XIV. THE CHEMICAL NATURE OF THE SUB-CUTANEOUS FAT IN THE NORMAL AND SCLEREMATOUS INFANT.

BY HAROLD JOHN CHANNON (*Beit Memorial Research Fellow*) AND GEOFFREY ARTHUR HARRISON.

From the Departments of Physiology and Biochemistry, University College, London, and the Biochemical Department, the Hospital for Sick Children, Great Ormond Street, London.

(*Received December 17th, 1925.*)

SCLEREMA NEONATORUM is a rare but interesting condition peculiar to infants and is characterised by the appearance of hard well-defined thickenings of the subcutaneous tissue. It is with the material composing these swellings that this investigation is concerned. Frozen sections of the tissue show large numbers of sheaves of crystals which, seen through crossed Nicol prisms, are doubly refracting. It was the observation of the presence of these crystals which led to this work being undertaken, for their doubly-refracting nature suggested that they were probably cholesterol or its esters. Subsequent work proved that this view was probably incorrect, and the work finally took the form of an enquiry into the nature of the subcutaneous fat in normal children and in those suffering from sclerema.

Observations of the behaviour of these crystals in the frozen sections of sclerema cases towards various solvents under the polarising microscope were made. By direct observation in this way, they appeared to be insoluble in ether, acetone, light petroleum, pyridine and alcohol; likewise in acid alcohol, alcoholic sodium hydroxide, water, hydrochloric acid and sodium hydroxide. It is to be noted, however, that the temperature of a volatile solvent rapidly evaporating on a slide is probably well below 0°. On slightly warming, the crystals were soluble in ether, acetone, light petroleum and pyridine, but insoluble in water, aqueous hydrochloric acid and sodium hydroxide; in the other reagents they were slightly soluble. Between 40° and 50° the crystals melted, to reappear *in situ* on cooling and in the original form. There was no liquid crystal formation. Similar crystals with like behaviour were observed in ether extracts of the tissue. They appeared therefore to be ether-soluble and of fatty nature. Frozen sections and ether extraction of normal subcutaneous tissue revealed that there were present similar doubly-refracting crystals with like solubilities but a lower melting point. Experiments with pure tripalmitin showed that, on slow crystallisation from ether at 0°, tufts of anisotropic, acicular crystals could be obtained, which, as regards solubility and melting point behaved similarly to the crystals from subcutaneous tissue. Further consideration of the nature of these crystals is made later, but it is to be noted that the assumption is generally made by histologists that anisotropic, acicular crystals are cholesteryl esters; the possibility of their being neutral fats has scarcely been considered. As these crystals were obtainable in the ether extract, it was decided to make a study of the ether extract in cases of sclerema and in normal cases with a view to attempting to determine (*a*) the nature of the crystals, (*b*) the reasons for the fat in the subcutaneous tissue in cases of sclerema having a melting point well above that of the body fat.

Chemical Investigation of Subcutaneous Tissues.

The ether extract of the tissue.

Although the first two cases were not studied chemically in any detail, they suggested the procedure which was adopted in the later ones. In these two cases the fat was melted out of the portions of the subcutaneous tissue and dried. Determinations of melting point, iodine value (Hübl), and free and total cholesterol were made by Dr J. W. McNee and similar treatment was given to the fat from two control cases (Table I).

In the remainder of the work (Tables II and V), the tissue was exhaustively extracted with ether, after sufficient grinding with sand and anhydrous sodium sulphate to produce a fine powder. Every precaution was taken to ensure that no oxidation of the extracted material should occur during the process of removal of the solvent and drying. As many determinations of the fat constants, etc., were made as was possible. The limiting factor in all cases was the small amount of material available, which in no case exceeded 7·25 g. As the melting point is of some importance, as the later results will show, it is necessary to state the exact procedure adopted in its determination. The fat, after being sucked up into an open capillary tube, was allowed to stand for 24 hours on ice; the melting point was taken as that temperature at which the fat began to rise in the open capillary; the determination was carried out at least twice on each sample of fat.

