An Investigation of SCLEREMA NEONATORUM; with Special Reference to the Chemistry of the Subcutaneous Tissues. (Part II.)

BY

G. A. HARRISON, B.A., M.D.

(Biochemist, The Hospital for Sick Children, Great Ormond Street, London.)

CHEMICAL INVESTIGATION OF SUBCUTANEOUS TISSUES.

A few preliminary determinations only were made by McNee in Cases I. and II. These will be given first. As a result of the experience gained in these two cases, a definite plan of campaign was outlined for the investigation of Cases III. and IV. Case V. recovered spontaneously and no biopsy was made.

A. Cases I. and II. and Controls.

The fat was melted out of a mass of subcutaneous fat and dried. The melting point of this separated fat was taken by the capillary method; and the iodine value was determined by Hübl's method.

	Case I.	Control I.	Case II.	Control II.
Age	6 weeks	1 year	12 weeks	9 weeks
M.P. of separated fat, °C. ...	50·5	33·5	38·5	32·5
Iodine value of ditto (Hübl) .	42·1	45·7	52·0	57·5

Control I. died from capillary bronchitis and Control II. from gastro-enteritis.

Cholesterol determinations were made in Case I. and Control I. with the following results (method of Windaus):—

	Case I.	Control I.
Percentage of :—		
Free cholesterol	0·25	Trace
Cholesterol as esters	0·20	0·82
Total cholesterol	0·45	—

B. *Cases III. and IV. and Controls.**Methods.*

The subcutaneous fat removed at autopsy or biopsy from the chest, abdominal wall, arms, or thighs, was cut into small pieces with a scalpel, and then treated in one of two ways. (a) The small pieces were spread in a thin layer on a clock glass, and dried to constant weight over calcium chloride in a vacuum desiccator. This usually occupied two or three weeks. (b) The small pieces were ground with sand and sufficient anhydrous sodium sulphate to make an "easy flowing" powder. The dry subcutaneous fat, or the powder, was extracted continuously with ether in a Soxhlet apparatus for three or four days (total hours always exceeded 18). Both methods were sometimes used, each on half of the available fat. On comparing the two methods the use of anhydrous sodium sulphate resulted in a slightly better extraction, but the difference was very slight—less than 2%. It was of course essential to employ (a) when determining the percentage loss of moisture.

At the end of the three or four days' extraction most of the ether was removed from the ether-soluble portion ("ether extract") on the sand bath, the process being completed in CO_2 . Access of air and moisture was prevented by storage in a vacuum desiccator over paraffin wax shavings (to remove last trace of ether) and calcium chloride. When constant weight was attained the analyses were started. The residue in the Soxhlet thimble ("ether-extracted residue") was left in the air till the ether evaporated and was then stored similarly.

As many of the following determinations as possible were made upon the "ether extract":—Melting point, acid value, saponification value, iodine value, total cholesterol, and proportion of unsaponifiable matter. The fatty acids were separated, and the following determinations made upon them:—Melting point, neutralisation value (and mean molecular weight) and iodine number. The methods used were the standard ones employed in the determination of fat constants, and need no description beyond the following notes. Melting point: open capillary method; temperature when fat first started to rise in capillary taken as melting point. Iodine value by Wij's method. Total cholesterol by colorimetric method (technique of Bloor, or Myers and Wardell), or using digitonin (Windaus).

Results.(a) *Examination of the ether extract.*

Our results for cases of sclerema are given in Table VI. and for control cases in Table VII. The melting point values are the mean of two or more determinations. The olein and oleic acid percentages are calculated from the iodine values, on the assumption that they alone were responsible for taking up iodine. This is probably justifiable in the control cases, but not necessarily so in sclerema. In fact there is sufficient cholesterol to increase slightly the iodine value, but only slightly. In Case IV. by means of the

"solid bromide test," traces of highly unsaturated fatty acids were demonstrated, but in such minute amounts as to have no appreciable influence on the iodine value. Bearing in mind the slight increase due to the cholesterol (particularly in the autopsy material from Case III.), even in sclerema it is probably fair to take the iodine value as a measure of the olein or oleic acid content.

TABLE VI.

Results of examination of "ether extract" of subcutaneous fat in sclerema.

Determination.	CASE III.		CASE IV.
	Biopsy material. Age: 5 months.	Autopsy material. Age: 6 months.	Autopsy material. Age: 3 months.
Total: cholesterol %			
by digitonin method ...	1.5 (approx)	6.0	2.1
by colorimetric method ...	2.2	11.0	3.2
Unsaponifiable matter % ...	—	13.6	3.1
M.P. of ether extract, °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
Calculated olein % ...	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
Calculated oleic acid % ...	—	54.3	51.8

The ether extract from the biopsy material in Case III. only amounted to $\frac{1}{2}$ gm. The results for several of the analyses can therefore only be approximate. The autopsy material from Case III. yielded just over 5 gms., and from Case IV. $7\frac{1}{2}$ gms. of ether extract. It is considered that the results in these instances are reasonably accurate.

