pregnancy and increased to a maximum in the first two weeks post-partum (Vernet and Smith 1961). Its appearance was not associated with poor diabetic control, but it appeared earlier in pregnancies which terminated unsuccessfully.

Alterations in the Foetal Environment During the Early Stages of Gestation

In many ways, it seems unlikely that events at the end of gestation have very much to do with any of the abnormalities in either the infants of diabetic or toxaemic mothers or those of mothers who have had a previous abortion. In the first of these, evidence has been put forward suggesting that the lipoprotein lipid abnormality is one of the actual constitution of the lipoprotein molecule. In the last, the impression is received that the changeover from the foetal lipoprotein pattern to that obtaining in adults has begun in utero instead of after birth.

The original causes of these abnormalities may have existed very early in gestation, particularly during the

first trimester. At this time the foetus is most susceptible to teratogenic agents of all kinds, for this is the period of maximum differentiation and rate of growth. Youngstrom (1941) found that concentrations of acetyl choline esterase are closely related to the development of neur muscular function during this period. Flexner, Flexner and Strauss (1941) found that the concentrations of cytochrome C and cytochrome oxidase in the foetal pig increased during this, and other peak periods of differentiation. Any defect, even in only one enzyme system, would have far reaching effects.

The Effects of Known Teratogenic Agents

It is during the first trimester of gestation that infection of the mother with rubella virus leads to cataract, congenital heart disease, deafness and defects of the central nervous system. Similar effects may be obtained by subjecting animals to a remarkable variety of experimental procedures at this time. Maternal deficiency in Vitamin E (Cheng, Chang and Bairnson, 1957), in pantothenic acid (Nelson, Wright, Baird and Evans, 1957), in nicotinamide (Pinsky and Fraser, 1960), and in folic acid (Asling, 1961) have all been used in this way. So have excessive intakes of Vitamin A (Cohlan, 1953), ACTH and cortisone (Fraser and Feistat, 1951) as well as treatment

with trypan blue (Gilman, Gilbert, Spence and Gilman 1951) and nitrogen mustard (Haslin, 1948). The ill-effects of X-rays and other radiations are well known in this respect, and those of the sedative thalidomide have recently received considerable publicity in the national press.

Maternal Serum Proteins and the Development of Congenital Anomalies in the Infant

Langman, Drunen and Bouman (1959) have made an interesting survey of the serum protein fractions of pregnant women in relation to the development of congenital anomalies in their infants. Only one estimation was made on each woman but information was obtained for the whole gestational period from different women. Of the 178 normal pregnant women who all had normal serum proteins at the time of analysis, all had normal infants. Another group of 265 pregnant women was selected in that each one had had either previous abortions or one or more children with congenital malformations or infants who were abnormal in any other way. Mothers who were diabetic, syphilitic or had rhesus negative blood groups were excluded. Of these 265 mothers, 41 of them either had an abortion, or immature or premature infants, or full-term infants with congenital malformations. Abnormal serum protein curves had been found in 19 cases and 15 of these had abnormally ending pregnancies.

Since only one serum protein estimation was made on each of these mothers at widely differing times during pregnancy, (blood was taken the first time the mother visited the ante-natal clinic), this is quite an impressive total. It emphasizes the fact that an apparently normal person may have metabolic abnormalities which are quite undetectable by physiological examination.

It gives no clue to the cause of these abnormalities in the absence of any of the vitamin deficiencies or hormonal excesses which have been mentioned above, nor to the way in which such abnormalities might affect the foetus. It has already been remarked that maternal γ -globulins can cross the placenta and that there is some evidence that other maternal proteins can also do this.

Abnormalities of the serum protein, glycoprotein and lipoprotein patterns are of course well known in overt diabetes. The increase in β-lipoprotein typical of pregnancy is enhanced in diabetic pregnancy. Ditzel and Moinat (1957) report increases in β -glycoprotein in addition to β -lipoprotein in such cases. The appearance of "pre-beta lipoprotein" identified by Vernet and Smith (1961) has already been mentioned on page 116. How far these abnormalities affect the foetus is unknown.

If serum proteins can be altered in this way, it seems likely that serum lipoproteins will also show some

abnormalities. The alterations in serum cholesterol concentrations, which have been observed most commonly in the infants who were themselves abnormal or whose mothers were abnormal during pregnancy, may be the first or the most obvious sign of constitutional abnormalities in the lipoproteins.

The Necessity for Further Investigations into this Problem

The main result of this investigation is to pose many problems and to answer none. Far more sensitive methods are obviously necessary if details of constitutional abnormalities of lipoproteins are to be investigated. The concentrations of the various hormones in umbilical cord blood should be measured and their effects, in physiological combinations and concentrations, on serum lipid and lipoprotein concentrations established unequivocally. Longitudinal studies of the serum lipids and lipoproteins should be carried out in normal and abnormal mothers. The effects of placental dysfunction should be elucidated. But most of all, the actual function of the lipoproteins must be found. When all of these are done, not only may the questions posed here be answered, but also the reasons for some of the abnormalities observed in some new-born infants may be found.

CONCLUSIONS

1. The mean concentrations of the lipids and lipoprotein lipids in the umbilical cord blood are between one half and one third of those present in maternal venous blood.
2. The pattern of lipoprotein distribution in umbilical cord blood is different from that in maternal venous blood. In the cord blood, α -lipoprotein lipid is dominant; in maternal venous blood the β -lipoprotein lipid is dominant.
3. The relative proportions of the individual cord blood lipids are not completely accounted for by the differences in the proportions of the lipoprotein lipid fractions. This could indicate some slight difference in the constitution of the lipoproteins in the cord blood compared with those in maternal venous blood.
4. The concentrations of some of the lipids and lipoprotein lipids in cord blood have been found to be related to each other in a manner which is to be expected from the constitution of the different lipoproteins and which is susceptible to statistical analysis.
5. Some infants, who are themselves abnormal or whose mothers showed some abnormality during pregnancy, have been found to show certain abnormalities in their cord blood lipid and lipoprotein lipid concentrations.
6. The lipid concentration most frequently found to be

abnormal is that of cholesterol. It is higher than normal in infants of diabetic mothers and in infants of mothers who have had a previous abortion. It is lower than normal in infants of toxaemic mothers.

7. The alteration in cord blood cholesterol concentrations is not accompanied by a parallel alteration in the other lipid concentrations or in the lipoprotein lipid concentrations in the infants of diabetic mothers.

8. Evidence is put forward suggesting that in the infants of diabetic mothers the lipid composition of one or both the lipoprotein fractions is abnormal. It seems likely that the abnormality is mainly concerned with the cholesterol content of the ($\beta + \gamma$)-lipoprotein fraction.

9. It is considered possible that in the infants of mothers who have had a previous abortion the transition from the lipoprotein pattern characteristic of cord blood to that characteristic of adult blood has begun to take place in utero. This is because there is a generalised increase in the three individual lipid concentrations and in the β-lipoprotein lipid concentration but not in the concentrations of α- and γ-lipoprotein lipid.

10. In the infants of toxaemic mothers there is no evidence

for any specific abnormality of the lipid composition of any of the lipoprotein fractions or for any difference in the lipoprotein pattern. There is a generalised decrease in the individual lipid concentrations which is probably accounted for by the parallel but smaller decreases in the lipoprotein lipid concentrations.

11. The abnormalities in the lipids and lipoprotein lipids in the cord blood of the three groups of abnormal infants have been discussed on the basis of other abnormalities known to occur in the placenta and in the concentrations of any serum constituents in either the mother or the infant. No conclusions were reached which might explain these abnormalities because insufficient data are available.
12. Suggestions have been made on the nature of further investigations which might lead to an explanation of these problems.

CHAPTER V

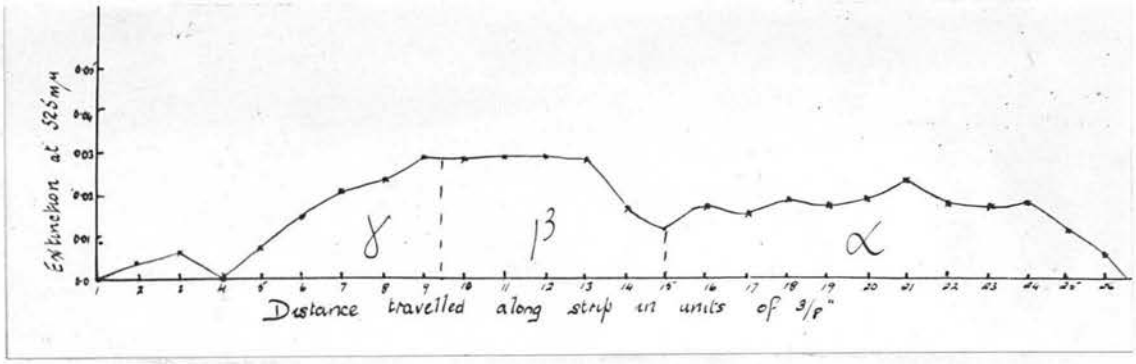
SERUM LIPIDS AND LIPOPROTEIN LIPIDS IN THE INFANTS OF DIABETIC MOTHERS DURING THE FIRST WEEK OF LIFE

INTRODUCTION

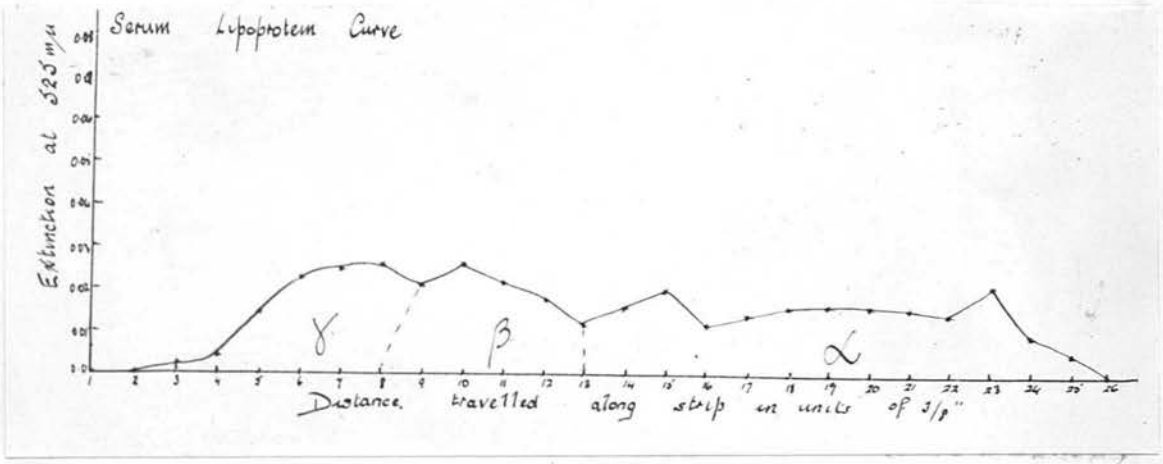
Infants born to diabetic mothers commonly exceed the mean body weight and crown to heel length for their gestational age (Farquhar, 1958). The infants are peculiarly subject to pulmonary hyaline membrane disease, and respiratory distress with cyanotic attacks are common. (Ibid). A prolonged Moro reflex produces the effect of twitching and convulsions. Physiological jaundice is common and, as with other premature infants, is often deep and prolonged (Ibid).

They appear oedematous at birth but are reported to have reduced extra-cellular and total body water (Osler, 1960). Opinions differ on the occurrence of increased urine production. Post-natal weight loss is not abnormal. Osler (1960) considers that the apparent oedema is caused by excessive deposition of glycogen and fat as a result of hyperinsulinism and also as a result of the non-utilization

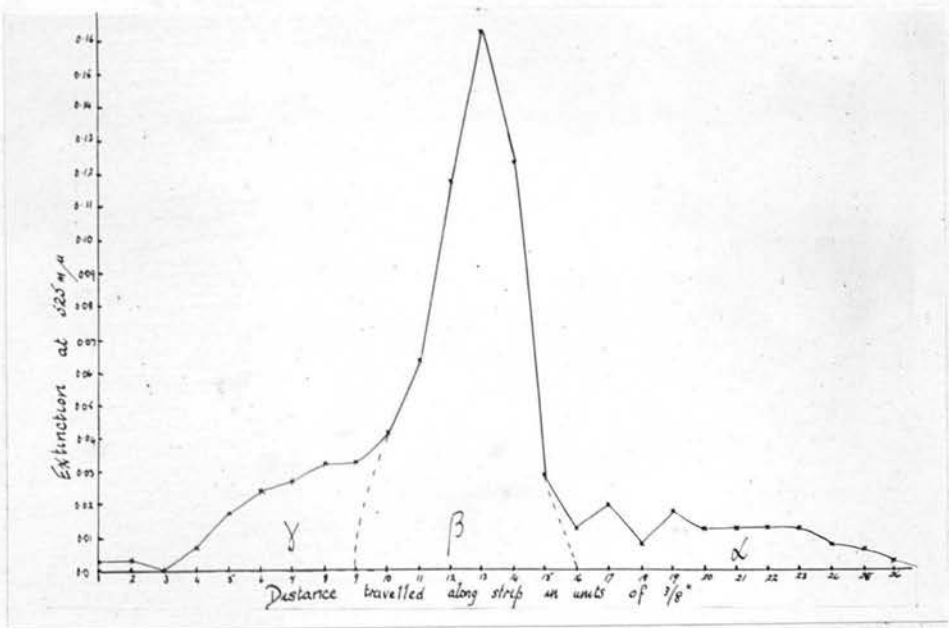
Figure 14 (cont.) Case Co.



c. Infant : Venous Blood. Day 1.



d. Infant : Venous Blood. Day 5.

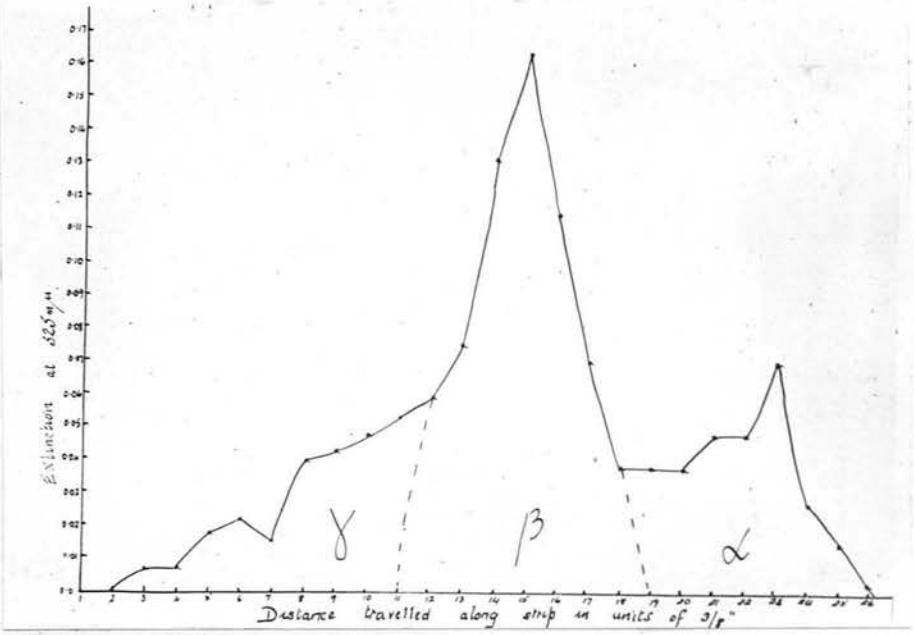


e. Infant : Venous Blood. Day 7.

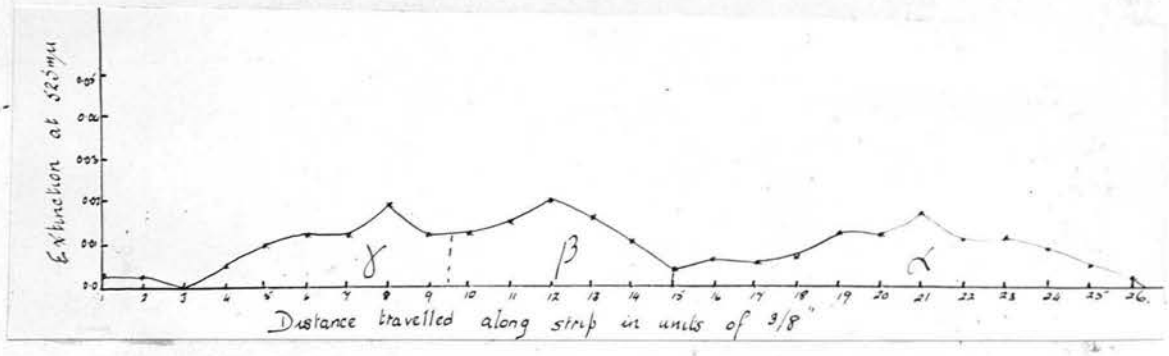
Figure 14

Serum Lipoprotein Curve Obtained by the Electrophoretic Separation of Lipoproteins in the Blood of Infants of Diabetic Mothers During the First Week of Life and in That of the Mother at the Time of Delivery of the Infant.

Case Co.



a. Maternal Venous Blood at the Time of Delivery.

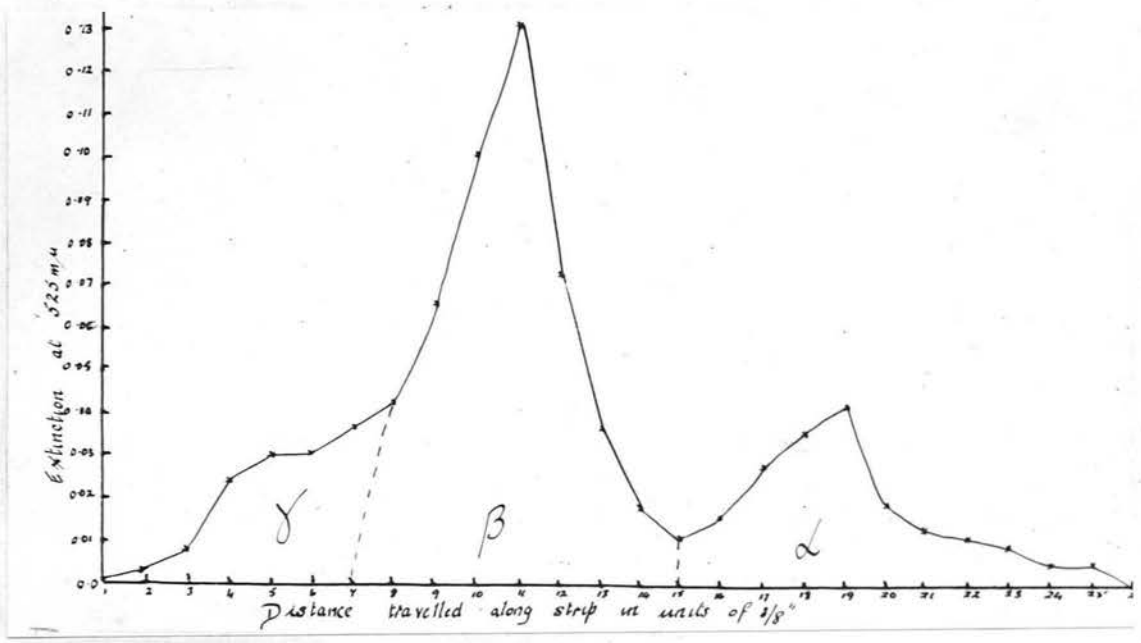


b. Infant : Cord Blood

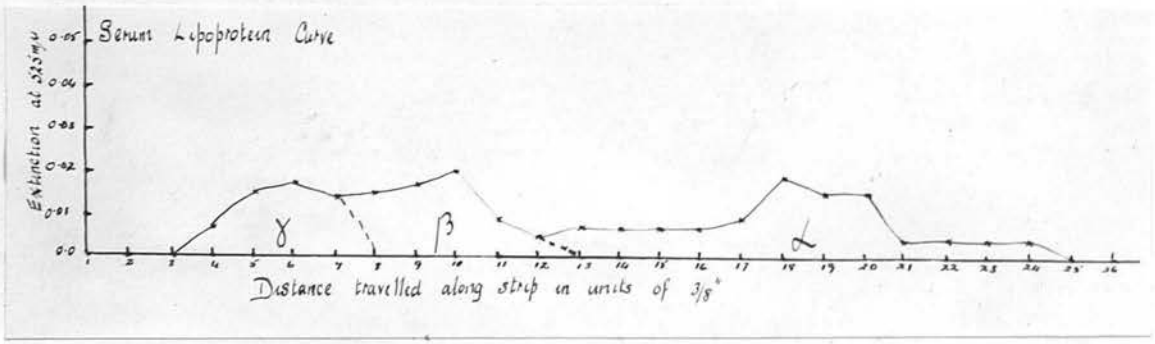
Figure 15

Serum Lipoprotein Curves Obtained by Electrophoretic Separation of the Lipoproteins in the Blood of Infants of Diabetic Mothers During the First Week of Life and in That of the Mother at the Time of Delivery of the Infant.

Case Do.

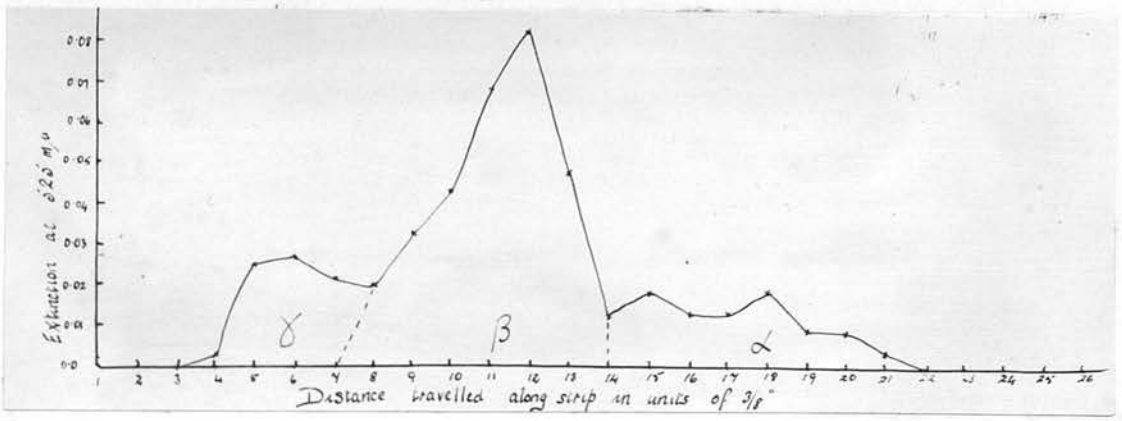


a. Maternal Venous Blood at the Time of Delivery.

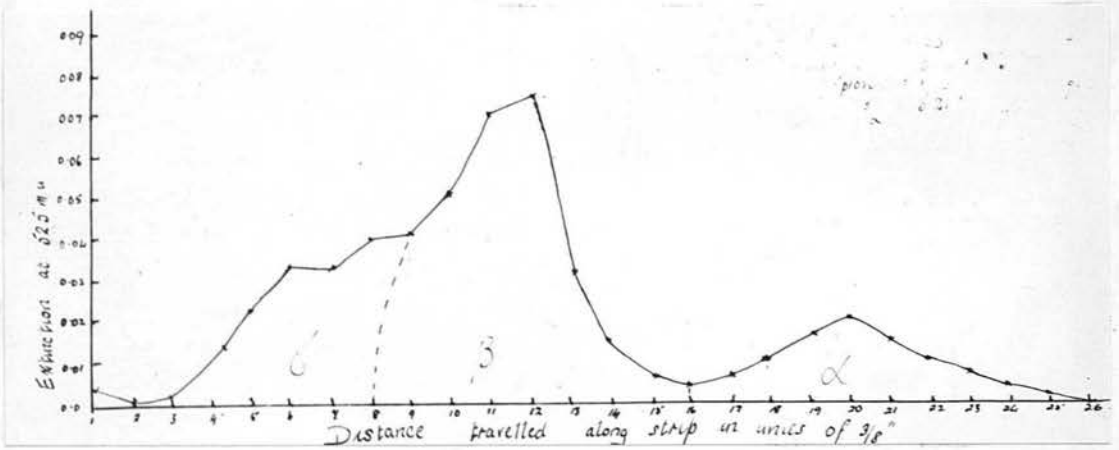


b. Infant : Cord Blood.

Figure 15 (cont.) Case Do.



c. Infant : Venous Blood. Day 5

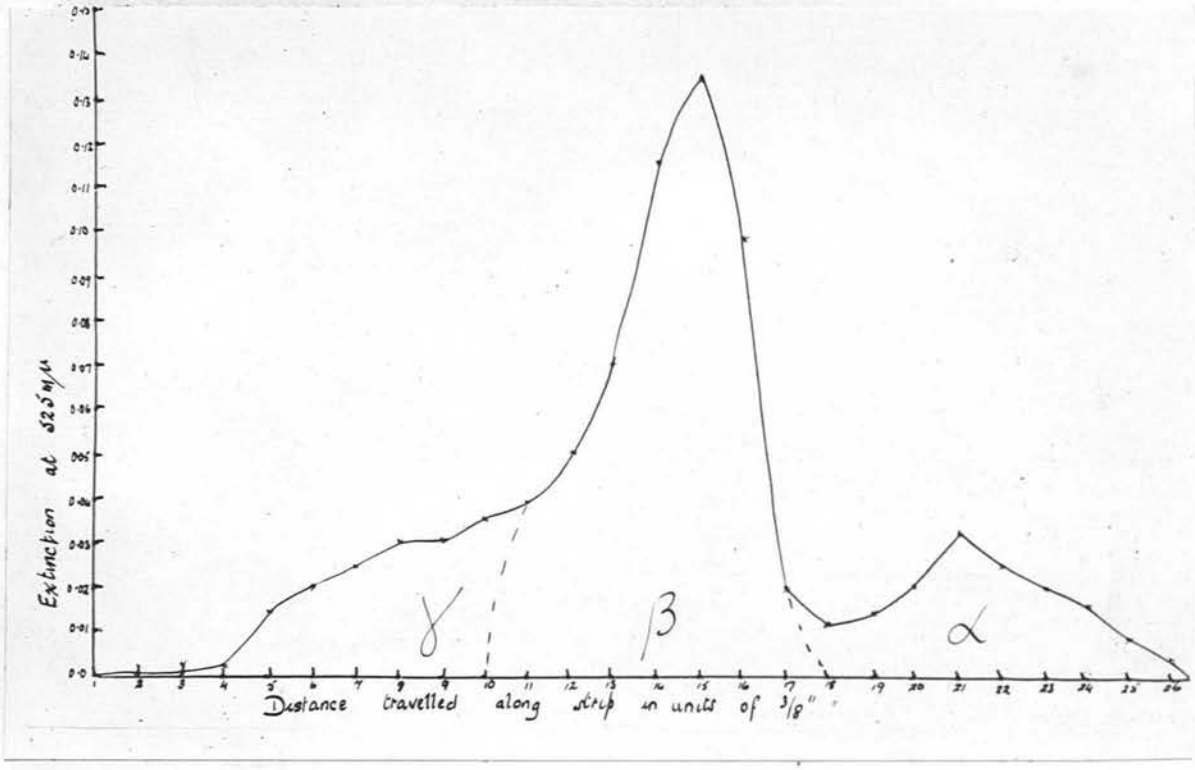


d. Infant : Venous Blood. Day 7.

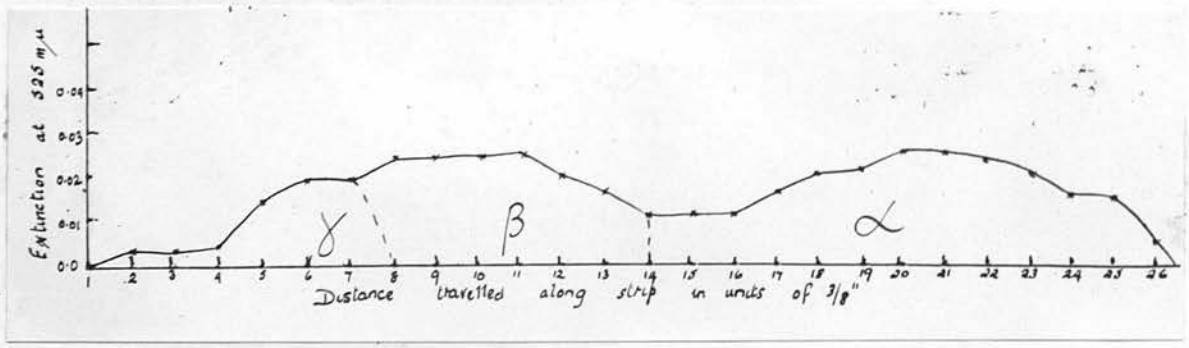
Figure 16

Serum Lipoprotein Curves Obtained by Electrophoretic Separation of the Lipoproteins in the Blood of Infants of Diabetic Mothers During the First Week of Life and in that of the Mother at the Time of Delivery of the Infant.

Case Sa.

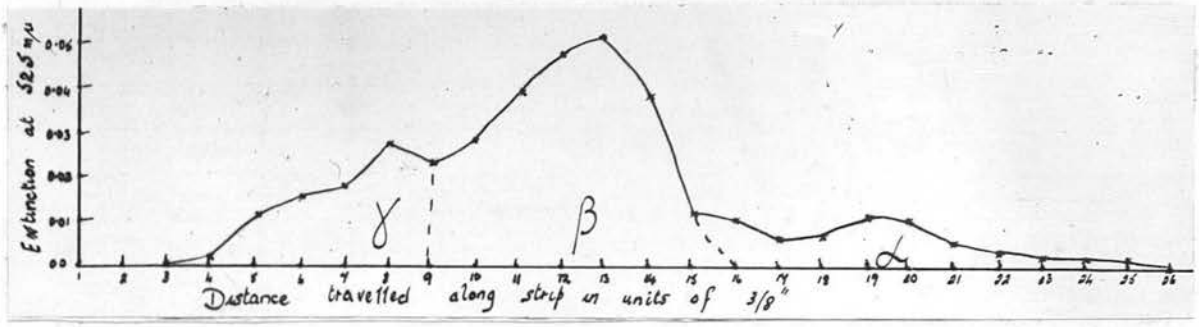


a. Maternal Venous Blood at the Time of Delivery.

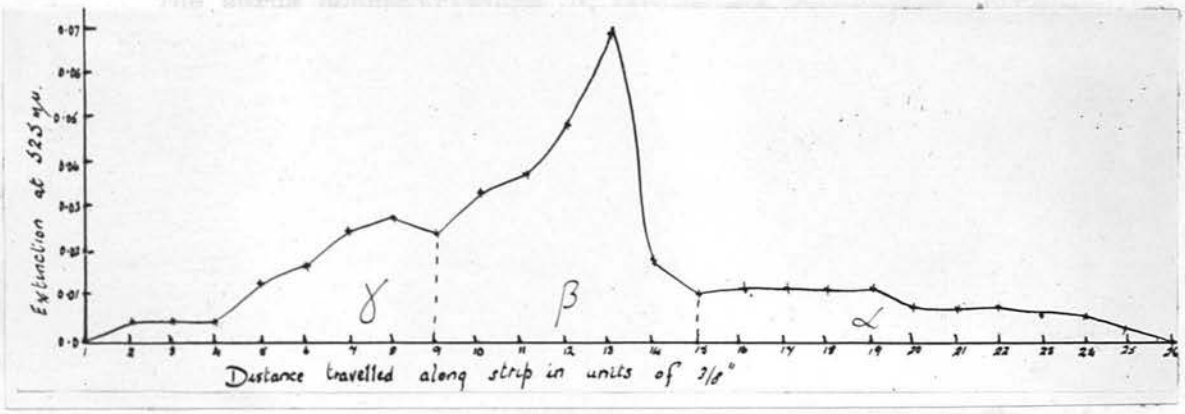


b. Infant : Cord Blood.

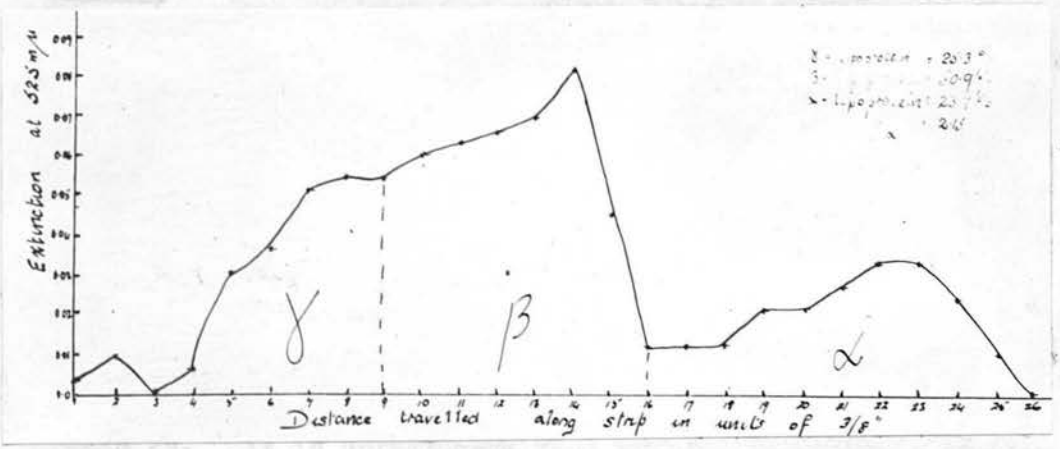
Figure 16 (cont.) Case Sa.



c. Infant : Venous Blood. Day 1.



d. Infant : Venous Blood. Day 3.



e. Infant : Venous Blood. Day 7.

of the fat depots during the pre-feeding period.

