CCXXI. OBSERVATIONS ON THE BLOOD FATS IN DIABETIC LIPAEMIA.

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THE present study is concerned with two cases of diabetes mellitus with intense lipaemia. At the height of the lipaemia, the plasma fat ranged from 9 to 22 %, and these extraordinarily high values made it possible to extract and analyse the fat by large-scale methods. Such opportunities rarely occur, and it seemed worth while to study the composition of the fat, and in particular, to determine the iodine value of the separated fatty acids in the hope of obtaining some information as to the source of the fat in diabetic lipaemia. Comparisons were made between the fats of the food and those of the blood, but as the lipaemia diminished very quickly under treatment, these experiments were made during the period of recovery and not at the height of the lipaemia.

Methods.

Extraction and estimation of total fat. A measured volume of plasma was diluted to 20 ml. with water, and an equal volume of alcohol was added. This was extracted with a mixture of equal parts of ether and light petroleum. The ether-petroleum layer was separated, the extraction was repeated twice more, and the extracts were combined. The solvent was removed by distillation followed by storage of the residue in a vacuum desiccator until constant weight was reached. The total fat was calculated from the weight of this extract.

Saponification and separation of fatty acids and unsaponifiable matter. A weighed amount of the extracted fat (usually 0.2–0.6 g.) was dissolved in neutral alcohol. An attempt was made to titrate any free fatty acid in this solution, but none was found. The material was saponified by heating with alcoholic potash under a reflux condenser ($\frac{3}{4}$ –4 hours) and the saponification value was estimated by titration of the excess KOH. The solution containing the soap and unsaponifiable matter was concentrated and transferred to a separating funnel, using alternately water and alcohol, so that after complete transference the mixture contained approximately equal parts of water and alcohol. The unsaponifiable matter was removed by three successive extractions with a mixture of equal parts of ether and light petroleum. The combined solution containing the unsaponifiable matter was washed by shaking with water, the aqueous washings being added to the main solution of soaps. The solvent was removed from the unsaponifiable matter and the extract weighed.

The alcohol-water solution containing the soaps was next acidified, and the fatty acids extracted three times with ether and petroleum. The combined solutions were washed with water, the solvent was removed and the extract weighed.

Cholesterol. For the majority of analyses the colorimetric method of Myers and Wardell¹ [1918] was used. In some instances the results were confirmed by the digitonin method as used by Gardner and Gainsborough [1927].

Lipin phosphorus. A known amount of the fat was digested by heating with sulphuric acid and hydrogen peroxide (perhydrol), the peroxide being added cautiously in successive small amounts until the digestion was complete. The resulting solution was diluted and treated with an excess of sodium hydroxide. The solution was evaporated to dryness and the residue ignited. The final residue was dissolved in water and the phosphorus was estimated colorimetrically by the method of Kuttner and Cohen [Kuttner and Lichtenstein, 1930].

Iodine values were determined by the method of Wijs.

Clinical and experimental notes.

Case 1. R. W., male, age 25, was admitted to the Newcastle General Hospital in March 1934. He had been treated for diabetes for 6 months with insulin and dietetic measures, but shortly before admission had indulged in large amounts of carbohydrate. No unusual amount of fat was taken. On admission, he had glycosuria and intense ketonuria, and the fasting blood sugar was 0.312%. The whole blood was the colour of chocolate. The plasma separated quickly and had the appearance and consistency of cream. The plasma fat was 22% and the cholesterol 1.41% (Table I). Under

