BIOCHEMICAL STUDIES IN IDIOPATHIC HYPERCALCAEMIA OF INFANCY

BY

JOHN O. FORFAR, S. L. TOMPSETT and W. FORSHALL

From the Departments of Paediatrics and Biochemistry, Edinburgh Northern Group of Hospitals, and the Departments of Child Life and Health and Clinical Chemistry, University of Edinburgh

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Part I. Serum Calcium and Cholesterol Levels from the First to the Fifteenth Month of Life

Idiopathic hypercalcaemia tends to develop between the first and fifteenth months of life and diagnosis rests primarily on biochemical findings. We have determined for this age period the normal values for serum calcium, serum cholesterol and the serum cholesterol fractions by the methods which we use.

Over a period of four years samples of blood were taken from 110 infants in hospital, or attending hospital, who required venipuncture. None was suffering from idiopathic hypercalcaemia, diabetes, nephrosis or hypothyroidism.

Methods

Serum Calcium. Initially the method of Kramer and Tisdall (1921) and Tisdall (1923) was employed in which calcium is precipitated as calcium oxalate and titrated with potassium permanganate. Two ml. of serum are required. Later Trinder's (1957) method was used in which calcium is precipitated with chloranilic acid; the precipitate is dissolved in a colour reagent consisting of ferric nitrate and sulphuric acid and the result determined colorimetrically by comparison with a similarly treated standard calcium solution. This method requires only 0.5 ml. of serum.

Serum Cholesterol. Total serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953), in which a direct colour reaction is obtained by the addition of a ferric chloride/glacial acetic acid/concentrated sulphuric acid mixture. No preliminary extraction is necessary and only 0.1 ml. of serum is required.

Free cholesterol was estimated by the method of Zak, Dickenman, White, Burnett and Chemey (1955) in which free cholesterol is precipitated from an alcohol-acetone extract with digitonin, the resulting digitonide separated and its cholesterol content estimated by the method of Zlatkis *et al.* (1953). One ml. of serum is required.

Cholesterol esters which are not precipitated by digitonin were estimated by difference.

Results

A comparison between the Kramer and Tisdall and Trinder methods of calcium estimation is given in Table 1. Both methods were applied to random

TABLE 1

COMPARISON BETWEEN KRAMER AND TISDALL AND TRINDER METHODS OF ESTIMATING SERUM CALCIUM. CARRIED OUT ON 30 RANDOM SAMPLES OF ADULT BLOOD

Serum Calcium (mg. %)	
Kramer and Tisdall	Trinder
13.9	13.2
7.7	7.6
9.1	9.2
8.3	7.6
8.8	9.1
8.7	8.8
9.0	9.0
7.1	6.6
13.3	13.4
9.4	9.2
14.5	14.5
9.4	9.8
8.8	9.2
9.4	9.5
9.9	9.4
8.9	9.0
9.0	8.8
8.8	8.8
10.2	9.9
9.0	8.5
10.5	10.3
8.6	9.0
8.8	8.7
8.2	8.5
8.7	8.5
10.1	10.1
10.1	9.9
14.5	13.7
9.1	9.0
9.2	9.2
Average 9.7	9.6