Because of their size and a tendency towards intra-uterine death after the thirty sixth week of gestation, parturition is often induced or a caesarian section performed at this time (Farquhar, 1958). The infants of diabetic mothers are therefore usually from three to four weeks premature by date but not by size.

The serum concentrations of lipids and lipoprotein lipids were measured in the cord blood of infants of diabetic mothers and in their venous blood at intervals during the first week of life. Blood was taken by vene-punctures or by posterior fontanelle punctures on the first, third and seventh day of life in most cases. In three of the infants, a different time interval resulted from difficulties in obtaining blood.

RESULTS

The data obtained are given in Table 28 and some of these are illustrated in Fig.17 Some of the lipoprotein distribution curves obtained by electrophoresis of blood serum taken at the different time intervals are given in Figs. 14 - 16.

The mean concentrations of the serum lipids and lipoprotein lipids and their standard errors are given in Table 29. It is unfortunate that efforts to obtain a similar series of data from normal infants produced only a few blood samples from six day old infants. For this reason, the data

TABLE 28

Serum Lipid and Lipoprotein Lipid Concentrations in the Blood of 12 Infants of Diabetic Mothers during the first week of life

Case	Day of Life	Placental Weight. lbs.ozs.	Birth Weight. lbs.ozs.	Sex	Maternal Age	Parity	Duration of Pregnancy	Duration of Diabetes	Serum Total Bilirubin mg/100 ml.	Total Lipid mg/100 ml.	Total Esterified Fatty Acid. Mg/100 ml.	Lipid Phosphorus mg/100 ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	C/P	γ-Lipoprotein %	β-Lipoprotein %	α-Lipoprotein %	γ-Lipoprotein lipid. mg/100 ml.	β-Lipoprotein lipid. mg/100 ml.	α-Lipoprotein lipid. mg/100 ml.	β/α	Calculated Total Lipid mg/100 ml.	Notes
Gor.	0	1.3	6.3	F	41	4	36	9y		316	166	5.2	135	79	0.59	14.0	34.6	51.4	44	109	162	0.67	286	Maternal palpable thyroid. 4th infant diabetic with muscular paralysis.
	1									406	172	5.0	130	103	0.79	-	-	-	-	-	-	-	314	
	3	Fed on 2nd.day								534	216	6.1	159	150	0.94	25.5	30.5	44.0	136	163	235	0.69	414	Caesarian section.
	7									584	344	9.4	244	187	0.77	31.3	38.0	30.7	183	222	179	1.24	604	
	13									493	119	9.7	252	172	0.68	34.4	35.1	30.5	170	173	150	1.15	367	
Rei	0	1.6	7.6	F	25	0	36	1y		228	116	3.7	96	66	0.69	26.9	28.4	44.6	61	65	102	0.64	211	Well controlled.
	1	Fed on 3rd.day								472	210	6.0	156	130	0.83	26.0	48.6	25.3	123	229	119	1.92	387	Spontaneous vertex delivery.
	10									468	223	6.5	169	180	1.07	30.9	40.6	28.5	145	190	133	1.43	454	
Gl	0	0.15	6.4	M	19	0+1	36	2-3y		270	144	4.6	120	80	0.67	20.0	46.4	33.6	54	125	91	1.37	260	Previous incomplete abortion at 12/52.
	4	Fed on 3rd.day							13.6	444	266	5.9	154	114	0.74	15.5	44.6	39.9	69	198	177	1.12	426	Spontaneous vertex delivery.
	7									452	222	7.3	190	158	0.83	15.2	61.4	23.4	69	278	106	2.62	437	
Mo	0	1.4	6.12	M	34	0	35	15y		280	93	3.5	90	84	0.83	12.9	37.1	49.9	36	104	140	0.74	204	Well controlled.
	1									452	166	5.4	140	146	1.04	17.6	51.2	31.2	79	238	141	1.69	354	Caesarian section.
	3	Fed on 2nd.day								548	231	7.2	187	176	0.94	30.9	35.2	33.9	169	193	186	1.04	453	
	7									632	225	8.8	228	241	1.06	36.5	40.9	22.6	231	258	143	1.80	534	
No	0	1.6	8.4	F	34	4	38	4/52		336	180	3.8	100	88	0.88	11.4	26.2	62.4	38	88	210	0.42	298	Uneventful.
	1									332	206	6.0	155	108	0.70	37.9	27.9	34.2	126	93	114	0.82	361	Forceps delivery.
	3	Fed on 2nd.day								486	192	7.9	205	148	0.72	31.7	38.0	30.3	154	185	147	1.25	402	
	7									468	162	7.0	181	139	0.78	21.4	33.6	45.1	100	157	211	0.74	355	
Ro	0	1.2	7.7	F	36	6	37	2y		316	121	4.4	114	121	1.06	21.0	30.1	48.9	66	95	154	0.62	276	Mother obese. Dietary diabetic control. 1st infant, died at 20 minutes. 6th stillborn.
	1									340	143	6.8	176	125	0.71	30.6	32.9	36.5	104	112	124	0.90	321	Caesarian section.
	3	Fed on 2nd.day								544	237	6.7	175	165	0.94	22.9	51.6	25.5	125	281	139	2.02	455	
	7									586	304	9.0	233	221	0.95	18.7	60.1	21.2	109	352	124	2.83	595	
Co	0	1.3	6.5	M	22	0	36	11y		310	177	5.1	133	90	0.68	27.0	30.1	42.8	84	93	133	0.70	307	Mother badly controlled. Admitted for stabilisation at 16/52.
	1	Became jaundiced								400	128	6.0	156	112	0.72	23.0	34.4	42.6	92	137	170	0.81	287	Forceps delivery.
	3	Fed this day after blood collection							13.6	270	55	3.1	81	74	0.91	24.9	37.3	37.8	67	101	102	0.99	153	
	7	Developed parotitis							17.8	1096	515	18.9	491	366	0.74	12.9	69.6	17.5	141	763	192	3.97	1028	
	14									1256	536	17.0	422	458	0.96	9.3	73.6	17.1	117	924	213	4.29	1121	
Sc	0	1.4	6.8	M	32	2+2	36	14y		292	122	4.2	109	75	0.68	19.0	42.8	38.2	55	125	111	1.12	230	1st pregnancy abortion at 16/52.
	1									508	170	6.0	155	98	0.63	29.8	48.4	21.8	151	246	111	2.22	315	4th pregnancy abortion at 5/12.
	3	Fed this day before blood collected. Jaundiced								583	309	7.8	204	133	0.65	22.0	47.7	30.4	128	278	177	1.57	503	Caesarian section.
	7									784	260	8.9	230	234	1.02	22.1	50.2	27.7	173	394	102	1.81	563	
Do	0	1.0	7.5	F	34	4+1	37	8y		340	159	5.0	130	68	0.52	23.8	29.5	46.6	81	101	158	0.63	266	Mother Rh.-ve. Pregnancy 5 aborted at 18/52.
	3	Fed this day. Time unknown								797	512	5.7	147	143	0.97	16.5	64.3	19.1	132	512	152	3.37	699	One infant - paroxysmal auricular tachycardia.
	7									693	360	8.9	231	165	0.91	25.7	56.6	17.6	178	392	122	3.21	594	Caesarian section.
Sa	0	1.11	6.12	F	32	1+1	38	7y		328	185	5.0	129	55	0.43	15.0	34.8	50.2	49	97	165	0.69	266	Badly stabilised. Frequent hypoglycaemic coma at 34/52. Pregnancy 1, stillbirth. Pregnancy 2, abortion at 24/52.
	1									668	307	9.0	234	42	0.18	26.0	58.0	16.0	174	387	107	3.60	419	
	3	Fed on 2nd.day								761	192	9.8	255	87	0.34	20.0	59.1	20.9	152	450	159	2.80	356	
	7									849	277	11.7	304	258	0.85	25.3	50.9	23.7	215	439	201	2.15	620	Forceps delivery.
Bu	0	1.6	5.15	F	27	1	36	9y		276	106	4.2	109	86	0.79	24.0	25.3	50.6	65	68	137	0.50	225	Badly stabilised at 32/52.
	1	Cyanotic attack								424	161	6.3	164	109	0.66	20.6	42.9	36.5	87	182	155	1.18	319	Caesarian section.
	3	Fed this day before blood collected								468	149	4.6	120	106	0.88	19.9	30.7	49.3	93	184	231	0.62	291	
	7								9.6	599	222	7.3	190	161	0.85	16.4	45.7	37.9	98	274	227	1.21	440	
Ph	0	1.6	7.6	F	25	0	37	1y		238	127	3.8	98	74	0.80	19.3	24.6	56.1	46	58	133	0.44	230	Well controlled.
	1									490	249	6.1	157	123	0.78	28.4	43.9	27.7	139	215	136	1.58	419	Caesarian section.
	3	Fed this day after blood collected								524	226	8.2	214	178	0.83	32.7	43.1	24.2	171	226	127	1.78	468	
	7									644	298	11.7	303	268	0.88	26.3	42.9	30.8	169	276	198	1.39	657	

given by Rafstedt (1955), for normal full term and premature infants have been used as a basis for comparison with the data obtained in this investigation. Rafstedt's data for the mean concentrations of serum lipids and lipoprotein lipids in fifteen full-term and fifteen premature infants are given in Table 30.

It has been said previously in Chapter IV, pages 62-69, that the mean serum lipid and lipoprotein lipid concentrations in the cord blood of infants of diabetic mothers differ somewhat from those of normal infants. The concentration of total cholesterol is 83.83 $\pm$ 5.29 (18) mg./100 ml. ($0.02 > p > 0.01$), compared with a normal value of 71.82 $\pm$ 2.05 (50) mg./100 i.e. it is significantly higher than normal. The concentration of γ-lipoprotein lipid is 61.76 $\pm$ 4.91 (18) mg./100 ml. ($0.01 > p > 0.001$), which is lower than the normal value of 81.78 $\pm$ 3.90 (50) mg./100 ml. Both the concentrations of α- and of β-lipoprotein lipids are normal and the data suggest that at least one of the lipoprotein lipid fractions, probably the ($\beta + \gamma$)-lipoprotein lipid fraction carries more cholesterol than normal. These data refer to a group of eighteen infants.

Of these eighteen, only twelve were studied during the first week of life. The other six were born before this part of the investigation had begun and it is perhaps unfortunate that some of these six had higher cord blood

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T A B L E 29

Mean Serum Lipid and Lipoprotein Concentrations in Normal Infants at birth and in Infants of Diabetic Mothers during the first week of life.

	Total Lipid	TEFA	Phospho-lipid	Choles-terol	C/P	α-LP	β-LP	γ-LP	β/α
Cord Blood of Normal Infants (50)	317.7 ±8.8	148.3 ±4.8	115.2 ±2.9	71.8 ±2.1	0.64 ±0.02	141.0 ±4.9	95.5 ±5.2	81.8 ±3.9	0.76 ±0.06
Cord Blood of Infants of Diabetic Mothers (18)	284.2 ±9.7	142.3 ±7.6	112.8 ±5.6	83.8 ±5.3	0.77 ±0.07	139.7 ±6.6	97.3 ±7.3	61.2 ±4.9	0.76 ±0.09
Cord Blood of Infants of Diabetic Mothers (11)	292.7 ±11.5	138.1 ±9.2	110.3 ±4.9	79.6 ±5.1	0.72 ±0.05	130.0 ±16.4	76.0 ±10.1	54.1 ±4.0	0.71 ±0.9
Venous Blood of Infants of Diabetic Mothers on									
Day I	454.7 ±30.4	198.2 ±15.7	152.4 ±10.2	109.3 ±8.9	0.70 ±0.07	108.9 ±14.2	152.9 ±17.4	122.9 ±10.8	1.55 ±0.27
Day III	568.9 ±33.7	253.0 ±35.7	178.0 ±11.7	140.0 ±9.1	0.79 ±0.06	160.3 ±19.6	169.6 ±15.4	132.9 ±10.8	1.63 ±0.27
Day VII	629.1 ±37.4	267.4 ±18.5	223.7 ±14.9	203.2 ±14.0	0.89 ±0.03	141.5 ±20.3	210.0 ±21.7	152.5 ±18.5	1.90 ±0.24

lipid concentrations than the other twelve. For this reason, the mean concentrations of cholesterol and β - and γ - lipoprotein lipid are lower in the cord blood cholesterol concentrations is within the normal range; that of β -lipoprotein lipid is slightly below normal, and that of γ -lipoprotein lipid is even lower than it was in the original eighteen infants.

It is at once obvious, from Tables 29 and 30 and Fig. 17 that, with the exception of α -lipoprotein lipid, there is a great increase in the concentrations of all the serum lipids and lipoprotein lipids immediately after birth. Table 31 gives the mean increment in each lipid in mg./100 ml. of serum compared with the original concentrations in cord blood and Table 32 the mean percentage increment based on the concentration of the previous occasion on which it was measured. These data may be compared with those of Rafstedt which are also given in Table 31. Data for Baby Co. in this investigation, have been omitted from the increment calculations because parotitis developed between the third and seventh day. By this date the serum lipid concentrations had reached adult values in this infant and the lipoprotein pattern had assumed the adult form.

SERUM LIPID CONCENTRATIONS

TOTAL LIPID

The mean serum total lipid concentration, which is

TABLE 30

Mean Serum Lipid and Lipoprotein Lipid Concentrations in mg/100 ml. in Normal Infants at Different Times in the First Week of Life. Taken from Rafstedt(1955)

	Total Lipid	TEFA	Phospho-lipid	Chole-sterol	C/P	α-Lipo-protein	β-Lipo-protein	γ-Lipo-protein	β/α
Cord Blood of Normal Full-term Infants (15)	314 ±14	-	124 ±6.7	74 ±3.5	0.60	134 ±9.0	103 ±9.0	77 ±7.2	0.77
Venous Blood of Normal Full-term Infants (15) at 18 - 24 Hours	443	-	188	99	0.53	159	168	116	1.06
at 3 - 10 Days	608 ±30	-	207 ±8.1	134 ±4.9	0.63	194 ±12	277 ±11.5	138 ±7.4	1.59
Cord Blood of Premature Infants (15)	306 ±13	-	102 ±6.4	67 ±3.5	0.65	122 ±5.3	98 ±6.3	86 ±3.6	0.80
Venous Blood of Premature Infants (15) at 3 - 26 Days	537 ±22	-	191 ±10	117 ±6.6	0.61	156 ±9.6	247 ±13	133 ±5.7	1.59

within the normal range in the cord blood, increases rapidly on the first day of life. There is a smaller increase during the next six days when a concentration of 639 ± 37 (11) mg./100 ml. is reached. The data are very similar to those of Rafstedt for normal infants, in whom a concentration of 608 ± 30 (15) mg./100 ml. was found between the third and tenth day. Rafstedt reports a slower rate of increase in premature infants compared with full-term ones, the former reaching values at eight days comparable with those of normal infants on the fourth day. Infants of diabetic mothers do not behave like premature infants in this respect.

TOTAL ESTERIFIED FATTY ACIDS

The mean serum concentration of total esterified fatty acid in the cord blood is normal. The initial rapid increase in the concentration ceased by the seventh day. Rafstedt does not give data for this lipid fraction.

PHOSPHOLIPID

There is the same rapid increase in the mean serum concentration of phospholipid from an initially normal cord blood concentration. This has slowed down by the third day and the percentage increase is smaller between the first and third day than during the other intervals. The mean concentration on the seventh day is 224 ± 15 (11) mg./100 ml. In Rafstedt's series there is a steady decline in the increment

TABLE 31

The Mean Increases in the Serum Lipid and Lipoprotein Lipid Concentrations in mg./100 ml. during the First Week of Life, compared with the original Concentrations in the Cord Blood of 15 Normal Full-term and 15 Premature Infants (taken from Rafstedt, 1955) and in 11 Infants of Diabetic Mothers.

	Total Lipid mg/100 ml.	Total Esterified Fatty Acid. mg/100ml.	Phospholipid mg/100 ml.	Cholesterol mg/100 ml.	α-Lipoprotein Lipid mg/100 ml.	β-Lipoprotein Lipid mg/100 ml.	γ-Lipoprotein Lipid mg/100 ml.
Original Cord Blood Concentrations							
Normal Infants	314 ± 14	-	124 ± 7	74 ± 4	134 ± 9	103 ± 7	77 ± 5
Premature Infants	306 ± 13	-	102 ± 6	67 ± 4	122 ± 5	98 ± 6	86 ± 4
Infants of Diabetic Mothers	293 ± 12	138 ± 9	110 ± 5	80 ± 5	130 ± 16	76 ± 10	54 ± 4
Mean Increases Observed							
Normal Infants 18 - 24 Hours	+139	-	+ 64	+ 25	+25	+ 65	+39
Infants of Diabetic Mothers. 1 Day Old	+162	+ 60	+ 42	+ 30	-21	+ 77	+69
Infants of Diabetic Mothers. 3 Days Old	+274	+117	+ 68	+ 61	+30	+ 94	+79
Normal Infants 3 - 10 Days Old	+294	-	+ 83	+ 60	+60	+174	+61
Premature Infants 7 Days Old	+231	-	+ 51	+ 86	-	-	-
Infants of Diabetic Mothers. 7 Days Old	+335	+131	+113	+124	-21	+144	+98

rate and the mean concentration between the third and the tenth day is 207 ± 8 (15) mg./100 ml. This is of the same order as the corresponding one for infants of diabetic mothers.

CHOLESTEROL

The mean serum cholesterol concentration in the cord blood of this series of 11 infants of diabetic mothers is 80 ± 5 (11) mg./100 ml. This is above the normal value of 72 ± 2 (50) mg./100 ml. but the numbers are insufficient to make the difference significant. It increases rapidly and by the seventh day the mean concentration is 203 ± 14 (11) gm./100 ml. compared with a value of 134 ± 5 (15) mg./100 ml. which Rafstedt found in his normal series. As in the case of phospholipid concentrations, the increment rate is least between the first and third day. There appears to be no relation between the increment rate in cholesterol concentrations and the development of raised serum bilirubin concentrations in the infants who became jaundiced.

RATIO OF CHOLESTEROL TO PHOSPHOLIPID CONCENTRATIONS

The ratio of cholesterol to phospholipid concentrations in normal infants is 0.64 ± 0.02 (50); in the larger group of infants of diabetic mothers it is 0.77 ± 0.07 (18) and in this group of infants of diabetic mothers it is 0.72 ± 0.05 (11). The former value is significantly different from normal but the numbers in the latter group are too small to allow the

TABLE 32

THE MEAN PERCENTAGE INCREASES IN THE SERUM CONCENTRATIONS OF LIPIDS AND LIPOPROTEIN LIPIDS IN THE INFANTS OF DIABETIC MOTHERS DURING THE FIRST WEEK OF LIFE COMPARED WITH THE CONCENTRATION WHEN PREVIOUSLY MEASURED.

	Total Lipid	Total Esterified Fatty Acid	Phospholipid	Cholesterol	α-Lipoprotein Lipid	β-Lipoprotein Lipid	γ-Lipoprotein Lipid
Cord Blood Concentration in mg/100 ml.	292.7	138.1	110.3	79.6	130.0	76.0	54.1
Day I % Increase	+55%	+43.5%	+38.1%	+36.2%	-16.0%	+100.0%	+127.0%
Day III % Increase	+25%	+27.7%	+11.7%	+28.4%	+46.8%	+1.11%	+8.1%
Day VII % Increase	+10.6%	+5.5%	+18.4%	+45.0%	-11.6%	+24.0%	+14.8%

demonstration of a significant difference. The value of this ratio rises progressively until, by the seventh day, it is 0.89 ± 0.03 (11) which is highly significantly above the value of 0.63 (15) quoted by Rafstedt for his normal series between the third and tenth day of life. This increase is almost entirely due to the abnormally great increase in the serum cholesterol concentration.

It will be remembered that a theoretical value for this ratio in cord blood was calculated on the basis of the observed lipoprotein distribution on the assumption that the lipoproteins had a normal lipid constitution (See Chapter IV page 68). The calculated value was considerably lower than the observed value, the discrepancy being 0.11. If the ratio is calculated in the same way from the data for the seventh day of life, a value of 0.76 is obtained compared with an observed value of 0.89. It has been suggested that this discrepancy in the data for cord blood is a result of an abnormality of the lipid constitution of the lipoproteins and it seems from this that it is maintained after birth. In fact the discrepancies observed between the two values for the ratio in both cord blood and at seven days are the same as that in the data for maternal blood. The same calculations have been made for Rafstedt's series of normal infants at birth and between the third and tenth day of life. The data is summarised in Table 33.

T A B L E 33

	Calculated C/P	Observed C/P	Observed-Calculated
Cord blood of infants of diabetic mothers	0.66	0.77 ± 0.07(18)	-0.11
Infants of diabetic mothers at seven days	0.76	0.89 ± 0.03(11)	-0.13
Cord blood of normal infants	0.67	0.64 ± 0.02(50)	+0.03
Cord blood of normal infants (Rafstedt)	0.69	0.60 (15)	+0.09
Normal infants at seven days (Rafstedt)	0.75	0.63 (15)	+0.12
Diabetic mothers	0.83	0.94 ± 0.05(12)	-0.11
Normal mothers	0.81	0.93 ± 0.04(20)	-0.12

It will be observed from this Table that if any change occurs in the lipid constitution of the lipoproteins in the normal infants it is in the opposite direction from that in the infants of diabetic mothers at birth and during the first week of life. In fact, the discrepancy between the observed and calculated values in Rafstedt's normal infants is of the same order at both of these times. More information is obviously required before any conclusions can be drawn from this.

SERUM LIPOPROTEIN LIPID CONCENTRATIONS

α-Lipoprotein Lipid

The mean concentrations of α-lipoprotein lipid in the cord blood of infants of diabetic mothers is within the normal range. It is very similar to that in Rafstedt's normal series. In the present series it decreased by 16 per cent on the first day and then increased again by 50 per cent. A further small decrease produced a concentration of 142 ± 20 (11) mg./100 ml. on the seventh day. This is considerably lower than the concentration of 194 ± 12 (15) mg./100 ml. which Rafstedt found in his normal infants between the third and tenth day of life. In his series there was a steady increase in the concentration of α-lipoprotein lipid throughout the first week of life as the increasing concentration of total lipid compensated for the gradual decrease in the proportion of the lipoprotein fraction. In Rafstedt's series of premature

infants there was the same steady increase but the final concentration of 156 ± 10 (15) mg./100 ml. bears more resemblance to that of the infants of diabetic mothers than does the value for his normal series.

The fall and subsequent rise in α -lipoprotein lipid concentrations in the infants of diabetic mothers are not statistically significant changes because they do not all take place on the same days. The fall in concentration occurs in three out of nine cases on the first day and in two others by the third day. In two there was no change and in only two did it increase. In three other cases there were no data for one or both of these times. If the differences in the time that it occurs are allowed for in the calculation, it is found that the fall in α -lipoprotein lipid concentration is a significant one.

As far as can be determined from Rafstedt's data this fall did not occur in his normal infants. For this reason it cannot be ascribed to an imbalance of the increasing total lipid concentration and the decreasing proportion of α -lipoprotein lipid. It cannot be ascribed to alterations in the packed cell volume of the blood either, because this would be reflected in all the serum lipid concentrations.

There does seem to be a connection between the changes in the concentration of the α -lipoprotein lipid and the time elapsing between birth and the first feed. This interval is variable. The timing is given in each case in Table 28.

The infants who were first fed on the second day showed an increase in α -lipoprotein lipid concentrations by the third day. One who was first fed on the third day showed an increase by the fourth day (Baby Gl.). In Baby Ph. and Baby Co. who were first fed on the third day after that day's blood sample had been taken, the initial fall in α -lipoprotein lipid concentration must have occurred between the times of blood collection on the first and third days. Baby Bu. and Baby Sc. who were first fed before blood was collected on the third day, already showed an increase in the α -lipoprotein lipid concentration in that day's blood specimen.

In seven out of ten cases, the concentration of α -lipoprotein lipid had fallen again by the seventh day. In three it had increased. Of the other two of the total of twelve cases, Baby Co. had developed parotitis by this time and there are insufficient data for Baby Re. to judge the behaviour of the lipids.

β-Lipoprotein Lipid

The mean serum concentration of β -lipoprotein lipid in the cord blood of this group of eleven infants of diabetic mothers is 76 $\pm$ 10 (11) mg./100 ml. which is below that of 97 $\pm$ 7 (18) mg./100 ml. found in the original group of eighteen infants of diabetic mothers. (See page 126). There is an increase of 100 per cent on the first day of life

and after this the increment rate falls sharply to 1.0 per cent and then rises to 6.0 per cent per diem. By the seventh day there is considerably less β-lipoprotein lipid in the serum than in Rafstedt's normal series and less even than in hid group of premature infants. In the infants of diabetic mothers the final concentration is 210 $\pm$ 22 (11) mg./100 ml. and in Rafstedt's series it is 277 $\pm$ 12 (15) mg./100 ml. for normal infants and 247 $\pm$ 13 (15) mg./100 ml. for premature infants.

γ-Lipoprotein Lipid

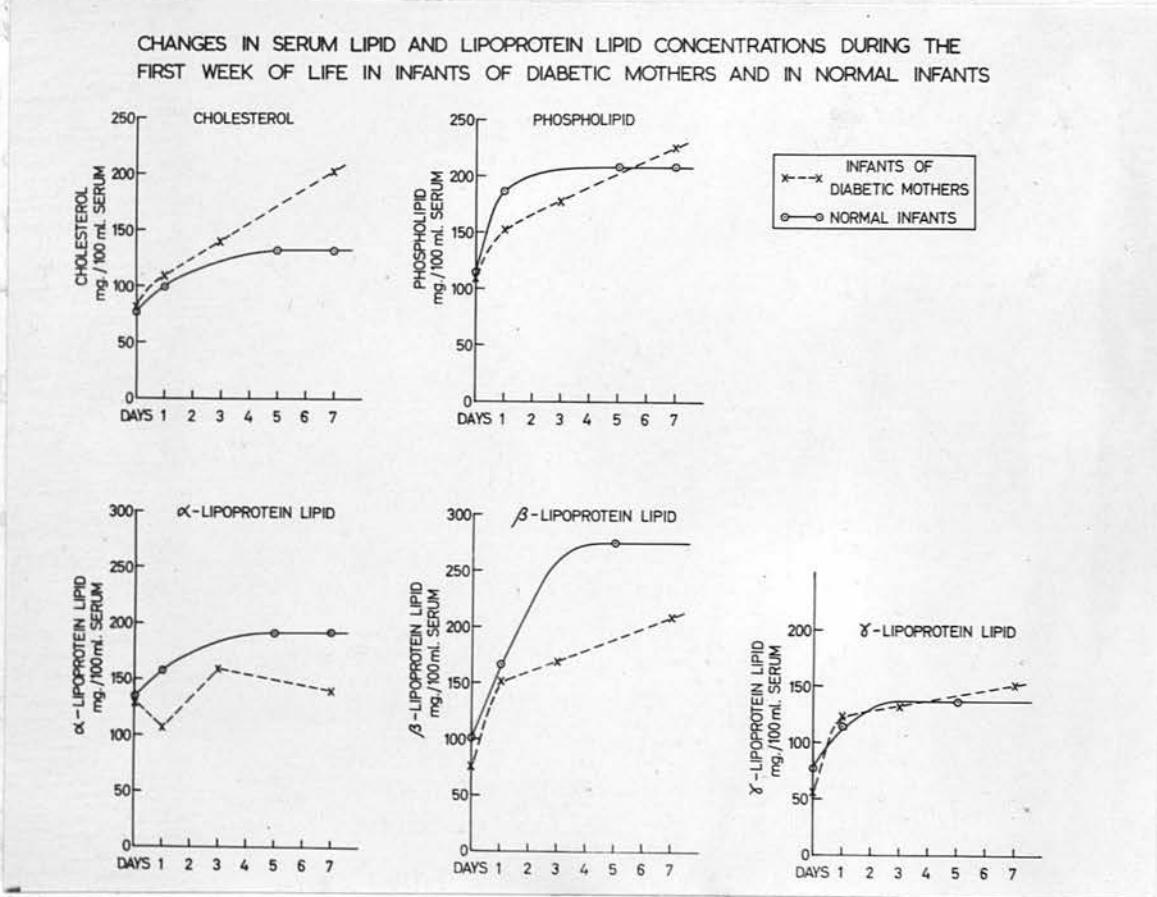
The mean serum concentration of γ-lipoprotein lipid in the cord blood of these infants of diabetic mothers is 54 $\pm$ 4 (11) mg./100 ml. This is significantly below the normal values of 82 $\pm$ 4 (50) mg./100 ml. found in this investigation and 77 $\pm$ 5 (15) mg./100 ml. found by Rafstedt ($P < 0.001$). In the infants of diabetic mothers there is an increase of 127 per cent on the first day of life and by the seventh day there is no difference between them and Rafstedt's normal infants in this respect.

Ratio of β-Lipoprotein Lipid to α-Lipoprotein Lipid Concentrations

In the cord blood of normal infants and in that of the original group of eighteen infants of diabetic mothers, the value for this ratio is almost identical. It is 0.76 $\pm$

Figure 17

Changes in Serum Lipid and Lipoprotein Lipid Concentrations During the First Week of Life of Infants of Diabetic Mothers and in Normal Infants.



NOTE.

Values for the infants of diabetic mothers are the mean concentrations found in this investigation in cord blood and in venous blood on days 1. 5, and 7. Values for normal infants are the mean concentrations found by Rafstedt (1955) for cord blood, venous blood at 18 - 24 hours and venous blood between the age of 5 to 7 days.

0.06 (50) and 0.76 ± 0.09 (18) respectively. The value found in the group of eleven infants of diabetic mothers is 0.71 ± 0.09 (11) which is of the same order. By the seventh day of life the ratio is 1.90 ± 0.24 (11) which is above the value of 1.60 found by Rafstedt. The concentrations of the two lipoprotein lipid fractions are below normal in the infants of diabetic mothers and the increase in the ratio is a result of the more rapid decline in the concentration of the α-lipoprotein lipid.

DISCUSSION

The main points of interest in this comparison between normal infants and infants of diabetic mothers appear to be the differences in the behaviour of the concentration of α- and β-lipoprotein lipid and of cholesterol, with increasing age. This is illustrated in Fig. 17.

The data for the infants of diabetic mothers may be summarised as follows. The concentration of α-lipoprotein lipid in the umbilical cord blood does not differ from normal. Thereafter it falls, rises again, apparently after the infants have been fed, and subsequently falls again. The concentration on the seventh day is below that found in normal infants in whom it rises steadily throughout the first week of life. The concentration of β-lipoprotein lipid is normal in the umbilical cord blood and in the venous blood on the first day of life. Thereafter the rate of increase declines so that by

the seventh day the concentration is significantly below normal. The concentration of cholesterol is normal for the first three days of life. Thereafter its rate of increase continues to rise instead of falling off as it does in normal infants, and by the seventh day of life its concentration is considerably above normal.

The cause of these differences seems likely to be a complex interaction of hormonal effects and nutritional status. A review of the literature on the various agents which affect the metabolism of adipose tissue and the concentrations of lipids and lipoproteins in the blood has been given in Chapter II. The data collected there will be summarised briefly, once more.

THE REGULATION OF THE METABOLISM OF ADIPOSE TISSUE

Dependence on a Normally Functioning Carbohydrate Metabolism

Insulin and a normally functioning carbohydrate metabolism, particularly that of glucose, are required for the normal synthesis of fatty acids in adipose tissue and in the liver. In their absence there is a release of free fatty acid from the fat depots and a consequent rise in the concentrations of free fatty acid in the blood. This is followed by a rise in blood glucose concentrations and a delayed rise in the concentrations of lipids and lipoproteins in the blood.

Insulin Antagonists

The suggestion by Himsworth and Scott (1938), that an adequate diet, low in carbohydrate, causes the secretion of an insulin antagonist by the pituitary deserves further mention in this context. Plasma insulin antagonists have been detected in the albulin of untreated or uncontrolled insulin-requiring diabetics. (Vallance-Owen, 1960): in obese diabetics and in persons in a clinically pre-diabetic state i.e. showing typically diabetic glucose tolerance curves during pregnancy, infection, or after oral treatment with cortisone or prednisolone (Vallance-Owen and Lilley, 1961). The antagonist disappears if sufficient insulin is given to return the blood sugar concentrations to normal. (Ibid). It was also shown to be present, in a far less active state, in the plasma albumin fractions of normal persons. (Vallance-Owen, Dennes and Campbell, 1958): to be absent from hypophysectomized cats: and to appear in adrenalectomized non-diabetic patients stabilized with cortisone (Vallance-Owen and Lilley, 1961).