In Case III. three weeks elapsed between biopsy and autopsy. The patient died from intercurrent gastro-enteritis and was very emaciated at death, whereas when the biopsy was performed there was no emaciation. It is possible that the greater concentration of cholesterol (Table VI.) and of calcium (Table IV.) in the autopsy material is largely accounted for by the extreme degree of emaciation that occurred during the three weeks. Fat was absorbed from the subcutaneous depôts during that period, but cholesterol and calcium would be absorbed very much more slowly; in fact it is more than possible that during these three weeks more cholesterol and more calcium may have been laid down.

On ashing the "ether extract" there was no residue, thus excluding the presence of calcium or other inorganic substances.

TABLE VII.
Results of examination of "ether extract" from a series of control cases (autopsy material throughout).

Case No.	...	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 yrs. 10 mths.	40 years
Cause of death	...	Acute gastro-enteritis.	Gastro-enteritis.	Influenzal meningitis.	Abscess of neck and Septicæmia.		Congenital heart disease.	Osteomyelitis. Purulent arthritis. Gastro-enteritis.	Generalised tuberculosis.	Osteomyelitis.	Placenta prævia.
					Gluteal region.	Chest and abdominal wall.					
Total cholesterol % by colorimetric method.		—	—	—	0.18	0.19	—	(x)	—	—	0.26
M.P. of ether extract °C		36.8-37.8	30.5	27.9	28.4	29.4	32.7	22.0	29.0	21.5	27.3
Acid value	...	2.0	1.7	1.2	1.1	0.6	2.7	0.4	0.9	Nil.	Nil.
Saponification value	...	206	203.6	199.7	190.3	203	203.3	197.7	198.3	199.4	199.0
Iodine value	...	53.8	56.9	60.6	68.7	56.6	57.4	53.3(2)	61.0	67.5	65.0
Calculated olein %	...	62.5	66.1	70.4	79.7	65.7	66.8	61.9(?)	70.8	78.3	75.5
Separated fatty acids*											
M.P. °C	...	—	38.6	36.1	—	—	39.9	34.8	35.8	34.0	33.5
Neutralisation value	...	—	214.5	209.6	—	—	209.7	209.0	206.5	206.6	207.0
Mean molecular wt.	...	—	261.8	267.7	—	—	267.5	268.8	271.8	271.5	271.0
Iodine value	...	—	59.6	62.4	—	—	59.8	57.6	62.9	70.3	65.7
Calculated oleic acid %	...	—	66.2	69.3	—	—	66.5	64.0	69.9	78.1	73.0

* The separated fatty acids include the unsaponifiable matter.
† 2 portions of fat (x) and (y) both from chest and abdominal wall, each extracted and analysed separately.

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(b) *Examination of the "ether-extracted residue."*

Reference to the estimations of calcium and of total phosphorus has already been made (Tables IV. and V.).

C. *Special experiments.*

(1) The effect of added cholesterol on the melting point of an ether extract of subcutaneous fat:—

				M.P. °C.
Control fat (non-sclerematous)	...	...	...	28.4
plus 2% cholesterol	...	...	...	28.5

The addition of 2% cholesterol had no effect.

(2) The effect of added palmitin on the melting point of an ether extract of subcutaneous fat:—

		Added palmitin, as per cent. of resultant mixture.	Average M.P. °C.	Elevation of M.P. °C.
Control fat (non-sclerematous)	...	Nil	27.0	—
" " plus pure palmitin	...	3.0	35.9	8.9
" " " "	...	7.1	44.0	17.0
" " " "	...	28.4	53.7	26.7

The addition of tripalmitin had a marked effect on the melting point of the resultant mixture. Or to put this in another way the artificial reduction of the percentage of olein greatly increased the melting point.

LITERATURE.

It is convenient to give a summary of the literature on the chemistry of the subcutaneous fat under two headings: (a) Non-sclerematous patients, (b) Cases of sclerema.

(a) *Non-sclerematous patients.*

Langer⁽¹⁵⁾ noted the difference between the subcutaneous fat of infants and that of adults as regards melting point and consistency. He found that infants' fat yielded 31% solid-fatty acids, whereas the figure for adults' fat was less than 10%. He stated that a large part of the fat in the fat-cells of the infant was solid and opaque above body temperature.