Table I. R. W. *First series of observations.*

Conditions	...	...	4-5 hours after breakfast and insulin			
Date	...	...	22. iii. 34	23. iii. 34	10. iv. 34	14. iv. 34
Total fat, % of plasma			22.0	18.7	2.71	1.87
"Lecithin," % of plasma (calculated from lipin P)			0.95	—	—	—
Cholesterol, % of plasma			1.41	1.31	0.75	—
Cholesterol, % of corpuscles			—	—	0.16	—
Composition of plasma fat:						
Free fatty acids			Absent	Absent	Absent	
Total fatty acids, % of total fat			—	80.1	53.3	
"Lecithin," % of total fat			4.1	—	—	
Total cholesterol, % of total fat			7.3	7.0	25.8	
Unsaponifiable matter, % of total fat			—	12.6	38.4	
Saponification value			206	201	—	
Separated fatty acids: Mean mol. wt.			—	276	—	
I.v.			—	68.5	82.6	
I.v. of fatty acids in diet (approximately)				?	44	

treatment by diet and insulin the lipaemia and the hyperglycaemia were controlled. During the period of recovery a special diet was given in order to compare the fats of the food with those of the blood. This was started on Aug. 7th and continued until Aug. 14th, two blood samples being taken during this time (Table I). During the same period the average daily excretion of fat in the faeces was 5.5 g. The experimental diet contained 55 g. protein, 129 g. carbohydrate and 106 g. fat daily. Of the fat, 84 g. were given as butter, 18 g. were derived from eggs and the remainder from vegetable sources. The butter was analysed; the daily ration would yield 80 g. fatty acids of i.v. 39.1. The eggs were not analysed, but on the basis of figures given by Needham [1931] it was calculated that the daily ration of eggs would yield 15 g. fatty acids of mean i.v. 70. The mean i.v. of the fatty acids in the food would be 44.

The patient recovered and was discharged when a proper balance of diet and insulin had been established.

¹ It was noted that the rate of colour development was sometimes more rapid in the chloroform solutions of blood-fat than in pure cholesterol solutions. Erroneous results were obtained if the estimations were made after 10 minutes' colour development. At 15 minutes the colour was maximum in both the "standard" and "unknown" solutions provided that the solutions were perfectly free from moisture.

In the following August he again took excessive carbohydrate, but no undue amount of fat, and was re-admitted to hospital in coma with severe ketosis and a fasting blood sugar of 0.562%. There was lipaemia again, the plasma fat being 9.12% and the cholesterol 1.1% (Table II). For the first 4 days he was treated with insulin and glucose, and as soon as he began to take food he was given the same experimental diet as had been used before. This was continued throughout the period of the analyses given in Table II, except for an interval between August 7th and 10th when some bacon fat was given instead of butter.

Table II. *R. W. Second series of observations.*

Conditions		...	No food.	5½ hours after breakfast and insulin				
			Insulin and glucose					
Date	...	...	30. vii. 34	3. viii. 34	7. viii. 34	13. viii. 34	17. viii. 34	
Total fat, % of plasma			9.12	4.08	4.27	2.86	2.45	
"Lecithin," % of plasma			0.57	—	—	—	—	
Free cholesterol, % of plasma			0.52	—	—	—	—	
Total cholesterol, % of plasma			1.10	0.64	0.64	0.52	0.41	
Composition of plasma fat:								
Free fatty acids			Absent	Absent	Absent	Absent	Absent	
Total fatty acids, % of plasma fat			75.7	60.9	62.0	65.2	—	
"Lecithin," % of plasma fat			6.21	—	—	—	—	
Free cholesterol, % of plasma fat			5.64	—	—	—	—	
Total cholesterol, % of plasma fat			12.1	15.7	14.9	18.1	16.8	
Unsaponifiable matter, % of plasma fat			19.8	27.9	22.9	24.8	22.6	
Saponification value			198	—	—	—	—	
Separated fatty acids: i.v.			86.8	52.8	46.7	92.2	62.1	
i.v. of fatty acids in diet (approx.)			?	44	44	44	44	

Again, the condition was controlled by treatment and the lipaemia diminished. The patient was discharged on a balanced régime of diet and insulin.

In March 1935 another breach of diet was followed by coma. A blood sample taken during recovery under treatment with glucose and insulin showed only faintly turbid plasma and the cholesterol was 0.349%.