Hormones Other than Insulin

A low blood sugar concentration also promoted the secretion of adrenaline and nor-adrenaline which in their turn promote the secretion of corticosteroids. Adrenaline, nor-adrenaline and ACTH produce, separately, a mobilization of

free fatty acid within a few minutes, and an increase in the metabolic rate. Adrenaline produces a hyperglycaemia almost simultaneously with the elevation of plasma free fatty acid concentrations. It also produces a delayed increase in serum lipid and lipoprotein concentrations. Nor-adrenaline does not produce the hyperglycaemia but the elevation of plasma free fatty acids which it produces is more long lasting. Both effects of adrenaline are potentiated by cortisone which apparently does not affect normal serum lipid concentrations on its own. It will reduce them in a hypercholesterolaemic state. Thyroxine also potentiates the mobilization of free fatty acid and the subsequent increase in serum lipid and lipoprotein concentrations. Like cortisone, it will reduce the cholesterol concentrations in such hypercholesterolaemic conditions as hyperthyroidism. That is, cortisone and thyroxine seem to have a dual role. They potentiate an increase in serum lipid concentrations produced by adrenaline but reduce them in hyperlipaemic conditions.

THE HORMONAL AND NUTRITIONAL STATUS OF INFANTS OF DIABETIC MOTHERS

The data summarized above may now be applied to the conditions existing in the infants of diabetic mothers during the first week of life. Since insulin concentrations and nutritional status will be discussed in more detail, they will be dealt with last.

Adrenaline and Nor-Adrenaline

The concentrations of adrenaline and nor-adrenaline in the blood of new-born infants are at present unknown. Although it might be thought possible that these hormones would be produced in large amounts by the mother during labour and cross the placenta, the evidence to date suggests that the maternal serum adrenaline and nor-adrenaline concentrations are not above normal at this time. (Stone, Pilliero, Hammer and Portnoy, 1960). This does not have any bearing on whether the infants themselves produce either or both of these hormones in excess, in response to the stress of delivery.

Corticosteroids

The possibility that the infants of diabetic mothers may possess abnormally high serum concentrations of corticosteroids is discussed in detail in Chapter VI, Pages 165.

Thyroxine

Pickering et.al. (1958) report that thyroxine concentrations are raised in the blood of normal infants during the first few days of life. It is likely that the same is true for the infants of diabetic mothers.

Insulin

The large size and considerable fat reserves of the infants of diabetic mothers have been considered for some

time to be a result of hyperinsulinism, (Farquhar 1958; Osler, 1960). Hyperplasia of the pancreatic islet cells has been observed by many investigators (Cardell 1953, Woolf and Jackson 1957, Driscoll, Benirschke and Curtis, 1960).

Baird and Farquhar, (1962) measured insulin activity in the umbilical cord blood of normal infants and of infants of diabetic mothers at birth and at intervals between the third and fifth hours of life. The plasma insulin activity was measured by estimating the rate of glucose uptake in isolated rat diaphragm. There was no significant difference between the two groups at birth either in plasma insulin activity or in blood glucose concentrations. It is difficult to see how this is compatible with the hypothesis of foetal hyperglycaemia in the infants of diabetic mothers which is considered to be a result of maternal hyperglycaemia and a cause of the hyperinsulinism (Pedersen 1952). Both plasma insulin activity and blood glucose concentrations fell markedly between birth and the start of the experiment. A glucose load was given by umbilical catheterization at the third hour of life and five minutes later there was a striking difference between the two groups. The mean level of plasma insulin activity in the infants of diabetic mothers was approximately ten times that in normal infants. The infants of diabetic mothers showed a high glucose tolerance. The normal infants developed poor glucose tolerance curves which

were comparable to those observed in adults suffering from mild stable diabetes.

In the absence of a glucose load during the first few hours of life in normal infants, blood glucose concentrations are reported to fall to about half the level existing in cord blood at birth, which is not greatly different from the concentrations existing in the maternal blood. (Cornblath, Odell and Levine, 1959; Farquhar, 1961). Komrower (1954) considered that the fall in blood glucose concentrations in the infants of diabetic mothers during this period was greater than that in normal infants.

Since the normal infants do not respond to glucose loading with the development of a high plasma insulin activity and good glucose tolerance, Baird and Farquhar (1962) suggest that either their pancreatic islet cells are peculiarly unresponsive to glucose loading or that the rate of destruction o of insulin is very high. If this is another instance of insulin antagonism, it is completely different from that obtaining in the infants of diabetic mothers. The latter infants appear to have developed high concentrations of insulin antagonists which are very sensitive to glucose concentrations.

There is some evidence that albumin can cross the placenta from the maternal to the foetal circulation (Abbas and Tovey, 1960), so that insulin antagonists could be

transferred from a diabetic mother to her infant. Alternatively, since the antagonist appears to be a low molecular weight polypeptide it might cross the placenta in the free state (Vallance-Owen and Lilley, 1961). But, since the antagonism actually develops during the first three hours after birth, this does not seem likely. Such antagonists might however develop in response to a combination of raised concentrations of corticosteroids and thyroxine and of falling blood glucose concentrations. Vallance-Owen and Lilley (1961) consider that the effect of the antagonist is dependent upon adrenal corticosteroids.

Nutritional Status

The infants of diabetic mothers are not fed until at least two and sometimes three days after delivery. They may be regarded as having fasted for a period sufficient to alter their lipid metabolism. This, of course, could not contribute to any greater development of insulin antagonism than in normal infants during the first few hours of life, but in fact the fasting state might have begun prior to delivery. The exact date of the onset of labour is considered, as mentioned before, to be partly hereditary and partly caused by a diminution in the secretion of progesterone by the mother. This results in the degeneration of the placenta (Clifford 1957). There is evidence that such degeneration begins

earlier than normal in diabetic pregnancies (Farquhar, 1958), which would result in a reduction in the supply of food to the foetus. In view of the disagreement over the effects of starvation on the serum lipid concentrations which has been mentioned previously, (see Chapter II Page 15) it is difficult to say whether early placental degeneration would lead to reductions or increases in the serum lipid and lipoprotein lipid concentrations.

The falling blood glucose concentrations during the first few hours of life in both normal infants and in the infants of diabetic mothers could be expected to elicit an increase in the release of free fatty acid into the blood, then of glucose and subsequently an increase in the serum concentrations of lipids and lipoproteins. This effect could be mediated by the secretion of adrenaline as well as by the development of insulin antagonism. Whatever the cause, this increase does occur in all the serum lipids and lipoprotein lipids in normal infants and in all of them except α-lipoprotein lipid, in the infants of diabetic mothers.

Possible Reasons for the Difference in Serum Lipid and Lipoprotein Concentrations between Normal Infants and the Infants of Diabetic Mothers

The situation is more complicated in the infants of diabetic mothers than in normal infants. The possible presence of raised corticosteroid concentrations in the

infants of diabetic mothers would be expected to enhance any lipid response to fasting and to adrenaline or to both. But if they have been subjected to a reduction in the supply of maternal nutrients by a placenta which has started to degenerate early and if, as suggested by Osler (1960), they are unable to utilize their fat reserves prior to feeding, a condition may have been achieved similar to that in the fasted dogs of Mann and White (1953) in which serum lipid concentrations fell. (See Chapter II page 30). This might explain why the increase in the serum concentrations of cholesterol and β-lipoprotein lipid are normal, instead of above normal on the first day of life, while that of α-lipoprotein lipid actually decreases, because simultaneously with any changes peculiar to infants of diabetic mothers, the normal change over from the foetal lipoprotein pattern with predominant α-lipoprotein to the adult pattern with predominant β-lipoprotein is also taking place. Feeding, especially with small amounts of glucose at frequent intervals, would be expected to stop the secretion of insulin antagonists and adrenaline and so promote the uptake of fat instead of its release. Therefore, once feeding has begun, one would expect there to be a transitory alimentary rise followed by a hormonal fall dependent upon insulin, in the rate of increase in the serum lipid and lipoprotein lipid concentrations.

This is, in fact, what is observed in the behaviour of α -lipoprotein lipid concentrations. The concentrations of cholesterol however, continue to rise throughout the first week of life. It has been suggested previously that there is some abnormality in the lipid constitution of the lipoproteins of infants of diabetic mothers. (See Chapter III page 48 and Chapter IV page 69). The evidence suggests that this is connected with a higher cholesterol content than normal. It is possible that the increased rates of cholesterol synthesis typical of overt diabetes also exist in the infants of diabetic mothers and are connected in some way with the maternal hyperglycaemia. If these hypotheses are correct, it would be possible to have increased cholesterol concentrations co-existing with decreased lipoprotein concentrations.

Obviously far more work is required before any firm conclusions can be drawn on this topic. It might however be of some interest, not only from the point of view of diabetic mothers butalso from that of lipoprotein metabolism in general, for it suggests that α -lipoprotein is more easily affected by the nutritional status than the other lipoprotein fractions. Of course that fraction

designated " α -lipoprotein" during electrophoretic separation includes both α_1-and α_2-lipoprotein, which have widely differing constitutions and metabolic roles, and this investigation provides no information as to whether one or both of them are responsible for the alterations observed.

CONCLUSIONS

1. The mean concentrations of lipids and lipoprotein lipids in the umbilical cord blood of infants of diabetic mothers differ in certain respects from those in normal infants.
2. As in normal infants, there is a considerable and rapid increase in the concentrations of total lipid, cholesterol, phospholipid, and β - and γ -lipoprotein lipid during the first week of life and especially on the first day. It is likely that the increase found in total esterified fatty acid concentration is a perfectly normal one since approximately 50 per cent of it is present in phospholipid, although no comparable data for normal infants is available.
3. Whereas the concentration of α -lipoprotein lipid in the cord blood of infants of diabetic mothers is little different from that in normal infants, it decreases on the first day of life instead of increasing as it normally does. By the seventh day its concentration is considerably below that found in normal infants.
4. The concentration of β-lipoprotein lipid, although within the normal range in cord blood, is also considerably lower than normal by the seventh day.

5. The concentration of cholesterol in the cord blood is also within the normal range but it is above normal by the seventh day.
6. The high cholesterol concentrations might be a result of increased synthesis similar to that found in the diabetic mother.
7. The combination of raised cholesterol concentrations with reduced concentrations of β-lipoprotein lipid might be accounted for by the possibility that this lipoprotein carries more cholesterol in the infants of diabetic mothers than in normal infants.
8. The reduced concentrations of α- and β-lipoprotein lipid existing by the end of the first week of life could be the result of a complex series of reactions to inanition, antagonism to increased insulin concentrations and its subsequent removal and to increased serum concentrations of corticosteroids.

CHAPTER VI.

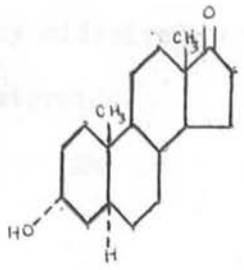
URINARY STEROID EXCRETION IN THE NEONATAL PERIOD.

The concentrations of 17-ketosteroids and 17-ketogenic steroids have been measured in the urine of 40 normal infants during the first day of life. The data are given in Table 34. In Table 35 are the data for the excretion of the same steroids by 5 infants of diabetic mothers. For 3 of these, analyses were made on each of the first three days of life and for 1 on the first four days. The data are illustrated in Fig. 19.

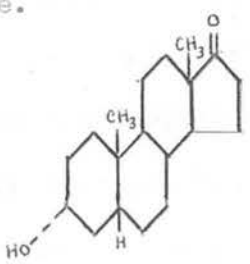
The Nature of the Steroids Estimated by the Method Employed.

The Zimmerman reaction, (Zimmerman, 1935) is positive for 19 carbon atom steroids having a $C{=}O\text{-}CH_2$ grouping at the C_{17} position. It is also given by steroids having a carbonyl group at the C_5 and C_{20} positions, but the colour obtained is less intense and these steroids are usually important only during pregnancy. The principal steroids giving this reaction are:

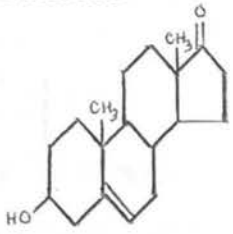
ANDROSTERONE.



Aetiocholanone.



Dehydroisoandrosterone.



The only other steroids of importance which give a positive reaction in the Zimmerman procedure are the oestrogens: but these are phenols and are removed when the benzene extracts containing the ketosteroids are washed with sodium hydroxide.

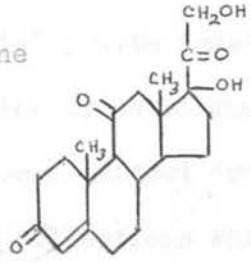
The Origin of the Steroids Measured

The sources of the 17-ketosteroids in the male are the adrenal cortex which contributes two thirds and the gonads which contribute one third of the total (Loraine 1958). In females, the main source is the adrenal cortex. The adrenal 17-ketosteroids are produced partly by oidative removal of the side chain from 21-carbon atom steroids.

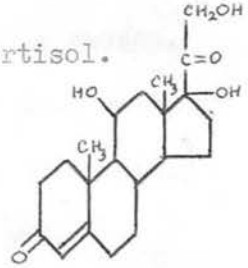
The conversion of 21-carbon atom steroids to those

having the 19-carbon atom skeleton in the adrenal cortex is the biological parallel of the chemical reaction used in the estimation of 17-ketogenic steroids. Corticosteroids are those 21-carbon atom steroids having at least one of the following side chains: α-ketol; α-glycol; 11-oxy; and 17-hydroxy. The 17-ketogenic steroids are those corticosteroids having either a 17: 21 dihydroxy: a 20-keto: a 17:20:21-trihydroxy: or a 17:20-dihydroxy: 21-deoxy grouping. These include cortisone and cortisol but not corticosterone, 11- dehydrocorticosterone, or aldosterone.

Cortisone



Cortisol.



During oxidation with sodium bismuthate the 17-hydroxy group and the side chain are converted to the 17-keto group which then reacts positively in the Zimmerman procedure. The Porter-Silber chromogens (Porter and Silber, 1950), queted by many authors, are those giving a colour reaction with phenyl hydrazine instead of m-dinitro-benzene. They may be regarded

as equivalent to 17-hydroxy steroids and both give results similar, but nor quite equal to those obtained by estimating 17-ketogenic steroids. Data expressed in terms of formaldehydogenic steroids may be regarded in the same way. (Marrian 1951).

Sources of Error in the Method of Estimation Employed.

The estimation of 17-kesteroids and 17-ketogenic steroids is subject to considerable and various errors. Firstly the completeness of a twenty four hour urine collection is essential. To obtain such a collection from new-born infants is fraught with difficulties, especially with female infants. For this reason, very low urine volumes with very low steroid content have been omitted from the data. They were mainly those collections which were less than 5ml.

Secondly the hydrolysis of conjugated steroids with hydrochloric acid causes some loss, especially of corticosteroids. Methods of enzymatic hydrolysis with β -glucuronidase are available which reduce this loss considerably. (Talalay, Fishman and Huggins, 1946). This was not attempted because of the decision to use only those methods prevalent in routine clinical laboratories.

TABLE 34

Steroid concentrations in the urine of 40 normal infants during the first day of life.

Case	Birth Weight lbs. ozs.	17-Ketosteroids mg/24 Hours.	17-Ketogenic steroids.mg/24 Hours.	Urine Volume ml/24 Hours.
Ca.	8.8	0.92	2.34	20
Ma.	7.5	1.14	2.57	28
Sc.	6.8	0.53	0.91	15
Hu.	7.3	1.55	1.68	9
Ha.	7.4	0.54	0.42	15
La.	5.7	0.89	1.70	24
Gr.	6.11	0.73	0.90	18
Hu.	8.2	0.51	1.73	6
Do.	7.6	0.39	0.45	19
Bl.	7.15	0.89	0.71	14
Og.	7.8	0.99	0.85	17
Si.	9.4	0.52	1.35	9
McD.	8.8	2.29	3.65	24
Dr.	7.9	1.44	1.23	22
Ca.	8.3	1.34	2.36	12
Br.	6.14	1.09	2.61	67
Fe.	9.2	0.70	1.70	19
An.	8.4	0.58	1.03	21
Co.	7,6	0.68	2.63	48
Ca.	7.13	0.69	1.07	67
Cl.	8.4	1.78	3.66	29
Ew.	8.9	1.23	1.12	28
Fr.	8.9	0.75	1.27	15
Fe.	7.9	0.69	2.93	52
Ke.	6.6	1.24	1.78	23
Ma.	6.7	0.81	1.93	16
Mar.	7.2	0.85	1.31	39
Mu.	7.4	0.48	0.73	34
McN.	8.4	2.64	3.48	39
McK.	7.15	0.84	3.62	26
O'H	7.6	0.43	0.82	28
Ra.	9.2	1.10	3.34	71
Sca.	7.9	0.66	0.84	52
St.	7.12	1.30	3.99	28
Sk.	7.6	0.44	0.56	6
Ti.	7.2	1.03	1.89	16
Te.	7.10	0.29	0.55	20
Wa.	7.2	0.44	0.60	16
Wi.	8.2	0.48	1.37	6
We.	8.4	1.68	2.19	40

The 17-ketogenic steroids are also subject to considerable loss during the bismuthate oxidation. The figures given in the tables have been corrected, as far as possible, to allow for this error. The amount of loss was calculated from the recovery experiments performed on each new stock of sodium bismuthate. (See appendix I page 211).

Another source of error is the Zimmerman reaction which is not as specific as could be desired. It gives a colour reaction with certain non-steroid chromogens which are particularly prominent in the urine of newborn infants. The application of the Allen equation (Allen 1950) to the calculation of the results of the estimations goes a long way to eliminating this error.

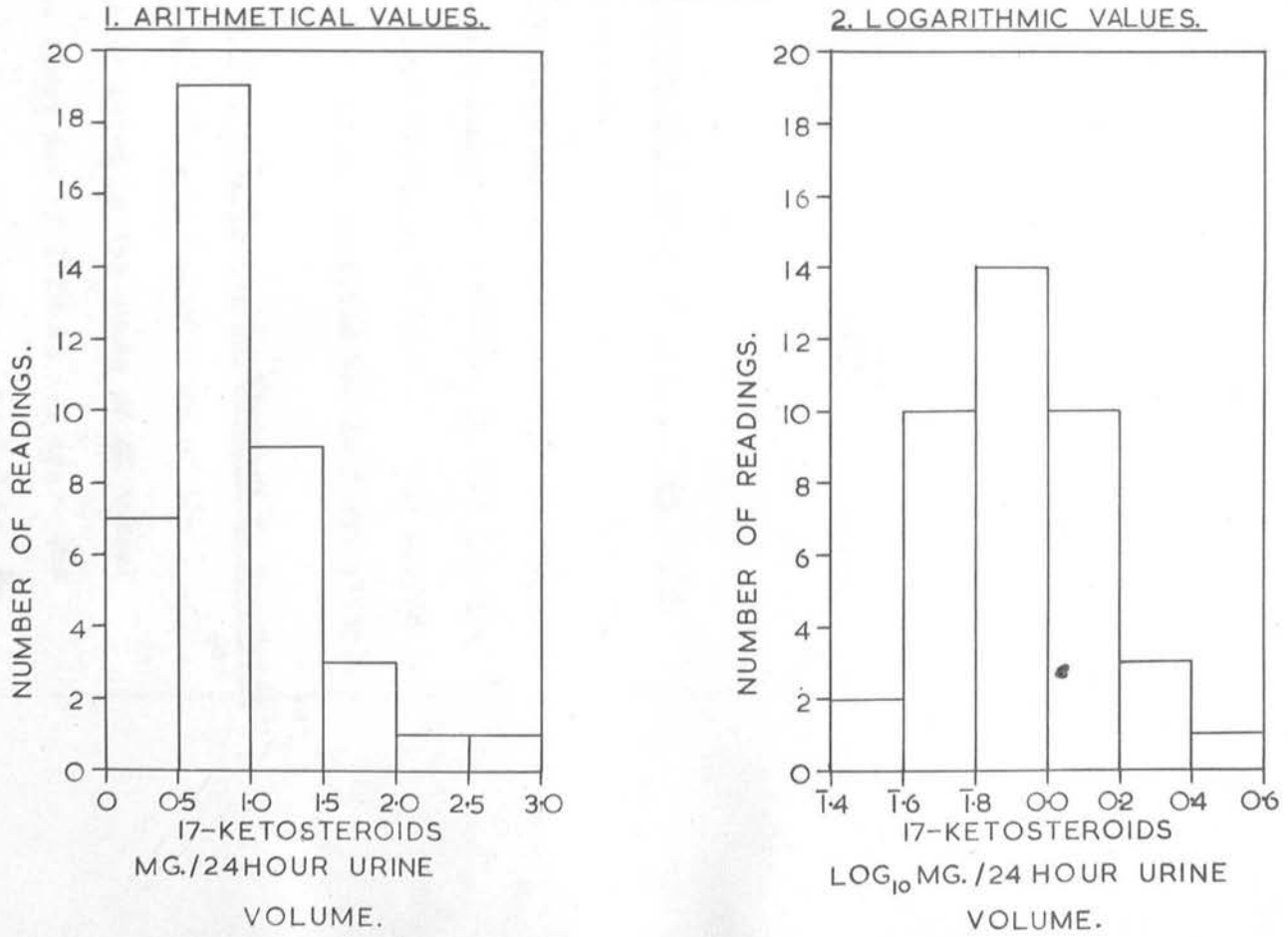
Finally there is always considerable daily variation in steroid excretion. This was magnificently illustrated by Hamburger (1954) who did daily estimations of 17-ketosteroid excretion in an adult male for six years. Apart from the daily variation, an annual cycle was apparent, excretion being highest in September and lowest in May.

Results.

When considering the tables of data it should be noted that a normal distribution curve is not obtained

FIGURE 18

HISTOGRAMS SHOWING THE VARIATION IN THE EXCRETION OF 17-KETOSTEROIDS IN THE URINE OF 40 NORMAL NEWBORN INFANTS.



unless logarithmic values are substituted for the arithmetical ones. This is illustrated in Fig. 17 . This was first noted by Dicsfalusy, Planin, Burke and Westman (1955). The mean values reported in this investigation are therefore the arithmetical values of the logarithmic standard deviation is used to obtain a range of arithmetical values which corresponds to the standard deviation. This is referred to as the small range of variation.

(See Appendix II page 216 for the methods of statistical analysis).

The Mean Concentrations of 17-Ketosteroids in the Urine

The mean concentration of 17-ketosteroids excreted in the urine on the first day of life of 40 normal infants is 0.8 mg. The small range of variation is from 0.5 mg. to 1.4 mg. in the first twenty four hours. This agrees well with the data on Bogin, Gottfried and Levjcky. (1955).

The Mean concentrations of 17-Ketogenic Steroids in the Urine

The mean value for the concentrations of 17-ketogenic steroids excreted in the urine of 40 normal infants during the first day of life is 1.5 mg. The small range of variation is from 0.8 mg. to 2.7 mg. in the first twenty four hours. This is slightly higher

than the data given for 17-hydroxy corticosteroids by Salmi, Pekkarinen and Meikkila (1957), and Cranny and Cranny (1960). However, Cranny and Cranny (1960) give average values for the steroid excretion during the first three days of life. There is good evidence that steroid excretion falls rapidly during the first week of life and that the decrease is noticeable even on the second day in some cases (Bjorklund and Jensen, 1955; Gardiner 1956; and Gottfried, Bogin and Levycky 1957). Assuming this to be true, the average concentration of steroids excreted over the three day period is not truly representative of the first day of life.

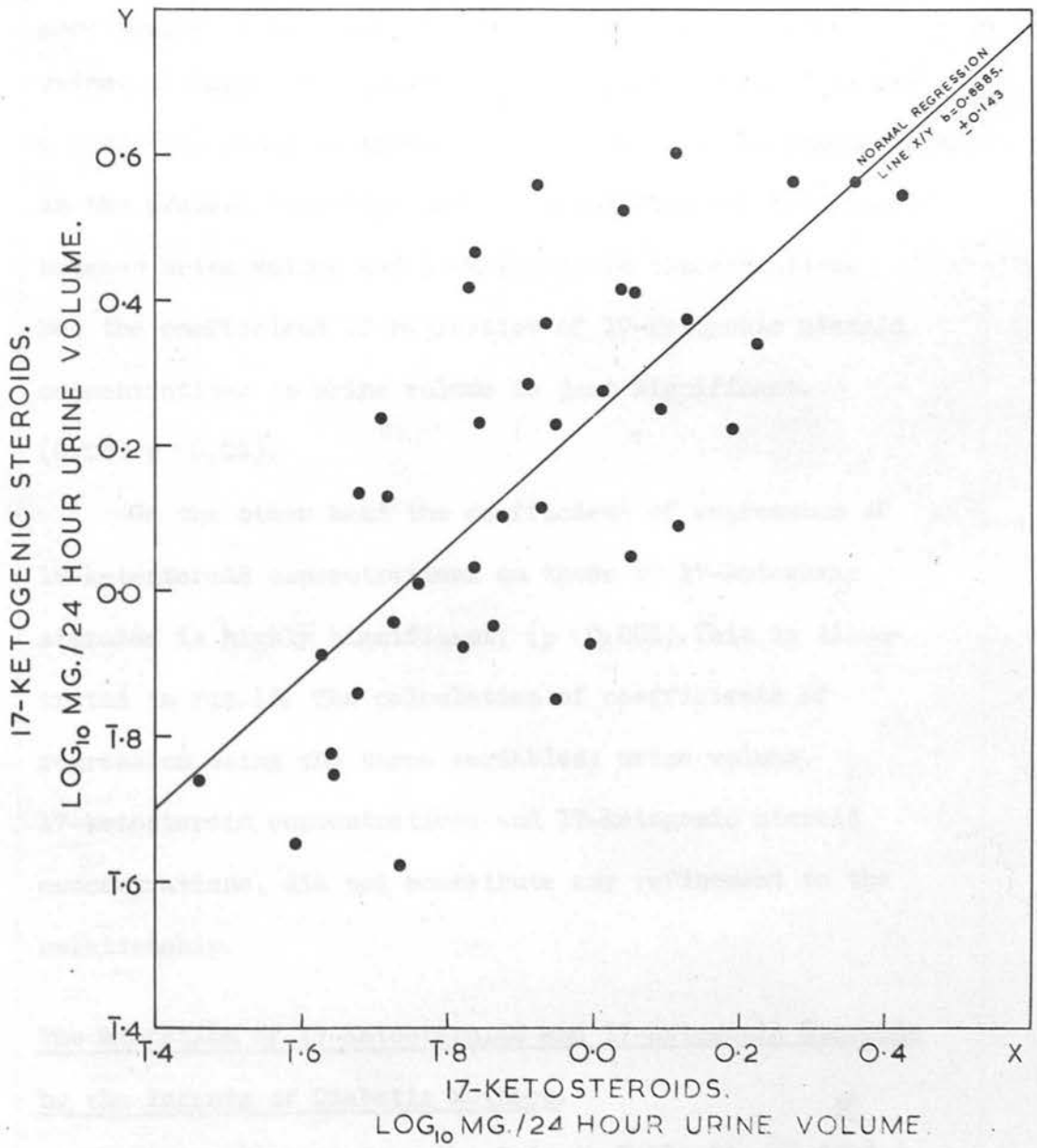
The Mean Volume of Urine Produced on the First Day of Life.

The mean volume of urine produced by the 40 normal infants on the first day of life is 21.9 ml. in twenty-four hours. The small range of variation from 11.71 ml. to 41.4 ml. This is considerably below the volumes quoted by many American authors. The reason for this could be the routine use of intravenous saline drips during labour in America. This loading with extra fluid would be reflected by the foetus fairly rapidly.

FIGURE 19

REGRESSION DIAGRAM OF

LOG_{10} 17-KETOSTEROIDS/LOG_{10} 17-KETOGENIC STEROIDS.



Inter-relationships between Urinary 17-Ketosteroid and 17-Ketogenic Steroid Concentrations and between both of these and Urine Volume.

Cranny and Cranny (1960) found that 17-hydroxy corticosteroid excretion was not connected with urine volume, although in a previous paper (1958), using data for a different group of infants, a relationship was demonstrated. In the present investigation, no connection has been found between urine volume and 17-ketosteroid concentrations but the coefficient of regression of 17-ketogenic steroid concentrations on urine volume is just significant. ($0.05 > p > 0.02$).

On the other hand the coefficient of regression of 17-ketosteroid concentrations on those of 17-ketogenic steroids is highly significant; ($p < 0.001$). This is illustrated in Fig. 18. The calculation of coefficients of regression using the three variables: urine volume, 17-ketosteroid concentrations and 17-ketogenic steroid concentrations, did not contribute any refinement to the relationship.

The Excretion of 17-Ketosteroids and 17-Ketogenic Steroids by the Infants of Diabetic Mothers.

It is apparent from the data in Table 33 and from Fig. 19 which illustrates these data, that the infants of diabetic mothers have a higher excretion of 17-ketogenic steroids on the first day of life than the infants

FIGURE 20

EXCRETION OF STEROIDS IN THE URINE OF NORMAL INFANTS ON THE FIRST DAY OF LIFE AND OF INFANTS OF DIABETIC MOTHERS ON THE FIRST THREE DAYS OF LIFE

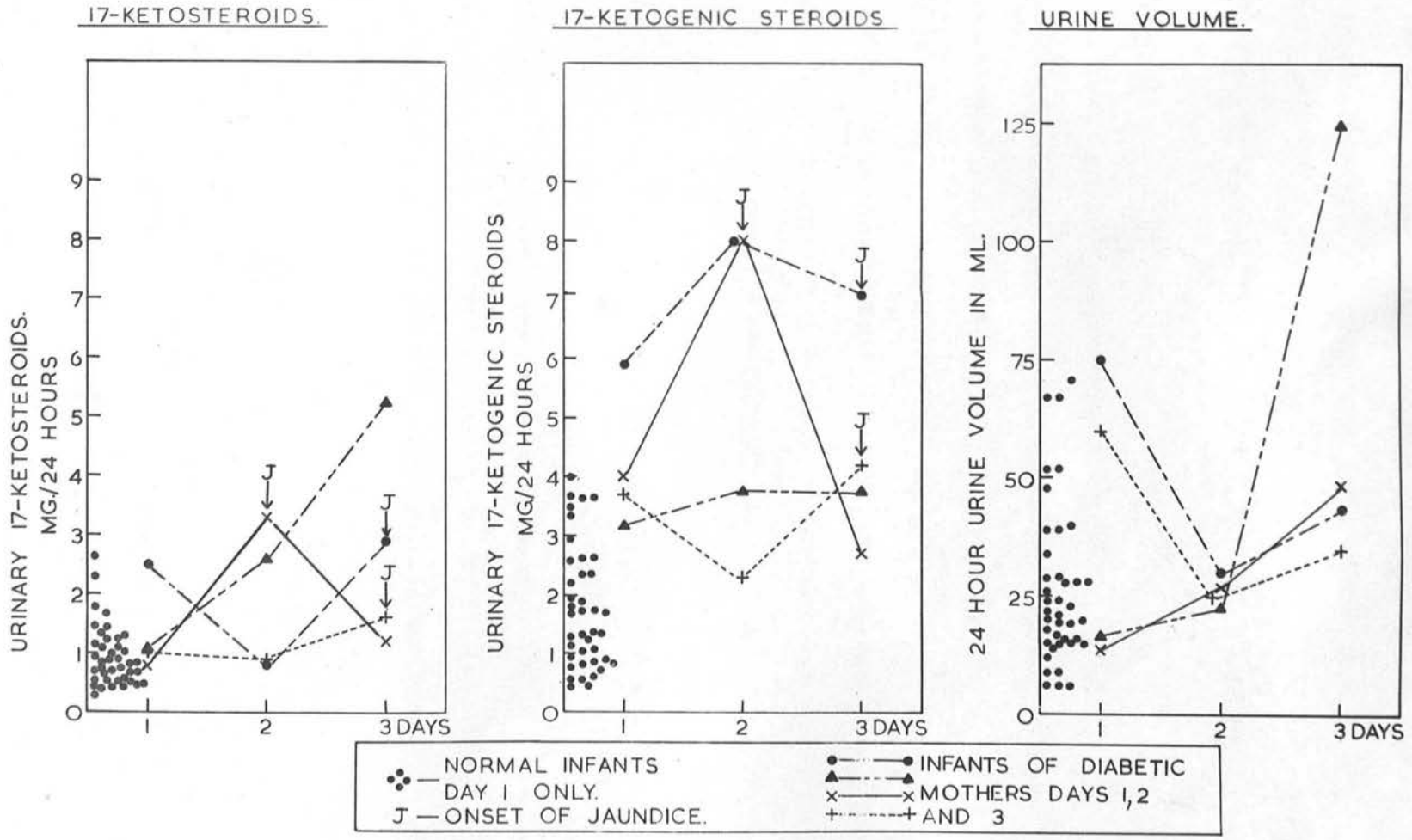


TABLE 35

Concentrations of 17-Ketosteroids and 17-Ketogenic Steroids in the Urine of Infants of Diabetic Mothers on the First Three Days of Life.

Baby	Weight lbs.ozs.	Sex	Day	17-Ketosteroids Mg./100 ml. Urine	17-Ketosteroids Mg./24 Hours	17-Ketogenic Steroids Mg./100 ml. Urine	17-Ketogenic Steroids Mg./24 Hours	Total Mg./24 Hours	Urine Volume ml.
Sa.	6.12	M	I	2.9	1.9	4.9	3.2	5.1	65
Co.	6.5	M	I	5.6	0.8	28.2	4.0	4.8	14
Became jaundiced			II	12.3	3.3	29.7	8.0	11.3	27
Bilirubin 13.6mg.			III	2.6	1.2	5.7	2.7	3.9	48
Bu.	5.15	F	I	6.1	1.1	17.7	3.2	4.3	18
Cyanotic attack			II	11.1	2.6	16.3	3.8	6.2	23
on Day I			III	4.2	5.3	3.1	3.8	9.1	125
Sc.	6.8½	M	I	3.3	2.5	7.9	5.9	8.4	75
			II	2.5	0.8	26.5	8.0	8.8	30
Became jaundiced			III	6.6	2.9	16.1	7.1	10.0	44
Gl.	6.4	M	I	1.7	1.0	6.2	3.7	4.7	60
			II	3.6	0.9	8.7	2.3	3.1	26
Became jaundiced			III	4.5	1.6	12.0	4.2	5.8	35
Bilirubin 13.6mg.			IV	1.5	1.6	5.1	5.3	6.9	103

of normal mothers. The lowest value obtained in the infants of diabetic mothers is outside one standard deviation from the normal mean concentrations. The 17-ketosteroid concentrations are not so markedly high, three of them being within one standard deviation from the normal mean concentration and three outside it.