Lebedeff⁽¹⁷⁾ stated that Chevreul found that the melting point of human subcutaneous fat was 20-22°C. Lebedeff analysed the subcutaneous fat from a man who died from apoplexy. He separated the liquid fatty acids by the lead-salt-ether method, and was careful to avoid oxidation by working in a current of CO₂. He found 80 and 78.6% oleic acid respectively in duplicate analyses.

Raudnitz⁽²⁰⁾ determined the melting point of the subcutaneous fat from an infant of 2 days and from a child of 2 years. In the former he found a range of 42.3 to 44.6°C. (fat of back 43.8, of chest 44.6, and of gluteal region 42.3°C.), and in the latter a range of 28.7 to 30.2°C.

Mitchell⁽²²⁾ who is often quoted in German literature, worked with perirenal and not with subcutaneous fat.

Knöpfelmacher⁽¹⁴⁾ found that the iodine value of the subcutaneous fat of a new-born child was lower than that of an adult. On the basis of analyses in seven cases (Table-VIII.) he suggested that the olein percentage of infant's fat increases until at the age of about 12 months it reaches the "adult" level. He made the interesting observation (which so far as I know has never been confirmed) that the heel fat (and palmar fat) was peculiar in that it, in contrast with the fat from the chest, back and thigh, at birth already contained the full complement of olein.

TABLE VIII.
Knöpfelmacher's results in non-sclerematous patients.

Case No.	Age.	Cause of death.	Neutral fat.			Separated fatty acids.			Calculated oleic acid per cent.	Solid fatty acids (by difference.)
			M.P. °C.	F.P. °C.	Iodine value.	M.P. °C.	F.P. °C.	Mean iodine value.		
1	New-born	—	43	38	39.05	48 (Heel)	44	40.08	49.49	55.41
2	7 weeks	Lues hereditaria	37	30	—	43	39	42.84	47.56	52.44
3	7 "	" "	34	28	—	44	37.5	50.35	55.9	41.1
4	6 months	Diphtheria ...	31	25	—	41.5	37	50.98	56.6	43.4
5	10½ "	" "	—	—	—	39	35	55.53	61.09	38.91
6	12 "	Tuberculous meningitis	—	—	—	37 (Heel)	33.5	63.75	70.77	29.23
7	17 "	Purpura hæmorrhagica	—	—	59.25	37	32	64.53	71.64	28.36

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Thiemich ⁽³⁴⁾ determined the iodine value of the fatty acids separated from the subcutaneous fat of 17 non-sclerematous infants of different ages. He confirmed the fact that very young infants' fat is poorer in olein than that of adults. He was, however, unable to confirm Knöpfelmacher's suggestion that there was a regular increase in the iodine value from birth till the age of 12 months.

Siegert⁽³¹⁾ likewise determined the iodine values of the separated fatty acids in 26 non-sclerematous infants. He combined his own results with those of Knöpfelmacher and of Thiemich. Unfortunately, however, he included the sclerema cases in his table. In Table IX. these findings are reproduced, but with omission of the sclerema results.

TABLE IX.

Non-sclerematous cases of Knöpfelmacher, Thiemich and Siegert.
Iodine value of separated fatty acids according to age.

Age.	No. of cases.*	IODINE VALUE.		
		Lowest.	Highest.	Average.
Premature. 8 months ...	2	46.8	49.4	48.1
New-born	12	38.8	49.2	43.3
1 day to 1 month	5	38.1	48.7	43.9
1 month to 2 months ...	5	38.45	51.4	46.7
2 months „ 3 „ ...	2	41.5	53.5	47.5
3 „ „ 4 „ ...	6	41.4	58.9	53.2
4 „ „ 5 „ ...	3	42.3	47.8	45.5
5 „ „ 6 „ ...	4	47.0	51.47	49.9
6 „ „ 8 „ ...	4	46.1	52.9	48.9
8 „ „ 10 „ ...	3	51.7	61.6	57.0
10 „ „ 12 „ ...	3	55.5	63.7	60.1

* Total number of cases, 49.

Siegert concluded that it could be definitely accepted that the fat of young infants contains less olein than that of adults. He pointed out that, allowing for the difficulties of analysis and the fact that the number of cases tested was small, the results for the first eight months of life were fairly uniform. Between the eighth and twelfth months the iodine value rapidly reached the adult level of about 65. He suggested that the explanation

might lie in the change from a purely milk diet to a mixed diet which usually occurs at this period of infancy. In Table X. are included the results published since Siegert's paper. It is felt that this larger group bears out his contention.

TABLE X.