TABLE 2

SERUM CALCIUM, TOTAL CHOLESTEROL, FREE CHOLESTEROL AND CHOLESTEROL ESTER ESTIMATIONS IN 110 INFANTS AGED 1-15 MONTHS

No.	Age (mth.)	Weight (lb. oz.)	Sex	Serum Calcium (mg. %)	Total Chol. (mg. %)	Free Chol. (mg. %)	Chol. Ester (mg. %)	Free Chol. Chol. Ester	Ca × Total Chol.	Ca × Free Chol.	Ca × Chol. Ester
1	6	12.0	M	9.5	162	—	—	—	1,539	—	—
2	9	24.6	M	9.5	106	—	—	—	1,007	—	—
3	10	21.2	M	12.2	165	—	—	—	2,008	—	—
4	7	16.5	F	10.0	133	56	77	.727	1,330	560	770
5	10	14.10	M	8.3	135	35	100	.350	1,120	290	830
6	11	20.8	M	12.5	196	83	113	.735	2,450	1,037	1,412
7	11½	20.9	M	10.6	208	83	125	.664	2,204	879	1,324
8	8½	17.14	F	11.7	233	61	172	.355	2,726	713	2,010
9	9	19.0	F	—	237	67	170	.394	—	—	—
10	5	8.8	M	9.3	144	87	57	1.526	1,340	809	530
11	15	22.0	M	—	183	48	135	.356	—	—	—
12	13	18.0	M	9.8	212	81	131	.618	2,076	794	1,284
13	9	15.5	F	9.4	204	89	115	.775	1,916	837	1,081
14	5½	13.3	M	9.7	139	63	76	.824	1,348	611	737
15	4½	12.4	M	10.7	175	79	96	.823	1,874	846	1,027
16	11	18.2	M	10.2	204	100	104	.962	2,081	1,020	1,060
17	2	7.5	M	10.4	166	71	95	.748	1,725	738	988
18	6	16.12	M	10.2	179	42	137	.307	1,826	428	1,396
19	12	14.9	M	10.0	129	31	98	.316	1,290	310	980
20	6	21.4	M	—	200	52	148	.352	—	—	—
21	13	18.12	F	10.1	162	46	116	.397	1,636	464	1,170
22	12	16.7	F	10.4	157	39	118	.331	1,634	406	1,226
23	15	16.9	M	10.2	208	50	158	.316	2,120	510	1,611
24	11½	20.0	M	10.9	237	79	158	.500	2,582	861	1,722
25	6	11.14	M	11.2	174	46	128	.359	1,950	515	1,434
26	13	21.5	F	8.8	226	58	168	.345	1,988	511	1,478
27	7	14.2	M	10.0	148	38	110	.345	1,480	380	1,100
28	4	10.0	F	10.4	159	41	118	.348	1,654	436	1,227
29	2	9.14	F	10.3	222	59	163	.362	2,286	605	1,677
30	15	18.11	M	10.3	231	59	172	.343	2,376	605	1,770
31	3	8.11	M	9.5	150	38	112	.339	1,424	361	1,063
32	6	12.11	M	10.2	150	34	116	.293	1,529	347	1,182
33	12	21.11	M	10.3	211	47	164	.287	2,172	484	1,688
34	3½	13.2	F	10.9	177	79	98	.806	1,928	861	1,068
35	15	24.5	M	10.1	—	—	—	—	—	—	—
36	12	18.1	M	10.6	218	89	129	.690	2,311	943	1,366
37	9	13.8	F	10.7	258	102	156	.654	2,757	1,091	1,669
38	8	14.12	F	9.5	162	—	—	—	1,538	—	—
39	9	14.4	F	10.7	171	50	121	.413	1,828	535	1,294
40	2½	6.6	F	9.6	164	74	90	.828	1,573	710	864
41	15	24.6	M	10.4	—	—	—	—	—	—	—
42	8	17.0	F	—	289	74	215	.344	—	—	—
43	12	17.8	F	10.4	233	56	177	.316	2,421	582	1,840
44	13	18.0	F	11.0	200	—	—	—	2,200	—	—
45	5	14.0	F	10.4	144	60	84	.715	1,496	624	873
46	3½	12.3	M	11.0	154	16	138	.116	1,694	176	1,518
47	8	17.6	M	9.8	210	35	175	.200	2,056	343	1,715
48	3	6.13	F	10.1	175	21	154	.138	1,768	212	1,554
49	4	10.0	M	10.1	202	33	169	.196	2,040	333	1,705
50	7	12.14	F	9.9	202	—	—	—	2,000	—	—
51	7	16.0	F	10.0	280	73	207	.353	2,800	730	2,070
52	3	7.3	F	11.0	154	38	116	.328	1,694	418	1,276
53	10½	12.12	M	10.0	241	67	174	.385	2,410	670	1,740
54	10½	13.13	M	9.3	—	—	—	—	—	—	—
55	3	12.15	F	10.5	237	23	214	.108	2,488	241	2,246
56	9	15.4	M	9.6	183	54	129	.418	1,756	518	1,238
57	10	13.13	M	10.8	233	79	154	.513	2,515	853	1,663
58	5½	13.8	M	10.6	229	75	154	.487	2,425	795	1,632
59	3	13.0	F	10.5	229	71	158	.444	2,401	745	1,659
60	11	16.3	M	10.0	—	—	—	—	—	—	—
61	9	16.6	M	11.0	171	65	106	.613	1,881	715	1,166
62	9	11.12	M	10.4	192	—	—	—	1,996	—	—
63	8	16.4	F	9.7	217	46	171	.269	2,104	446	1,659
64	4	13.8	M	10.8	200	54	146	.370	2,160	583	1,576
65	2	10.0	F	10.2	133	—	—	—	1,356	—	—
66	3	9.8	M	9.9	183	52	131	.397	1,810	515	1,296
67	6	10.2	M	9.8	142	38	104	.366	1,392	372	1,019
68	1	8.4	F	11.5	188	56	132	.424	2,160	644	1,517
69	1	7.0	F	—	275	—	—	—	—	—	—
70	5	16.0	M	9.9	178	40	138	.290	1,762	396	1,366
71	5	14.1	M	10.0	250	56	194	.289	2,500	560	1,940
72	3	10.12	M	9.7	188	54	134	.403	1,823	523	1,299
73	1	5.12	M	9.3	204	46	158	.291	1,897	428	1,470
74	7	24.13	M	9.6	—	—	—	—	—	—	—
75	5	11.8	M	—	209	47	162	.291	—	—	—
76	6	12.6	M	10.1	149	—	—	—	1,503	—	—
77	3	10.6	F	10.9	217	50	167	.300	2,365	545	1,819
78	5	12.0	F	10.7	192	—	—	—	2,053	—	—
79	2	10.13	F	9.4	217	—	—	—	2,040	—	—
80	7	18.0	M	9.7	158	52	106	.491	1,532	504	1,027

(contd.)

TABLE 2—*contd.*

No.	Age (mth.)	Wt. (lb. oz.)	Sex	Serum Calcium (mg. %)	Total Chol. (mg. %)	Free Chol. (mg. %)	Chol. Ester (mg. %)	Free Chol. Chol. Ester	Ca × Total Chol.	Ca × Free Chol.	Ca × Chol. Ester
81	7	14·11	M	10·2	171	71	100	·710	1,743	724	1,020
82	2½	9·5	M	9·7	171	42	129	·326	1,659	407	1,250
83	8	10·6	M	8·9	160	33	127	·260	1,424	294	1,130
84	10	14·4	F	9·7	237	—	—	—	2,298	—	—
85	7	18·4	M	10·0	194	42	152	·277	1,940	420	1,520
86	13	21·0	M	9·6	258	104	154	·676	2,475	999	1,478
87	8	13·5	M	9·5	131	34	97	·351	1,244	323	922
88	2	6·8	M	8·9	177	52	125	·416	1,574	463	1,112
89	5½	11·8	F	10·1	155	83	72	1·154	1,564	838	727
90	8	11·8	F	10·3	219	76	143	·532	2,255	783	1,472
91	1	7·7	M	9·9	196	84	112	·751	1,940	832	1,108
92	6	13·2	M	9·8	196	40	156	·256	1,920	392	1,528
93	15	23·0	M	8·5	217	52	165	·316	1,844	442	1,401
94	1	8·15	M	8·9	190	65	125	·520	1,690	578	1,112
95	1½	5·12	M	9·8	196	58	138	·420	1,921	568	1,352
96	13	16·12	M	10·6	233	73	160	·457	2,368	773	1,698
97	14	23·7	M	10·2	279	—	—	—	2,845	—	—
98	9½	22·0	M	11·7	167	—	—	—	1,954	—	—
99	6	11·10	F	9·9	179	56	123	·455	1,771	554	1,217
100	13	17·0	F	9·3	175	46	129	·357	1,627	427	1,199
101	10½	18·3	F	9·3	171	—	—	—	1,590	—	—
102	4	10·3	F	9·9	218	46	172	·267	2,159	455	1,703
103	15	13·7	F	9·9	282	54	228	·237	2,794	535	2,256
104	5	11·11	F	10·7	192	63	129	·489	2,052	674	1,379
105	4	11·0	F	9·0	250	—	—	—	2,250	—	—
106	4	8·0	M	9·8	202	54	148	·365	1,978	529	1,449
107	4	7·14	F	11·1	217	68	149	·456	2,408	755	1,653
108	2	12·8	M	9·1	250	—	—	—	2,274	—	—
109	5½	18·0	M	9·8	225	50	175	·286	2,204	490	1,715
110	4	10·8	M	10·3	—	—	—	—	—	—	—