The results are given in Tables I and II. The unsaturated acid has been calculated from the iodine value, the assumption being made that oleic acid is the only unsaturated acid present. This would appear to be justifiable in control cases, but may not be entirely so in cases of sclerema, because traces of a precipitate were obtained on carrying out the bromide test. Owing to the very small amount of material available, it was not possible to prove definitely that this precipitate was due to the presence of acids more unsaturated than oleic, and even if it were, the relative amount in which they were present allows their effect on the iodine value to be neglected without introducing any considerable error.

Table I.

	Age (weeks)	Melting point ° C.	Iodine value (Hübl)	Free cholesterol %	Ester cholesterol %
Case 1	6	50·5	42·1	0·25	0·20
„ 2	12	38·5	52·0	—	—
Control 1	52	33·5	45·7	trace	0·82
„ 2	9	32·5	57·5	—	—

Table II.

Determination	Case 3 Biopsy material (age: 21 weeks)	Case 3 Autopsy material (age: 24 weeks)	Case 4 Autopsy material (age: 12 weeks)
Total cholesterol % (digitonin)	1·5	6·0	2·1
Unsaponifiable matter	—	13·6	3·1
Melting point ° C.	38·5	42·9	47·3
Acid value	5·4	9·0	5·9
Saponification value	211	206	195
Iodine value	52·3	54·0	45·5
Olein % (calc.)	60·8	62·7	52·8
Separated fatty acids.			
M.P. ° C.	—	45·1	49·7
Neutralisation value	—	216	206
Mean molecular weight	—	260	272
Iodine value	—	48·9	46·7
Oleic acid % (calc.)	—	54·3	51·8

Remarks.

The results obtained with the ether extract from the biopsy material in case 3 are not more than approximate because only 0·5 g. of ether extract was obtained; the corresponding autopsy material weighed 5 g. and that from case 4, 7·25 g.

There is a marked difference in the total cholesterol content of the autopsy and biopsy material in case 3. The patient was very emaciated at death, but there was no emaciation at the time of biopsy. It would seem probable that during this interval of 3 weeks, while fat was being absorbed from the subcutaneous depôts at a rapid rate, cholesterol was being absorbed much more slowly. This hypothesis would seem to receive some confirmation from the fact that there was a similar difference in the calcium content of the ether-extracted residue of the biopsy and autopsy materials. It would seem reasonable to suggest therefore that cholesterol and calcium were absorbed much more slowly, if at all, during the period when emaciation was taking place.

As will be seen from a comparison with the melting points, given later in the paper, for subcutaneous fat of normal children, the melting points of the sclerematous fat seem to be raised to a definite if limited extent. As the amount of cholesterol present was much above normal, it seemed of interest to see whether the addition of small amounts of cholesterol to normal fat would affect the melting point of that fat to any appreciable extent. Accordingly to normal fat (M.P. 28·4°) was added 2 % of cholesterol; the melting point of the resultant mixture was 28·5°, the addition having raised the melting point by 0·1° only. Additions of tripalmitin to normal fat caused, however, marked elevations in the melting point as will be seen from the following table:

Table III.

	Added palmitin as % of resultant mixture	Average M.P. °C.	Elevation of M.P. °C.
Control fat	—	27·0	—
Control fat + palmitin	3·0	35·9	8·9
,, ,,	7·1	44·0	17·0
,, ,,	28·4	53·7	26·7

Further reference will be made to these results later.

The ether-extracted residue from sclerematous tissue.

It was of interest to enquire into the reasons for the opacity of the subcutaneous tissue which X-ray photographs had demonstrated. Estimations of calcium and phosphorus in the ether-extracted residue gave the following results:

	Case 3		Case 4	Control
	Biopsy	Autopsy	Autopsy	case
Ca as % dry residue	0·26	2·62	0·25	0·07
P (total) dry residue	—	1·41	0·47	—
Ratio Ca/P in dry residue	—	1·85	0·54	—

Ratio Ca/P for $Ca_3(PO_4)_2$ is 1·965.

There is a definite excess of calcium in the tissue in sclerema cases. Calcium is generally deposited in pathological tissues as phosphate or carbonate. In these cases there was a relative excess of phosphorus. Whilst, therefore, there is no evidence as to the form in which the calcium was actually combined, there is nothing in the figures contrary to the assumption that it was in the form of phosphates.