Non-sclerematous cases of Knöpfelmacher, Thiemich, Siegert, Jæckle, Bardisian, Finkelstein & Sommerfeld, and Lasch.

Iodine value of separated fatty acids according to age

Age,	No. of cases.	IODINE VALUE.		
		Lowest.	Highest.	Average.
Premature. 8 months ...	2	46.8	49.4	48.1
New-born	16	36.9	49.2	42.2
1 day to 1 month	7	38.1	60.7	47.1
1 month to 2 months ...	5	38.45	51.4	46.7
2 months „ 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.—Total number of cases, 69. In the majority of instances the determination of the iodine value was made directly on the separated fatty acids. In a minority of cases the determination was made on the neutral fat (glycerides). In these latter cases the approximate corresponding value for the separated fatty acids has been calculated, and these calculated figures have been included in the above table. In the case of Lasch's results, his findings for the subcutaneous fat from the thigh or, if no analysis is given for the thigh, the results for the fat from the cheek have been taken. Case No. 8 is omitted since Lasch found an iodine value of only 22.6, whereas that of the abdominal fat of the same patient was 54.0.

Jæckle⁽¹²⁾ questioned the absolute accuracy of the methods of previous workers, but accepted the general conclusions of Siegert. He recorded his own analyses in two infants and seven adults. He found that human fat was composed essentially of the glycerides of oleic, palmitic and stearic acids, with but a mere trace of lower acids. He noted the influence of the state of nutrition on the results of analysis, and stated that the fat of man and

of other higher animals was of practically the same composition. Likewise he found the composition of lipoma fat to be very similar to that of subcutaneous fat.

Bardisian⁽³⁾ compared the composition of fat from three sources (subcutaneous, mesenteric and perirenal) in each of five children to see if there were any differences. The idea was suggested to him in view of the fact that lard from different sources varies in composition. He did find slight differences, but one is here concerned solely with his results for subcutaneous fat. Only one of his patients was under 1 year of age, and in this case only the iodine value of the neutral fat was low (Age 3 months: iodine value 50.3. Ages 19 months to $5\frac{1}{2}$ years: iodine value 60.1 to 63.2).

Finkelstein and Sommerfeld⁽⁶⁾ summarised the previous work of German investigators and added their own results in two non-sclerematous patients.

Lasch⁽¹⁶⁾ studied the composition of the fat from different parts of the body particularly in regard to the iodine value. From analyses in 16 cases of ages between birth and $10\frac{1}{2}$ months, he concluded that there was no significant difference in the iodine values of fat taken respectively from the cheek, sucking pad, thigh and abdomen.

The values for the fat constants of the subcutaneous fat of children and of adults recorded in the literature, may conveniently be summarised in the following notes:—

Neutral Fats (glycerides).

(i.) Melting point.

There is a general agreement that the melting point of the fat of newborn and very young infants is higher than that of adults, but the recorded figures do not decrease regularly with increase in age. Highest figure recorded $44.6^{\circ}\text{C}.$, in an infant 2 days' old (Raudnitz). Figures for the first six months of life vary between 44.6 and $23^{\circ}\text{C}.$ Thereafter the melting point usually lies between 30 and $19^{\circ}\text{C}.$

(ii.) Solidification point.

Again there is a general agreement as to the difference between infants and adults, with irregular levels as for the melting point. Highest figure (Knöpfelmacher) at birth $38^{\circ}\text{C}.$ Most writers regard the solidification point as too indefinite to be of any value.

(iii.) Iodine value.

From birth to the end of the eighth month the iodine value varies between 35 and 55 , though occasionally higher figures have been recorded. From the end of the eighth month to about the twelfth month the iodine value rapidly rises to the adult level. The value in adults most commonly lies between 57 and 65 , though figures as high as 73 have been recorded.

(iv.) Saponification value.

There are very few recorded analyses in children. Jæckle⁽¹²⁾ found 204.4 in each of his two infants under 1 month, and figures varying from 193.3 to 199.9 in his seven adult patients. Bardisian's⁽³⁾ figures ranged from 212 to

228 in his five children of 3 months to 5½ years. Bardisian's results are higher than those given by most authorities. An average figure for adults may be taken as 195 with a range commonly between 193 and 200.

(v.) Acid value.

The amount of free acid in human subcutaneous fat usually amounts to a mere trace. The acid value is generally less than two.

(vi.) Cholesterol.

Unna and Golodetz⁽³⁵⁾ found that the subcutaneous fat contained the following:—

Free cholesterol	...	...	...	...	0.14%
Cholesterol as esters	...	...	...	...	0.04%
Total cholesterol	...	...	...	...	0.18%

Separated fatty acids.

(i.) Melting point.