An increased excretion of steroids was found in the infants of diabetic mothers by Marrian (1951) who measured formaldehydogenic steroids: by Tompsett (1954) who measured 17-ketosteroidsand formaldehydogenic steroids: and by Bjorklund and Jensen (1955) who measured 17-ketosteroids only. Farguhar (1958) reported similar findings.

It should be remembered that these infants of diabetic mothers are all four weaks premature by date, although not by weight. They are also more or less distressed at birth. Cranny and Cranny (1960) report that premature infants with an uneventful meonatal history excrete less Porter-Silber chromogens than full term infants. Distressed premature infnats who survived, excreted still less and distressed premature infnats who eventually died, least of all. Therefore, if the infants

of diabetic mothers are to be regarded as premature in function, their steroid excretion is even more abnormal when compared with the premature infants in the Cranny and Cranny (1960) series. On the other hand, Gardiner (1956) reported that premature infants excrete more 17-ketosteroids than full-term infants so that the infants of diabetic mothers in the present series cannot be regarded as abnormal in this respect.

DISCUSSION.

The Abnormality of the Steroid Excretion of the Infants of Diabetic Mothers.

It is difficult to comment on the steroid excretion of the infants of diabetic mothers during the second and third days of life in the absence of normal vaules. A consideration of the lietrature suggests that it continues to be in excess of the normal amounts.

The Origin of the Steroids Excreted in the Neonatal Period.

Gardiner (1956) considers that the 17-ketosteroid concentrations in the urine are higher in the first few days of life than in any subsequent period in infancy and childhood. Other investigations confirming this have already been quoted on Page 157 . For many

years it was thought that the foetus and the new-born infant were incapable of synthesizing their own steroids and that the high excretion of these compounds was solely due to the excretion of steroids of maternal origin. The placenta is permeable to many steroids (Klein, 1957; Soule, 1938). Very high maternal steroid concentrations such as those produced during prolonged labour are reflected in the cord blood steroid concentrations of the infant (Klein, 1957). A proportion of the neonatal steroid excretion is undoubtedly a result of this.

The existence of steroid biosynthesis in the foetus has appeared to be a possibility for some time. The concentration of steroids in umbilical cord blood is generally above that of the maternal blood. This is particularly so when the mother has been adrenalectomized. Recently, steroid biosynthesis has been demonstrated in the foetal zone of the neonatal adrenal gland. (Bloch, Benirschke & Rosemberg 1958; Bloch and Benirschke 1959; Villee, Engel and Villee 1959). Migeon (1959) demonstrated that cortisol production in the new-born infant is as high as, or higher than it is in the mother. This foetal zone of the neonatal adrenal gland contributes 80 per cent of the mass of the gland and is so large that it causes the gland to represent 0.5 per cent of the total body weight

compared with 0.01 per cent in the adult. It involutes rapidly after birth and at the same time the definitive or permanent zone is proliferating. The steroids excreted on the first day of life will therefore include those of maternal origin and those of foetal origin. Once the maternal steroids have been removed, the urinary steroids of the infant will be restricted to those synthesized in situ and the amounts will be expected to parallel the relative rates of involution of the foetal zone of the adrenal gland and the proliferation of the permanent zone.

Possible Reasons for the Abnormally High Urinary Steroid Excretions in the Infants of Diabetic Mothers During the First Three Days of Life.

The total steroid excretion in three out of four of the infants of diabetic mothers is still rising on the third day of life, instead of showing some signs of falling. Theoretically this could be a result either of daily variation, or of the existence of some abnormality in the infant. There could be:

1. An Increase in the Efficiency of Renal Function

This is unlikely to be the most important factor because renal clearance increases by an average of only 40 per cent at birth and probably even less in the premature

infant (Cranny and Cranny, 1958). These infants of diabetic mothers sometimes show more than 100 per cent increases in steroid excretion from one day to the next.

2. Alterations in the rate of development of the adrenal gland.

Either the proliferation of the definitive zone of the adrenal cortex could be more rapid than normal or the involution of the foetal zone could be retarded or both of these factors could operate simultaneously. More sites of steroid biosynthesis would thus be made available.

3. An increased production of ACTH by the pituitary and a proportionately great response by the adrenal cortex.

Lanman (1961) considers that the source of adrenal stimulation in the foetus is the pituitary gland just as it is in the adult. In support of this he sites

(a) the atrophic adrenal glands in

1. anencephalic infants
2. infants of mothers who have been treated with very large amounts of cortisone during pregnancy;

(b) the very large adrenal glands found in cases of adreno-genital syndrome. In (a) 1 he deduces the absence of any pituitary stimulation and in (a) 2, an inhibition of the action of ACTH by the large amounts of steroids of maternal origin which have reached the foetal circulation.

In (b) he suggests the secretion of excess ACTH to compensate for reduced steroid biosynthesis. Even in normal mothers, serum corticosteroid concentrations are high at term and especially during labour. In the diabetic mother, serum corticosteroid concentrations are even higher than in normal mothers, particularly because their diabetic control tends to become unstable during pregnancy. (Hoet 1951). It is possible that, on the removal at birth of inhibition of foetal ACTH secretion by elevated concentrations of steroids of maternal origin, the neonatal pituitary is secreting at its maximum rate. This could explain the absence of a stress response by the adrenal cortex during the first few days of life. (Klein and Hansen, 1951; Ullstrom, Colle, Burley and Gunville, 1960). In the infants of diabetic mothers, the effect of the removal of such an inhibition could be quite dramatic. Peel, (1951) described the post mortem findings on an infant of a diabetic mother who died during the first week of life. Death was attributed to an adrenal crisis resulting in dyspnoea, pseudopneumonia infantum and adrenal haemorrhage. The adrenal crisis was attributed to the sudden removal, at birth, of over-stimulus of the foetal adrenal cortex by maternal steroids. In fact, the opposite conclusion that there was a sudden removal of inhibition at birth which caused over-stimulation of the

neonatal adrenal would be as valid and would still fit the post mortem data.

Thus one can suggest that in the infant of the diabetic mother there could be an increased stimulation of the adrenal cortex during the first few days of life. This would increase the concentrations of corticosteroids in the blood, over and above the high concentrations of corticosteroids of maternal origin. This would result in a delay in the onset of the fall in the concentration of steroids excreted in the urine, which normally begins on the second or third day of life.

Concentrations of Corticosteroids in the Serum of Infants of Diabetic Mothers.

There is little data, as yet, on the concentration of corticosteroids in the serum of the new-born normal infants or the infants of diabetic mothers and none suggesting that it is higher than normal. Gemzell (1954) reported the presence of approximately 28 gms. 17-hydroxy corticosteroids/100 ml. of serum in the cord blood of infants of primaparous mothers and approximately 16 gms./100 ml. in the cord blood of infants of multiparous mothers. He reports that these values are of the same order as the concentrations in maternal serum. Migeon, Nicolopoulos and Cornblath (1960) report normal concentrations of corticosteroids in the blood of their series of infants of diabetic

mothers. Only indirect evidence of raised serum-corticosteroid concentrations can be advanced in the present investigation.

When the urine output of excreted 17-ketosteroids and 17-ketogenic steroids is at its peak in this series of infants of diabetic mothers, its total varies from approximately 7.0 to 11.0 mg./24 hours. Of this total, 5.0 mg. to 9.0 mg. consist of 17-ketogenic steroids. Assuming a surface area of 0.2 square metres for these infants (Farquhar, 1958), the glomerular filtration rate would be of the order of 20 litres per diem. Assuming the absence of any tubular secretion of corticosteroids, a serum concentration of 45 mg./100 ml. would be required to produce an output of 9.0 mg./24 hours. This value does not include any steroid which is bound to protein and is thus incapable of excretion. Therefore, unless the amount of steroid reaching the urine by tubular secretion is greater than the amount bound to serum proteins, the concentration of corticosteroids in the serum of the infants of diabetic mothers is likely to be higher than that in normal infants.

Steroid Excretion and the Development of Neonatal Jaundice.

An examination of the data suggests the possibility that there is a relationship between the concentration of

17-ketosteroids and 17-ketogenic steroids in the urine and the onset of neonatal jaundice in the infants of diabetic mothers. The day on which the presence of jaundice was first noted in the case report of each infant is marked on Fig. 19 and also noted in Table 33. It must be emphasized that this date is the one on which the jaundice became particularly noticeable.

One infant-Baby Bu.- was not reported as becoming jaundiced. The other three were all deeply jaundiced on the day on which there was a sudden rise in the concentration of 17-ketosteroids in the urine. The same is true of the 17-ketogenic steroids except in the case of Baby Sc, in whom jaundice was reported on the day following the peak 17-ketogenic steroid excretion.

Conjugation of Steroids and Bile Pigments by a Common Enzyme System.

Steroids and bile pigments are both conjugated by a common enzyme system prior to excretion, i.e. the glucoronyl transferase system. This is known to be deficient at birth (Brown and Zuelzer, 1958; Lathe and Walker, 1958). Lathe and Walker (1958) demonstrated the inhibition of bilirubin conjugation in rat liver slices by human pregnancy serum, neonatal serum and by a variety

of steroid compounds. Hsia, Dowben, Shaw and Grossman (1960) demonstrated a similar inhibition by progestational agents in pregnancy serum. Competitive inhibition has apparently been excluded by Lathe and Walker (1958) because they could obtain no inhibition of bilirubin conjugation in broken cell preparations of liver.

In their search for a specific inhibitor, Lathe and Walker (1958) have established certain structural requirements for the compound. They are possessed by progesterone, 17-ketosteroids and 17-ketogenic steroids. However, all of these are rejected on the grounds that the concentration of the inhibitor on both sides of the placenta and in the neonatal blood at different times, bears no relationship to the concentrations of the steroids mentioned. This could be unwarranted because an individual steroid may not behave in a manner exactly reflected by the group of 17-ketosteroids or 17-ketogenic steroids as a whole. Lathe and Walker do consider that the inhibitor, whatever its nature, would contribute to the height to which the hyperbilirubinaemia rises, but would not be the cause of it.

The concentration of 17-ketosteroids and 17-ketogenic steroids excreted by infants of diabetic mothers is higher than in normal infants. It is possible that the

concentrations of corticosteroids in the blood are also higher than normal. Even in the absence of a specific inhibitor, this sudden loading of the glucuronyl transferase system, which is deficient in the infants of diabetic mothers as in all premature infants, might strain it severely.

Bilirubin Concentrations in the Infants of Diabetic Mothers.

Data for the separate concentrations of conjugated and non-conjugated steroids in the blood of new-born infants does not exist. Data for the concentrations of conjugated and non-conjugated bilirubin, where known for the infants of diabetic mothers, are given in detail. In Baby Co., the concentration of total bilirubin, already rising on the first day of life, rose to 15.7 mg./100 ml. of serum on the second day when steroid excretion was at a maximum. Of the total bilirubin, only 0.21 mg./100 ml. was conjugated. On the third day, when steroid excretion was falling, the concentration of non-conjugated bilirubin had fallen slightly while that of conjugated bilirubin had not altered.

No measurements of bilirubin concentrations were made on Baby Sc. until the fourth day. By that time the concentrations of the conjugated and non-conjugated forms

were 0.81 mg. and 12.49 mg./100 ml. of serum respectively. The concentration of the conjugated bilirubin then increased slowly and that of non-conjugated bilirubin decreased. Measurements of urinary 17-ketosteroid and 17-ketogenic steroid concentrations ceased on the third day, on which they were still rising.

It does seem possible that the presence of high concentrations of steroids in the blood of new-born infants of diabetic mothers could contribute to the development of the very deep jaundice to which they are peculiarly prone. It would be interesting to investigate the concentrations of steroids in both the blood and the urine in greater detail, not only in these infants, but also in those who develop "physiological jaundice" and in cases of kernicterus.

CONCLUSIONS.

1. Measurements have been made of 17-ketosteroids and 17-ketogenic steroids in the urine of 40 normal infants and 5 infants of diabetic mothers on the first day of life, in infants of diabetic mothers on the first three days of life, and in 1 on the first four days of life.
2. A normal distribution curve is only obtained from these values in the group of normal infants if all the values are converted to their logarithmic form.
3. There is a close relationship between the concentrations

of 17-ketosteroids and of 17-ketogenic steroids excreted. There is little or no relationship between these values and urine volume.

4. Infants of diabetic mothers excrete higher concentrations of these steroids on the first day of life than normal infants.

5. It is likely that the steroid excretion of the infants of diabetic mothers is still raised on the second and third day of life. This could be a result of an increase in the efficiency of renal function, of alterations in the rate of development of the adrenal gland, or of an increased production of ACTH by the pituitary.

6. The possibility of the existence of a relationship between the increased urinary concentrations of 17-ketosteroids and 17-ketogenic steroids and the onset of jaumdice in the infants of diabetic mothers has been suggested.

Figure 21.a

Amino Acid Chromatogram Obtained From the Urine of an Infant on the First Day of Life.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

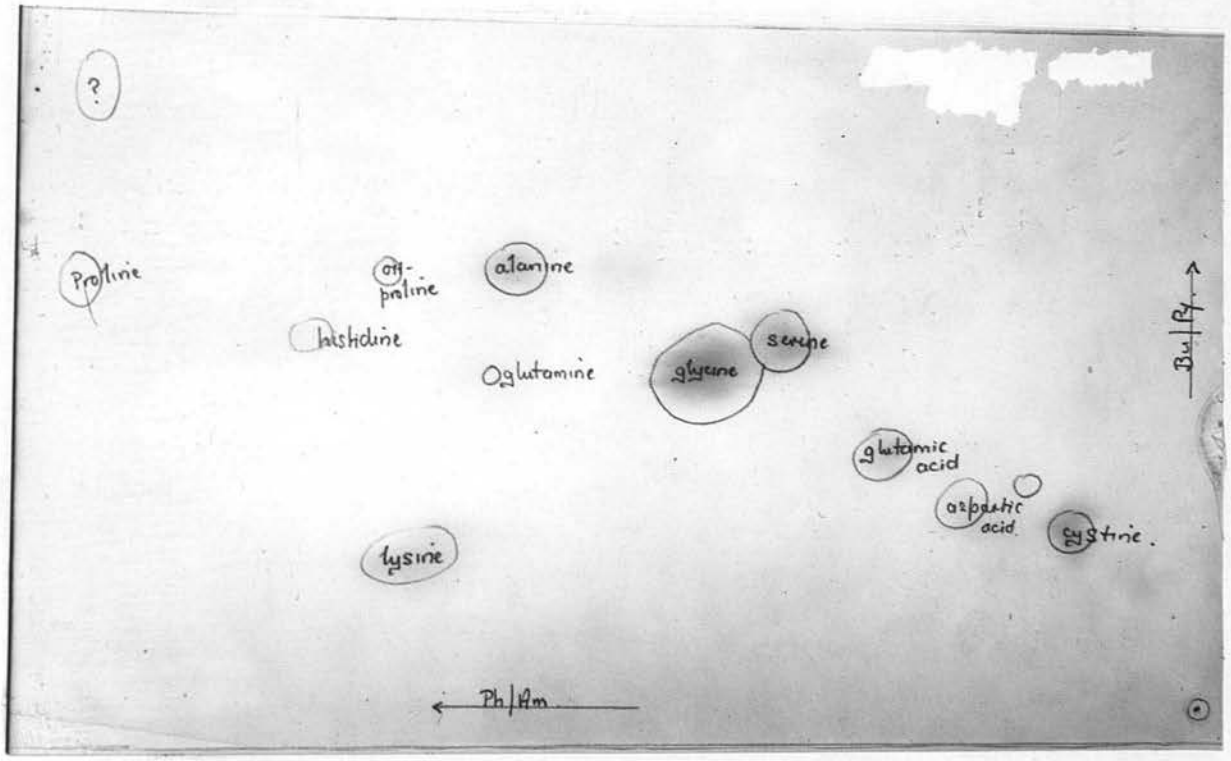


Figure 21 *b*

Amino Acid Chromatogram Obtained From the Urine of an Infant on the First Day of Life.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

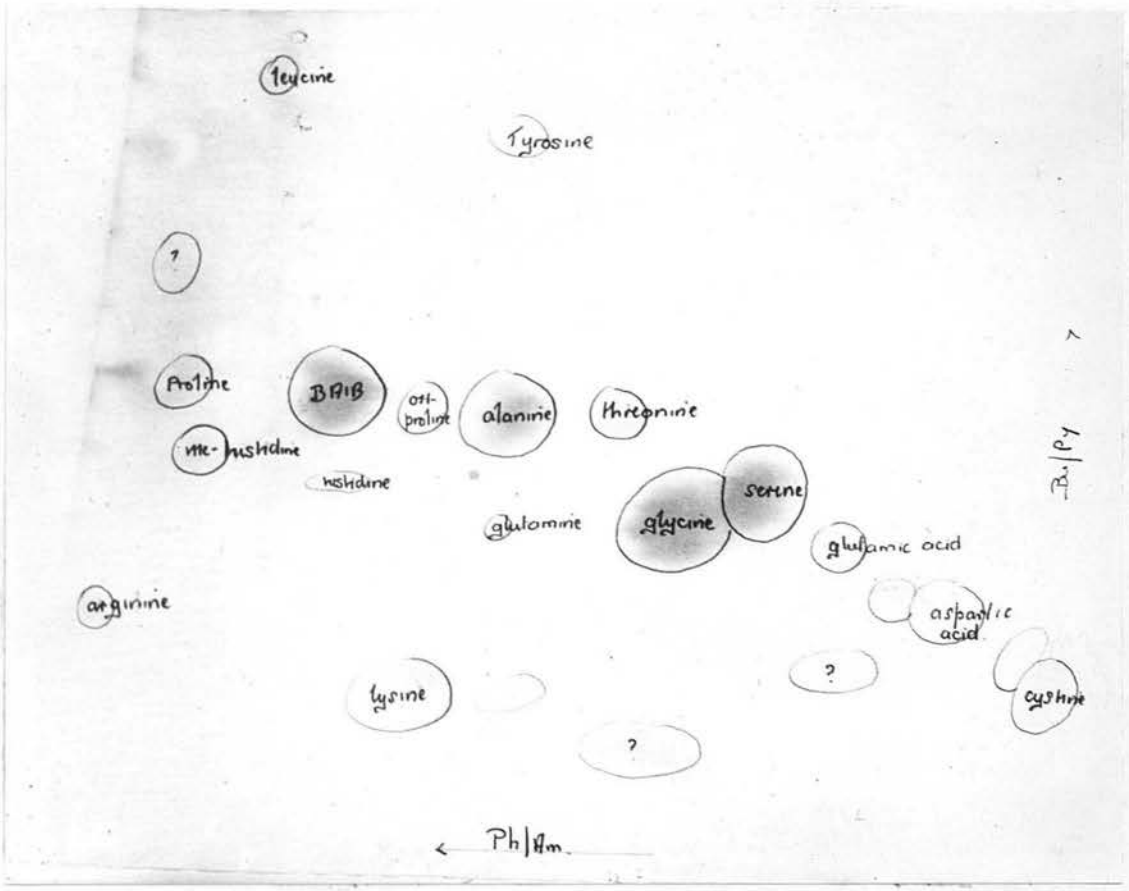


Figure 22.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Two Weeks.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

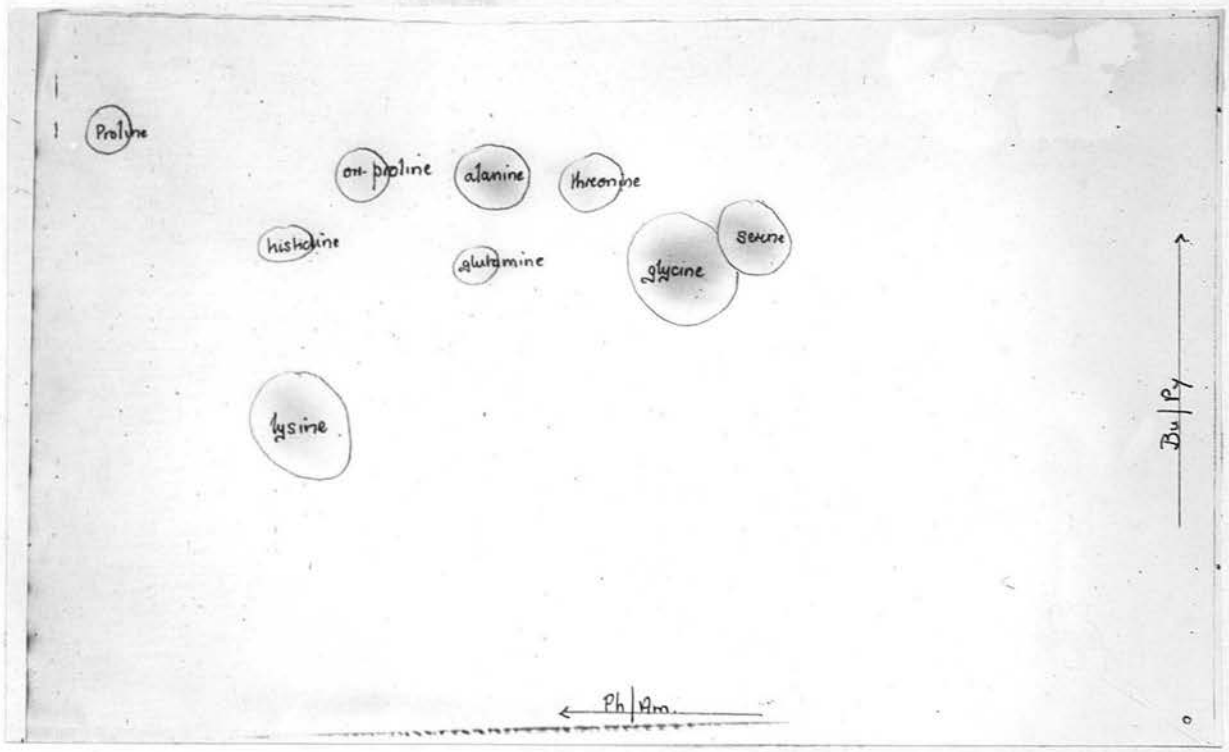


Figure 23.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Six Weeks.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

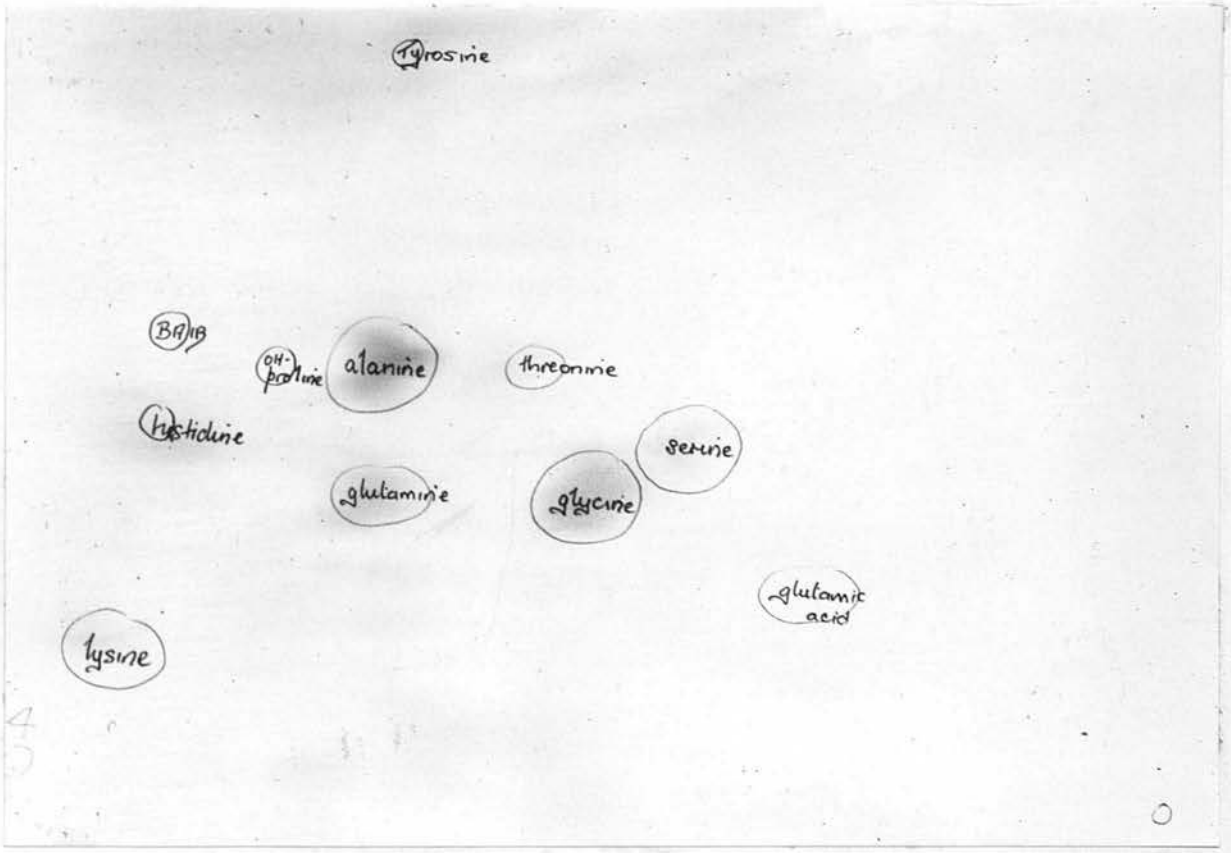


Figure 24.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Seven Weeks.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

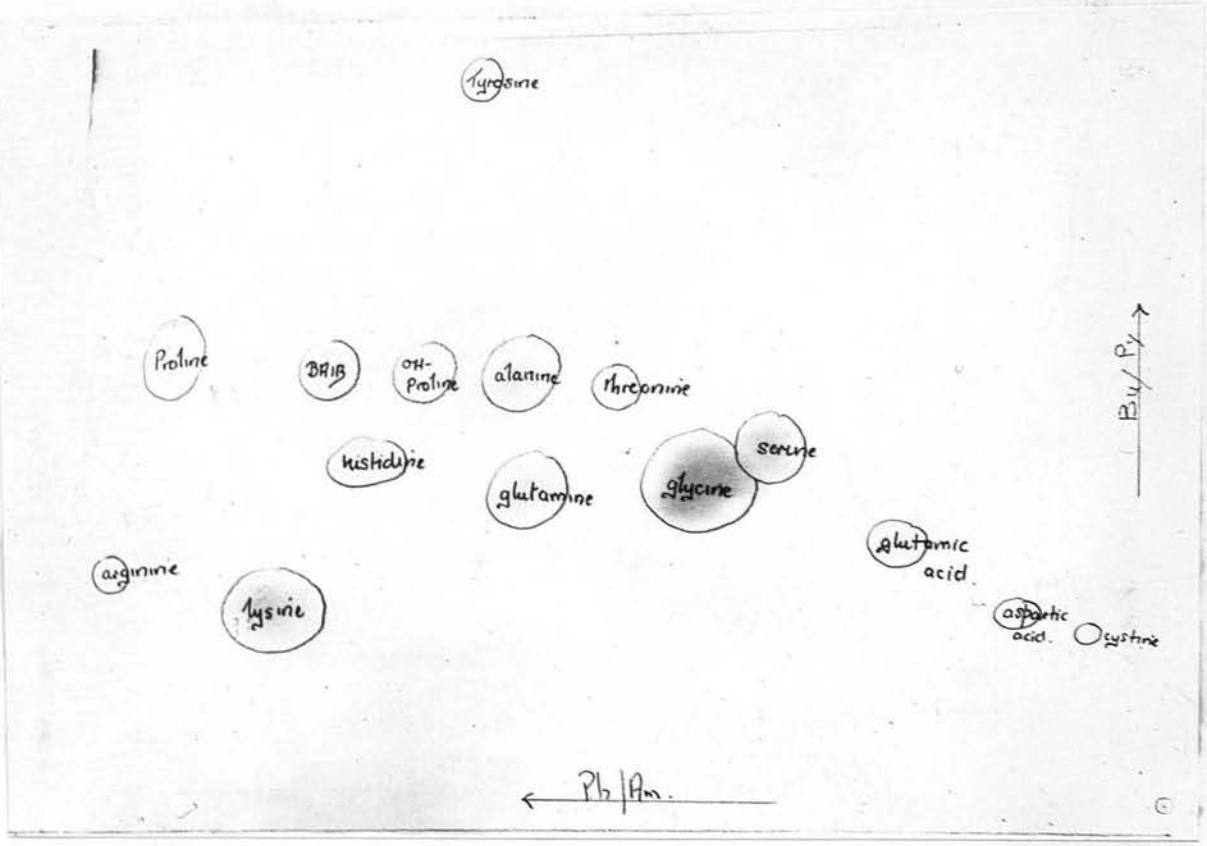


Figure 25.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Two Months.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

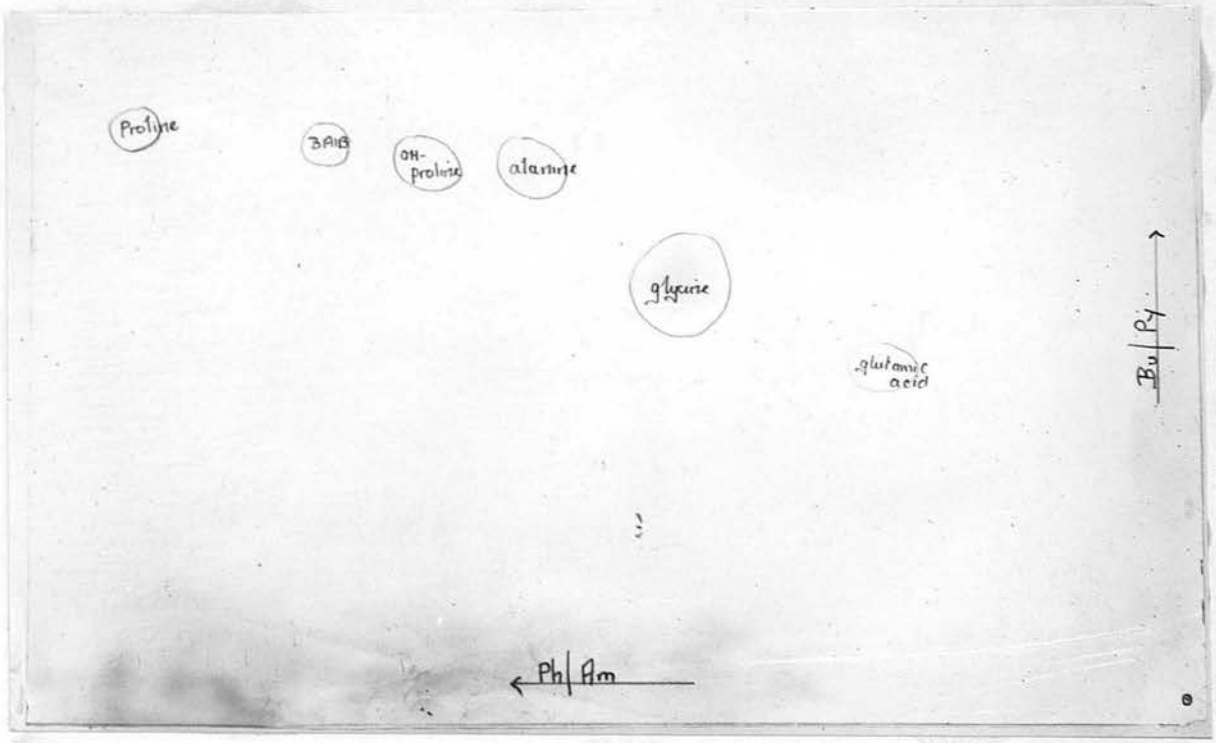


Figure 26.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Four Months.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