Case 2.¹ K. U., male, aged 9 years, was admitted to the Royal Victoria Infirmary in June 1934. He had been treated for diabetes mellitus for 4 years. On admission he had extensive cutaneous xanthomatosis. There was glycosuria and ketonuria. The blood sugar 5½ hours after the morning dose of insulin was 0.198%, and there was intense lipaemia. The plasma fat was 9.59% and the cholesterol 1.44% (Table III).

Table III. *K. U.*

Conditions		...	...	5½ hours after break-fast and insulin		3½ hours after break-fast and insulin	
Date	...	...	...	20. vi. 34	26. vi. 34	30. vi. 34	16. vii. 34
Total fat, % of plasma				9.59	3.43	3.05	1.57
"Lecithin," % of plasma				0.74	—	—	—
Total cholesterol, % of plasma				1.44	1.06	0.92	0.46
Total cholesterol, % of corpuscles				0.23	—	—	—
Composition of plasma fat:							
Free fatty acids				Absent	Absent	Absent	Absent
Total fatty acids, % of blood fat				73.0	54.6	53.0	51.3
"Lecithin," % of blood fat				7.7	—	—	—
Total cholesterol, % of blood fat				14.3	29.2	29.2	29.1
Unsaponifiable matter, % of blood fat				19.7	37.0	43.6	40.1
Saponification value				196	—	—	—
Separated fatty acids: i.v.				54.5	65.6	67.1	74.2
i.v. of fatty acids in diet (approx.)				?	43	43	43

¹ A clinical account of this case has already been published [Bolam and Herbert, 1934].
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On the day after these figures were obtained, the patient was put on a special diet containing 102 g. carbohydrate, 66 g. protein and 112 g. fat. Of the fat, 87.5 g. were given as butter, 16.5 g. were derived from eggs, 6.2 g. from milk and the remainder from vegetable sources. The fatty acids separated from a sample of the butter had i.v. 38.7 and the mean i.v. of the fatty acids of the food was calculated as 43. This diet was maintained throughout the whole period of study.

Under treatment the hyperglycaemia was controlled, the ketosis disappeared, the lipaemia diminished, and the cutaneous deposits of cholesterol were completely re-absorbed. The patient remained well, under treatment, for some months, but later died of scarlet fever.

DISCUSSION OF RESULTS.

It has been pointed out by Bloor [1921] that in diabetic lipaemia the increase in fats and lipoids occurs almost entirely in the plasma. This is illustrated in the present work by the data for the distribution of cholesterol between plasma and corpuscles (Tables I and III).

The fat in the plasma exists in an extremely fine emulsion, and shows no tendency to separate out on standing. Microscopic examination shows that the particles of fat are comparable in size with bacteria.

The high figures for fat and cholesterol here recorded—up to 22 % for fat, and up to 1.41 % for cholesterol in the plasma—are very unusual, but even higher figures have occasionally been reported. Among the highest in the literature are the following: for total fat, 23.7 % of whole blood [Fischer, 1903], 19.7 % of whole blood [Neisser and Derlin, 1904], 27 % [Frugoni and Marchetti, 1908]; and for cholesterol, 1.5 % of serum [Imrie, 1915], 2.14 % [Ringer, 1917], 2.6 % and 3.6 % [Adler, 1909].

At the height of the lipaemia, the most striking feature is the enormous increase in total fat, with relatively less increase in cholesterol and phospholipin, and the abnormally high proportion of fatty acids. The figures suggest the presence of a large amount of triglyceride, and this is confirmed by a consideration of the percentage composition of the fat in the only instance where the analysis is sufficiently complete to permit of such a calculation—namely, case R. W. July 30th (Table II). It is convenient to take the molecular weight of the fatty acids as 276, the figure obtained for the blood of the same patient on an earlier occasion, but the calculation is not materially affected by the choice of this figure rather than the molecular weight of any of the higher fatty acids ordinarily occurring in the body.