TABLE 3

SERUM CALCIUM, CHOLESTEROL, FREE CHOLESTEROL, CHOLESTEROL ESTERS, FREE CHOLESTEROL/CHOLESTEROL ESTERS RATIO, CALCIUM×CHOLESTEROL, CALCIUM×FREE CHOLESTEROL, CALCIUM×CHOLESTEROL ESTERS—MEANS, STANDARD DEVIATIONS AND RANGES

Estimation	Observations (no.)	Mean	S.D.	Range Mean±S.D. × 2 (P=0·05)	Range Mean±S.D. × 2·57 (P=0·01)
Calcium (all results)	104	10·11 mg. %	0·707	8·7–11·5 mg. %	8·3–11·9 mg. %
Calcium (Kramer and Tisdall method)	61	10·26 mg. %	0·693	8·9–11·6 mg. %	8·5–12·0 mg. %
Calcium (Trinder method)	43	9·88 mg. %	0·699	8·5–11·3 mg. %	8·2–11·6 mg. %
Total cholesterol	104	194 mg. %	38·2	118–270 mg. %	96–292 mg. %
Free cholesterol	86	57 mg. %	18·6	20–94 mg. %	9–105 mg. %
Cholesterol esters	86	137 mg. %	33·3	70–204 mg. %	51–223 mg. %
Free cholesterol/cholesterol esters ratio	86	0·453	0·229	0–0·9	0–1·0
Calcium × total cholesterol	98	1,947	403	1,141–2,753	914–2,980
Calcium × free cholesterol	81	583	205	173–993	56–1,110
Calcium × cholesterol esters	81	1,374	348	678–2,070	481–2,267

samples of blood from adult patients. No significant difference was observed in the results obtained by the two methods.

Table 2 gives the results of the estimation of serum calcium, cholesterol, free cholesterol and cholesterol esters in 110 infants aged 1 to 15 months. In each infant these estimations were carried out on the same specimen of blood. In the first 65 infants calcium was estimated by the Kramer and Tisdall method, thereafter by the Trinder method. The Table also gives the age, weight and sex of the infants and the arithmetical products of calcium and total cholesterol, calcium and free cholesterol and calcium and

cholesterol esters. These products are included in view of the suggestion that they might be of value in the diagnosis of idiopathic hypercalcaemia of infancy (Forfar, Balf, Maxwell and Tompsett, 1956).

There is no significant sex difference between serum calcium, total cholesterol, free cholesterol and cholesterol esters levels.

Table 3 gives means, standard deviations and ranges for calcium, total cholesterol, free cholesterol, cholesterol esters, free cholesterol/cholesterol esters ratio, calcium×cholesterol, calcium×free cholesterol and calcium×cholesterol esters.

TABLE 4
CORRELATIONS

Correlation	Observations (no.)	Coefficient of Correlations (r)	P
Age: calcium	104	+0.023	0.81
Age: cholesterol	104	+0.116	0.075
Age: free cholesterol	86	+0.181	0.095
Age: cholesterol esters	86	+0.178	0.10
Age: free cholesterol/ cholesterol esters ratio	86	-0.021	0.85
Calcium: cholesterol	98	+0.081	0.42
Calcium: free cholesterol	81	+0.199	0.075
Calcium: cholesterol esters	81	+0.044	0.69
Calcium: free cholesterol/ cholesterol esters ratio	86	-0.082	0.45
Free cholesterol: cholesterol esters	86	-0.126	0.245

In Table 4 correlations have been calculated between age, calcium and the various cholesterol fractions.

Discussion

Serum calcium levels quoted by different authors show some variation according to the method used. There is general agreement that the level tends to be higher in infancy than in later life. Jaffe and Bodansky (1943) give a range for infancy (excluding the first week of life) of 10.5–12.0 mg./100 ml. By the two methods we used we found a somewhat wider range but a similar upper limit of normal.

Although there was no significant difference in the results obtained by the Kramer and Tisdall and the Trinder methods when both were carried out on random samples of adult blood it will be observed (Table 3) that at the idiopathic hypercalcaemia age period the 61 results obtained by the Kramer and Tisdall method gave a slightly higher average result (10.3 mg./100 ml.) than the 43 results obtained by the Trinder method (9.9 mg./100 ml.). The difference is significant ($P=0.01$).

Serum cholesterol levels in the first year of life have been estimated by Hodges, Sperry and Andersen (1943) and by Rafstedt (1955). The former found a mean level of 200 mg./100 ml. for the age period 2–6 months and 207 mg./100 ml. for the period 7–12 months. Their results were thus similar to ours, as too was the range which they observed. Rafstedt (1955) for the period 1–12 months found a mean total cholesterol level of 130 mg./100 ml. with a range of 69–173 mg./100 ml. Rafstedt's results for free and combined cholesterol in infancy were also lower than ours, the mean levels being 40 mg./100 ml. (range 27–66 mg./100 ml.) for free cholesterol and 90 mg./100 ml. (range 51–132 mg./100 ml.) for cholesterol esters. He found the mean free cholesterol/cholesterol ester ratio between 1–12 months to be 0.45, a figure almost identical with ours. His range was narrower (0.31–0.77) but also revealed

the considerable variation in the ratio which we found.

As regards the correlations shown in Table 4 it will be observed that none is significant. The probability value of three of these correlations is less than 0.1, however, i.e. age: cholesterol, age: free cholesterol and calcium: free cholesterol. It is possible that with a larger number of estimations these correlations might have become significant. Positive correlation between age and cholesterol would be in keeping with the findings of György (1926) and Rafstedt (1955) (who in childhood observed a rise in total serum cholesterol with increasing age) but not with those of Hodges *et al.* (1943). So far as we are aware it has never been suggested that there might normally be a correlation between serum calcium and serum free cholesterol levels at this age.

Summary

A group of 110 infants aged 1–15 months has been examined.

Estimations of the serum calcium level (using two different methods) showed a range of 8.5–12.0 mg./100 ml. A difference was observed in the mean results obtained by the two methods.

Total serum cholesterol, free cholesterol and cholesterol esters were estimated and mean levels and ranges obtained.