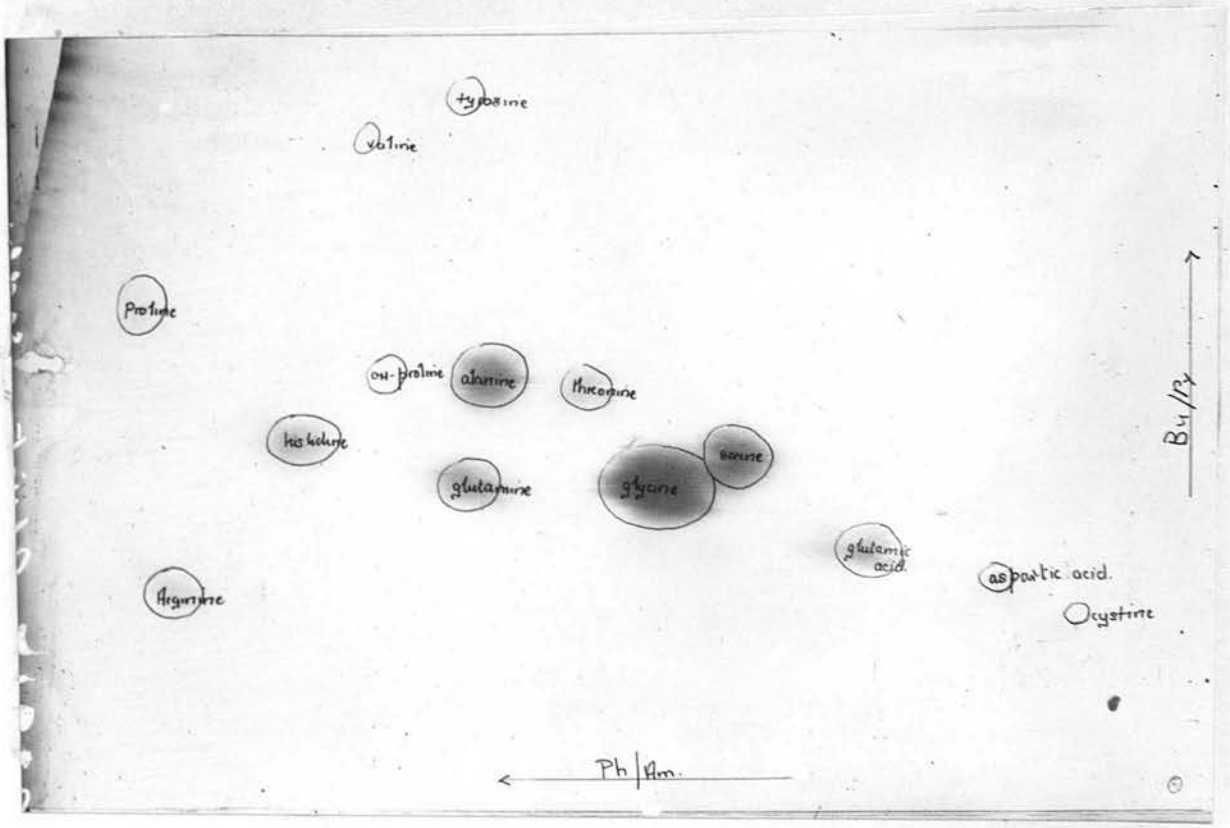


Figure 27.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Six Months.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

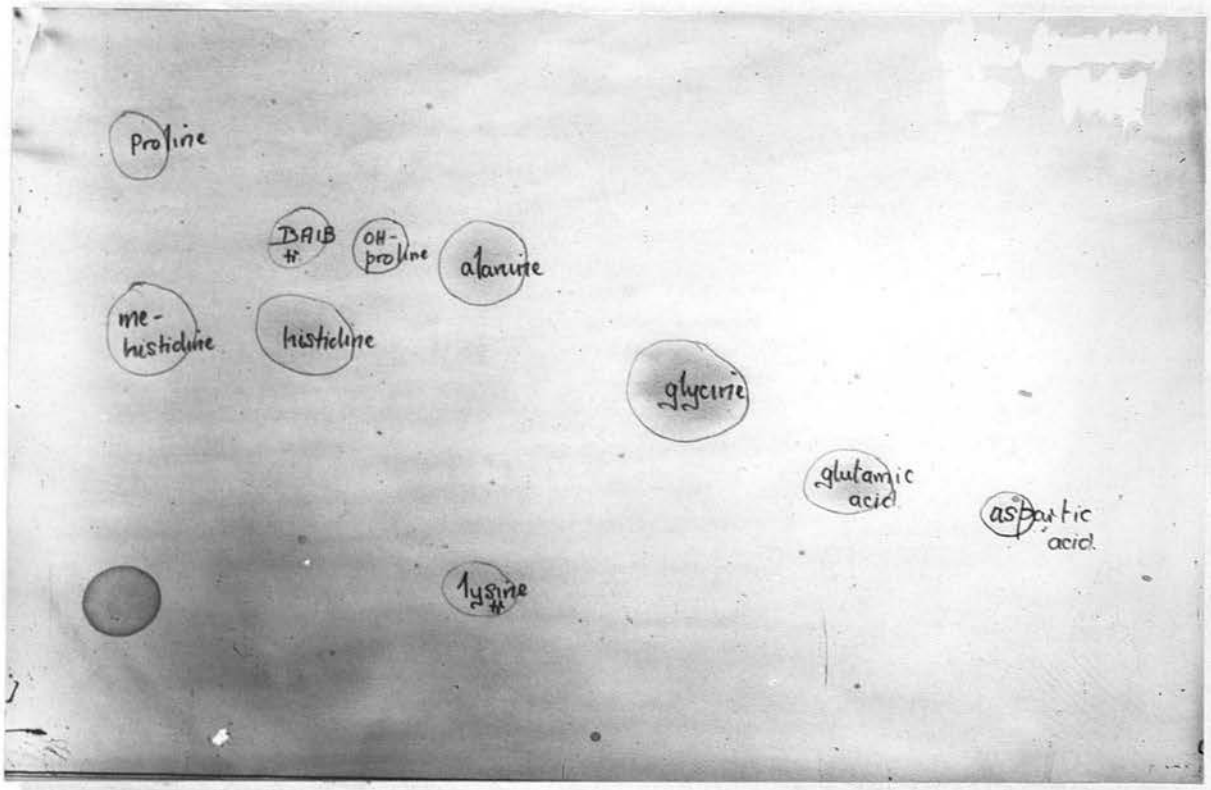


Figure 28.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Eighteen Months.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

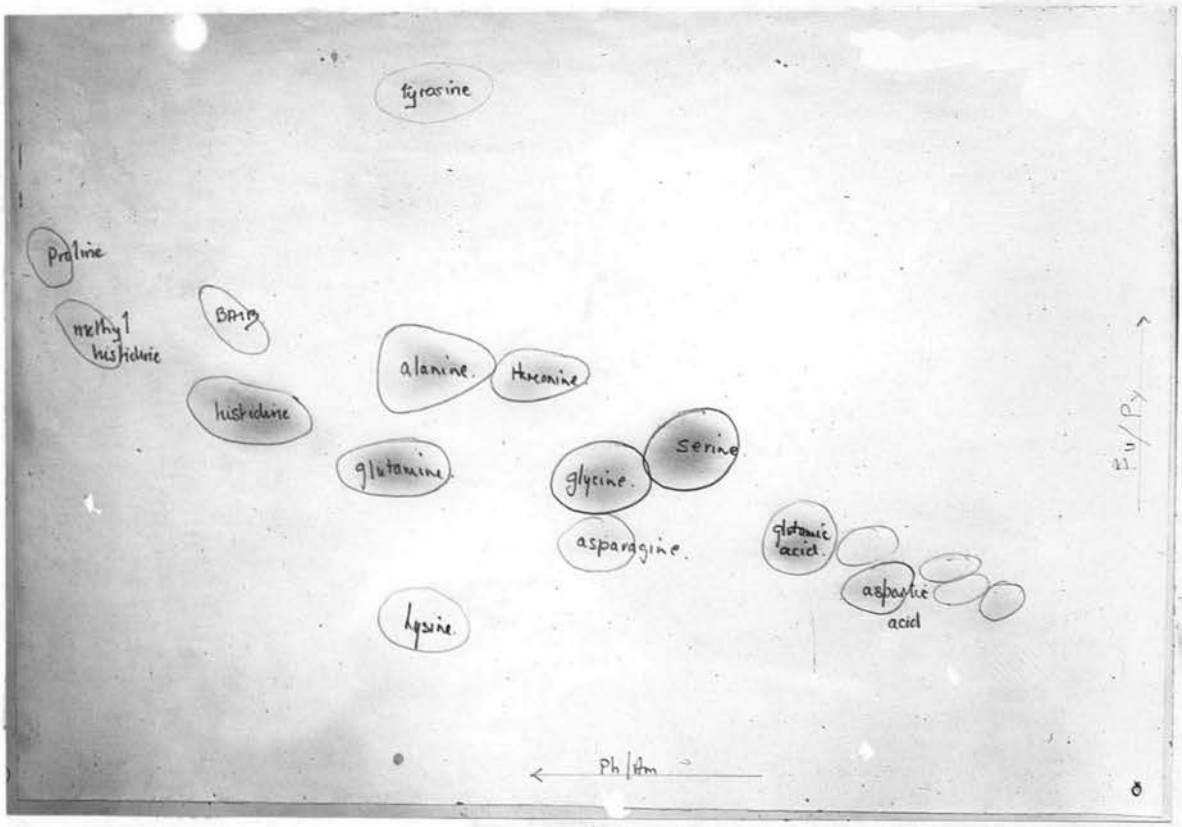
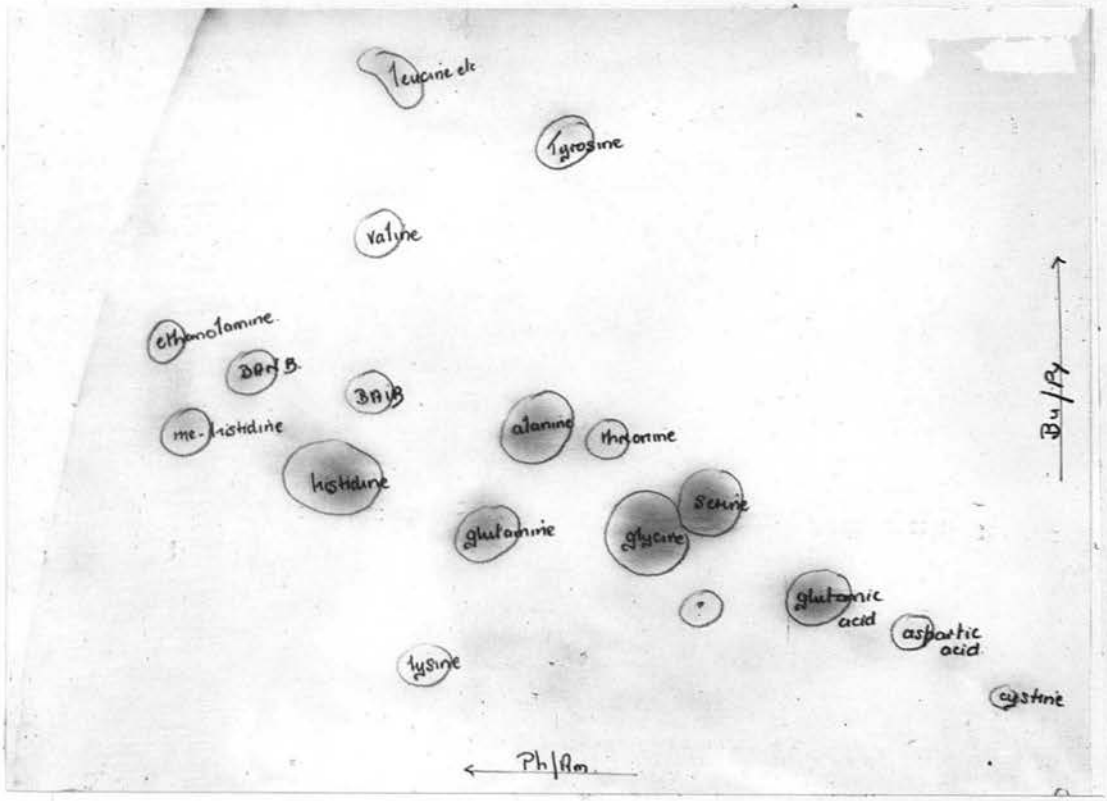


Figure 29.

Amino Acid Chromatogram Obtained From the Urine of an Infant Aged Four Years.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.



CHAPTER VII

AMINO ACID PATTERNS IN URINE AND SERUM IN THE NEONATAL PERIOD

Paper chromatography has been employed to identify amino acids occurring in urine and serum. This method is capable of detecting a) amino acidurias and amino acidaemias and b) the presence of abnormal amino acids in the urine and the serum.

PATTERNS OF AMINO ACID EXCRETION IN THE URINE OF NORMAL INFANTS

Some examples of urinary amino acid chromatograms are given in Figs 20-29. They illustrate normal variations at a given stage of development, and differences in the excretion pattern which occur at different stages of development.

The amounts of each amino acid present in a given sample of urine (judged purely by visual examination of the stained chromatogram) have been divided into three categories:

1. High or large amounts.
2. Medium amounts.
3. Small or trace amounts.

The infants investigated have been divided into three groups according to their age.

(a) Aged one to two days.

(b) Aged two to eight weeks.

(c) Aged six to eighteen months.

The number of infants excreting a particular amino acid at each of these three levels has been expressed as a percentage and these percentage values have been illustrated in the form of block diagrams in Fig. 30 a. The numerical values are given in Table 36.

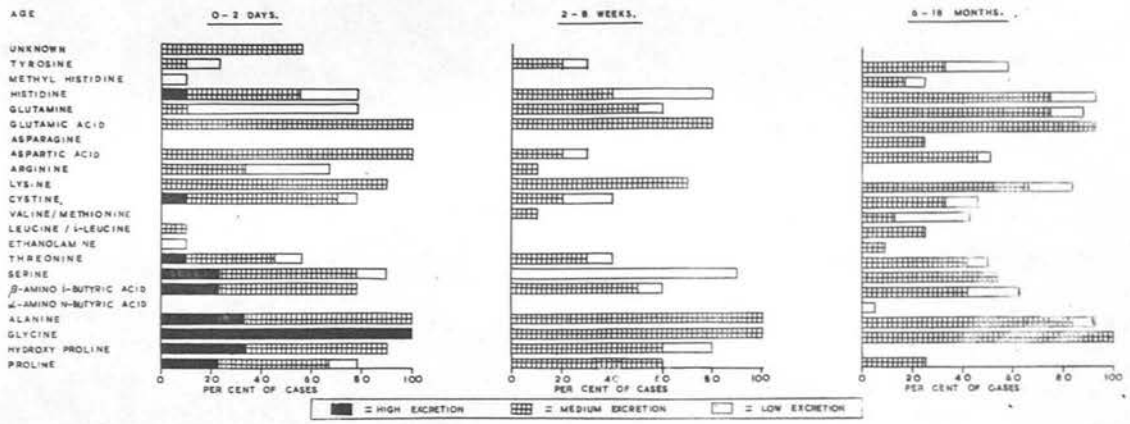
Figure 30b has been constructed by devising a points system, by which means a picture is obtained combining both the frequency of appearance, and the amount of each amino acid in the urine in the different age groups. If 100 per cent of the infants in a particular age group excreted large amounts of a particular amino acid, 100 points are allowed. If 100 per cent of the infants in a particular age group excreted medium amounts of a particular amino acid, 50 points are allowed. If 100 per cent of the infants excreted only trace amounts of a particular amino acid, 25 points are allowed. Different percentages are scored in proportion to these values. The scores obtained in each age group and in two groups of abnormal infants are given in Table 37.

In all the figures and tables, the amino acids have been tabulated according to the different side chains

FIGURE 30

PATTERNS OF URINARY AMINO ACID EXCRETION AT DIFFERENT AGES

A. ACCORDING TO THE NUMBER OF CASES EXCRETING HIGH MEDIUM AND LOW AMOUNTS OF EACH ACID.



B. ACCORDING TO A POINTS SYSTEM.

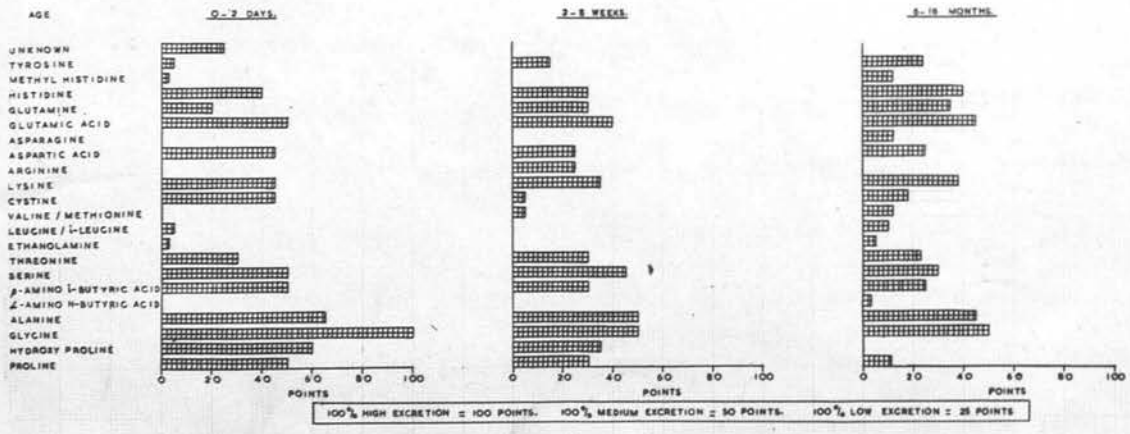


TABLE 36

The Urinary Excretion of Amino Acids Expressed as a Percentage of the Number of Cases Excreting Each Amino Acid at One of Three Different Levels: High, Medium and Low. Data for Normal Infants: a) Aged 1 - 2 Days (9); b) Aged 2 - 8 Weeks (10) and c) Over 6 Months (10).

	1-2 Days			2-8 Weeks			Over 6 Months		
	High	Medium	Low	High	Medium	Low	High	Medium	Low
Proline	23	56	11	0	60	0	0	30	0
Hydroxy Proline	33	56	0	0	60	20	0	0	0
Glycine	100	0	0	0	100	0	0	100	0
Alanine	33	67	0	0	100	0	0	90	10
α-Amino Butyric Acid	0	0	0	0	0	0	0	10	0
β-Amino-iso-Butyric Acid	23	56	0	0	0	0	0	50	10
γ-Amino Butyric Acid	0	0	0	0	0	0	0	0	0
Serine	22	67	11	0	90	0	0	60	0
Threonine	11	33	11	0	30	10	0	30	10
Ethanolamine	0	0	11	0	0	0	0	10	0
Leucine/iso-Leucine	0	11	0	0	0	0	0	10	0
Valine/Methionine	0	11	0	0	10	0	0	10	0
Cystine	11	56	11	0	20	20	0	30	0
Aspartic Acid	0	89	0	0	20	10	0	40	0
Asparagine	0	0	0	0	0	0	0	30	0
Glutamic Acid	0	100	0	0	80	0	0	100	0
Glutamine	0	11	67	0	50	10	0	70	20
Lysine	0	89	0	0	70	0	0	50	30
Arginine	0	33	33	0	10	0	0	0	0
Histidine	11	44	23	0	40	40	0	70	20
Methyl Histidine	0	0	11	0	0	0	0	20	0
Tyrosine	0	11	11	0	20	10	0	40	20
Unknown - Ninhydrin gives blue colour	0	56	0	0	0	0	0	0	0

incorporated into the amino acid molecule. Table 36 gives a complete list of all the different amino acids found to be excreted at the different ages.

THE INDIVIDUAL AMINO ACIDS

1. Imino Acids - Proline and Hydroxy Proline

These two acids are remarkable. They are both excreted in the urine in large amounts at birth but thereafter there is a rapid decrease in excretion. Hydroxy proline has disappeared completely by the age of six months and proline is excreted only in small amounts at that age. It is worth noting that nearly all the hydroxy proline in the body and a high proportion of the proline is found in collagen. The only source of hydroxy proline is thought to be hydroxylation of collagen proline is situ. Linstedt and Prockop (1961) found only insignificant incorporation of administered hydroxy proline into collagen hydroxy proline. Its excretion may be regarded as a measure of collagen turnover. The high rate of excretion of this amino acid in the neonatal period points to a higher rate of collagen turnover than at any other age except in such collagen diseases as arachnodactyly.

2. Glycine, Alanine and the Amino Butyric Acids

The excretion of glycine is particularly high during

TABLE 37

The Urinary Excretion of Amino Acids by three groups of normal infants at different ages and two groups of abnormal infants, described in terms of a points system, calculated on the following basis:

100% high excretion = 100 100% medium excretion = 50 100% low excretion = 25

	Normal Infants				
	1 - 2 Days	2 - 8 Weeks	Over 6 Months	Epileptics all ages	Failure to Thrive 2 - 12 Weeks
Proline	50	30	10	38	50
Hydroxy Proline	60	35	0	13	50
Glycine	100	50	50	65	80
Alanine	65	50	45	53	60
α-Amino Butyric Acid	0	0	2.5	0	40
β-Amino-iso-Butyric Acid	50	30	25	38	30
γ-Amino Butyric Acid	0	0	0	10	100
Serine	50	45	30	55	70
Threonine	30	30	23	30	50
Ethanolamine	3	0	5	5	20
Leucine/iso-Leucine	5	0	10	11	0
Valine/Methionine	0	5	12	30	5
Cystine	43	5	18	25	20
Aspartic Acid	45	25	25	35	50
Asparagine	0	0	12	15	50
Glutamic Acid	50	40	45	60	60
Glutamine	20	30	35	53	40
Lysine	45	35	38	45	45
Arginine	0	25	0	6	20
Histidine	40	30	40	58	50
Methyl Histidine	3	0	10	23	5
Tyrosine	5	15	24	20	25
Unknown - Ninhydrin gives blue colour	25	0	0	0	20

the first two days of life. That of alanine and β-amino-iso-butyric acid is also raised. By the age of two weeks, glycine excretion has fallen to half its original value and does not alter greatly thereafter. Alanine excretion only decreases a little, whilst that of β-amino-iso-butyric acid resembles glycine in falling to half the original amount. This decrease is more gradual than that of glycine and it continues for longer. Only in one infant aged six months did α-amino butyric acid ever appear in the urine, and γ-amino butyric acid was never found in the urine of any normal child of any age. The urine of none of these infants contained β-amino-n-butyric acid, although it is occasionally found in that of much older children.

3. Leucine and iso-Leucine, Valine and Methionine and Cystine

With the solvent system used in this investigation, it is impossible to distinguish between leucine and iso-leucine and between valine and the sulphur-containing amino acid, methionine. There is a slight increase in the excretion of both of these pairs of amino acids with increasing age but it is not high at any time. Cystine, the other important sulphur-containing amino-acid appears to a greater extent in the urine of the new-born infants,

and the amount excreted decreases fairly rapidly with increasing age.

4. Basic Amino Acids - Lysine and Arginine

The excretion of lysine and arginine is linked to that of cystine, especially in some diseases such as cystinuria (Chisholm, 1959). This does not appear to be important in infancy. Lysine excretion, like that of cystine, decreases with increasing age but to a far less extent. Arginine was never found in the urine of new born infants and in only one eight weeks old infant.

5. Hydroxy Amino Acids - Serine and Threonine

Serine and threonine excretion follow the same pattern as β-amino-iso-butyric acid. They are both excreted in large amounts by some new-born infants but only in medium amounts by others and in medium amounts by all infants of other ages.

6. The Amino Alcohol Ethanolamine

Ethanolamine is occasionally found in the urine of children of all ages.

7. Dicarboxylic Acids - Aspartic Acid, Glutamic Acid and their Amides

The excretion of aspartic acid is high in some new born infants and decreases with age. That of glutamic

acid, appears in the urine in increasing amounts, and more frequently, with increasing age.

8. Aromatic and Heterocyclic Amino Acids - Tyrosine, Histidine and Methyl Histidine

The excretion of tyrosine presents a curious picture. It is known to be excreted in such large amounts by premature infants, that a tyrosyluria has been described. (Dustin, Moore and Bigwood, 1955). In this series, which did not include any premature infants, tyrosine excretion increased uniformly with increasing age. It is not a constant component of the urine, even in the new-born period.

Histidine, an imidazole compound, is excreted in large amounts by some new-born infants. The amounts decrease with increasing age. The excretion of methyl histidine is slight until an age is reached when meat is included in the diet. This is to be expected because meat is the main source of this amino acid in the urine.

9. Unidentified Substance giving a Positive Reaction with Ninhydrin

A substance giving a bright blue colour with ninhydrin was found in the urine of half of the new-born infants. It also appears in the urine of some rats but has not been seen on any other occasion. Its Rf value

in butanol/ pyridine is approximately 0.50 and in phenol/ ammonia it is approximately 0.90. It was not identified, but it could correspond to α-amino caprylic acid which has similar Rf values in these solvents.

SUMMARY

The results of this investigation of the patterns of amino acid excretion in the urine of normal infants will be summarised briefly.

1. A larger amount of amino acid is excreted in the urine during the first two days of life than in any subsequent period. Proline, hydroxy proline, glycine, alanine, β-amino-iso-butyric acid, cystine, serine, threonine and histidine are all excreted in particularly large amounts.
2. There is a decrease in the excretion of nearly all the amino acids during the first eight weeks of life and subsequently it either increases slightly or does not alter further.
3. Hydroxy proline is only found normally in the urine of infants under six months old.
4. Proline excretion decreases dramatically but does not cease altogether.
5. In this investigation, tyrosine excretion increases

Figure 31.

Amino Acid Chromatograms Obtained From Cord Blood Serum.

Solvent 1. Butanol / Pyridine.

Solvent 2. Phenol / Ammonia.

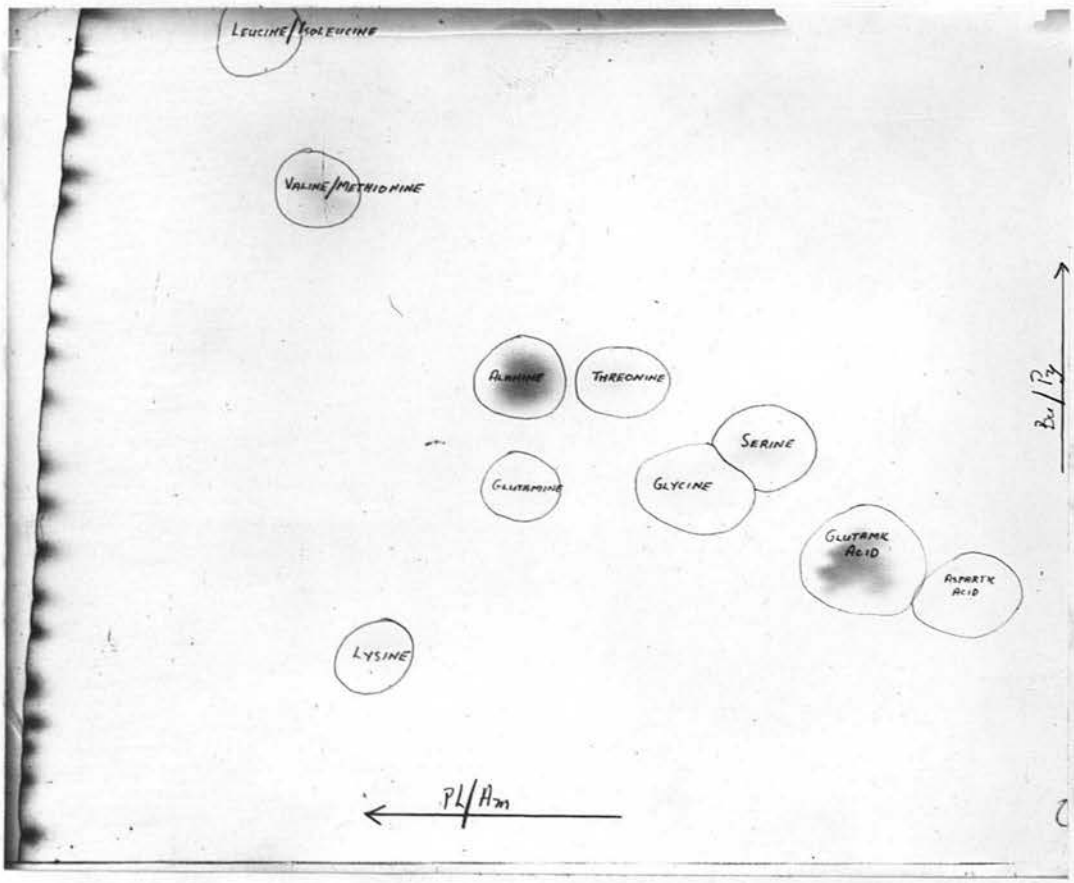


Figure 32.

Amino Acid Chromatogram Obtained From Cord Blood Serum.

Solvent 1. Butanol / Pyridine.

Solvent 2. Phenol / Ammonia.

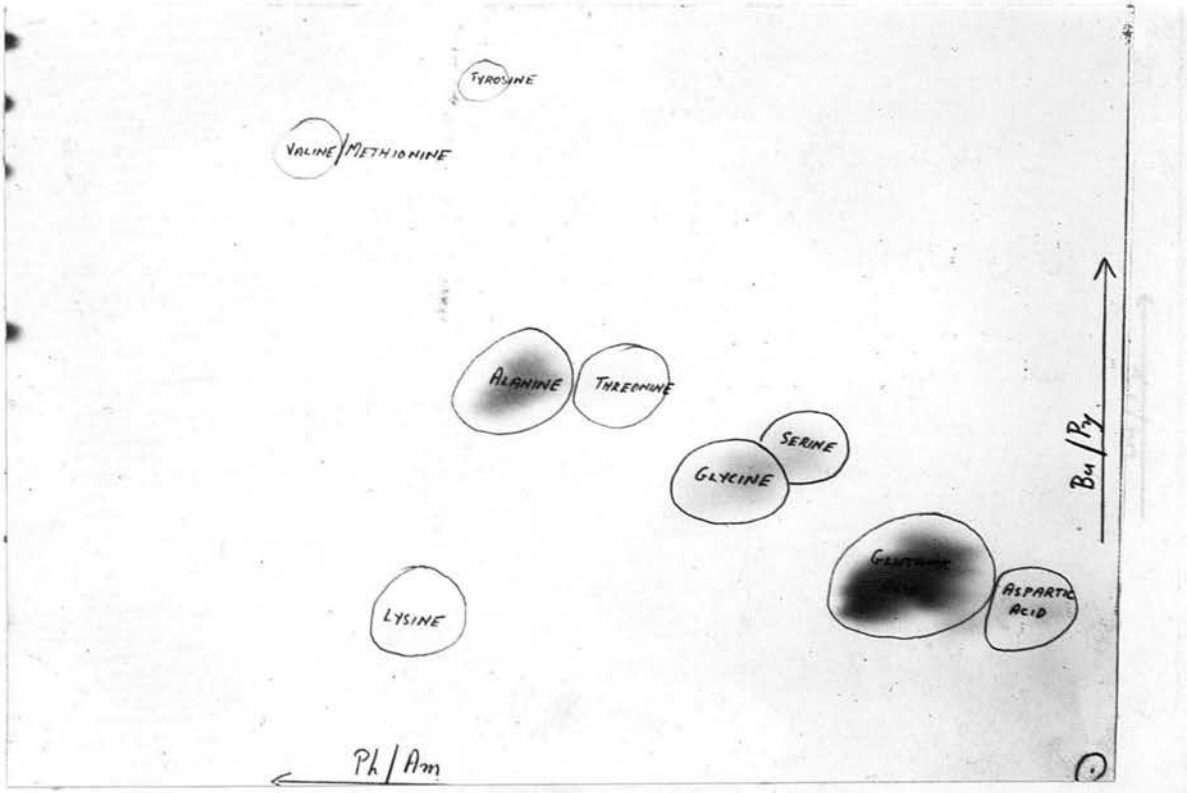
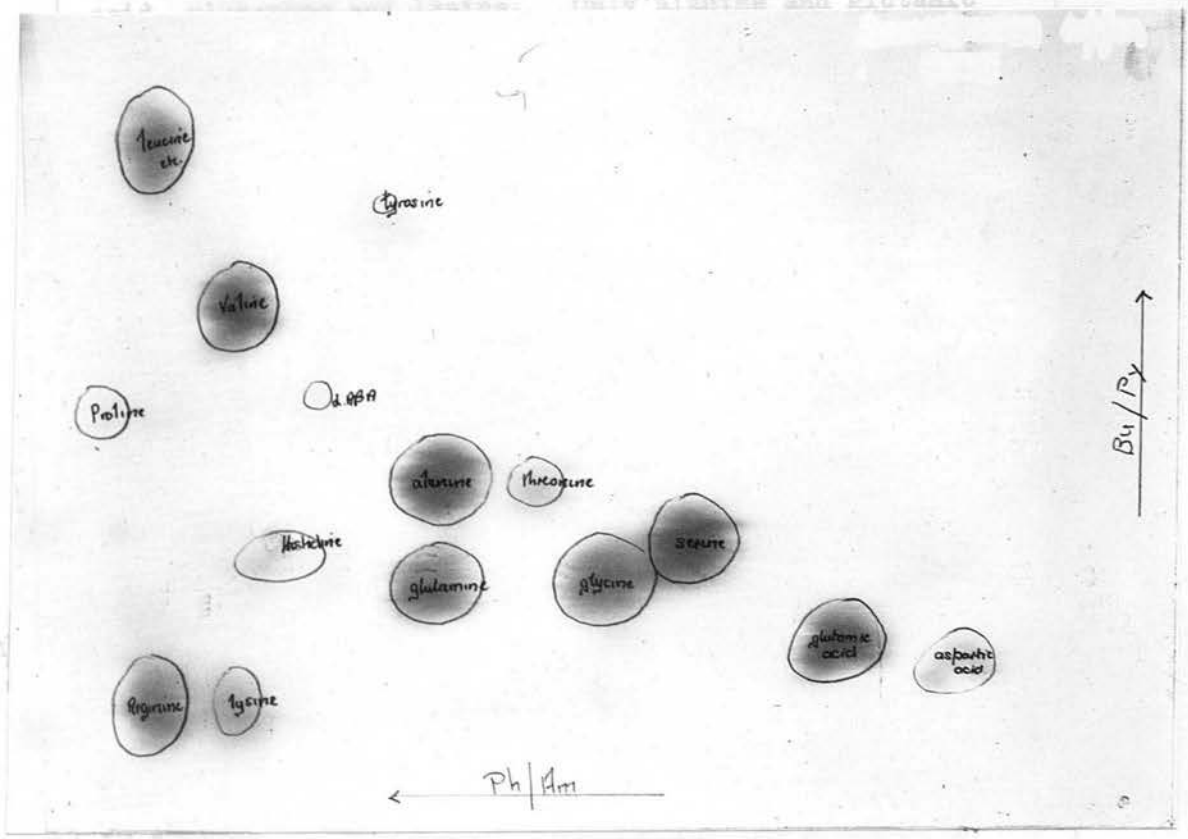


Figure 33.