Of 100 g. total ether-soluble material, the following constituents were determined: phospholipin 6.33 g., free cholesterol, 5.64 g., cholesteryl ester, 10.76 g. (corresponding to 6.45 g. cholesterol in combination with fatty acid of mean molecular weight 276) and non-sterol unsaponifiable matter, 7.70 g. The sum of these constituents is 30.4 g. The “residual fat”, which may be glyceride, amounts to 69.6 g.

As regards the fatty acids, the amount present in 100 g. fat is 75.7 g., and of this 4.42 g. are accounted for by lecithin and 4.65 g. by cholesteryl ester. The remainder of the fatty acids, which may be in combination with glycerol, amounts to 66.6 g.

This shows that 69.6 g. “residual fat” yield 66.6 g. fatty acid; that is to say, the residual fat is a substance yielding 95.7 % of its weight of fatty acid. This corresponds closely to the figure expected on the assumption that the residual fat is triglyceride, the theoretical value for which would be 96.7 %.

At the height of the lipaemia therefore the greatest increase is in the neutral fat. As a result of treatment, the total fat and cholesterol fall, but the fall of cholesterol is relatively less rapid than that of total fat, so that the cholesterol

forms an increasing proportion of the total fat. At the same time the fatty acid forms a decreasing proportion of the total fat. The results show that neutral fat diminishes more rapidly than cholesterol. Similar results have been reported by Bloor [1921].

In the present analyses, the cholesterol constitutes only 56–79 % of the total unsaponifiable matter. It is possible that some of the non-sterol unsaponifiable matter may be an artefact, derived from corks used during saponification and distillation. No tap-grease was used at any stage. Saponification of the cholesterol esters was proved to be complete, for the whole of the cholesterol in the unsaponifiable matter could be precipitated by digitonin. It is of interest that the sum of fatty acids and unsaponifiable matter constituted from 85 to 95 % of the total fat. The amount lost during saponification was approximately what would be expected to disappear (glycerol, choline and phosphoric acid). However, it is possible that the real loss was rather greater but was partly balanced by the introduction of extraneous material. Imrie [1915] has described a case of diabetic lipaemia in which the fat amounted to 14.06 % of the serum, and the cholesterol to 1.5 %; he found that the cholesterol accounted for 98 % of the unsaponifiable matter.

As regards the i.v. of the fatty acids, it is impossible to generalise, and the three series of data must be considered separately.

In the first series of data on R. W. (Table I), the i.v. at the height of the lipaemia was 68.5. This figure was obtained about 4 hours after a meal containing fat, but since at that time the total fat in circulation must have been about 560 g., the fat derived from the recent meal must have been a very small proportion of the whole. No food had been taken on 21. iii. 34 and the fats taken on the next two days were the fats of a mixed diet. The i.v. 68.5 is distinctly higher than one would expect from a mixture of the food fats, and it seems probable that the fat came from the patient's own tissues, probably from fat depots in adipose tissue, or partly from those depots and partly from the more unsaturated tissue fats. The results during the period of the experimental diet, when recovery was well-advanced, show that the i.v. of the plasma fatty acids rose during recovery, although the patient had for some days taken a diet in which the more saturated fats predominated, and although the blood was taken during fat absorption. The balance experiments made at this time showed that at least 96 % of the fat in the food was absorbed.

When R. W. was admitted for the second time (Table II) the first blood sample was taken during recovery from coma under treatment with glucose and insulin. The i.v. of the separated fatty acids was 86.8. The total fat in circulation would then be about 270 g. The patient had not taken any gross amount of fat before admission, and the i.v. is higher than that of mixed food fats. The fat must have come from the patient's tissues, and as the i.v. is higher than that of storage fat, some at least was probably derived from the more unsaturated tissue fats.