Free cholesterol/cholesterol esters ratios, calcium $\times$ total cholesterol, calcium $\times$ free cholesterol and calcium $\times$ cholesterol esters products, and correlations between age and the cholesterol fractions, calcium and the cholesterol fractions and between the cholesterol fractions themselves have been calculated.

Part II. Serum Calcium and Cholesterol Levels in Idiopathic Hypercalcaemia of Infancy

Idiopathic hypercalcaemia of infancy presents the paediatrician with a particular problem in management and therapy. To him as well as to the biochemist, to those interested in calcium and cholesterol metabolism and to the nutritionist, the disease has even wider implications not least in its possible relationship to vitamin D activity and the light which it may shed on the ill-understood metabolism of this vitamin. As the incidence of idiopathic hypercalcaemia appears to be waning, opportunities for studying the disturbed biochemical relationships which occur may be diminishing.

It is generally accepted, and implied in the title, that the serum calcium level is raised in idiopathic hypercalcaemia. A raised blood cholesterol level

TABLE 1

RESULTS OF SERUM CALCIUM AND CHOLESTEROL ESTIMATIONS CARRIED OUT ON 19 INFANTS IN THE NATURAL STATE OF IDIOPATHIC HYPERCALCAEMIA

Case	Calcium (mg. %)	Chol. (mg. %)	Free Chol. (mg. %)	Chol. Esters (mg. %)	Free Chol. Chol. Ester
1	15.6	281	—	—	—
	10.8	217	—	—	—
	10.4	167	—	—	—
	—	—	—	—	—
2	12.2	306	—	—	—
	11.4	184	106	78	1.36
	11.9	186	—	—	—
	10.4	197	—	—	—
3	11.2	265	—	—	—
	10.0	194	—	—	—
	10.3	198	52	146	.357
	—	—	—	—	—
4	16.2	243	—	—	—
	15.5	238	141	97	1.454
	12.3	145	—	—	—
	12.5	202	84	118	.712
	14.1	262	127	135	.942
	14.6	256	—	—	—
	13.3	237	—	—	—
	10.2	229	100	129	.776
5	12.1	249	—	—	—
	13.5	213	123	90	1.367
	13.6	158	—	—	—
	10.8	179	75	104	.722
	11.0	196	50	146	.343
	10.6	155	38	117	.325
6	14.8	216	152	64	2.375
	14.0	206	112	94	1.192
	12.9	175	102	73	1.399
	10.7	161	39	122	.320
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—
7	12.5	217	100	117	.855
	11.9	133	37	96	.386
	10.8	126	29	97	.299
	10.6	183	46	137	.336
	13.6	165	41	124	.331
	11.0	230	46	184	.250
	11.0	168	52	116	.449
9	11.0	251	124	127	.997
	11.7	316	126	190	.664
	12.0	370	135	235	.575
	10.7	320	102	218	.468
	10.2	292	28	264	.106
	10.7	229	75	154	.487
10	11.8	267	—	—	—
11	13.0	245	91	154	.591
	11.8	179	67	112	.599
	10.8	188	61	127	.481
	10.4	217	56	161	.348
	12.6	254	100	154	.650
12	9.5	198	75	123	.610
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—
13	12.8	229	60	169	.355
	11.1	229	58	171	.339
	10.7	176	63	113	.557
	10.2	225	54	171	.316
	11.9	238	50	188	.266
	12.9	233	104	129	.807
14	12.0	320	130	190	.684
15	12.2	250	54	196	.276
	10.6	192	58	134	.433
	14.1	200	52	148	.352
	12.8	292	188	104	1.810
	11.0	158	42	116	.362
16	13.4	210	63	147	.428
	—	—	—	—	—
	—	—	—	—	—
	—	—	—	—	—
17	13.8	307	—	—	—
	17.5	270	—	—	—
	9.9	160	60	100	.666
	10.3	130	—	—	—

(contd.)

TABLE 1—contd.

Case	Calcium (mg. %)	Chol. (mg. %)	Free Chol. (mg. %)	Chol. Esters (mg. %)	Free Chol. Chol. Ester
17	12.7	197	59	138	.429
	10.9	116	36	80	.450
	15.2	181	45	136	.331
	12.4	242	71	171	.415
	12.0	200	50	150	.333
18	12.2	233	—	—	—
	11.4	202	55	147	.375
	10.4	221	113	108	1.046
19	15.3	285	85	200	.425
	15.1	257	83	174	.477
20	11.8	250	73	177	.413
	9.6	240	55	185	.298
Mean	12.08	219.4	76.4	140.6	.612
Normal mean	10.1	194	57	137	.453

has also been a frequent finding (Lowe, Henderson, Park and McGreal, 1954; Creery and Neill, 1954; Dawson, Craig and Perera, 1954; Macdonald and Stapleton, 1955; Forfar *et al.*, 1956).

We have studied the relationship between calcium and cholesterol in the blood in 20 infants with idiopathic hypercalcaemia, and in addition have sought to establish certain diagnostic criteria based on calcium and cholesterol levels.

Serum Calcium, Serum Cholesterol and Serum Cholesterol Fractions

Forms of treatment such as a low calcium diet and steroid therapy can significantly lower the serum calcium level in idiopathic hypercalcaemia and may have other biochemical effects. In studying the biochemical relations pertaining in the 'natural' state of the disease therefore it is necessary to exclude estimations made under any treatment which might modify these.

Table 1 gives the results in 19 cases, of serum calcium, serum cholesterol, serum free cholesterol and serum cholesterol esters estimations carried out (on the same specimen of blood) in the 'natural' state of the disease. Any treatment given had been discontinued for at least a week before the specimens were taken.

There is, as expected, a significant rise in the mean serum calcium level and in addition a significant rise in the total cholesterol and free cholesterol levels. The cholesterol esters level, on the other hand, shows no change from normal. The rise in total cholesterol is thus determined by the rise in its free cholesterol component.