The melting point of the separated fatty acids runs parallel with, and at a slightly higher level than, the melting point of the corresponding neutral fats. The remarks made in connection with the latter apply equally to the former. The highest recorded melting point of subcutaneous fatty acids is 48°C. (Knöpfelmacher) in a new-born infant.

(ii.) Solidification point.

This lies at a slightly higher level than that of the corresponding neutral fat. The highest recorded figure is 44°C. (Knöpfelmacher) in a new-born infant.

(iii.) Iodine value.

This runs parallel with, and at a slightly higher level than, that of the corresponding neutral fat:—

Birth to end of eighth month	...	...	...	36 to 58
Ninth month to one year	...	...	...	50 to 65
Average adult level	...	...	...	65
Usual range in adults	...	...	...	62 to 70

(iv.) Neutralisation value and mean molecular weight.

No data appear to have been recorded in children or in adults.

All the available literature on analyses of the subcutaneous fat in children has been reviewed. For further accounts of similar analyses in adults, the reader is referred to the papers of Partheil and Ferié⁽²⁶⁾ and of Unna and Golodetz⁽³⁵⁾ and to the works of Abderhalden⁽¹⁾ and of Lewkowitsch and Warburton⁽¹⁸⁾. On the whole, very little work on human subcutaneous fat has been reported.

(b) *Cases of Sclerema.*

In the literature there are 18 cases of sclerema neonatorum in which chemical analyses are recorded. These are summarised in Table XI. It will be seen at a glance how meagre are the chemical data on which hypotheses as to the causation of sclerema have been based. Apart from estimations of the iodine values and of the oleic acid percentages, very little has been done.

TABLE XI.
Summary from literature of chemical analyses of subcutaneous fat in sclerema neonatorum (sclerema adiposum).

Authority.	No. of Case.	Age.	Neutral Fats (Glycerides)						Separated Fatty Acids				
			M.P. °C.	F.P. °C.	Iodine Value.	Calcd. Olein %	Acid Value.	Saponification No.	M.P. °C.	F.P. °C.	Iodine Value.	Calcd. Oleic Acid %	Estimated Oleic Acid (Lead Salt Method)
Knöpfelmacher	1	25 days	44	36	—	—	—	—	48	44	38.53	42.8	—
	2	3 weeks	43	36	—	—	—	—	48	42 (Heel fat : 60.73)	39.7	44.1 67.5)	—
Thiemich	3	1½ months	—	—	—	—	—	—	—	—	48.1	53.4	—
	4	3½ months	—	—	—	—	—	—	—	—	53.2	59.1	—
	5	3½ months	—	—	—	—	—	—	—	—	53.3	59.2	—
Siegert ...	6	Prem. 8/12	—	—	—	—	—	—	—	—	35.6	39.6	—
	7	" "	—	—	—	—	—	—	—	—	37.7	41.9	—
Miura ...	8	—	—	—	—	—	—	—	—	—	—	—	40.32* (control new-born 41.31)
Sarvonat	9	—	23	—	43	49.9	—	—	—	—	—	—	56.0*
Carpenter & Neave (°)	10	6 weeks	—	—	—	—	—	205	—	—	—	—	—
	11	Twin : 9 days	52	Instant	Instant	—	—	—	—	—	—	—	23.4
Bayer ...	12	" 15 days (Control twin 35)	70	"	"	—	—	—	—	—	—	—	24.0 (control twin 62.0)
Smith ...	13	20 days	37.5	—	44-48	51-56	163	190	—	—	—	—	—
Finkelstein u.	14	6 days ...	—	—	55	63.8	—	—	—	—	—	—	60
Sommerfeld	15	4 weeks	—	—	53	61.6	—	—	—	—	—	—	64
	16	6 weeks	—	—	60	69.7	—	—	—	—	—	—	70
	17	3 months	—	—	58	67.4	—	—	—	—	—	—	68
	18	10 months	—	—	61	70.8	—	—	—	—	—	—	73

* Method of Estimation of Oleic Acid not known because papers not available. These figures are quoted from Smith's paper.

Langer⁽¹⁵⁾ suggested that in diseases of infants a preferential oxidation of olein might occur. The resulting increase in palmitin and stearin might easily lead to a hardening of the subcutaneous fat even in the presence of hyperpyrexia. When there was a fall in body temperature the fats might readily solidify.

Knöpfelmacher⁽¹⁴⁾ believed that sclerema was due to a combination of three factors, (a) loss of fluidity of the subcutaneous fat, (b) lowering of body temperature, and (c) the high level of the solidification point of the fat of infants. He considered that sclerema was seldom possible after the second, and never possible after the sixth month. Subsequent clinical observations have proved this latter statement to be incorrect. Knöpfelmacher suggested that the freedom of the soles and palms from sclerema might be accounted for by his observation that the heel fat contained its full complement of olein already at birth. (*See* Tables VIII. and XI.)