Chemical Constants of the Subcutaneous Fat of Normal Children.

It was hoped that a comparison of the results obtained by us with the fat of sclerematous patients and with normal subcutaneous fat, would throw some light on the abnormalities of the former, and provide an explanation of its occurrence and nature. The literature however contains but little information concerning subcutaneous fat of normal children. Such as there is concerns almost exclusively the degree of unsaturation determined by the iodine value or by the lead salt-ether method, and the melting point. Thus Langer [1881] noted the difference in melting point and consistency of the subcutaneous fat of infants and that of adults; he obtained 31 % solid fatty acids in the former and 10 % from the latter. Raudnitz [1888] found the melting point of the subcutaneous fat from an infant of 2 days and a child of 2 years to be 43·6° and 29·4° respectively. These are averages of the figures he obtained for the fat from various regions.

Knöpfelmacher [1897] found that the iodine value of the subcutaneous fat of a new-born child was lower than that of an adult. He determined the melting points and iodine values of the fatty acids obtained from seven children varying in age from 0–17 months. The melting points on the whole decreased with increase in age (from 48° to 37°), and the iodine values showed a progressive rise from 40·1 to 64·5. As a result of this work he made the interesting suggestion that the percentage of triolein in infant body fat is at its lowest value at birth and increases until the twelfth month when it reaches "adult" level.

Thiemich [1898] and Siegert [1902] also observed the smaller triolein content of child fat as compared with adult fat; the former was however unable to confirm Knöpfelmacher's hypothesis that the iodine values increased *regularly* to a maximum at the age of 12 months. Siegert suggested that the increase in the iodine values was due to a change from a milk to a mixed diet at the age of from 8 to 12 months. The following table contains the results obtained with the fat of non-sclerematous cases by Knöpfelmacher [1897], Thiemich [1898], Siegert [1902], Jaeckle [1902], Bardisian [1921], Finkelstein and Sommerfeld [1923] and Lasch [1925], and will serve as a rapid survey of the literature on this point:

Table IV. *Iodine values of the separated fatty acids according to age.*

Age	No. of cases	Iodine values		Average
		Lowest	Highest	
Premature	2	46·8	49·4	48·1
Newborn	16	36·9	49·2	42·2
1 day–1 month	7	38·1	60·7	47·1
1– 2 months	5	38·5	51·4	46·7
2– 3 "	6	41·5	58·7	50·1
3– 4 "	9	41·4	75·5	57·4
4– 5 "	4	40·6	47·8	44·3
5– 6 "	6	47·0	53·3	50·9
6– 8 "	7	46·1	56·2	49·6
8–10 "	3	51·7	61·6	57·0
10–12 "	4	55·5	63·9	61·0
Adult	—	—	—	65·0

NOTE. In those cases where the authors have made their determinations of the iodine values on the fat itself, the approximate corresponding values for the fatty acids have been calculated and have been included in the above table.

There is general agreement in the literature that the melting point of the fat of young infants is higher than that of adults and decreases in general with increase in age up to about one year. The iodine values as will be seen from Table IV behave in the reverse sense (iodine values from first to eighth month, 37–55, increasing rapidly to "adult" level, 60–65).

The only recorded saponification values are those of Jaeckle [1902], 204 for two infants of under one month and 193·3 to 199·9 as the extremes in seven adult cases; Bardisian [1921] gives 212–228 for children aged 3 months to 5½ years. Unna and Golodetz [1909] give the free cholesterol as 0·14 % and that as ester 0·04 %, total 0·18 %.

As the bulk of the work on normal subcutaneous fat was carried out prior to 1902, and as there are but few recorded figures to serve as a basis for comparison with those obtained from the fat of sclerematous patients, it was deemed wise to make a study of subcutaneous fat of normal infants. This was obtained by the method already described for sclerematous fat, and the results are given in Table V.