The correlation co-efficients between calcium and cholesterol fractions have been calculated as shown in Table 2. There is a significant positive correlation

TABLE 2
CORRELATIONS

Correlation	Observations (no.)	Coefficient of Correlation (r)	P
Calcium: total cholesterol ..	75	+ .280	0.015
Calcium: free cholesterol ..	57	+ .361	>0.01
Calcium: cholesterol esters ..	57	- .118	0.37
Free cholesterol: cholesterol esters ..	57	- .150	0.26
Calcium: free cholesterol/cholesterol esters ratio ..	57	+ .405	>0.01

between calcium and both total cholesterol and free cholesterol levels; also between calcium and the free cholesterol/cholesterol esters ratio. There is no significant correlation between calcium and cholesterol esters or between free cholesterol and cholesterol esters. The critical correlation therefore is that between calcium and free cholesterol.

Calcium and Cholesterol Levels in Diagnosis

The diagnosis of idiopathic hypercalcaemia of infancy is based on clinical, radiological and especially biochemical findings. By the methods employed in our laboratory the upper limits during the age period 1-15 months for various biochemical estimations are as follows (Part I, Table 3):

Estimation	Mean + S.D. $\times 2.57$	Mean + S.D. $\times 2$
Serum calcium	12.0 mg. %	(11.6 mg. %)
Serum cholesterol	292 mg. %	(270 mg. %)
Serum free cholesterol	105 mg. %	(94 mg. %)
Serum cholesterol esters	223 mg. %	(204 mg. %)
Calcium $\times$ cholesterol	2,980	(2,753)
Calcium $\times$ free cholesterol	1,110	(993)

It has been suggested previously (Forfar *et al.*, 1956) that as both serum calcium and cholesterol tend to be raised in idiopathic hypercalcaemia the use of their product might be of value in diagnosis where neither is in itself raised above the upper limit of normal. In addition to the basic estimations of calcium and cholesterol we have used these products as diagnostic criteria as shown in Table 3.

This Table presents the results of simultaneous (on the same specimen of blood) serum calcium, cholesterol and free cholesterol estimations carried out on 131 blood samples at all stages of the disease. It also gives the highest serum calcium level obtained at any time.

In 15 of the 20 cases there would be no doubt on biochemical grounds of the diagnosis of idiopathic hypercalcaemia. Stapleton and Evans (1955) have put the diagnostic level at above 12.5 mg./100 ml.

These fifteen cases show serum calcium levels greater than this. Among them it will be observed that in Cases 2 and 5 the serum free cholesterol level and the calcium $\times$ free cholesterol product were both raised at a time when the serum calcium level was within normal limits, and in Case 19 that total cholesterol and the calcium $\times$ cholesterol product on one occasion, and the free cholesterol and calcium $\times$ free cholesterol product on another were raised in the presence of a normal calcium level. Thus the cholesterol and free cholesterol levels and/or their products with calcium may be diagnostic when the serum calcium level itself is not significantly raised.

Turning to the other five cases it will be observed that in two (Cases 10 and 18) the highest serum calcium level was greater than 12.0 mg./100 ml., i.e. above our upper limit of normal, and that in addition the calcium $\times$ cholesterol product in Case 10 and the free cholesterol level and calcium $\times$ free cholesterol product in Case 18 were above normal.

In two of the remaining three cases the highest serum calcium level reached was 12.0 mg./100 ml. In both of these (Cases 9 and 13) the serum cholesterol level, serum free cholesterol, calcium $\times$ cholesterol product and calcium $\times$ free cholesterol product were raised.

The remaining case is Case 3 whose highest serum calcium level was 11.2 mg./100 ml. His calcium $\times$ cholesterol product reached 2,968, practically the upper limit of normal. He suffered from an illness which, although comparatively short-lived and mild, was clinically suggestive of idiopathic hypercalcaemia and he was the identical twin of Case 2, who undoubtedly suffered from this disease. His serum calcium fell as he recovered.

Serum cholesterol, serum free cholesterol and their products with serum calcium are thus of value in the diagnosis of idiopathic hypercalcaemia where the calcium level itself does not establish diagnosis or on occasion when the serum calcium level has been lowered by treatment (as indicated in Table 3). With a low calcium diet, for instance, the serum calcium level may fall quickly but cholesterol and free cholesterol and their products with calcium may still remain elevated. A raised total or free cholesterol level is also confirmatory of a raised calcium level in this disease.

Of the 131 blood samples taken, 59 were positive in respect of one or more of the five biochemical criteria, yet in 14 (24%) of these positive samples the serum calcium level was not above normal. In four, total cholesterol, free cholesterol, calcium $\times$ cholesterol and calcium $\times$ free cholesterol conferred positivity; in one, free cholesterol, calcium $\times$ cholesterol and calcium $\times$ free cholesterol; in two,

TABLE 3

CALCIUM, CHOLESTEROL AND FREE CHOLESTEROL ESTIMATIONS FOR 131 BLOOD SAMPLES FROM 20 CASES OF IDIOPATHIC HYPERCALCAEMIA

Case (Highest Serum Calcium (mg. %))	Serum Calcium (mg. %)	Serum Cholesterol (mg. %)	Serum Free Cholesterol (mg. %)	Calcium × Cholesterol	Calcium × Free Cholesterol	Treatment
1 (16.4)	15.6 10.8 10.4	281 217 167	— — —	4,384 2,344 1,736	— — —	
2 (15.6)	12.2 11.4 11.9 11.5 15.1 10.4 11.4	306 184 186 156 206 197 204	— 106 — — — — 52	3,732 2,095 2,212 1,793 3,110 2,049 2,324	— 1,207 — — — — 593	
3 (11.2)	11.2 10.0 10.3	265 194 198	— — 52	2,968 1,940 2,039	— — 534	
4 (16.2)	16.2 15.5 12.3 13.4 15.5 12.5 14.1 14.6 10.7 13.3 10.2	243 238 145 226 261 202 262 256 197 237 229	— 141 — — — 84 127 — — — 100	3,936 3,687 1,783 3,026 4,047 2,526 3,692 3,735 2,107 3,151 2,334	— 2,185 — — — 1,050 1,791 — — — 1,020	Cort.
5 (13.6)	12.1 13.5 13.6 9.8 10.8 11.4 9.8 11.0 10.6	249 213 158 155 179 206 260 196 155	— 123 — 71 75 141 136 50 38	3,013 2,876 2,148 1,520 1,932 2,346 2,546 2,156 1,643	— 1,661 — 696 810 1,608 1,333 550 404	Pred. LCD LCD LCD
6 (14.8)	14.8 14.0 12.9 12.2 10.0 9.7 9.8 10.5 9.7 10.6 10.1 10.7	216 206 175 171 196 135 150 158 162 166 240 161	152 112 102 85 54 45 50 50 46 30 38 39	3,198 2,881 2,257 2,085 1,960 1,310 1,470 1,660 1,570 1,759 2,423 1,722	2,250 1,567 1,315 1,037 540 437 490 525 446 318 384 417	LCD LCD + Pred. LCD LCD + Pred. LCD + Pred.
7 (13.6)	12.5 11.9 10.8 10.6 10.6 13.6 11.0 11.0	217 133 126 192 183 165 230 168	100 37 29 50 46 41 46 52	2,711 1,582 1,361 2,035 1,939 2,243 2,530 1,847	1,250 440 313 530 487 557 506 572	Pred.
8 (17.0)	15.1 10.9	247 172	88 86	3,728 1,874	1,328 937	LCD LCD
9 (12.0)	11.0 11.7 12.0 10.7 10.2 10.4 9.9 10.7 11.4 9.6 10.3	251 316 370 320 292 283 254 229 286 342 272	124 126 135 102 28 58 81 75 142 131 73	2,760 3,700 4,438 3,424 2,975 2,941 2,515 2,448 3,259 3,282 2,832	1,364 1,475 1,620 1,091 286 603 802 802 1,618 1,257 741	LCD + Cort. LCD + Cort. LCD LCD LCD LCD
10 (12.2)	11.8 10.4	267 250	— 60	3,150 2,600	— 624	LCD