Amino Acid Chromatograph Obtained From the Blood Serum of a One Year Old Infant.



with increasing age although it is reported to be highest in premature infants at birth.

AMINO ACID PATTERNS IN THE UMBILICAL CORD BLOOD OF NORMAL INFANTS

The following amino acids were found in the umbilical cord blood: glycine, alanine, serine, threonine, leucine/iso-leucine, valine/methionine, aspartic acid, glutamic acid, glutamine and lysine. Only alanine and glutamic acid were found in consistently high amounts and the glutamic acid probably contained a certain amount which had been formed from glutamine during the desalting process. Little difference was observable between these and the chromatograms obtained from the serum of older children except that proline was unaccountably absent from all of them, although it is often present in the serum of older children and is reported to occur in all sera (Woolf and Norman, 1957). These data are illustrated in Figs. 31-33.

DISCUSSION

Previous Investigations of Urinary Amino Acid Excretion in Infants

Other investigators have obtained results similar to those in this investigation (Woolf and Norman, 1957; Sereni, McNamara, Shibuya, Kretchmer and Barnett, 1955;

Dustin, Moore and Bigwood, 1955).

Dustin et.al. (1955) point out that it is the neutral and acidic amino acids which emerge at relatively high levels during the neonatal period and decrease with maturation, while the basic amino acids are excreted at approximately adult levels throughout. These authors were investigating premature infants in whom the tendencies observed in full-term infants are intensified; i.e. they excrete even more glycine, serine, threonine, alanine and glutamine than full-term infants. Tyrosine was also found to be excreted in particularly large amounts and the excretion of valine, leucine/iso-leucine, phenyl alanine, proline, cystine and glutamic acid was found to be elevated.

Previous Investigations of Serum Amino Acid Patterns in Infants

Sereni et.al. (1955) confirm the results obtained in this investigation. They also reported that, in addition to the amino acids mentioned on Page 179, the plasma concentrations of glycine, serine, threonine, glutamine, tyrosine and phenyl alanine are elevated in the infants in the neonatal period compared with older children.

Woolf and Norman (1957) found that while the serum concentrations of leucine, valine, alanine, and proline

are the same in cord blood as in the blood of older children, those of taurine, lysine, ethanolamine, β-amino-iso-butyric acid, tyrosine and glutamic acid are much higher in cord blood. Glycine concentrations are highest of all. Traces of hydroxy proline are found in some neonatal sera but never in those of older children. Small premature infants have five times the concentrations of leucine, iso-leucine and valine as full term infants but in the latter, these amino acids increased rapidly during the first few days of life.

Relationships Between the Concentrations of Amino Acids in the Blood and in Urine

The relationship between the concentrations of amino acids in blood serum and in urine is a complex one because different amino acids have different clearance rates in the kidney. This is well illustrated in the data of Doolan, Harper, Hutchin and Shreeve (1959) who investigated the concentrations of seventeen amino acids in the plasma and the urine. They underline the fact that the tubular reabsorption of all amino acids is virtually complete so that only a small fraction of the filtered load of each amino acid is actually excreted in the urine. Glycine, the amino acid whose excretion is the greatest, is 96 per cent reabsorbed. There is considerable variation in both

plasma concentrations and endogenous clearance rates. These independent variables account for the fact that the normal distribution of amino acids in the urine is quite different from that in plasma.

Doolan (1955) also found that when the filtered load is increased, both excretion and reabsorption increase but that competition for reabsorption exists within certain groups of amino acids. When those amino acids with highly efficient reabsorption mechanisms are excreted in large amounts without parallel increases in plasma concentrations, it is considered that there must be an impairment in the reabsorption mechanism. (Snyderman, 1958).

The Reabsorption Mechanisms in the New-born Infant

New-born infants may well be described as having immature, rather than impaired, reabsorption mechanisms. A lower glomerular filtration rate is accompanied by a reduced reabsorption rate compared with older children and adults so that despite the lower glomerular filtration rate, the amount of amino acids excreted is increased. Since there is not a generalized increase in the excretion of all the amino acids, it seems likely that the kidney of the new-born infant and especially the premature infant is deficient in the carrier systems controlling the reabsorption of specific amino acids only. (Kretchmer, 1957).

TABLE 38

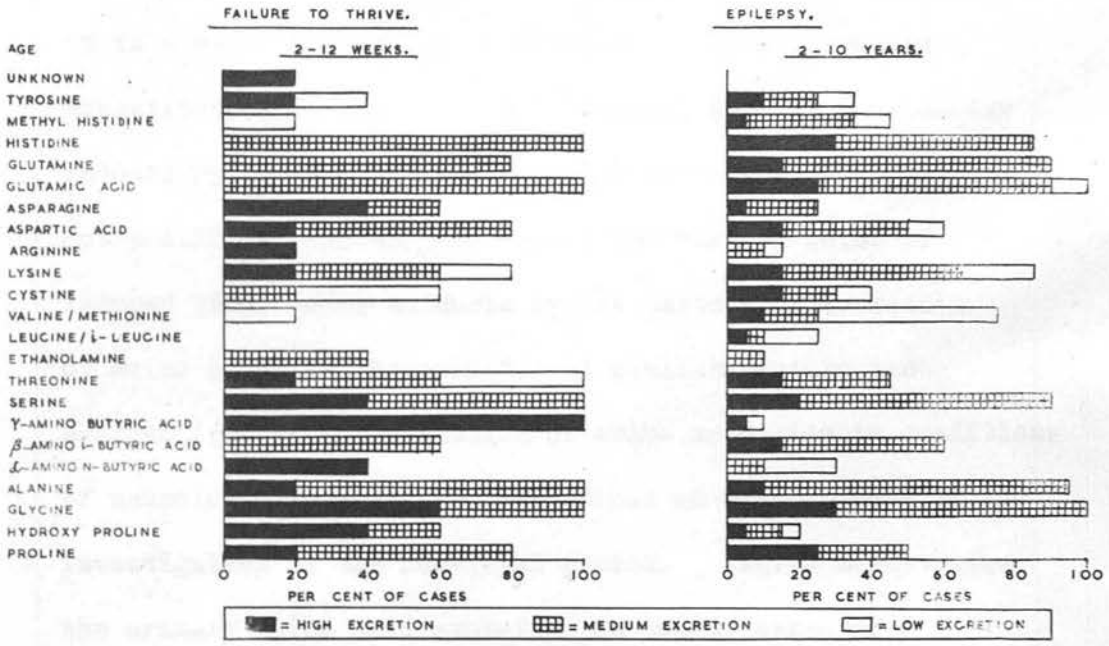
The Urinary Excretion of Amino Acids Expressed as a Percentage of the Number of Cases Excreting Each Amino Acid at One of Three Different Levels: High, Medium and Low. Data for 20 Cases of Epilepsy of Different Ages and 5 Cases of Idiopathic Failure to Thrive: Aged 2 - 12 Weeks.

	Epileptics(20)			Failure to Thrive(5)		
	High	Medium	Low	High	Medium	Low
Proline	25	25	0	20	60	0
Hydroxy Proline	5	10	5	40	20	0
Glycine	30	70	0	60	40	0
Alanine	10	85	0	20	80	0
α-Amino Butyric Acid	0	10	20	40	0	0
β-Amino-iso-Butyric Acid	15	45	0	0	60	0
γ-Amino Butyric Acid	5	0	5	100	0	0
Serine	20	70	0	40	60	0
Threonine	15	30	0	20	40	40
Ethanolamine	0	10	0	0	40	0
Leucine/iso-Leucine	5	5	15	0	0	0
Valine/Methionine	10	15	50	0	0	20
Cystine	15	15	10	0	20	40
Aspartic Acid	15	35	10	20	60	0
Asparagine	5	20	0	40	20	0
Glutamic Acid	25	65	10	20	80	0
Glutamine	15	75	0	0	80	0
Lysine	15	50	20	20	40	20
Arginine	0	10	5	20	0	0
Histidine	30	55	0	0	100	0
Methyl Histidine	5	30	10	0	0	20
Tyrosine	10	15	10	20	0	20
Unknown - Ninhydrin gives blue colour	0	0	0	20	0	0

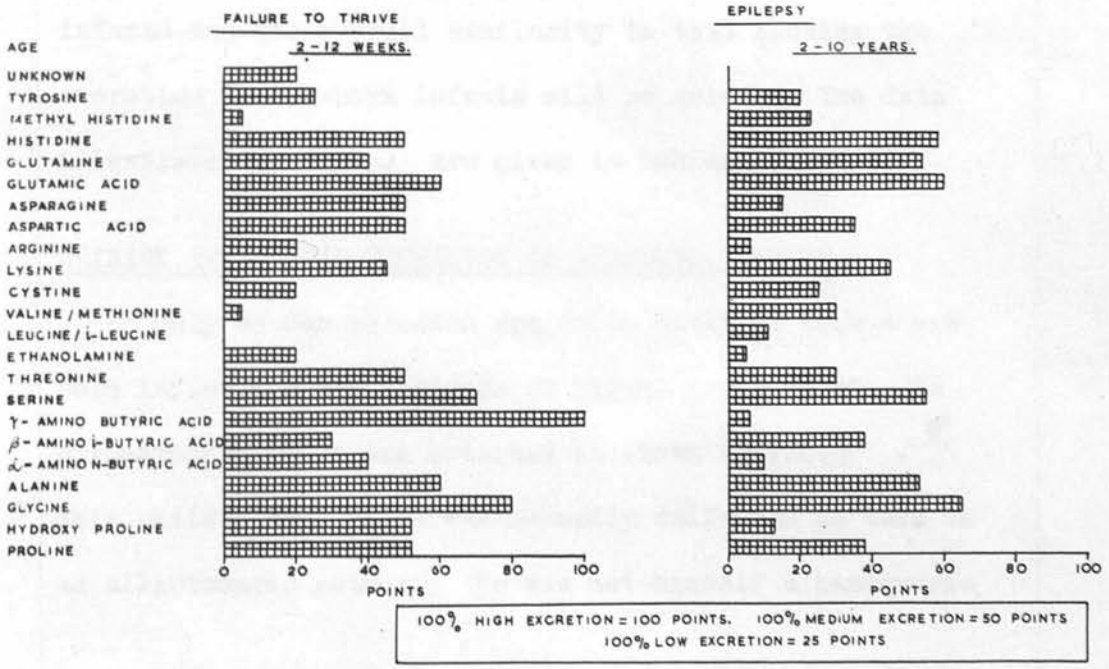
FIGURE 34

PATTERNS OF URINARY AMINO ACID EXCRETION IN CASES OF IDIOPATHIC FAILURE TO THRIVE AND OF EPILEPSY.

A. ACCORDING TO THE NUMBER OF CASES EXCRETING HIGH, MEDIUM AND LOW AMOUNTS OF EACH ACID.



B. ACCORDING TO A POINTS SYSTEM.



It seems likely that these carrier systems are very easily affected by a variety of conditions because in the majority of the amino acidurias described in the literature, it is always the same amino acids which have increased excretions. Harrison, (1957) compared the amino acidurias induced by six different causes and points out that "It is not possible to distinguish among the various forms of induced renal amino aciduria by the pattern of excretion of amino acids in the urine". A similar picture has emerged from an investigation of amino acidurias in conditions of neurological dysfunction which had no connection with the investigation of the neo-natal period. Fig. 34 illustrates the urinary amino acid excretion in twenty cases of epilepsy, and in ten cases of idiopathic failure to thrive. It is constructed in the same way as Fig. 30 for normal infants and its overall similarity to that showing the excretion in new-born infants will be noted. The data illustrated by Fig. 34 are given in Tables 37 and 38.

URINARY AMINO ACID EXCRETION IN ABNORMAL INFANTS

Only on one occasion was urine obtained from a new-born infant who was abnormal at birth. The amino acid chromatogram which was obtained is shown in Fig. 35 . This child - Baby Bl. - was normally delivered at term to an alkaptonuric mother. He was not himself alkaptonuric

Figure 35.

Amino Acid Chromatogram Obtained From the Urine of Baby Bl., Aged Two Days. This Infant Was Hydrocephalic. His Mother is Alkaptonuric.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

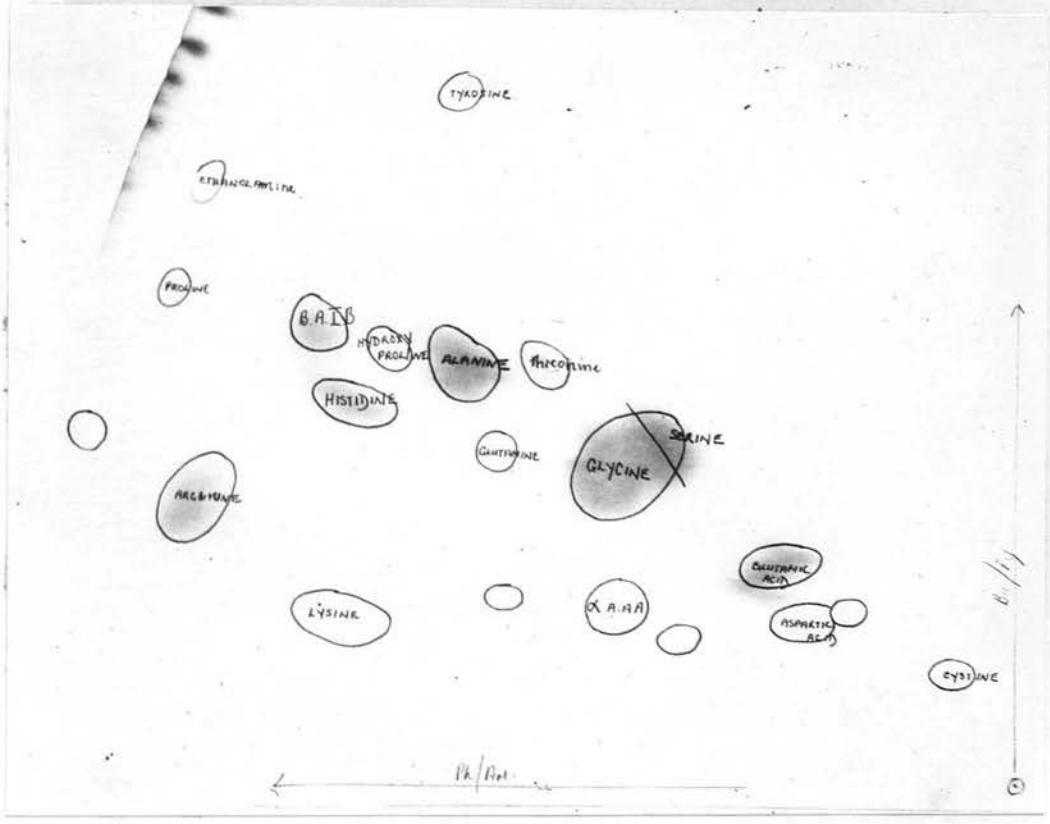
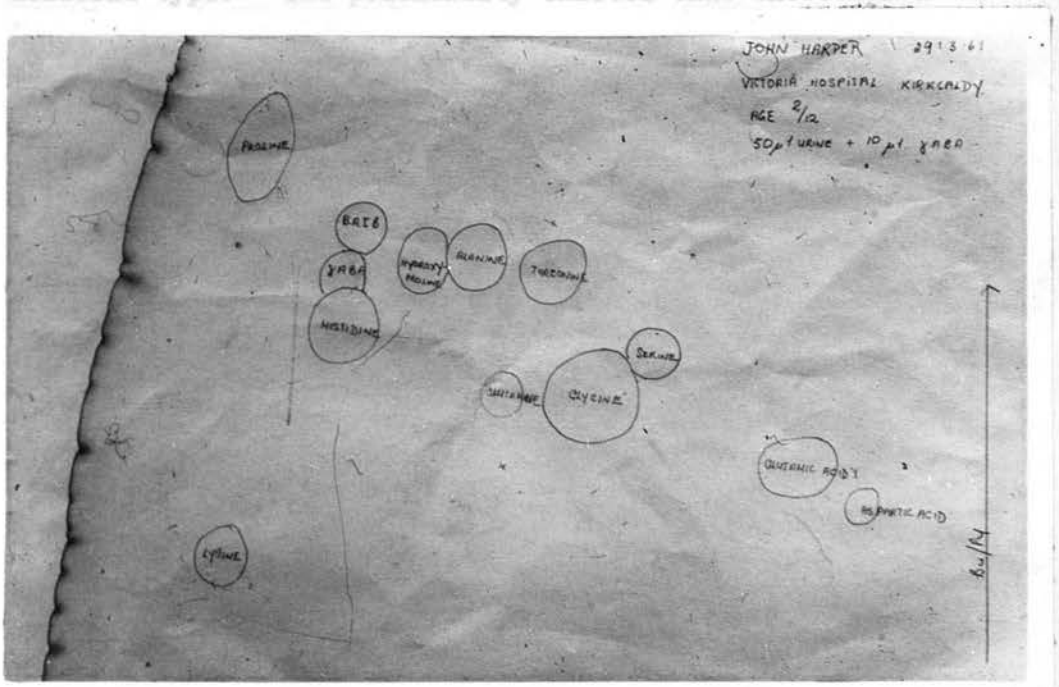


Fig. 36.

Amino Acid Chromatogram Obtained from the Urine of Baby Ha. aged Two Weeks. This Infant Died on the Following Day of a "Congenital Cerebral Disorder".

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.



but was hydrocephalic and had abnormally high concentrations of lipids and lipoprotein lipids in the cord blood. (See Chapter IV Page 82). A sibling had died when five days old, allegedly from "cerebral anoxia". The present infant is now apparently quite normal.

The amino acid excretion was considerably greater than that found in any other child during the first two days of life and it was of the generalized renal amino aciduria type. The possibility existed that there was a trace of γ-amino butyric acid showing on the chromatogram in between β-amino-iso-butyric acid and histidine. This is not shown in Fig. 35. This amino acid does not normally appear in the urine at all. It has been found in this work in two groups of children.

I. Those having a variety of neurological abnormalities and

II. Those diagnosed as "idiopathic failure to thrive".

The data obtained in Groups I and II are given in Tables 37 and 38, and illustrated in Fig. 34.

Group I - Infants having Neurological Abnormalities

Urine was obtained from an example of Group I when the infant was two weeks old and the chromatogram obtained is shown in Fig. 36. This infant died the following day. Already in an emaciated condition, despite his having been

Figure 57

Amino Acid Chromatograms From Baby Li., Aged 9 Months.

This Infant is an Epileptic.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

1. Urine Ammino Acid Chromatogram.

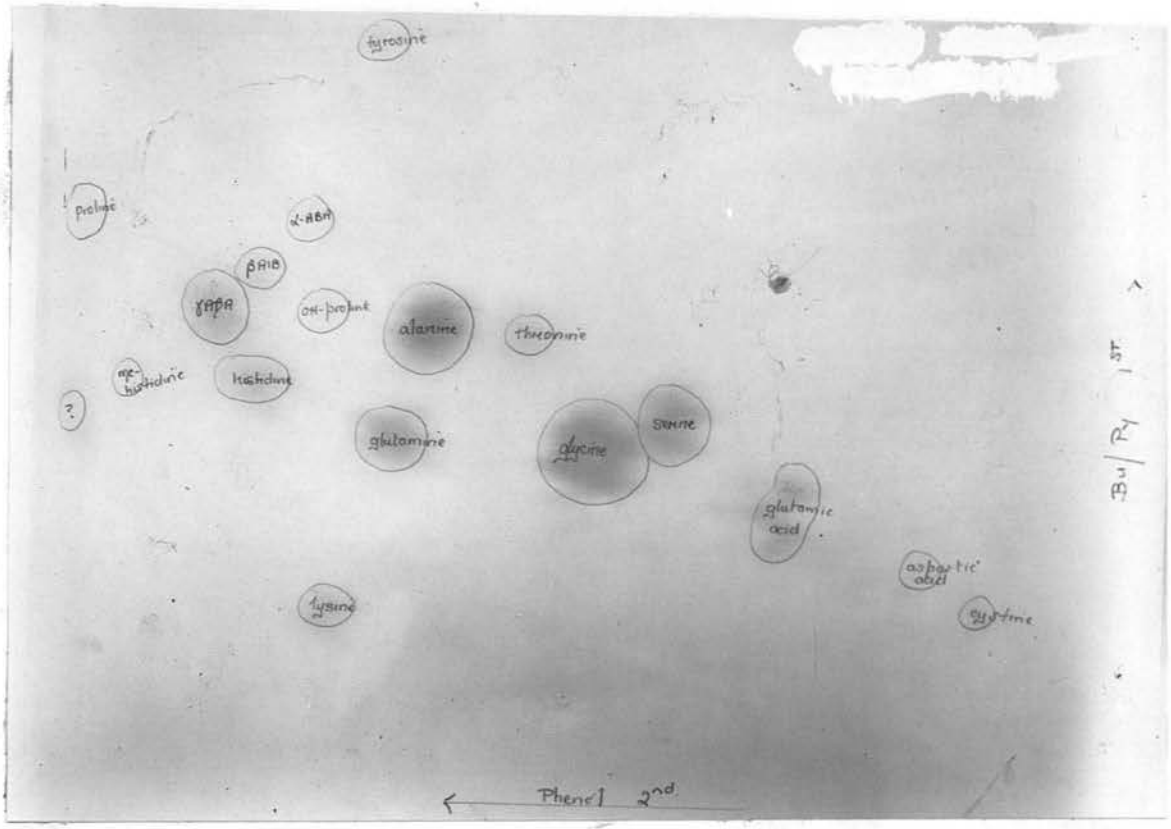


Figure 37 (cont.)

2. Serum Amino Acid Chromatogram.

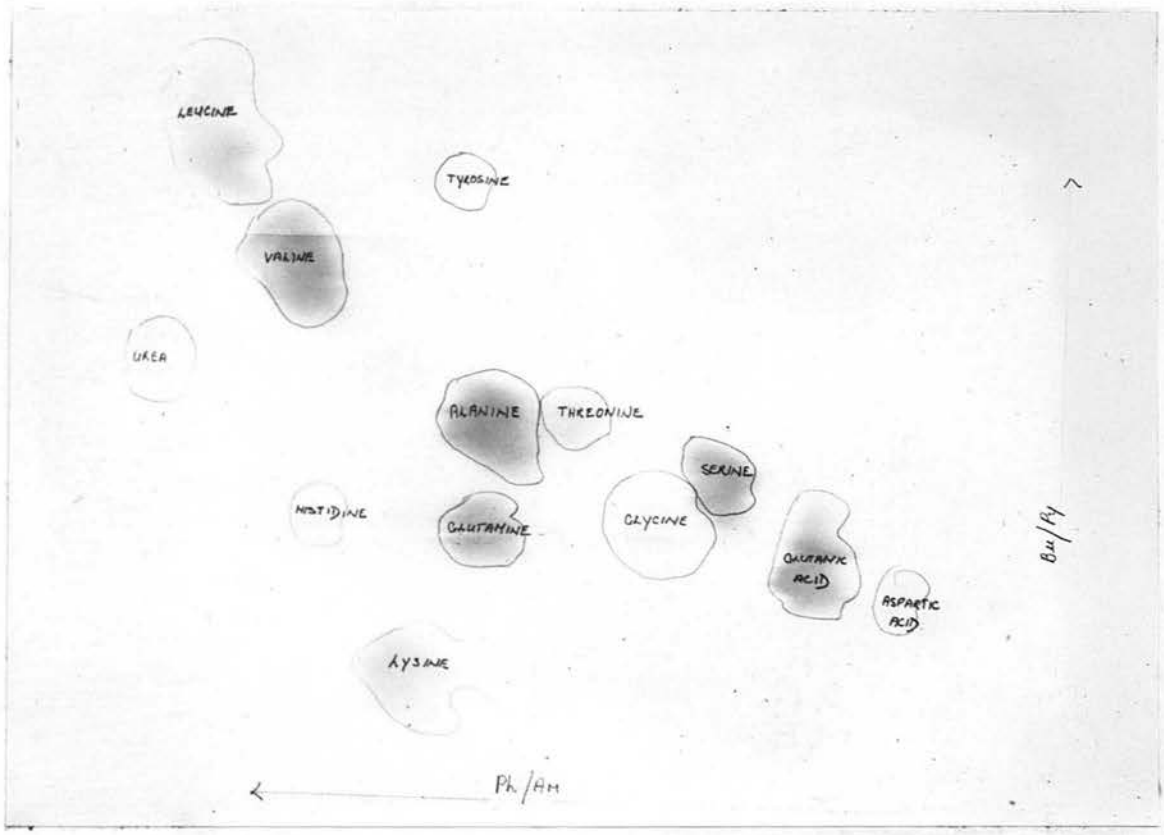


Figure 38.

Amino Acid Chromatograms From Baby Va., Aged Fifteen Weeks.

A Case of Failure to Thrive.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

1. Urine Amino Acid Chromatogram.

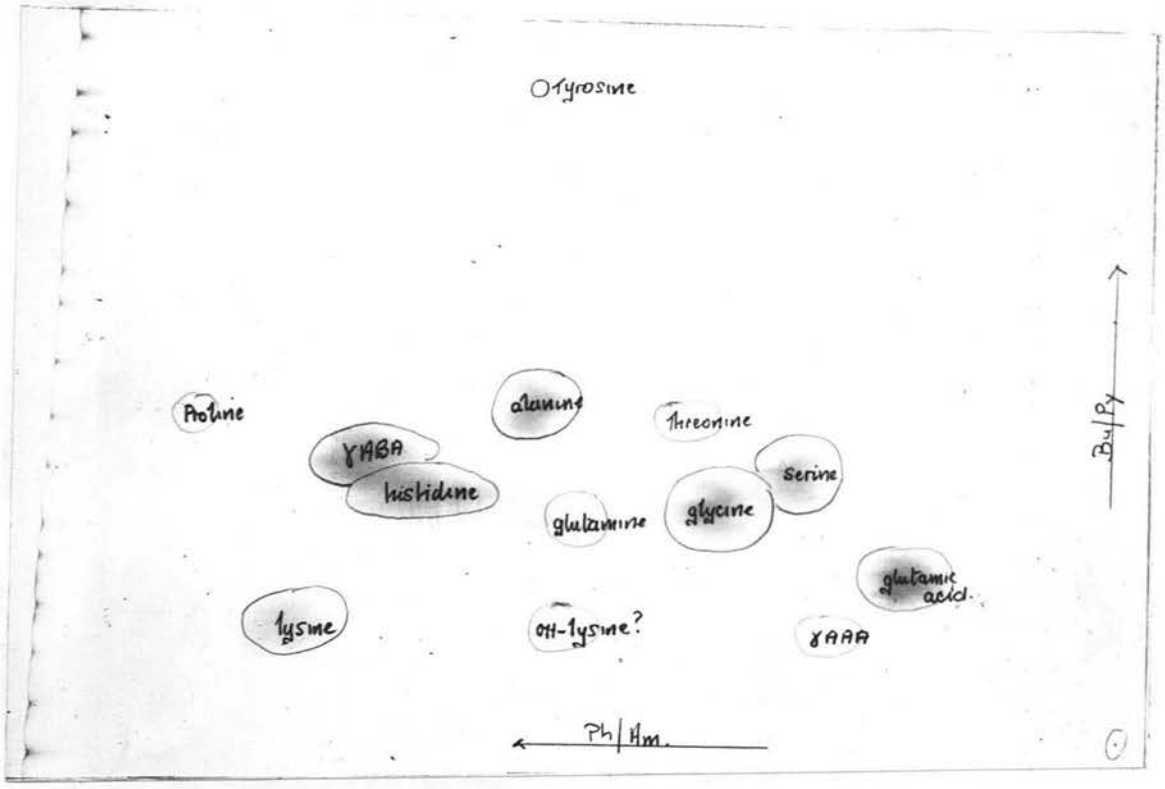


Figure 38 (cont.)

2. Serum Amino Acid Chromatogram.

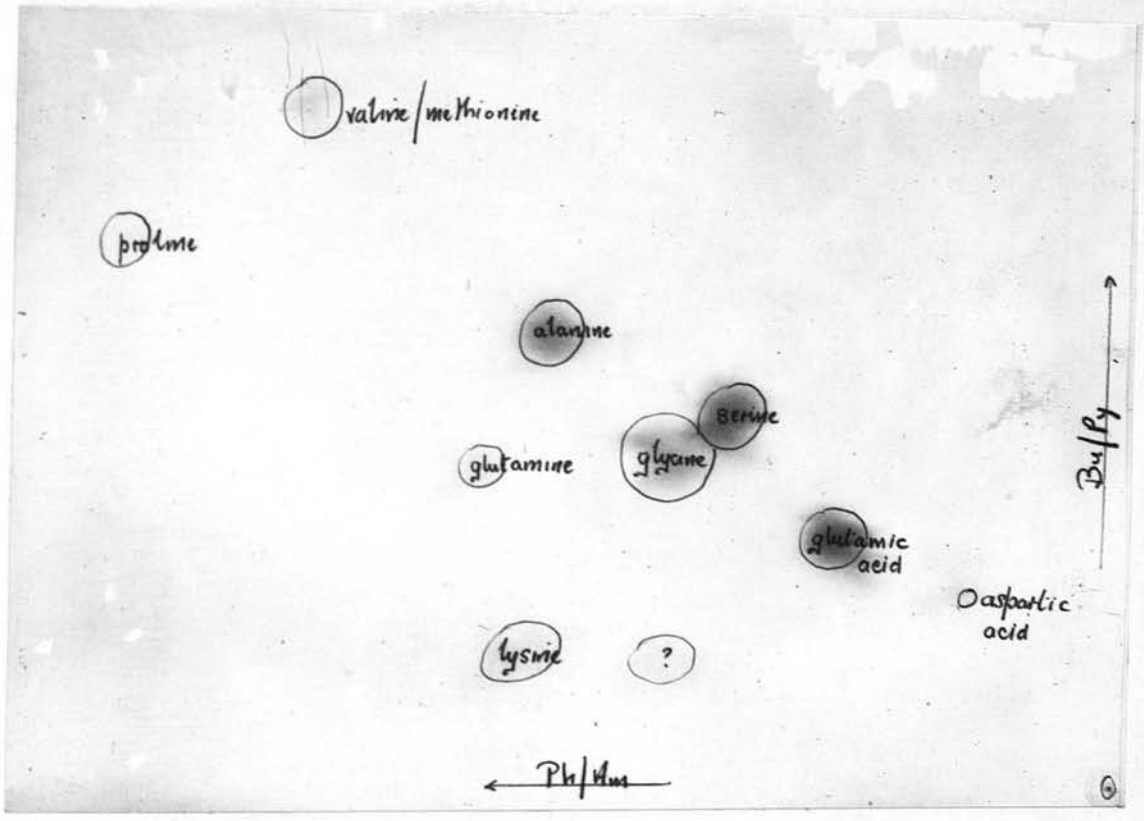


Figure 39.

Amino Acid Chromatograms From Baby Th., Aged Three Weeks.

A Case of Failure to Thrive.