Table V. *Results of examination of "ether extract" from a series of control cases (autopsy material throughout).*

Case	a	b	c	d	e	f	g	h	i
Age	3/12	4/12	8/12	10/12	10/12	1 year	14/12	2 years 10 months	40 years
Cause of death	Acute gastro-enteritis	Gastro-enteritis	Influenzal meningitis	Abscess of neck, and septicaemia	Congenital heart disease	Osteomyelitis Purulent arthritis Gastro-enteritis	Generalised tuberculosis	Osteomyelitis	Placenta praevia
Total cholesterol % by colorimetric method	—	—	—	0·19	—	—	—	—	0·26
M.P. of ether extract, ° C.	37·3	30·5	27·9	29·4	32·7	26·9	29·0	21·5	27·3
Acid value	2·0	1·7	1·2	0·6	2·7	0·9	0·9	Nil	Nil
Saponification value	206	203·6	199·7	203	203·3	201·8	198·3	199·4	199·0
Iodine value	53·8	56·9	60·6	56·6	57·4	58·4	61·0	67·5	65·0
Calculated olein %	62·5	66·1	70·4	65·7	66·8	67·8	70·8	78·3	75·5
Separated fatty acids:									
M.P. ° C.	—	38·6	36·1	—	39·9	37·8	35·8	34·0	33·5
Neutralisation value	—	214·5	209·6	—	209·7	209·2	206·5	206·6	207·0
Mean molecular weight	—	261·8	267·7	—	267·5	268·1	271·8	271·5	271·0
Iodine value	—	59·6	62·4	—	59·8	61·1	62·9	70·3	65·7
Calculated oleic acid %	—	66·2	69·3	—	66·5	67·9	69·9	78·1	73·0

These figures confirm the observations of other workers that the iodine value and melting point of the fat increase and decrease respectively from birth to about 12 months of age but without complete regularity. It is of interest to notice that there is however little variation in the saponification value which appears to approximate closely to 200, and to vary to a smaller extent than the iodine value. The obvious objection to these results is that they have been obtained from pathological cases, but it is virtually impossible to obtain post-mortem specimens from healthy infants, and the results do not contain any figures which appear so abnormal as to warrant their being viewed with suspicion.

With these figures for comparison, we may now consider the various hypotheses which have been put forward by various workers to account for the appearance of the exceptionally solid fat in the subcutaneous tissues of infants suffering from sclerema.

There are 18 cases of sclerema neonatorum in which chemical analyses have been reported in the literature, and the various theories put forward may be briefly reviewed. Langer [1881] suggested that in diseases of infants preferential oxidation of olein might occur, the resulting increase in the saturated acids and a lowering of the body temperature being responsible for the hardening of the fat. Knöpfelmacher [1897] believed that sclerema was due to a combination of three causes: loss of fluidity of the subcutaneous fat, lowering of body temperature, and the high temperature of solidification of the fat of infants. Siegert [1902] pointed out that the diminished olein hypothesis could not hold in view of the observations of Thiemich [1898] that there was no significant difference between the iodine values of the fat of two sclerematous infants and that of normal infants of the same age, and that it was mere assumption that olein was more readily oxidised than the saturated acids. Miura [1905] concluded that "the fat of scleremic infants did not differ materially from the fat of normal infants of the same age," and Sarvonat [1906] that "neither the body temperature nor the oleic acid content would explain the hardening of the tissues." Bayer [1908] maintained that a pathological reduction of the oleic acid was the cause of sclerema, and lastly Finkelstein and Sommerfeld [1923], as a result of work on five cases, suggested that the disease was due to some change in the colloids of the subcutaneous tissue.

Langer's hypothesis is obviously untenable and need not be discussed. The only other hypothesis is that founded on the observation that the melting point of the fat in sclerematous children is above normal (Knöpfelmacher, Bayer).

In every one of the five analyses made by us the melting point has been found to be greater than that for the fat from non-sclerematous children of the same age. Is this raised melting point due to a decrease in the amount of oleic acid present? Our results are set out in Table VI.

Table VI.