(contd.)

TABLE 3—*contd.*

Case (Highest Serum Calcium mg. %)	Serum Calcium (mg. %)	Serum Cholesterol (mg. %)	Serum Free Cholesterol (mg. %)	Calcium × Cholesterol	Calcium × Free Cholesterol	Treatment
11 (13.0)	13.0 11.8 10.8 10.4 12.6 10.0 9.5	245 179 188 217 254 276 198	91 67 61 56 100 83 75	3,184 2,112 2,029 2,257 3,198 2,760 1,882	1,182 791 659 582 1,260 830 713	LCD
12 (12.9)	12.8 11.1 10.7 10.2 11.9 10.4 12.9	229 229 176 225 236 267 233	60 58 63 54 50 38 104	2,928 2,540 1,882 2,295 2,832 2,777 3,001	768 644 674 551 595 395 1,340	
13 (12.0)	12.0 10.1	320 242	130 46	3,840 2,443	1,560 464	LCD
14 (14.1)	12.2 10.6 14.1 12.8 11.0 10.5	250 192 200 292 158 198	54 58 52 188 42 94	3,050 2,034 2,820 3,738 1,738 2,078	659 615 733 2,406 464 987	LCD
15 (13.4)	13.4	210	63	2,812	844	
16 (17.5)	13.8 17.5 11.4 11.4 11.5 15.8 16.6 14.9 16.6 12.3 12.0 10.2 9.9 10.3	307 270 220 156 146 160 163 150 140 156 138 190 160 130	— — — — 50 — 63 50 45 61 50 55 60 —	4,232 4,722 2,507 1,778 1,676 2,527 2,704 2,234 2,324 1,919 1,654 1,936 1,584 1,337	— — — — 575 — 1,045 745 747 750 600 561 594 —	LCD LCD LCD LCD LCD LCD LCD
17 (15.2)	12.7 10.9 15.2 12.4 12.0 10.9 10.4 10.3 10.1	197 116 181 242 200 174 192 225 232	59 36 45 71 50 — 50 96 98	2,501 1,263 2,748 3,000 2,400 1,896 1,996 2,317 2,343	749 392 684 880 600 — 520 989 990	LCD LCD LCD LCD
18 (12.2)	12.2 11.4 10.4	233 202 221	— 55 113	2,840 2,302 2,297	— 627 1,175	
19 (15.3)	15.3 15.1 12.1 9.4 12.0 12.1 11.2 12.0 10.8 11.2 10.0 11.0	285 257 178 283 215 207 172 320 234 250 228 —	85 83 50 158 50 45 36 — 68 53 65 78	4,358 3,880 2,154 2,660 2,580 2,503 1,925 3,840 2,525 2,800 2,280 —	1,299 1,252 605 1,484 600 545 403 — 734 593 650 858	LCD LCD LCD LCD + Pred. LCD + Pred. LCD LCD LCD LCD
20 (13.4)	11.8 9.6	250 240	73 55	2,950 2,302	862 528	

LCD=low calcium diet; Cort.=cortisone; Pred.=prednisolone

cholesterol and calcium × cholesterol; in six, free cholesterol and calcium × free cholesterol, and in one, calcium × cholesterol alone.

We have purposely set our upper limits of normal high at mean + standard deviation × 2.57 (P=0.01).

We may in fact have set the limit too high. Had we used the customary upper limit of normal, mean + standard deviation × 2 (as also given above), a considerably greater number of values in Table 3 would have been diagnostic.

Discussion

One of the most fascinating aspects of idiopathic hypercalcaemia concerns aetiology and possible relationship to vitamin D administration. Many reports testify that the dosage of vitamin D has often been no greater than that considered optimal and seldom greater than the average dosage received by the vast majority of healthy infants. The dosage has been in no way comparable to that known to cause frank vitamin D poisoning. Lacking evidence of gross overdosage the concept of undue hypersensitivity to vitamin D has been put forward by Lightwood (1952) and supported by Lowe *et al.* (1954), Bonham Carter, Dent, Fowler and Harper (1955) and Morgan, Mitchell, Stowers and Thomson (1956). On the other hand, administration of increased amounts of vitamin D to infants suffering from idiopathic hypercalcaemia may not cause any worsening or significant biochemical change (Creery and Neill, 1954; Forfar *et al.*, 1956).

Vitamin D poisoning in the adult may cause hypercholesterolaemia but the scanty evidence available on vitamin D poisoning in infancy suggests that at that age period it does not do so. Fanconi and de Chastonay (1950) found that hypocholesterolaemia occurred in three infants aged 9½, 6 and 11 months who had received grossly excessive dosages of vitamin D, but hypercholesterolaemia occurred in a 4½-year-old child in similar circumstances. These authors comment particularly on this age distinction. Jelke (1946) found no hypercholesterolaemia in vitamin D poisoning in an infant.