Solvent 1. - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

1. Urine Amino Acid Chromatogram.

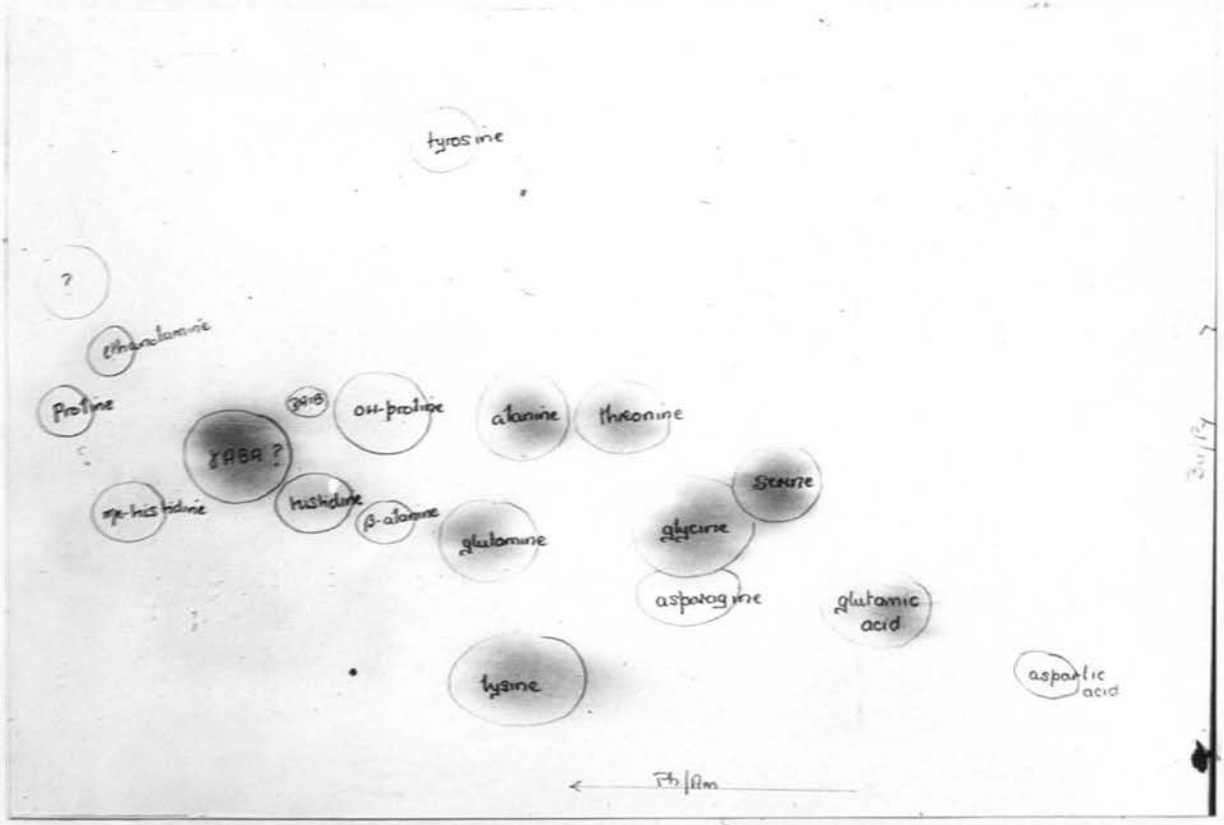


Figure 39. (cont.)

2. Serum Amino Acid Chromatogram.

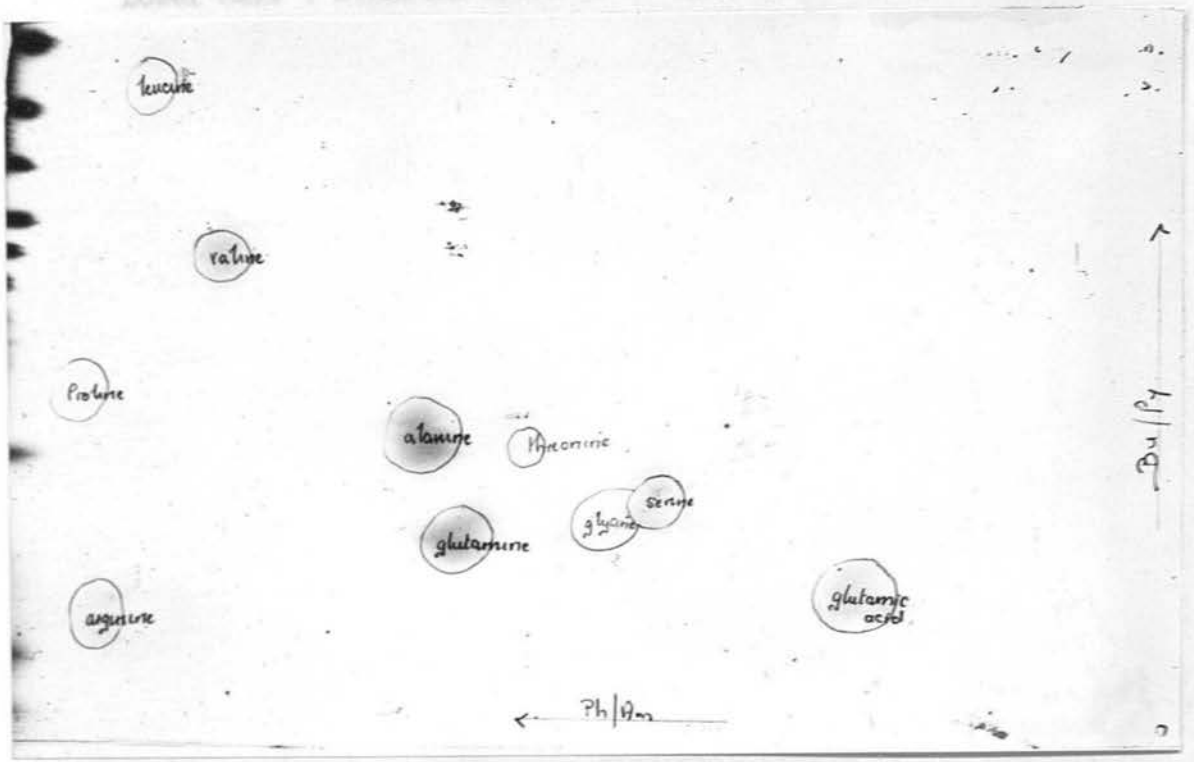


Figure 40.

Urine Amino Acid Chromstograms From Baby Bu., Aged Three Months.

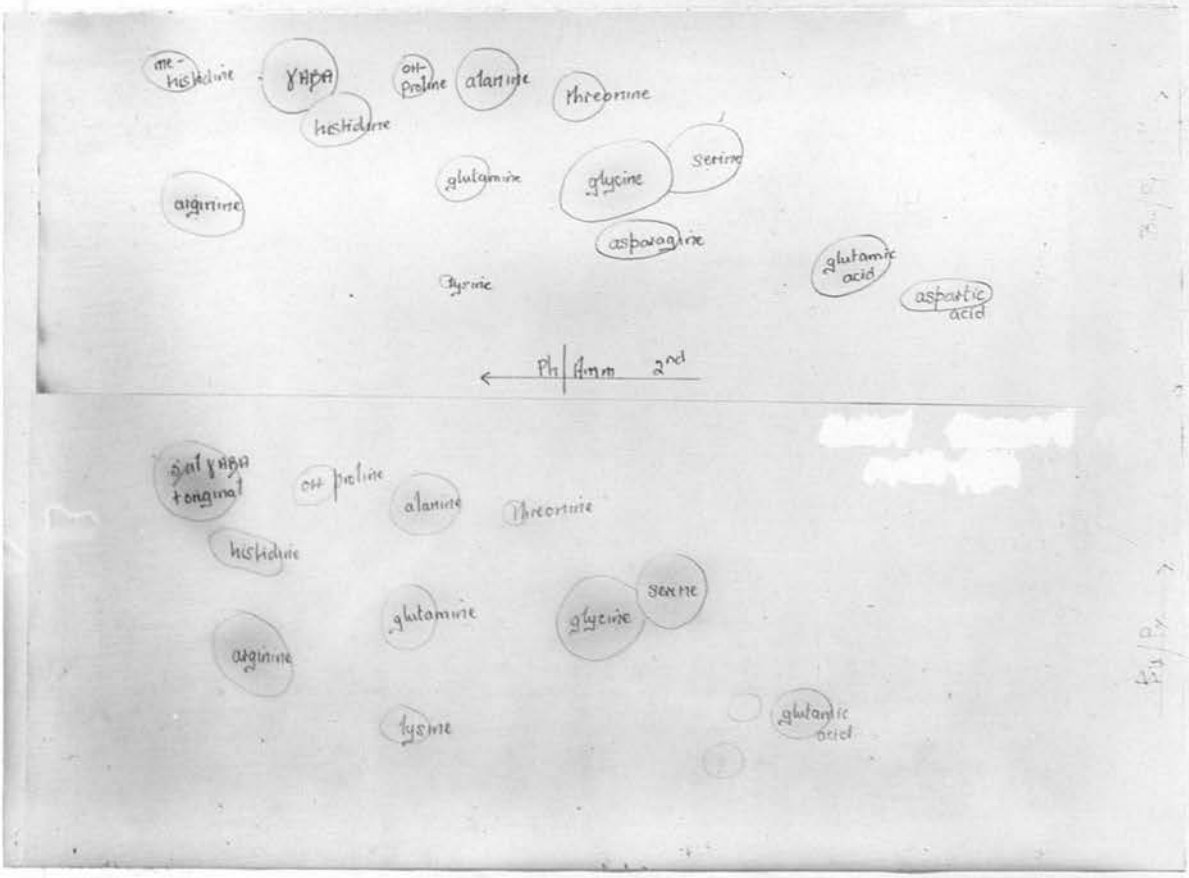
A Case of Failure to Thrive.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

Upper Half : Desalted Urine Only.

Lower Half : Desalted Urine plus 5 μ - Litres of γ- Amino Butyric Acid.



perfectly normal at birth (as far as could be seen) he developed severe convulsions prior to death and a "congenital cerebral disorder" was diagnosed after a post mortem examination. Not only was γ-amino butyric acid excreted by this infant but also enormous amounts of proline and hydroxy proline which suggests the co-existence of a collagen disease. There were apparently no grounds for diagnosing Wilson's disease or cystinosis in both of which proline excretion is increased.

Another case of the excretion of γ-amino butyric acid in a neurological disorder is illustrated by the chromatogram in Fig. 57. This was a case of myoclonic epilepsy at the age of five months.

Group II -"Idiopathic Failure to Thrive"

Examples of the chromatograms obtained from the cases of idiopathic failure to thrive in Group II are given in Figs. One of the, Baby Va. (Fig.) died in an emaciated condition when four months old during which time he had gained approximately two pounds over his birth weight, which was normal. The post mortem examination findings were negative.

The other cases in Group II were eventually described as "thriving" when they were approximately six months old. It was at this time that γ-amino butyric acid disappeared

from the urine. It was not present in detectable quantities in their plasma at any time. This was hardly to be expected since it did not appear in the plasma of an epileptic child treated with γ-amino butyric acid in a maximum dose of 2 grams per diem. This dose was sufficient to control her convulsions and a large amount of the amino acid appeared in the urine. This implies that the renal clearance of this amino acid, at least in this case, is very high, due to impaired reabsorption or to active tubular secretion.

No conclusions have been reached on the significance of the excretion of this amino acid. There are greater concentrations of γ-amino butyric acid in the brain than in any other part of the body and it is worth noting that it was also excreted in large amounts by a child who had a fatal sub-arachnoid haemorrhage. On the other hand it was never found in cerebro-spinal fluid. (C.S.F.). It is possible, however, that with the high turnover rate of C.S.F., it would usually be undetectable there.

It seems unwise to speculate on the possibility of a leakage of γ-amino butyric acid across a defective blood-grain barrier because there is no reason to suppose any brain injury in the cases of idiopathic failure to thrive, even if it is present in the neurological cases. The

problem is therefore put forward as one which might prove interesting on further investigation.

It will be noted from Fig.38-40 that the urinary amino acid patterns in these Group II infants resemble those of new-born infants rather than those in the 2 - 8 weeks age group into which they would normally fall.

CONCLUSIONS

1. Infants in the neo-natal period excrete larger amounts of amino acids than at any subsequent period in their development.
2. Serum amino acid concentrations in the neo-natal period are not greatly different from those in older children and in adults.
3. It is likely that the amino aciduria of the neo-natal period is the result of immaturity of the renal absorbtion mechanisms.
4. The presence of an abnormal amino acid - γ-amino butyric acid - has been described in the urine but not the serum, of two groups of infants:
   I. those with neurological abnormalities and
   II. those with an idiopathic failure to thrive.
5. It has proved impossible to find an explanation for this abnormality.

APPENDIX I

METHODS OF ESTIMATION

Throughout this investigation, the policy has been to use methods of investigation which are in present use in routine clinical laboratories - mainly the Royal Infirmary, Edinburgh and the Hospital for Sick Children, Birmingham.

SOURCE OF BLOOD SPECIMENS

Blood was obtained at delivery from infants and also from the mothers when possible, at two maternity hospitals. At one of these this was done for all cases delivered during the day but not at night. At the other, the only limitation was the availability of staff and time. There was necessarily, but unavoidably, a certain amount of selection of the cases in that the pressure on hospital beds restricts admissions to primagravid women, particularly the older ones and also women with complicated pregnancies. No attempt was made to classify the cases according to ante-natal history until a large number of blood specimens had been analysed. Classification was then carried out by an obstetrician and a paediatrician using the patients' record.

METHODS OF COLLECTION OF SPECIMENS

Umbilical cord blood was obtained from new-born infants by draining the cord either while the placenta was still in situ, or just after delivery. In one case, estimations were made on blood taken at both times. The differences were well within the experimental error.

Maternal blood was taken at the end of labour by vene-puncture. Blood was collected from infants during the first week of life either by vene-puncture or by posterior fontanelle punctures, and from older infants by vene-puncture only.

SOURCE AND COLLECTION OF URINE SPECIMENS

Urine was collected from new-born female infants in a metabolic bed and from new-born male infants by the use of Paul's tubing. Although both methods were expected to be efficient, the completeness of 24 hour specimens was sometimes suspect and specimens of very low volume which gave very low steroid concentrations were discarded. The male infants were more easily dealt with and form the bulk of the normal group.

Urine obtained for amino acid chromatography was also collected as 24 hour specimens as far as possible but this was not always the case for the older infants.

ESTIMATION OF SERUM LIPIDS

PREPARATION OF SERUM EXTRACT

The method is based on that of Bloor (1929).

Reagents :

Bloor's Reagent : absolute alcohol -diethyl ether A.R. Reagent (3 : 1 v/v).

Procedure

One ml. of serum was added drop by drop to 9.0 ml. Bloor's reagent in a stoppered tube graduated at 10 ml. The mixture was incubated in a water bath at 50 - 60°C for thirty minutes. After cooling to room temperature, the volume was made up to 10 ml. with more Bloor's reagent and the contents of the tube mixed by inversion.

This extract is stable in the refrigerator for several days. The protein precipitate was removed when estimations of the lipid content were made on the extract by using a pipette with a plug of cotton wool, which had been washed with Bloor's reagent, on the end. This avoids the losses due to evaporation which might result from filtration or centrifugation.

ESTIMATION OF TOTAL CHOLESTEROL IN SERUM

Total cholesterol was measured by the method of Sacket (1925), using the traditional Lieberman reaction

whereby a green colour is produced with glacial acetic acid and concentrated sulphuric acid. As far as is known this is not affected by the presence of bile pigments in the serum. Any yellow colour in the chloroform extract is removed when the sulphuric acid is added.*

Reagents

1. Chloroform A.R. Reagent.
2. Acetyl chloride A.R. Reagent.
3. Sulphuric Acid. Concentrated A.R. Reagent.
4. Standard cholesterol solution.

Cholesterol (0.1 gm. per cent) was dissolved in chloroform. This was kept in a dark, glass stoppered bottle in the regrigerator and was diluted to 0.008 gm. per cent with chloroform before use.

Procedure

Four ml. of Bloor's extract of serum were evaporated to dryness in a small beaker on a hot plate. Care was taken to avoid losses by spurting and by over-heating. The residue was extracted four times with 4.0 ml. hot chloroform and the extracts collected in a tube graduated at 5.0 ml. and at 7.0 ml. After cooling the volume was made

*Since the completion of this thesis evidence has been found that very high serum bilirubin concentrations also produce a blue green colour in the Lieberman reaction. This gives a falsely high result. (Wolff 1963).

up to 5.0 ml. with chloroform. Acetyl chloride was added to the 7.0 ml. mark followed by 0.1 ml. of concentrated sulphuric acid. The tubes were mixed by inversion, stoppered, and left in the dark for 10 minutes. The extinction of the colour developed was compared with that of pure chloroform in a spectrophotometer set at 625 mμ

This reading was compared with a standard on each occasion. The standard contained 5.0 ml. of cholesterol (0.008 gm. per cent) instead of the chloroform extract, and the concentration of cholesterol in the original serum was calculated.

The Standard Deviation of the Method

This was calculated statistically on the results of ten replicate estimations of the cholesterol content of each of two sera. One of these was from an adult and one from cord blood to give the different concentration ranges.

Adult serum: $\bar{x}$ = 178 ± 2.1 mg./100 ml. Coefficient of variation = 1.2%

Cord serum : $\bar{x}$ = 64 ± 2.7 mg./100 ml. Coefficient of variation = 4.2%

The coefficients of variation are sufficiently small to indicate that nay clinically significant changes would be detected by the method. In practice, duplicate estimations were made unless the amount of blood available was severely limited.

ESTIMATIONS OF LIPID PHOSPHORUS IN SERUM

Lipid phosphorus was measured by the method of Stewart and Hendry (1935). The phosphate is converted to ammonium phosphomolybdate which is reduced to a blue colour with aminonaphthol sulphonic acid.

Reagents

1. Sulphuric acid. 10 Normal solution of concentrated A.R. Reagent, in water.
2. Hydrogen peroxide. 100 volume, A.R. Reagent.
3. Ammonium molybdate solution 2.5 per cent w/v.
4. Reducing agent (Fiske and Subarrow).

Sodium metabisulphite (5.36 gms.) was dissolved in 39 ml. water and the solution filtered if it appeared doudy. 0.1 gm. 1 : 2 : 4 : aminonaphthol sulphonic acid was added and about 1.0 ml. of a 40 per cent solution of crystalline sodium sulphite, drop by drop, until all the sulphonic acid dissolved. The mixture was filtered and used for a period not exceeding three weeks.

5. Stock phosphate solution.

Potassium dihydrogen phosphate (4.934 gms. per cent) was dissolved in water and a few drops of chloroform added as a preservative. This was diluted 1 : 100 with water to give a solution containing 0.10 mg. phosphorus per millilitre.

Procedure

Four ml. of Bloor's extract of serum were delivered into a combustion glass tube 6" x 5/8" which was graduated at 10 ml. A glass bead was added and the extract was evaporated to dryness in a boiling water bath. After adding 1.0 ml. 10 n sulphuric acid, the mixture was heated until charring began. After cooling, one drop of hydrogen peroxide was added and the mixture was heated and cooled again. This was repeated until a clear and colourless solution resulted. Care was taken to avoid loss of acid by visible fuming and to remove all the hydrogen peroxide. The latter can be judged by the way in which the mixture bubbles on heating.

The walls of the tube were washed down with a little distilled water and the volume was made up to approximately 9.0 ml. with more water. A standard was prepared by taking 2.0 ml. dilute phosphate standard, adding 0.75 ml. sulphuric acid and a glass bead and diluting to 9.0 ml. To each tube were then added 0.5 ml. ammonium molybdate solution and 4.0 ml. reducing agent. The contents of the tubes were mixed by swirling them and they were heated in a boiling water bath for ten minutes. After cooling to room temperature, the volumes of the blue solutions were made up to 10 ml. with water and the extinction of the colour compared with that of water in the spectrophotometer set at

680 mμ. The concentration of lipid phosphurus in the original serum was calculated. This value was converted to mg. phospholipid/100 ml. of serum by multiplying by a factor of 26, which is equivalent to assuming a phospholipid molecular weight of approximately 806.

The Standard Deviation of the Method

This was calculated in exactly the same way as for the estimation of cholesterol concentration.

Adult serum : $\bar{x} = 215 \pm 5.4$ mg./100 ml. Coefficient of variation = 2.5%

Cord serum : $\bar{x} = 169 \pm 2.5$ mg./100 ml. Coefficient of variation = 1.5%

The coefficients of variation are sufficiently small to indicate that any clinically significant changes would be detected by the method.

ESTIMATION OF TOTAL ESTERIFIED FATTY ACIDS IN SERUM

The total esterified fatty acids in serum were estimated by the method of Stern and Shapiro (1953). Hydroxylamine reacts with the esters of fatty acids in an alkaline solution to form hydroxamic acids. These react with ferric chloride solution to give colour varying from brown to purple depending on the concentration.

Reagents

1. Hydroxylamine hydrochloride : 2 Molar solution in water. This is kept in the refrigerator.

2. Sodium hydroxide : 3.5 Normal solution in water.
3. Hydrochloric acid : one volume of concentrated. A.R. reagent acid (sp.gr.1.18) diluted in two volumes of water.
4. Ferric chloride solution : 0.37 Molar solution in 0.1. Normal hydrochloric acid.
5. Standard triacetin solution : 72.7 mg. triacetin in 25 ml. Bloor's reagent.
6. Diethyl Ether : A.R. reagent.

Procedure

Three ml. of Bloor's extract of serum were delivered into a 16 mm. tube. A blank was prepared with 3.0 ml. Bloor's reagent. To each tube was added 0.5 ml. hydroxylamine hydrochloride solution and 0.5 ml. sodium hydroxine solution. The contents were mixed by swirling, the tubes were stoppered, and left to stand for 20 minutes at room temperature.

After this interval, 0.6 ml. of hydrochloric acid was added. After mixing well, 0.5 ml. of ferric chloride solution was added. Because the extracts of maternal sera usually show a turbidity at this point, 1.0 ml. of ether was added routinely. The contents of the tubes were mixed by inversion and the extinction measured against that of water in a spectrophotometer at 525 mμ.

FIGURE 41

ESTIMATION OF TOTAL ESTERIFIED FATTY ACIDS

CALIBRATION CURVE

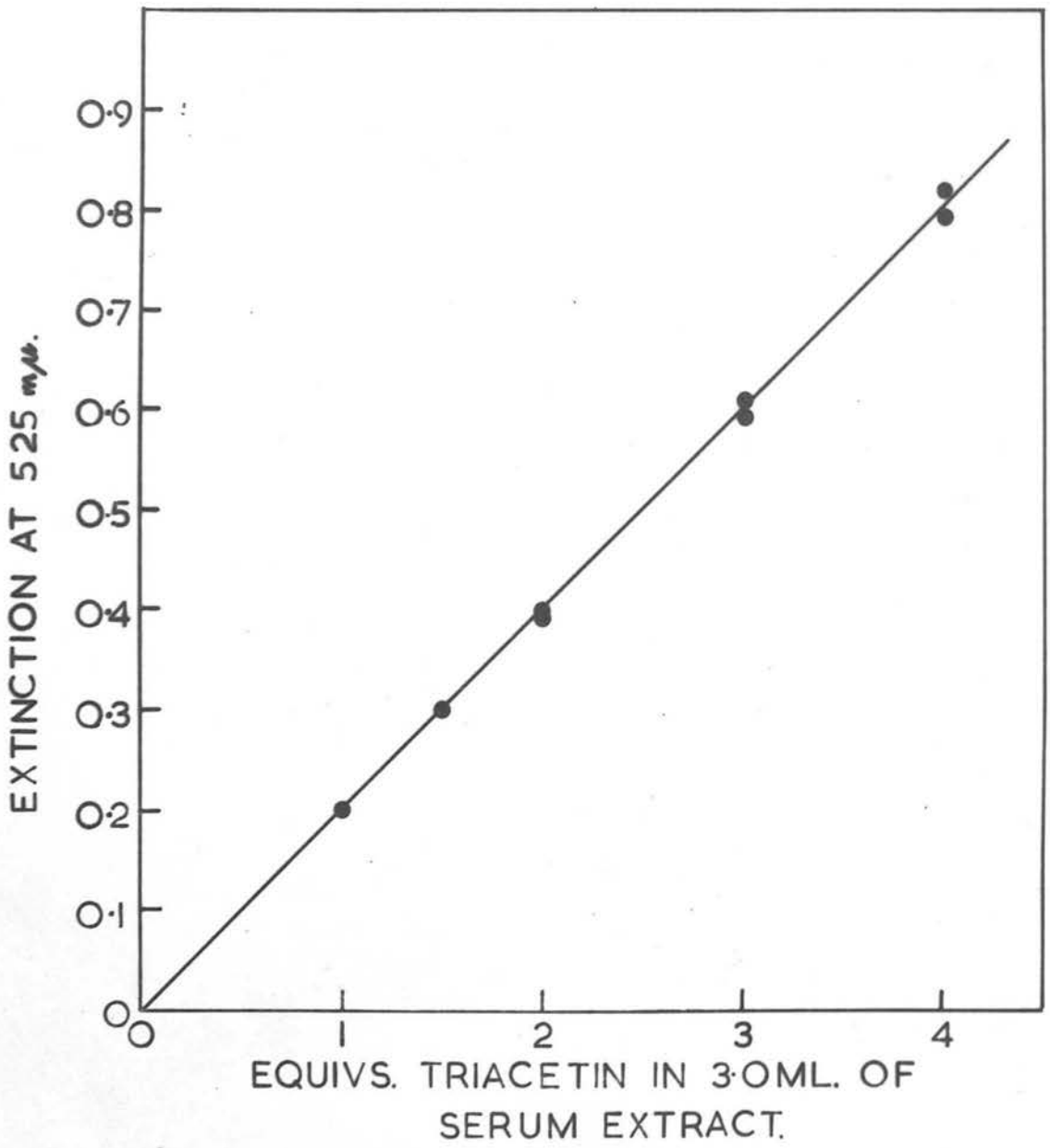


TABLE 39

Calibration of the Method of Estimation of Total Esterified Fatty Acids in Serum

equivs.Triacetin	Extinction at 525 mμ		Mg. Triacetin/100 ml. Serum	
1.0	0.200	0.200	185	185
1.5	0.300	0.300	277	277
2.0	0.395	0.400	364	369
3.0	0.595	0.605	549	558
4.0	0.795	0.810	734	748

Calibration of the Method

A calibration curve was constructed as follows: a standard solution of triacetin containing 2 μ equivs./ml. was prepared by diluting the concentrated standard solution twenty times with Bloor's reagent. Volumes varying from 0.5 ml. to 2.0 ml. were diluted to 3.0 ml. with Bloor's reagent. Each dilution was subjected to the estimation procedure. The details of the calibration are given in Table 39 and the curve obtained is shown in Fig. 41 .

The Standard Deviation of the Method

This was calculated in exactly the same way as before but four different sera were used to give a complete range of concentrations.

Serum A $\bar{x}$ = 485 $\pm$ 6.2 mg./100 ml. Coefficient of variation = 1.28%

Serum B $\bar{x}$ = 341 $\pm$ 5.7 mg./100 ml. Coefficient of variation = 1.67%

Serum C $\bar{x}$ = 256 $\pm$ 2.6 mg./100 ml. Coefficient of variation = 1.02%

Serum D $\bar{x}$ = 176 $\pm$ 2.6 mg./100 ml. Coefficient of variation = 1.48 %

The coefficients of variation are sufficiently small throughout the range of concentrations to indicate that any clinically significant changes would be detected by the method.

ESTIMATION OF TOTAL LIPID IN SERUM

The total lipid content of serum was measured by the

method of Huerga, Yesinick and Popper, (1953). It is a turbidimetric method which is calibrated gravimetrically.

Reagents

1. p-Dioxan : B.D.H. Ltd. - Di-ethylene Dioxide.
2. Sulphuric acid : 4 per cent v/v solution of concentrated A.R. reagent in water.
3. Methanol. A.R. Reagent.
4. Chloroform. A.R. Reagent.

Procedure

One ml. of Bloor's extract of serum was evaporated to dryness in a 5" x 3/8" tube in a boiling water bath. P-Dioxan (1.5 ml.) was added and the tube replaced in the water bath for one minute. After cooling to room temperature, 5.0 ml. of 4 per cent sulphuric acid were added. The tube was left to stand for thirty minutes. After careful inversion to remove the bubbles, the turbidity was measured by finding its extinction on the spectrophotometer at 650 mμ compared with that of water.

Standardisation

This is a modification of the method of Sperry and Brand (1954) in which the lipids are measured gravimetrically. The modifications have been developed at the Hospital for Sick Children, Birmingham.

Extraction

Methanol (8.3 ml.) was delivered into a 25 ml. volumetric flask. One ml. of serum was added, holding the tip of the pipette just above the surface of the methanol and the contents of the flask were swirled throughout the addition. Approximately 8 ml. chloroform were added and the solvent brought just to boiling point in a water bath. This takes practice if superheating and subsequent boiling over is to be avoided. The flask was cooled to room temperature and the volume made up to 25 ml. with chloroform. The contents of the flask were mixed by inversion.

Purification of the Lipid Extract to Remove Nitrogenous Substances

Twenty ml. of the serum extract obtained by the method described above were delivered into a flat bottomed glass tube 2 cm. x 8 cm. A plug of glass wool was put into the tip of the pipette before use to filter the extract. Water was added to the tube very gently until it was completely full and it was lowered very carefully into a litre beaker almost full of water. It was allowed to stand in the water for 18 hours.

The water was then siphoned off until the level was below that of the water in the tube. The tube was then removed without agitating the layer at the interface of the

ESTIMATION OF TOTAL LIPID.-CALIBRATION CURVE.

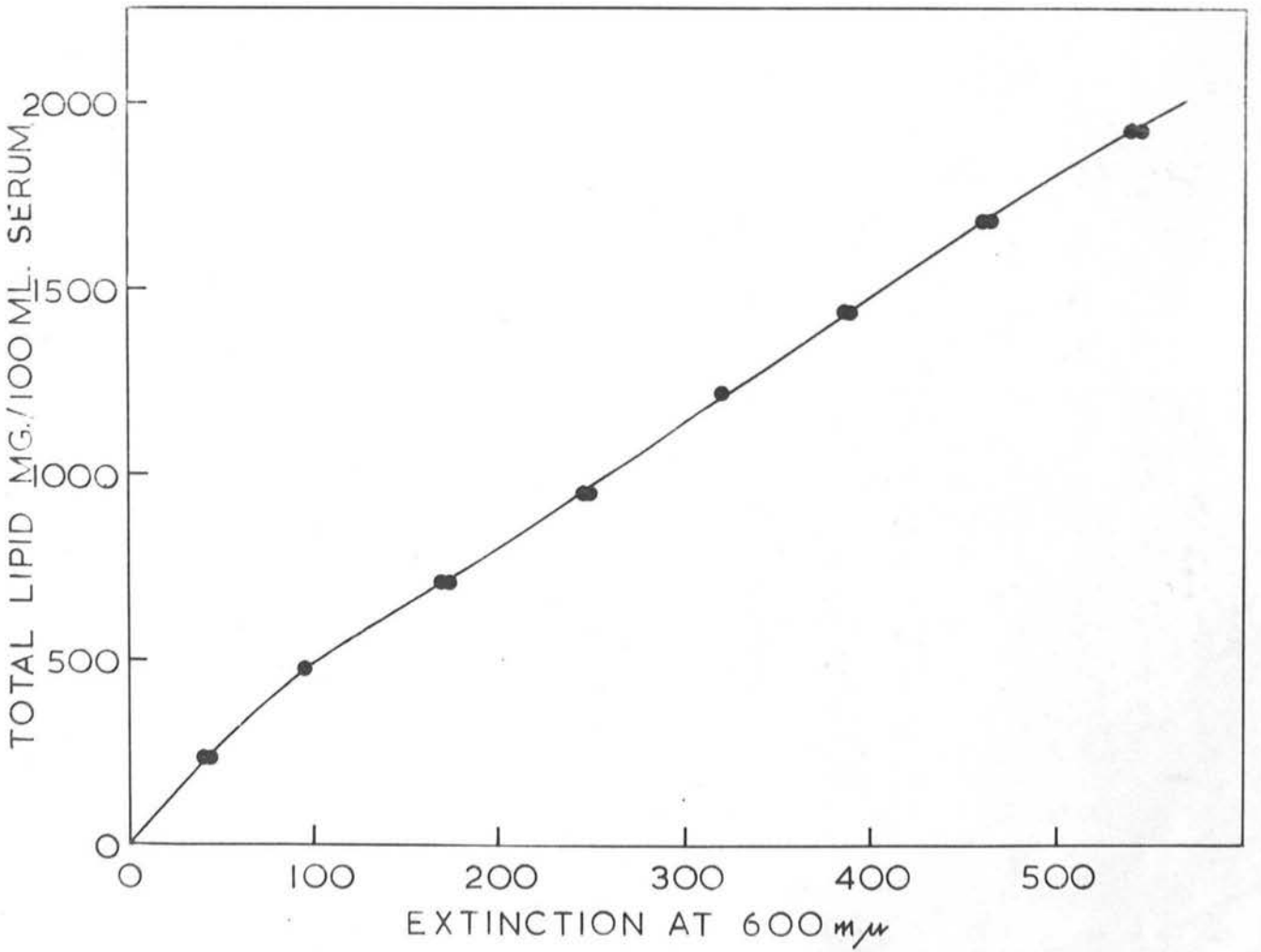


FIGURE 42

TABLE 40

Calibration of the Method used for the Estimation of Total Serum Lipids

Two 1.0 ml. samples (A and B) of the same serum each extracted in 25 ml. solvent. 20 ml. of each extract was evaporated and extracted with 5 successive 5 ml. volumes methanol-chloroform. Each sample corresponds to 0.8 ml. of serum.

Weight of lipid extracted in each of five extractions

	Sample A	Sample B	
	1) 0.0056 gms.	1) 0.0060 gms.	
	2) 0,0012 gms.	2) 0.0018 gms.	
	3) 0.0007 gms.	3) 0.0000 gms.	
	4) 0.0001 gms.	4) 0.0000 gms.	
	5) 0.0000 gms.		
Total	0.0076 gms.	0.0078 gms.	Mean = 0.0077 gms.

= 962.5 mg. lipid/100 ml. serum.

0.5 ml. serum containing 962.5 mg. lipid/100 ml. extracted in Bloor's reagent and the lipids estimated by the method of Huerga et.al.(1953).

Vol.Extract Used in ml.	Extinction Values at 650 m		Mg.lipid/100 ml. Serum.(Mean Value)
0.25	0.040	0.045	240
0.50	0.095	0.095	481
0.75	0.170	0.175	721
1.00	0.244	0.250	962
1.25	0.820	0.320	1225
1.50	0.385	0.390	1443
1.75	0.460	0.465	1683
2.00	0.540	0.545	1924

water and the methanol-chloroform. As much as possible of the upper aqueous layer was then removed by careful pipetting.