Age (weeks)	Iodine values		Melting points ° C.	
	Sclerema	Normal	Sclerema	Normal
6	42·1	—	50·5	—
12	52·0	53·8	38·5	37·3
12	45·5	53·8	47·3	37·3
21	52·3	56·9–60·6	38·5	30·5–27·9
24	54·0	56·9–60·6	42·9	30·5–27·9

In view of the fact that the iodine values in the control cases vary among themselves, it will be seen that the above table provides no great evidence in favour of the diminished olein hypothesis, for there are no significant differences in the iodine values of the sclerematous and normal cases, although the former tend to lower values. This however does not disprove the hypothesis, for but small alterations in the amount of olein in a fat have a marked effect on the melting point without affecting the iodine value to any appreciable extent (the addition of 3 % of palmitin to a normal fat raised the melting point by 8·9°; see Table III). Further if 3 % of olein present in a fat of iodine value 65 and saponification value 200 were replaced by tripalmitin, the iodine value of the resulting fat would be 62·4 and its saponification value 200·5, differences of 2·6 and 0·5 respectively. The fat would then have a definitely raised melting point, but the differences between the two constants mentioned would not be detected because those of normal infants' fat vary among themselves over a much greater range than this.

The literature records no serious attempt to determine the nature of the anisotropic crystals referred to earlier in this paper, although crystals have been observed by Knöpfelmacher [1897] and Lieberthal [1918]. It would appear probable that they are similar to those observed in normal subcutaneous fat, and in that case they are presumably tripalmitin or tristearin. Their solubility excludes the possibility of their being soaps or urates; nor can they be fatty acids on account of the low acid value of the fat and their behaviour towards alkaline alcohol. It is improbable that they are cholesteryl esters because (*a*) microchemical tests for cholesterol were negative; (*b*) on careful warming under the polarising microscope and cooling, no liquid crystal formation was ever observed; (*c*) the melting point of the sclerema crystals was lower than that of true cholesteryl esters as seen in frozen sections of cutaneous xanthoma; (*d*) the actual amount of cholesterol present was very much less than would have been expected from the microscopic appearance of the sections.

The solubility of the sclerema crystals in acetone, and the fact that on ashing they left no residue, rule out the possibility of their being cerebrosides.

We conclude, therefore, that the crystals are probably neutral fats, either palmitin or stearin or both, or glycerides of the higher fatty acids. Such a hypothesis will explain the raised melting point and it will fit in well with the observation that fat from sclerematous patients has an iodine value which, while not differing markedly from that of normal subcutaneous fat, tends to a somewhat lower value.

SUMMARY.

1. The analytical constants of the subcutaneous fat of eight infants and one adult have been determined. The iodine value is lowest at birth and increases to the adult value from the eighth to the twelfth month of life; there is a corresponding decrease of melting point with age. The saponification value is almost constant at 200.

2. Similar analyses have been made in four cases of sclerema neonatorum, in which the fat appears to have a higher melting point and a slightly lower iodine value than the corresponding values of the fat in infants of the same age.

3. The raised melting point is believed to be due to the presence of an excess of glycerides of the higher fatty acids—probably of palmitic acid—which causes a slight decrease in the oleic acid content of the fat.

4. Subcutaneous tissue in cases of sclerema contains an abnormal amount of cholesterol, calcium and phosphorus.

We wish to thank Dr J. W. McNee for permission to include his results in cases 1 and 2. A full account of the clinical, histological, X-ray findings, etc., will be published elsewhere by one of us (G.A.H.).

REFERENCES.

Bardisian (1921). *La Pediatria,* **29**, 156.
Bayer (1908). *Verhandl. deutsch. path. Ges.* **12**, 305.
Finkelstein and Sommerfeld (1923). *Monatsh. Kinderheilk.* **25**, 105.
Jaeckle (1902). *Z. physiol. Chem.* **36**, 53.
Knöpfelmacher (1897). *Jahrb. Kinderheilk.* **45**, 177.
Langer (1881). *Wien. med. Presse,* **22**, 1375, 1412.
Lasch (1925). *Jahrb. Kinderheilk.* **107**, 223.
Lieberthal (1918). *J. Cut. Dis.* **36**, 29.
Miura (1905). *Japan Z. Derm. Urol.* **5**, 412.
Raudnitz (1888). *Z. Biol.* **24**, 525.
Sarvonat (1906). *Arch. Med. Enfants,* **9**, 22.
Siegert (1902). *Beiträge chem. Physiol. Pathol.* **1**, 183.
Thiemich (1898). *Z. physiol. Chem.* **20**, 189.
Unna and Golodetz (1909). *Biochem. Z.* **20**, 469.