In view of the apparently increased vitamin D-like activity in idiopathic hypercalcaemia and the fact that some cholesterol derivatives have a vitamin D-like effect (e.g. vitamin D₃ is derived from 7-dehydrocholesterol), it has been suggested that this disease may be related primarily to a disorder of cholesterol metabolism with excess production of a cholesterol derivative with a vitamin D-like effect (Forfar *et al.*, 1956). The recent work of Fellers and Schwartz (1958) has lent some support to this view. They estimated the vitamin D-like activity in two severe cases of idiopathic hypercalcaemia by bio-assay methods and found increased activity which persisted unchanged for periods up to 17 months after all exogenous vitamin D had been withdrawn. The degree of activity was greater than that found in two children receiving 100,000 units of vitamin D daily.

Whether the synthesis or storage of a provitamin can occur in the human is uncertain but it can certainly occur in animals. Glover, Glover and Morton (1952) have shown that provitamin D₃ can be found in the tissues of certain animals even when the animals are fed on a practically sterol-free diet.

They concluded that the animal could synthesize provitamin D from cholesterol. In the guinea-pig infection of the liver with *Pasteurella pseudotuberculosis* was found to be associated with an increased concentration of provitamin in the intestinal mucosa, an interesting observation in view of the possible role of infection in idiopathic hypercalcaemia.

While activation of vitamin precursors, such as 7-dehydrocholesterol and ergosterol, is readily effected by irradiation, chemical activation is theoretically possible. Raoul, Le Boulch, Baron, Bazier and Guerilot-Vinet (1956) showed that cholesterol can be transformed to antirachitic compounds by a variety of natural earth, floridin. It has been suggested by Forfar (1958) that calciferol may act as a chemical activator in this disease; that apart from its own vitamin D effect calciferol may activate endogenous vitamin D precursors present in excess. A hypothesis of this sort would explain many of the features of idiopathic hypercalcaemia of infancy. Infants with an increased formation of endogenous vitamin D precursor would be those susceptible. Primarily, activation of that vitamin precursor would cause the disease. The dosage of exogenous vitamin D required to do this may not be large and dosage in excess of the activating amount will presumably merely aggravate the disease. This concept explains the comparatively small intake of vitamin D with which idiopathic hypercalcaemia may be associated and also the absence of a direct quantitative relationship between intake of vitamin D and severity of the disease. This theory could also explain the prolonged vitamin D-like activity which frequently persists in idiopathic hypercalcaemia after withdrawal of all vitamin D. As a corollary, variations in endogenous vitamin D precursor formation might explain the varying vitamin D needs in infancy to which Follis, Park and Jackson (1953) have drawn attention. Infants with low endogenous formation would be those liable to develop rickets, infants with high formation those requiring little or no exogenous vitamin D. It may be not without relevance that hypothyroid children, in whom there is also a disorder of cholesterol metabolism with a tendency to hypercholesterolaemia, are peculiarly resistant to rickets and peculiarly sensitive to vitamin D (Fanconi, 1956). If indeed idiopathic hypercalcaemia is associated with the use of the synthetic substance calciferol, as opposed to natural vitamin D, the advisability of using calciferol in place of natural vitamin may need further consideration.

It is doubtful whether idiopathic hypercalcaemia exists in two distinct types, the severe and the mild, or whether the difference is merely one of degree.

Reviewing the literature, Daeschner and Daeschner (1957) reported that the average interval between the time of onset of symptoms and diagnosis in the severe type of the disease was 13 months. In 52 reported cases of the mild type we have found that the corresponding average interval was less than six months. It seems possible that the severe type of the disease results essentially from delay in diagnosis. The use of total cholesterol and free cholesterol estimations in diagnosis in the manner outlined above may allow earlier positive recognition of the disease and the earlier institution of treatment and may allow recognition where diagnosis cannot be made on the basis of serum calcium estimations alone. Earlier diagnosis and treatment will presumably reduce the risks of mental retardation and permanent renal damage which accompany this disease. The use of the cholesterol factors may also be of some value in assessing progress during treatment.

Summary

An assessment has been made of the calcium, cholesterol, free cholesterol and cholesterol esters fractions of serum in 19 cases of idiopathic hypercalcaemia examined in the 'natural' (untreated) state of the disease.

Serum calcium, cholesterol and free cholesterol levels are significantly raised in the disease. Serum cholesterol esters level is not raised.

Calculation of correlation coefficients shows that there is a significant positive correlation between serum calcium, cholesterol and free cholesterol levels. The critical correlation is that between serum calcium and serum free cholesterol.

The results of simultaneous estimations of serum calcium, cholesterol and free cholesterol at all stages of the disease are presented. The role of the cholesterol fractions on their own and as a product with serum calcium in increasing the accuracy of diagnosis in idiopathic hypercalcaemia is indicated.

Disturbances of cholesterol metabolism are discussed in the light of the possible relationship of the condition to vitamin D, and an aetiological hypothesis is elaborated.

Part III. Blood Citrate Levels and Urinary Citrate Output in Idiopathic Hypercalcaemia of Infancy

Citrate occurs in high concentration in bone, probably 50–100 times the concentration found in other tissues (Dickens, 1941; Dixon, 1956). The general consensus of opinion is that citrate is actively involved in the metabolism of bone. Harrison (1954), for instance, considers that the serum citrate

level may be related to the activity of those metabolic processes in skeleton which actively destroy bone matrix and dissolve bone salts. Armstrong and Singer (1956), on the other hand, consider that the concentration of citrate in bone may be merely adventitious, body citrate being attracted to bone in virtue of the peculiar chemical and physical character of the latter.

We have made observations on the serum citrate levels and urinary citrate output at various stages of idiopathic hypercalcaemia. In addition we have assessed the normal serum citrate level in a group of 32 unaffected infants aged 2–15 months.

Methods

The method employed for the determination of citrate in serum and urine was predominantly that of Taylor (1953). Untreated urine and trichloroacetic extract of serum were used. Citrate was converted into pentabromoacetone (bromide/bromate/vanadate reagent plus sulphuric acid). After neutralization of the reaction mixture with ferrous sulphate, the pentabromoacetone was extracted with petroleum ether. A yellow colour was produced in the presence of a solution of borax and thiourea (Snell and Snell, 1953), the final estimation being made colorimetrically. Urine for citrate analysis was collected in a bottle containing dilute sulphuric acid to prevent loss of citrate.