Evaporation to Dryness

Six ml. methanol were added to the tube and the contents stirred with a glass rod which was then washed with a little more methanol. If the mixture was not clear, a little more methanol was added. The liquid was then transferred to a 50 ml. flask using more methanol for washing, and evaporated to dryness under reduced pressure at room temperature.

Estimation

The dry lipid was extracted 5 times with 5 ml. methanol - chloroform mixture (1 : 1 v/v), and each extract was transferred to a separate 10 ml. beaker which had been weighed earlier. The extracts were evaporated to dryness under reduced pressure at room temperature and the beakers were reweighed. The estimation was duplicated and a mean weight of lipid in 100 ml. of serum was obtained. The data obtained are given in Table 40.

Application of Gravimetric Standardisation to the Turbidimetric Estimation of Serum Lipids

An extract of 1.0 ml. of the original serum was made in 9,0 ml. Bloor's reagent. Duplicate aliquots of the serum extract ranging from 0.25 ml. to 2.0 ml. in steps of 0.25 ml. were subjected to the turbidimetric procedure for

estimating total lipid content. (see page 198). By calculation on a proportional basis, a graph of the extinction against mg. lipid/100 ml. of serum was obtained. This is shown in Fig. 42 . This was drawn on a large scale and a table of values derived from it.

The Standard Deviation of the Method

This was calculated in the same way as before using three different sera of different lipid content.

Serum A $\bar{x}$ = 681 ± 10.9. Coefficient of variation = 1.6%

Serum B $\bar{x}$ = 389 ± 8.3. Coefficient of variation = 2.2%

Serum C $\bar{x}$ = 202 ± 9.6. Coefficient of variation = 4.8%

The deviation is of the same order as that found in the other methods which have been used in this investigation.

THE ELECTROPHORETIC SEPARATION OF THE SERUM LIPOPROTEINS

The technique used was largely that described by Salt and Wolff (1957).

Procedure

A closed horizontal E.E.L. electrophoresis tank was used with a piece of polythene tubing inserted to maintain equal levels of buffer in the outer compartments. Barbital buffer, pH 8.6 and ionic strength 0.05, was made up freshly every second day by dissolving 20.6gms. sodium barbitone and 3.68 gms. di-ethyl barbiturie acid in 2 litres of water. On the intervening days the buffer used once was mixed with

that still unused and the bath refilled with the mixture.

Whatman No.3.MM paper strips 13.5" x 2" were used to obtain a distance of 12" between the two resevoirs of buffer. The serum was placed on a line drawn 4" from the cathode end. Six strips were run simultaneously even if some of them were blank. A constant current of 12 milliamps and a running time of 16 hours were used.

Before application of the serum, the paper was dipped in buffer and blotted firmly on filter paper. 0.15 ml. of serum was applied to the origin line in a regular manner and drops of buffer applied to the strip in such a way that the paper was saturated without the serum being disturbed. The strips were allowed to equilibrate in the closed apparatus for 30 minutes before the current was switched on.

After the overnight run (during which the albumin fraction migrates approximately 6"), the strips were removed and dried horizontally at 100° C for 30 minutes. They were then weighted with small glass rods at the anode end and suspended in a saturated solution of Oil Red O (George T. Gurr) in a 60 per cent solution of industrial methylated spirit in water. Staining was allowed to continue overnight after which the strips were washed in 60 per cent alcohol for half a minute and in water for thirty minutes. Both washes were performed by suspending the strips in the solvent.

The dye was made up at approximately one month intervals depending on the extent to which it was used.

Attempts at reducing the background colour according to the method of Strauss and Wurm (1958) were made but were discontinued. This consists of dipping the wet stained strip into a 0.1 per cent solution of aqueous 5 per cent sodium hypochlorite (Domestos) in 2 per cent acetic acid. Greater dilutions were tried and were ineffective. The dilution recommended bleached so quickly that the part of the strip entering the bleach first was more affected than the rest of the strip. Since satisfactory lipoprotein curves were obtained without this, the method was not pursued further.

After drying in air at room temperature the strips were cut into 26 portions each 3/8" wide starting 2" from the origin line in the direction of the cathode. Each segment was cut in half and placed in a 3/8" test tube. To this was added 5.0 ml. of a mixture of propanol and glacial acetic acid (4:1 v/v). After standing overnight, the contents of the tubes were mixed by inversion and the extinction of the eluted dye compared with that of the pure solvent in a spectrophotometer at 525 m μ. It is essential to use rubber gloves for this operation. The extinctions were plotted to give a lipoprotein curve.

The curve obtained in this way was then subdivided into three sections: α -, β - and γ -lipoprotein. The α -lipoprotein fraction migrates approximately to the same position as serum albumin : β -lipoprotein migrates to the β -globulin

position and the γ -lipoprotein fraction extends from the origin to the β -lipoprotein fraction.

When the demarcation was not obvious, the component curves were extrapolated and the contributory extinction values allocated to effect a subdivision. In the case of cord blood the distinction between γ - and β -lipoprotein was usually impossible to make visually. The division was therefore made by a combination of comparing it with the equivalent demarcation in an adult serum run simultaneously and, since adult and cord blood lipoproteins do not necessarily migrate at the same rates, by taking a relatively constant distance from the sub-division between the α - and the β -lipoprotein fractions which were always more clearly defined.

The Quantitative Evaluation of the Lipoprotein Lipid Concentrations.

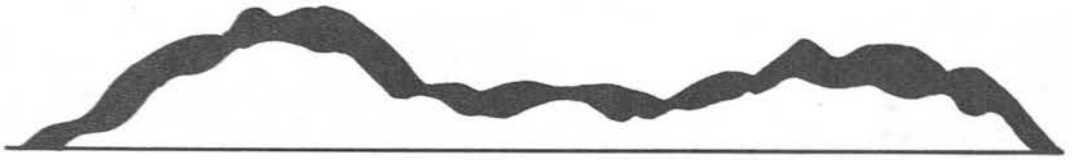
The extinctions of the extracts from all the strips carrying any one fraction of the lipoprotein were summed and expressed as a percentage of the overall sum of the extinctions. This was taken as equal to the percentage of the total lipoprotein falling into that fraction. This was then converted to mg. lipoprotein lipid by using the total serum lipid concentration which was estimated independently.

According to Langan, Durrum and Jenks (1955) no allowance need be made for differences in dye uptake by the different lipoproteins because this only becomes important at very high concentrations when the uptake by β -lipoprotein

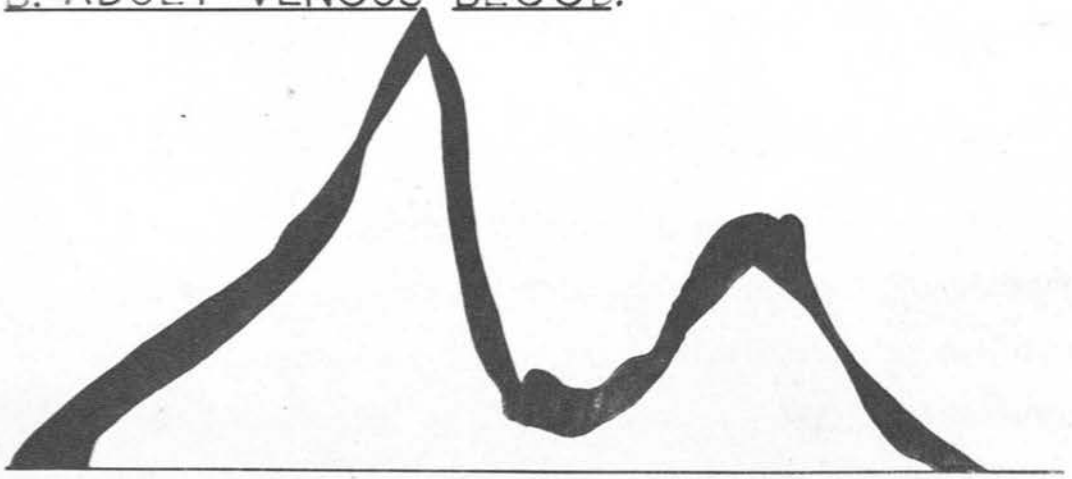
FIGURE 43

DIAGRAM SHOWING THE AREA OF OVERLAP IN FIVE REPLICATE LIPOPROTEIN CURVES

A. UMBILICAL CORD BLOOD.



B. ADULT VENOUS BLOOD.



is no longer strictly linear. There is thus a tendency to get systematically low β-lipoprotein values but the variation is no greater than the range of variation of individual values. Lanyan et.al. (1955) also found that the alcohol wash removed slightly more stain from the α-lipoprotein than from the β-lipoprotein so that at high concentrations the difference will be approximately cancelled out.

The Standard Deviation of the Method

Five replicate lipoprotein curves were obtained for six different sera and the error of the method was calculated from the results. The data for one sample of maternal blood and one sample of cord blood are given in Table 41 below. The variation is illustrated in Fig.43.

TABLE 41

One Maternal Blood Sample

α-lipoprotein - per cent of total. $\bar{x} = 36.5 \pm 3.5$ per cent.

β-lipoprotein - per cent of total. $\bar{x} = 46.8 \pm 2.1$ per cent.

γ-lipoprotein - per cent of total. $\bar{x} = 16.6 \pm 3.4$ per cent.

One Cord Blood Sample

α-lipoprotein - per cent of total. $\bar{x} = 53.1 \pm 1.4$ per cent.

β-lipoprotein - per cent of total. $\bar{x} = 37.8 \pm 1.7$ per cent.

γ-lipoprotein - per cent of total. $\bar{x} = 9.1 \pm 1.8$ per cent.

FRACTIONATION OF THE SERUM LIPOPROTEINS BY PRECIPITATION OF THE β-LIPOPROTEIN FRACTION

In a further attempt to differentiate between β - and γ-lipoproteins in cord blood, precipitation of the

β -lipoprotein fraction was performed on a few blood samples when sufficient serum had been obtained. The method of Burstein and Samaille (1958) was used in which dextran sulphate is the precipitant.

Reagents

1. Dextran sulphate : 5 per cent solution (May and Baker).
2. Calcium chloride : 2 Molar solution in water.

Procedure

To 1.0 ml. of serum were added 0.05 ml. of dextran sulphate solution and 0.2 ml. of calcium chloride solution. The mixture was centrifuged at 3000g for ten minutes, taking ten minutes each to speed up the centrifuge and to slow it down. The clear supernatant fluid was carefully removed with a Pasteur pipette into a clean tube. The β -lipoprotein precipitate was discarded.

Electrophoresis of the lipoproteins in the supernatant fluid and the estimation of the individual lipids was then performed and the original serum was also subjected to the same procedure. 0.18 ml. of the supernatant was used for electrophoresis instead of the normal 0.15 ml. to allow for dilution. To perform all these estimations 3 - 4 ml. of the original serum was required. This was not often available in cord blood because the packed cell volume of the blood is very high.

THE ESTIMATION OF 17-KETOSTEROIDS AND 17-KETOGENIC STEROIDS IN URINE

The method is based on that of Moxham and Nabarro(1952).

Collection of the Urine

The urine was collected in 24 hour samples, free from preservation, and it was analysed within two days of collection. During this period it was kept in the refrigerator. When the 24 hour volume was under 50 ml. it was diluted to 50 ml. When the volume was between 50 ml. and 100 ml. it was diluted to 100 ml.

Reagents

1. Acetic Acid: glacial A.R. reagent.
2. Sodium Bismuthate: A.R. reagent.
3. Benzene: A.R. reagent.
4. Hydrochloric Acid: concentrated A.R. reagent.(sp.gr.1.18)
5. Hydrochloric Acid: 0.5 Normal solution of concentrated A.R. reagent (sp.gr.1.18) in water.
6. Sodium hydroxide: Normal solution in water.
7. Sodium meta-bisulphite: 5 per cent solution in water.
8. Benzyl trimethyl ammonium hydroxide: aqueous solution (B.D.H.).
9. Absolute Alcohol: This was purified in the following manner.

Silver nitrate (4gm./l.) was dissolved in the alcohol. To convert the silver nitrate to silver oxide, a 40 per cent

aqueous solution of sodium hydroxide was added until no more precipitate appeared. This was allowed to stand overnight. After ensuring that all the silver oxide had been precipitated, the alcohol was filtered and distilled in an all glass still. The first and last 200 ml. of distillate were rejected. Distillation was repeated until the alcohol gave a low blank value in the Zimmerman procedure.

10. m-Dinitrobenzene: this was purified by sublimation and 0.100 gm. dissolved in 5.0 ml. of purified alcohol just before it was required for use.

Procedure

1. 17-Ketosteroids

Urine (30 ml.) and glacial acetic acid (10 ml.) were mixed in a flat bottomed 250 ml. flask. To this were added 7.5 ml. of concentrated hydrochloric acid, 25 ml. of benzene and two glass beads. The mixture was boiled under reflux for thirty minutes and then cooled to room temperature. The lower aqueous layer was then removed and extracted with a further 25 ml. of benzene. The two benzene extracts were combined in a 100 ml. separating funnel which was provided with a ground glass stopper.

The benzene extract was washed with two 15 ml. volumes of Normal sodium hydroxide solution, and one 15 ml. volume of 0.5 Normal hydrochloric acid and three 15 ml. volumes of water. The extract was then transferred to a dry 250 ml. flat

bottomed flask and the funnel washed out with more benzene. The benzene was distilled off under reduced pressure.

The dry extract was dissolved in 1.0 ml. or, in the case of urine collected from new-born infants, in 2.0 ml. of purified alcohol and the steroid content was measured by a modified Zimmerman procedure (Zimmerman, 1935; Medical Research Council, 1951; Salt, 1959).

The Zimmerman Procedure

To 0.2 ml. of the alcoholic extract of the steroids in a test tube were added 0.2 ml. of 2 per cent m-dinitrobenzene solution and 0.2 ml. of benzyl trimethyl ammonium hydroxide. The latter replaces alcoholic potash in the original method because it is difficult to maintain constant concentrations of potassium hydroxide throughout a long period.

A standard and a blank were set up simultaneously. The standard contained 0.2 ml of a solution of dehydro-iso-androsterone in purified alcohol containing 10 mg./100 ml. instead of the alcoholic steroid extract. The blank contained 0.2 ml. of purified alcohol instead of the alcoholic steroid extract.

The tubes were stoppered with waxed corks and incubated in a water bath at 25°C for one hour in the dark. After 5.0 ml. (or in the case of some neonatal urine, 10 ml.) of purified alcohol had been added, the contents of the tubes were mixed by inversion and their extinctions were measured

at 440 mμ., 520 mμ., and 600 mμ. Alcohol was used as a blank. The instances in which the larger volume of alcohol was added were those in which high concentrations of ketosteroid were expected. Care was taken to avoid any exposure to bright sunlight since this causes rapid fading of the purple colour.

The 17-ketosteroid content of the urine was calculated in mg./24 hours after correction for non-steroid chromogens which give a positive reaction in the Zimmerman procedure by the use of Allen's equation (Allen, 1950).

2. 17-Ketogenic Steroids Plus 17-Ketosteroids

Urine (20 ml.) and glacial acetic acid (20ml.) were delivered into a round bottomed 250 ml. flask with a ground glass stopper and a 4.5 gms. of sodium bismuthate were added. The stopper was firmly fixed with Cellotape and the flask shaken for thirty minutes in a Kahn shaker in the dark. This was repeated if the supernatant liquid was not completely clear.

The supernatant liquid was removed and 25 ml. transferred to a flat bottomed 250 ml. flask. Fifteen drops of a 5 per cent aqueous solution of sodium meta-bisulphite were added and the flask was swirled. This was followed by 25ml. of water, 7.5ml. of concentrated hydrochloric acid, 25ml. of benzene and two glass beads. The contents of the flask were boiled under reflux for thirty minutes. After cooling,

the steroids were extracted and were estimated in exactly the same way as that described for 17-ketosteroids alone.

In this estimation the ketogenic steroids are oxidised by the bismuthate to ketosteroids. The concentrations of the ketogenic steroids alone may thus be obtained by subtracting the concentration of the ketosteroids from that of the total steroid concentration.

Recovery Experiments

The oxidation of 17-ketogenic steroids to 17-ketosteroids with sodium bismuthate results in considerable loss and there is also some loss during the hydrolysis with concentrated hydrochloric acid. Recovery experiments were performed at approximately six months intervals when new stocks of sodium bismuthate were obtained.

An alcoholic solution was prepared containing 100 mg. each of dehydro-iso-androsterone and corticosterone in 100 ml. The 17-ketosteroid and 17-ketogenic steroid concentration of a very dilute sample of urine was measured.

The following mixtures were then prepared:

1. 10 ml. steroid solution + 90 ml. urine.
2. 5 ml. steroid solution + 95 ml. urine.
3. 2.5 ml. steroid solution + 97.5 ml. urine.

Each solution was subjected to the procedure for the estimation of their 17-ketosteroid and 17-ketogenic steroid concentration and the recovery values were calculated. The results of one

such experiment are given in Table 42.

T A B L E 42.

Results. Steroid Recovery Experiment, September 1960.

	Ketosteroids in Mg./100 ml.			Ketogenic Steroids in Mg./100 ml.		
	observed	calculated	recovery	observed	calculated	recovery
Original urine	0.33	-	-	1.46	-	-
Mixture 1	7.56	10.30	73%	12.02	21.46	56%
Mixture 2	3.85	5.32	72%	5.16	11.28	46%
Mixture 3	2.33	2.83	82%	3.12	6.42	49%

There was an average recovery of 75 per cent of the ketosteroids and 50 per cent of the 17-ketogenic steroids. This was allowed for in calculating the amount of steroid excreted in a 24 hour period.

THE IDENTIFICATION OF THE AMINO ACIDS IN URINE AND IN SERUM BY PAPER CHROMATOGRAPHY

Standard techniques, based on those of Dent (1948) were used throughout.

Serum was deproteinised by ultrafiltration through visking cellophane by the method described by Smith (1958). Urine was made neutral with dilute hydrochloric acid. Both urine and serum were then desalted on a cation exchange resin.

Desalting

Approximately 10 ml. of Amberlite IR-120 (H+) resin was washed by heating in 4 N.hydrochloric acid and then in water

until the supernatant was perfectly colourless. The resin was packed into a burette, the bottom of which had been blocked with glass wool. The amount of serum ultrafiltrate which was put on the column was not constant because it depended on the amount of blood which was available. The volume used was recorded in each case. Five ml. of urine were used in all cases.

The samples of serum ultrafiltrate or urine were dripped through the column at a rate of approximately one drop per 20 seconds. This was followed by 50 ml. of water at a slightly faster rate to wash out the salts. The amino acids were then eluted with 0.880 ammonia and the first 2 ml. of ammonia eluate were collected. The columns were then washed with 250 ml. of water to remove the excess ammonia, 100 ml. of 4 N.hydrochloric acid to replace the hydrogen ions in the resin, and finally another 250 ml. of water to remove the excess acid. The columns were repacked at approximately two monthly intervals. On these occasions the resin was completely renewed and blank elutions were made on the resin.

Chromatography

The eluate from the desalting column was applied to sheets of Whatman No. 1 chromatography (18" x 18") at a point 3.5" from one corner. The amount of urine eluate which was used was 50 μl. and of serum, 500 μl.

Micropipettes were used to spot the samples onto the paper and, by the use of a hair dryer, the size of the spot was not allowed to exceed 6mm. in the case of urine and 10 - 15mm. for serum.

Shandon glass chromatography tanks were used throughout with the solvent descending the paper. The papers were allowed to equilibrate for at least four hours in the solvent tanks, the bottom of which were lined with cotton wool soaked in the solvent. Two dimensional chromatography was always performed. The first solvent was either butanol-acetic acid-water (40-10-50) or, more frequently, butanol-pyridine-water (40-40-40). The second solvent was always phenol-ammona-water. This was prepared by adding 125 ml. of water to 500 gms. of phenol. The ammonia (a few drops only) was added to the cotton wool in the bottom of the chromatography tank.

The solvent was allowed to descend the paper for at least 16 hours but was not allowed to run off the paper. The sheets were dried between the two solvent runs and the final drying was conducted at 120°C and was continued until all the phenol had been removed. The amino acids were stained with a 0.1 per cent solution of ninhydrin in acetone and the colours developed by heating at a standard distance from an electric fire.

Reference chromatograms were prepared from standard amino acid solutions.* If unknown ninhydrin-positive substances were present in the urine or serum, duplicate chromatograms were prepared with the addition of a standard amount of the amino acid of the same calculated Rf value as the unknown, if it was available.

The assessment of the chromatograms is an operation in which experience plays a very large part. Variations in atmospheric conditions produced alterations in the solvent characteristics although these were kept to a minimum as far as was possible. The daily variation in the amino acid excretion of the same person is considerable. However any cases of amino aciduria or amino acidaemia are immediately obvious and so is the appearance of any amino acids not normally apparent.

* See Fig. 44. a and b.

Figure 44. a.

Amino Acid Chromatogram Obtained Using Standard Solutions of Known Amino Acids.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.

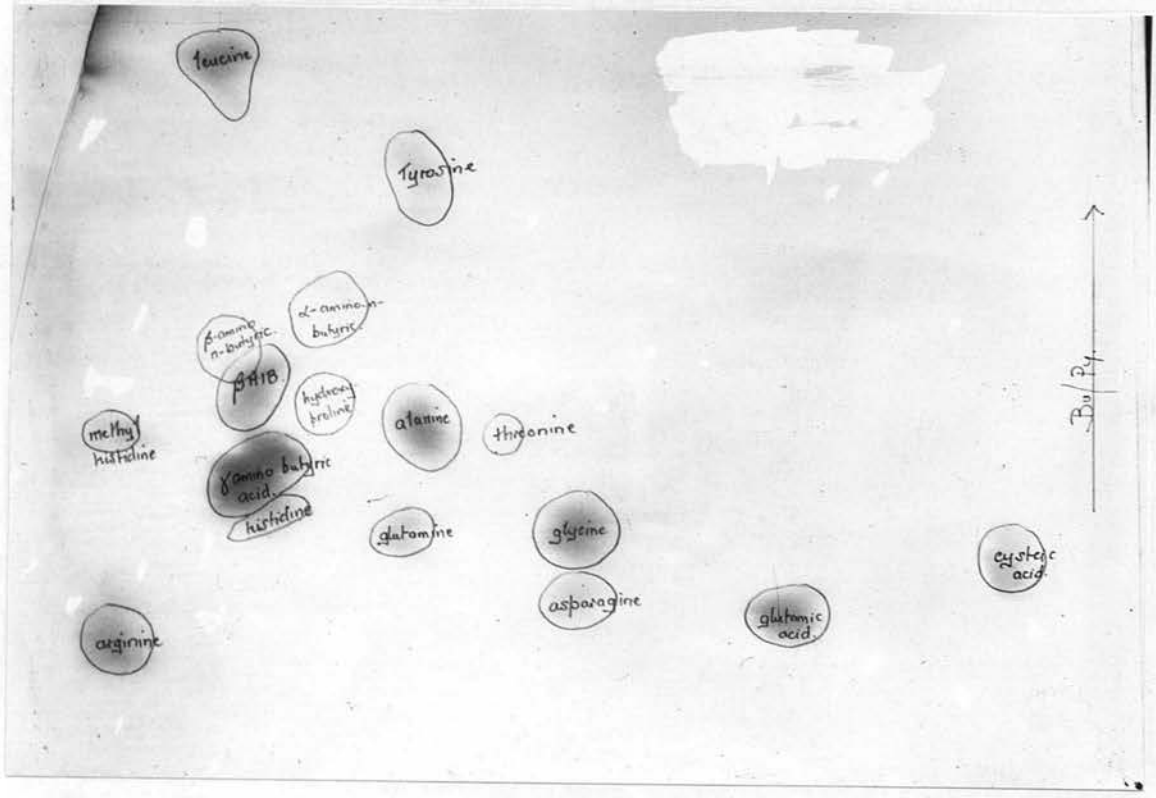
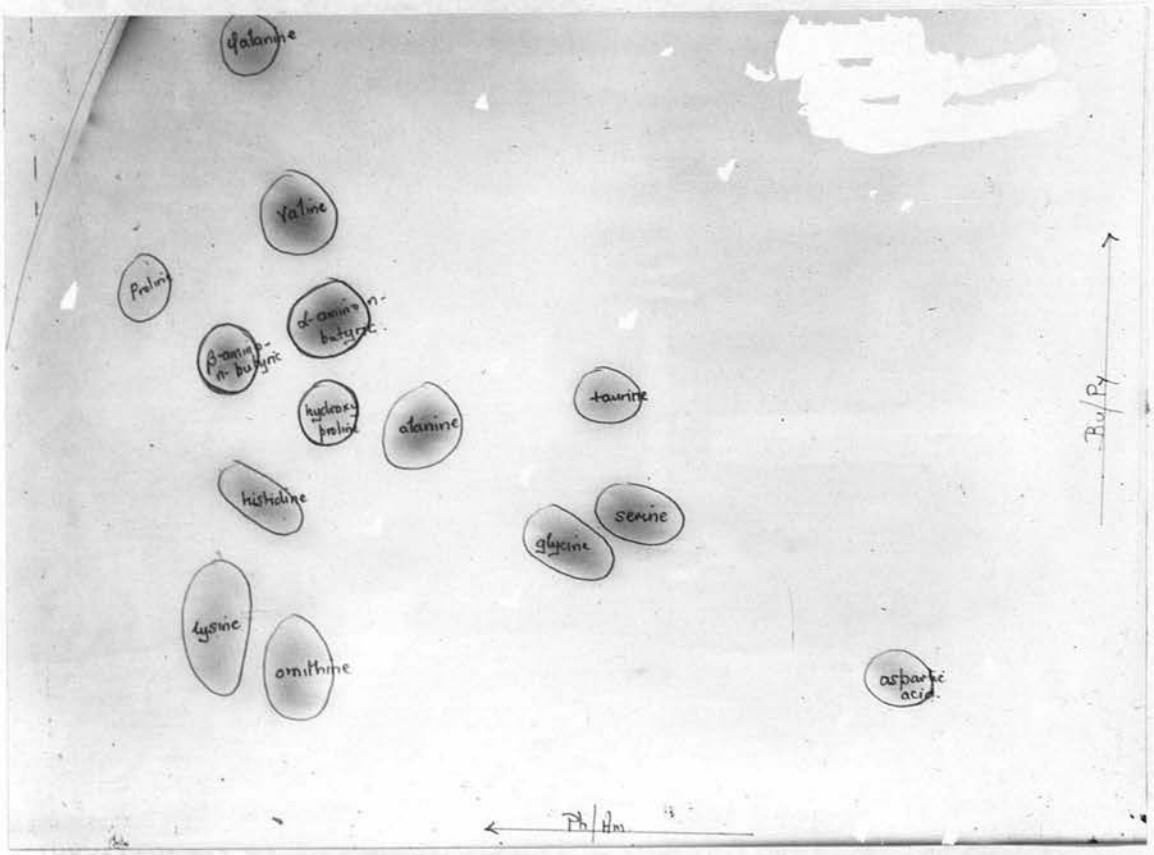


Figure b.

Amino Acid Chromatogram Obtained Using Standard Solutions of Known Amino Acids.

Solvent 1 - Butanol / Pyridine.

Solvent 2 - Phenol / Ammonia.



APPENDIX II

METHODS OF STATISTICAL ANALYSIS

1. CALCULATION OF MEAN VALUES, THE STANDARD DEVIATION AND THE STANDARD ERROR OF THE MEAN

For Example : The Mean Total Cholesterol Concentration in the Cord Blood of Normal Infants.

n = number of observations. x = individual observations.

S = sum of.

$n = 50$ $\quad \bar{x} = 71.82 = \frac{(Sx)}{n}$

$Sx = 3591$

$Sx^2 = 268151$

$\frac{(Sx)^2}{n} = 257905.62$

$S(x - \bar{x})^2 = 10251.38 = (Sx^2 - \frac{(Sx)^2}{n})$

$\frac{S(x - \bar{x})^2}{(n - 1)} = 209.2118$

$\sqrt{\frac{S(x - x)^2}{(n - 1)}} = 14.46$ = Standard Deviation. (S.D.)

$\sqrt{\frac{S(x - x)^2}{(n - 1)}} \div n =$ Standard Error of the Mean = 2.046.

Coefficient of Variation = $\frac{S.D.}{\bar{x}} \times 100 = 20.13$ per cent.

The mean total cholesterol concentrations in the serum of umbilical cord blood of normal infants is 71.82 + 2.05(50) mg./100 ml. The standard deviation of this value is 14.46 mg./100 ml. and the coefficient of variation is 20.13 per cent.

Where a logarithmic distribution is indicated the calculation is identical as far as (I) except that the logarithm to the base 10 is used throughout. The S.D. obtained is the logarithm of a factor which may be used to obtain ranges of values as follows:

Range equivalent to one S.D.=(mean/factor)to(mean x factor)

Range equivalent to 2xS.D.=(mean/factor^2)to(mean x factor^2)

Examples of a distribution curve which is normally logarithmically distributed is given in Fig.

2. CALCULATION OF THE SIGNIFICANCE OF THE DIFFERENCE BETWEEN TWO MEAN VALUES

For example : The Difference Between the Mean Cholesterol Concentrations in the Cord Blood of Normal Infants and the Infants of Diabetic Mothers.

n_1 = number of normal infants.

n_2 = number of infants of diabetic mother.

$\bar{x}_1$ = mean cholesterol concentration in the cord blood of normal infants.

$\bar{x}_2$ = mean cholesterol concentration in the cord blood of infants of diabetic mothers.

$$\text{Standard error of difference of means} = \sqrt{\frac{S(x_1 - \bar{x}_1)^2 + S(x_2 - \bar{x}_2)^2}{(n_1 - 1) + (n_2 - 1)} \left\{ \frac{1}{n_1} + \frac{1}{n_2} \right\}}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\text{Standard error of difference}} \quad \text{or} \quad \frac{\bar{x}_2 - \bar{x}_1}{\text{Standard error of difference}}$$

$n_1 = 50 \quad n_2 = 18 \quad \bar{x}_1 = 71.82$ mg. $\quad \bar{x}_2 = 83.83$ mg.

$$t = \frac{83.83 - 72.82}{4.64} = 2.588$$

The significance of the t value obtained may be found in standard statistical tables. In this case the t value of 2.588 corresponds to a P.value which is between 0.02 and 0.01 ($0.02 > p > 0.01$) and the difference between the two means is significant.

3. THE CALCULATION OF THE SIMPLE COEFFICIENT OF REGRESSION OF ONE VARIABLE UPON ANOTHER, TO FIT $Y = bx + A$

n' = number of pairs of values of variables x and y.

Sx; Sy; $(Sx)^2$; $(Sy)^2$; $Sx.Sy$; $\bar{x} = (Sx)/n'$; $\bar{y} = (Sy)/n'$;

(1)	(2)	(1-2)
Sx^2	$(Sx)^2/n'$	$S(x-\bar{x})^2$
Sy^2	$(Sy)^2/n'$	$S(y-\bar{y})^2$
Sxy	$(Sx.Sy)/n'$	$S(xy-\overline{xy})$

$$b = \frac{S(xy - \bar{x}\,\bar{y})}{S(x-\bar{x})^2} \qquad A = \frac{Sy - bSx}{n'} \qquad Y = bx + A$$

$$bS(xy - \bar{x}\,\bar{y}) = \frac{(S(xy - \bar{x}\,\bar{y}))^2}{S(x - \bar{x})^2}$$

$$S(y - Y)^2 = S(y - \bar{y})^2 - bS(xy - \bar{x}\,\bar{y})^2$$

$$S^2 = S(y - Y)^2/n' - 2$$

$$\text{Standard Error of } b = \frac{S^2}{S(x - \bar{x})^2}$$

$$t = \frac{b}{\text{S.E.of } b}$$

For Example : The Coefficient of Regression of β-Lipoprotein Lipid Concentrations on Cholesterol Concentrations in the Cord Blood of Normal Infants.

Cholesterol concentration = x

β-Lipoprotein lipid concentration = y

$n' = 50$

$Sx = 3591 \quad Sy = 4775 \quad (Sx)^2 = 12895281 \quad (Sy)^2 = 22800625$

$SxSy = 17147025$

	(1)	(2)	(1) - (2)
Sx^2	268157	257905.62	10251.38
Sy^2	521673	456012.50	65660.50
Sxy	351913	342940.50	8972.50

$b = \frac{8972.5}{10251.38} = 0.87524.$ $\quad A = 4775 - (3591 \times 0.87524)/50$

$$bSxy = \frac{\left(\frac{8972.5}{10251.38}\right)^2}{10251.38} = 7853.1628.$$

$S(y-Y)^2 = 65660.5 - 7853.163 = 57807.337.$

$$S^2 = \frac{57807.337}{48} = 1204.3195.$$

$$\text{S.E. of } b = \sqrt{\frac{1204.3195}{10251.38}} = \sqrt{0.11747} = \pm 0.2428.$$

$$t = \frac{0.87524}{0.2428} = 3.60477.$$

P is less than 0.001. This is very highly significant.

i.e. The coefficient of regression of β-lipoprotein lipid on cholesterol concentrations in the cord blood of normal infants is $0.87 \pm 0.24(50)$; $(p < 0.001)$.

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