The analyses of Thiemich⁽³⁴⁾ included observations on the iodine value of the separated fatty acids in three cases of sclerema. Thiemich found no significant differences between the iodine values of sclerematous infants and those of non-sclerematous infants of corresponding ages.

Siegert⁽³¹⁾ included two cases of sclerema in premature infants in his extensive investigation of the iodine value of the separated fatty acids of the subcutaneous fat. He pointed out that the diminished olein hypothesis of sclerema could not hold, in view of Thiemich's observations on two cases of sclerema whose ages were $3\frac{1}{2}$ months, and whose iodine values were 53.3 and 53.2 respectively (Table XI.). He further noted that the iodine value was often below 50 in patients who did not develop sclerema. Also in spite of a great loss of fluid, a low body temperature, and a low iodine value in many new-born and very young infants, the development of sclerema was exceptional. He further excluded the state of nutrition as the cause of low iodine values. Some of his very emaciated infants had just as high iodine values as some of his obese infants. Also he pointed out that it was a mere assumption that olein was more readily oxidised than stearin and palmitin in cases of rapid emaciation.

Miura⁽²³⁾ "concluded that the fat of scleremic infants did not differ materially from the fat of normal infants of the same age."

Sarvonat⁽²⁹⁾ "concluded that neither the body temperature nor the oleic acid content would explain the hardening of the tissues."

Bayer⁽⁴⁾ in a lecture maintained that sclerema was due to a pathological reduction of the content of oleic acid. He gave only a brief summary of his own work and no details of the methods he used. He found the melting point of the subcutaneous fat in sclerema very much raised (Table XI.). In fact one of his figures (M.P. 70°C.) is unique—so much so that one is tempted to question its accuracy. His figures for the oleic acid content in sclerema are very low in comparison with his control cases,

Smith⁽³²⁾ reviewed most of the previous literature, and added his own analyses in one case of sclerema. He made his determinations on the "normal" and "abnormal" fat from his case. It would seem to be impossible thus to separate with the unaided eye sclerema fat into two parts. A portion which is normal macroscopically often shows the typical changes in an earlier stage microscopically (compare remarks of Dr. McNee in histological report on Case I.). Smith did not support the diminished oleic acid hypothesis, and suggested tentatively that the changes in sclerema were similar to those in fat necrosis. Smith's acid values are extraordinarily high—so high in fact that one suspects that post-mortem hydrolysis took place.

Finkelstein and Sommerfeld⁽⁸⁾ summarised previous findings in tables, and added their own results in five cases of sclerema. They discussed previous hypotheses and concluded that most of these were negatived on clinical grounds. They suggested that sclerema was due to some change in the colloids of the subcutaneous tissues.

GENERAL REVIEW OF RESULTS.

Any hypothesis as to the pathology of sclerema neonatorum must be such as to embrace the following facts which have been found to be characteristic of the condition:—

- (1) Solid consistency of fat during life.
- (2) Histological picture of chronic inflammation with giant-cell formation and calcification.
- (3) Presence of anisotropic crystals in the frozen sections.
- (4) X-ray findings.
- (5) Excess of calcium and of phosphorus in the subcutaneous fat.
- (6) Excess of cholesterol in the subcutaneous fat.
- (7) Raised melting point of separated fat and of ether extracts of subcutaneous fat.
- (8) Iodine value and saponification value of subcutaneous fat within normal limits.

Previous work, and our own findings in the light of the above observations, may now be discussed.

The Solid Consistency of the Subcutaneous Fat.

It was suggested by Langer in 1881 that a fall in the surface temperature of the body in sclerema allowed the fats to come out of solution. Such a fall in body temperature might, he thought, be due to improper clothing or to exposure to cold air or water. This hypothesis scarcely requires discussion. In the first place a fall of more than a very few degrees in body temperature is incompatible with life. In the second place the fat of dead non-sclerematous infants (temperature that of the surroundings) does not have as solid a consistency as that of living sclerematous patients (temperature that of the body). In Langer's cases, which were of the severe type, the body temperature was subnormal. In our cases, which were of a milder type,

the body temperature was normal or raised, and yet the subcutaneous fat was of a solid consistency in patches. A number of previous investigators have discarded this hypothesis as untenable.

Practically the only other hypothesis that has been advanced was founded on the observation that the melting point of the subcutaneous fat in sclerema was higher than that of non-sclerematous infants of corresponding ages (Knöpfelmacher, Bayer). The next point for discussion therefore is the cause of this rise in the melting point of the subcutaneous fat.