The sample of 3. viii. 34 was obtained 5½ hours after the first meal taken after recovery from coma. The fat from the recent meal may have affected the figure somewhat, but in view of the total fat in circulation it could not have predominated. There must have been other relatively saturated fats beside those of the food. In the later samples as recovery advanced, the mean i.v. of the fatty acids in the plasma became definitely higher than the mean i.v. of the fatty acids in the food.

In the series of data for K. U. (Table III) the results are very regular. There was a relatively low i.v. at the height of the lipaemia (54.5) and the i.v. steadily

rose during recovery, although the mean i.v. of the fatty acids of the food was low.

Upon the whole, it seems that at the height of the lipaemia much of the fat is derived from the patient's own tissues. It is probable also that absorbed fat would remain in circulation longer than normally and contribute to some extent to the lipaemia. In the later stages of recovery the plasma contains a considerable amount of fatty acids more unsaturated than those of the food.

Other figures which have been obtained for the i.v. of the separated fatty acids in intense diabetic lipaemia are 73 [Imrie, 1915] and 60.6 [Fischer, 1903].

For normal human blood, there are few data for the degree of saturation of the fatty acids of plasma, and those that exist have been obtained by micro-methods of rather doubtful value. According to Boyd [1933] the i.v. of the fatty acids of plasma, in the fasting state, ranges from 64.5 to 102. For the fatty acids of whole blood, McClure and Huntsinger [1928] found a range from 27 to 81, and Page *et al.* [1930] a range from 98 to 132. Figures for animal blood are more reliable owing to the use of large-scale methods. It may be calculated from the data of Bloor [1923] that the i.v. of the fatty acids of the plasma of the pig is on the average 93.8, and of the plasma of the ox, 109. Most of the figures show that normal blood contains fatty acids of a mean i.v. intermediate between the fatty acids of adipose tissue and those of true tissue fats.

Himwich and Spiers [1931] have reported that in depancreatized dogs the development of diabetic lipaemia is associated with a fall in the i.v. of the total blood fat.

In any attempt to interpret changes in i.v. during the development or the subsidence of lipaemia, it must be remembered that any change in the proportions of neutral fat and phospholipin is likely to affect the i.v. Boyd [1933] has shown that in normal blood the more unsaturated fatty acids occur especially in the phospholipin fraction. At the height of diabetic lipaemia the proportion of neutral fat is high, and as the lipaemia subsides the neutral fat diminishes more rapidly than the phospholipin [Bloor, 1921]. An increase in the proportion of phospholipin would involve an increased proportion of unsaturated fatty acids. This may in part explain the results of Himwich and Spiers and some of the results of the present paper. On the other hand, the present work affords one example of the mobilisation of neutral fat of relatively high i.v. In the blood of R. W. taken on July 30th, the neutral fat constituted 69.6 % of the total, yet the mean i.v. of the fatty acids was 86.8.

The cause of the lipaemia is obscure. It would be natural to suppose that the fat accumulates in the blood because it cannot be adequately oxidised. If this is the whole explanation, it is difficult to understand why so many patients have ketosis without any appreciable lipaemia. The patient R. W. had three severe bouts of ketosis, and had marked lipaemia on two occasions, but no appreciable lipaemia on the third. It seems probable that some other disorder of fat metabolism may exist besides the failure in oxidation which leads to ketosis.

SUMMARY.

Observations have been made on the blood fats in two cases of intense diabetic lipaemia.

At the height of the lipaemia the neutral fat, cholesterol and phospholipin are all greatly increased, but the greatest increase is in the neutral fat.

As the lipaemia subsides under treatment, the neutral fat diminishes much more rapidly than the cholesterol.

A consideration of the i.v. of the separated fatty acids at the height of the lipaemia suggests that the fat is derived from the tissues.

Comparisons between the fats of the food and those of the plasma have been made during the subsidence of the lipaemia under treatment. The mean i.v. of the fatty acids of plasma is always higher than that of the fatty acids in the food, and on the whole the i.v. of the fatty acids of plasma tends to rise as the lipaemia subsides.

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