In the present series of cases of sclerema the melting point of the fat was found to be raised slightly, and this, together with the presence of crystals, an excess of fibrous tissue, and the patches of calcification, can be regarded as the cause of the solid consistency of the fat during life.

The Cause of the Raised Melting Point of the Subcutaneous Fat in Sclerema.

It was definitely shown by the work of Knöpfelmacher, Thiemich and Siegert, that the subcutaneous fat of normal young infants contains less olein than that of adults. Subsequent investigations and our own analyses (Table VII.) are in agreement with this. Is the slightly raised melting point in sclerema due to a further diminution in the content of olein? The work of Langer, Knöpfelmacher and Bayer is in favour of this hypothesis, the work of Thiemich, Siegert, Miura, Sarvonat, Smith, and Finkelstein and Sommerfeld is against it.

In our own work no *significant* differences between the iodine values of sclerematous and non-sclerematous fat respectively have been found (compare Table VI. with Table VII.), but it does not follow that this necessarily disproves the diminished olein hypothesis. It is well known that the melting point of fat mixtures may be altered considerably by relatively slight variations in the composition of the mixed fats. The experiment on the addition of palmitin to non-sclerematous ether extract illustrates this forcibly. One would hesitate before attaching any significance to variations of 3% in estimations of the iodine value, yet variations of the order of 3% in the olein content might influence very appreciably the melting point. In other words an estimation of the iodine value is a relatively crude measure of the olein content. In view of the absence of any other more likely hypothesis, one is inclined to agree that the raised melting point in sclerema may be due to an increase in the proportion of fats of higher melting point.

When we started this investigation⁽³⁷⁾ it was thought that the anisotropic crystals in the frozen sections would turn out to be cholesteryl esters, and that possibly an increase in the cholesterol content of sclerema fat might explain the raised melting point. The addition of 2% of cholesterol to non-sclerematous ether extract, however, did not alter the melting point.

The Nature of the Anisotropic Crystals.

On reference to the literature, a number of statements are found as to the presence of crystals in the fat both of sclerema and of normal infants,

Thus Knöpfelmacher⁽¹⁴⁾ states that each globule of normal infant's fat contains numerous "fatty acid" crystals, and Harris, in the discussion following Lieberthal's paper⁽¹⁹⁾, refers to a case of sclerema in which "he opened a couple of areas and pressed out a mass of sebaceous-looking material containing lobules of oil which, under the microscope, was found composed of needle-shaped crystals."

A careful search of the literature has been made, but no serious attempts to determine the nature of the crystals seen in body fats has been discovered. My own findings will be discussed.

It has been shown in the section on the histochemical examination of crystals in the subcutaneous fat, that the subcutaneous fat of normal (non-sclerematous) children contains an abundance of anisotropic acicular crystals. Presumably these "normal" crystals are either stearin or palmitin. Are the sclerema crystals also composed of neutral fats?

The solubilities of the sclerema crystals exclude the possibility of their being soaps or urates. The low acid values of the ether extracts makes it unlikely that the abundant sclerema crystals are free fatty acids, though it is to be noted that the acid values of sclerematous fat are slightly higher than those of non-sclerematous fat. The behaviour of the sclerema crystals towards alkaline alcohol however (*see* Table I. and contrast with experiments on palmitic acid crystals) rules out the possibility of their being free fatty acids.

It is unlikely that the sclerema crystals are composed of cholesteryl esters for the following reasons:—(a) Microchemical tests for cholesterol were negative; (b) on carefully warming the crystals under the polarising microscope, and then cooling, no evidence of the formation of fluid crystals was ever noted; (c) the melting point of the sclerema crystals was lower than the melting point of true cholesteryl esters (*e.g.*, as seen in frozen sections of cutaneous xanthoma); (d) had the sclerema crystals been cholesterol compounds, one would have expected, owing to the large quantity of crystals seen in the sections and ether extracts, that the cholesterol content of the ether extracts would have been much higher than that actually found.

The solubility of the sclerema crystals in acetone makes it unlikely that they are phosphatides or cerebrosides. The presence of these latter compounds is also negated by the absence of liquid crystal formation ("Maltese crosses"). Also the extract, made by means of ether, in which solvent the sclerema crystals are soluble, when ashed, left no residue.

It is concluded therefore that the sclerema crystals are in all probability neutral fats—either palmitin or stearin or both, or the glycerides of higher fatty acids. The observed properties (Table I.) fit in well with this hypothesis, and the experiments with palmitin provide no evidence against it.

If this work be confirmed, it should in future be made clear that the behaviour of *crystals* in polarised light is *not* a sure method of differentiating neutral fats ("true fats") from lipoids.

Do the Sclerema Crystals Exist as such during Life?

They probably do for the following reasons:—

(a) The melting point of the sclerema crystals as seen in frozen sections exceeds 40°C.

(b) The clefts seen in the paraffin sections and the arrangement of giant cells around some of these clefts is strongly suggestive of the existence of crystals *in vivo*.

(c) Masses of crystals were observed as little white lumps at the moment of aspiration of the fluid from the cysts in Case III.

Hypothesis as to the Pathology of Sclerema.

From a consideration of the evidence presented in this paper the following hypothesis is submitted tentatively.

Sclerema neonatorum is due to a peculiar inflammatory change which occurs in the subcutaneous tissues of infants, the cause of the inflammation being unknown. The condition is closely allied to "fat necrosis."

The histological examination of our cases presents undoubted evidence of a chronic inflammatory reaction. The question is whether this inflammation is of "chemical" or of "bacterial" or of some other origin. At first, owing to the presence of crystals in the frozen sections, and the clefts in the paraffin sections, one was inclined to look to some disturbance of the chemical metabolism of fat as the primary event, and to regard the inflammatory reaction as secondary to local irritation by the crystals. In view, however, of finding so little alteration in the chemical composition of the fat and in view of the conclusion that the sclerema crystals are composed of neutral fats, one is now more inclined to the view that the inflammatory reaction was primary and the chemical changes secondary to the inflammation. At the present moment any interpretation must remain a matter of personal opinion. At a later date it is hoped that the question may be submitted to bacteriological investigation and to experimental enquiry.

It is, however, necessary to discuss whether the above hypothesis could embrace all the observed facts.

If a primary inflammation of the subcutaneous fat be assumed there is no difficulty in interpreting the rest of the findings. Here and there the inflammation has been severe enough to lead to death of tissue. This in accordance with the generally accepted pathological "law" would be followed by deposition of cholesterol and calcification, thus explaining the chemical findings of an excess of cholesterol and calcium. The calcification would account for the X-ray findings. It is not unreasonable to assume that the mobilisation of fat from the subcutaneous depôts would be affected by the presence of a chronic inflammation, thus possibly leading to a slight preferential absorption of the fats of lower melting point. As a result there would be a tendency for the higher melting point fats to crystallise out.

There would seem to be no serious objection to supposing that the giant cells appear in response to irritation by the neutral fat crystals. As has already been noted, a preferential absorption of olein might easily account for the raised melting point, and yet be so slight as not materially to alter the fat constants. The raised melting point of the fat, with the crystalline deposits, possibly in conjunction with the products of inflammation and repair (fibrous tissue, cholesterol deposits and calcification) would readily account for the solid consistency of the fat observed during life.

In adopting tentatively the above as a working hypothesis of the pathology of sclerema one has no doubt been influenced by the reports in the literature. Thus Fabry⁽⁷⁾ describes a case of "Disseminated Subcutaneous Fat Necrosis occurring in an Infant without other lesions." A study of his report leads to the conclusion that his case was one of sclerema neonatorum. Smith⁽³²⁾ refers to Fabry's case also and suggests, indirectly at any rate, that it was an example of sclerema. Smith concludes that in his own case of sclerema the changes observed were similar to those of fat necrosis. Wells⁽³⁶⁾ in his section on "Fat Necrosis" also refers to Fabry's case and notes that it may have been one of "scleroderma."

SUMMARY.

(1) Five cases of sclerema neonatorum (adiposum) are reported, with post-mortem and histological findings in four. The fifth recovered. In two cases there were cysts in the subcutaneous fat.

(2) Histologically in the four cases the subcutaneous fat showed evidence of chronic inflammation and repair, with giant-cell formation. In each there were abundant anisotropic crystals in the fat and lying free.

(3) From histochemical studies it is concluded that the anisotropic crystals consist of neutral fats (glycerides).

(4) The X-rays revealed opaque deposits. Calcification is indicated histologically, and chemically there is an excess of calcium and of phosphorus in the subcutaneous tissue.

(5) The chemistry of the subcutaneous fat has been studied in four cases—extensively in two. The melting point was raised slightly, and there was an increase in the total cholesterol, but the iodine and saponification values were similar to control cases of corresponding ages.

(6) The literature of the chemistry of the subcutaneous fat in sclerema and in non-sclerematous cases is reviewed.

(7) The pathogenesis of sclerema is discussed, and the view that sclerema is a primary inflammation of the subcutaneous tissues of unknown cause is upheld.

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