Serum calcium was determined by the method of Trinder (1957).

Results

Serum Citrate Levels. The results of 32 serum citrate estimations carried out on controls, aged 2–15 months, are shown in Table 1. Serum calcium was estimated coincidentally with serum citrate. The mean serum citrate level was 2.4 mg./100 ml. and the range (mean $\pm$ S.D. $\times 2$) 1.2–3.5 mg./100 ml. For the age range 2 months–2 years Harrison and Harrison (1952) found the mean serum citrate level to be 2.5 mg./100 ml. with range 2.0–3.4 mg./100 ml. There is no evidence of correlation between calcium and citrate levels (correlation coefficient = -0.04).

The results of 28 coincident estimations of serum calcium and serum citrate at various stages of the disease in 11 cases of idiopathic hypercalcaemia in the 'natural' (see Part II) state are shown in Table 2. It will be seen that the mean citrate level is not significantly different from normal. The range however is wider than normal, being (mean $\pm$ S.D. $\times 2$) 0.6–4.8 mg./100 ml. Study of individual cases (the levels are in chronological order for individual cases) suggests that the serum citrate level is low in the acute phase of the disease and high in the recovery phase. Indeed the mean serum citrate level on the 10 occasions when the serum calcium was 11.6 mg./

TABLE 1
SERUM CALCIUM AND CITRATE LEVELS IN 32 CONTROL
INFANTS AGED 2-15 MONTHS

Age (mth.)	Serum Calcium (mg. %)	Serum Citrate (mg. %)
9	9.4	2.0
5½	9.7	1.8
12	10.4	2.4
15	10.2	2.8
6	11.2	2.8
13	8.8	2.4
7	10.0	3.5
4	10.4	2.0
2	10.3	2.1
15	10.3	1.8
3	9.5	2.3
6	10.2	2.0
12	10.3	2.1
3½	10.9	2.1
15	10.1	2.0
12	10.6	2.4
9	10.7	2.6
8	9.5	1.6
9	10.7	3.0
2	10.2	2.2
7	9.6	3.4
5	10.7	2.5
2	9.4	0.8
8	8.9	3.3
13	9.6	3.4
5½	10.1	2.5
8	10.3	1.9
6	9.9	2.5
13	9.3	1.6
4	9.9	2.3
4	9.8	2.9
4	11.1	1.6
Mean	10.1	2.35

TABLE 2
SERUM CALCIUM AND CITRATE LEVELS IN IDIOPATHIC
HYPERCALCAEMIA

Serum Calcium (mg. %)	Serum Citrate (mg. %)
11.4	2.5
10.3	3.1
12.0	1.8
11.6	2.4
13.5	0.7
10.8	1.8
11.0	3.3
10.6	3.7
14.8	0.25
14.0	2.8
12.9	1.3
10.1	4.7
10.7	2.9
12.0	1.9
11.9	2.3
10.8	2.1
10.6	3.5
13.6	2.4
11.0	3.0
11.0	3.0
11.4	2.6
9.6	4.4
10.3	3.4
10.6	2.7
12.4	2.5
10.4	2.4
9.6	5.4
10.0	2.5
Mean 11.4	2.7

100 ml. or above (we have taken 11.6 mg./100 ml. as this is the upper limit of normal for the method of calcium estimation we were using) was 1.8, and on the 18 occasions when the serum calcium level was below 11.6 mg./100 ml. it was 3.1 mg./100 ml.

The correlation coefficient between serum calcium and serum citrate levels for the results shown in Table 2 is -0.732 ($P > 0.01$). There is thus a highly significant negative correlation between calcium and citrate levels in idiopathic hypercalcaemia.

In Fig. 1 the results of all serum citrate levels in 14 cases before, during and after treatment have been charted. Points plotted along the time abscissa have been dated from time of admission to hospital with the object of indicating the pattern of citrate level during the course of the disease. As the early cases were treated less efficiently than the later ones and as some cases were already undergoing some spontaneous improvement by the time of admission to hospital, this method of representing the course of the disease is of necessity somewhat artificial. It does again suggest, however, that the serum citrate level tends to be low in the early stages of the disease and rises with improvement. Recovery indeed seems to be associated with a rise up to or beyond the upper limit of normal with a later fall to average levels.

Urinary Citrate. Seventeen estimations of urinary

citrate were made at various stages of the disease on seven patients. These are charted in Fig. 2, indicating that urinary citrate was low in the acute stage of the disease, rose with recovery and later tended to fall. We have taken the lower limit of normal to be in the region of 50 mg./24 hr.

Discussion

Much of our knowledge of citrate metabolism has been derived from animal experiments. It has been established that levels are low in rickets and that citrate will promote healing, that the administration of vitamin D to rats receiving normal or rachitogenic diets increases tissue citrate content including that of blood and that concurrent elevations of serum calcium and serum citrate levels occur under the influence of vitamin D (Armstrong and Singer, 1956). The level of calcium in the diet also appears to have some influence on the citrate content of bones, higher citrate concentrations being observed with high calcium diets as opposed to calcium-deficient diets (Steenbock and Bellin, 1953; Carlsson and Hollunger, 1954).

FIG. 1.—Serum citrate levels in 14 patients during the course of idiopathic hypercalcaemia.

FIG. 2.—Urinary citrate levels in seven patients during the course of idiopathic hypercalcaemia.

In the human, low blood citrate levels and a low urinary output of citrate have been observed in rachitic children. The levels rise with vitamin D administration (Harrison and Harrison, 1952). In hypervitaminosis D raised serum citrate levels up to 6 mg./100 ml. have been observed (Harrison, 1954).

Our findings of lowered serum citrate levels in blood and urine in the acute phase of idiopathic hypercalcaemia with a rise on withdrawal of vitamin D and recovery from the illness, and our evidence that the serum calcium level and serum citrate level are inversely related are thus apparently the reverse of those which would be expected with increased vitamin D activity. They appear to argue against increased vitamin D activity (when there is so much other evidence that it is present) or to suggest that the vitamin D-like action operates differently in respect of citrate metabolism. One should be very cautious, however, about applying to the period of infancy findings relevant to an older age group. Idiopathic hypercalcaemia is a peculiarly age-limited disease. At the age period at which it occurs hypervitaminosis D has indeed been reported to produce lowered serum citrate levels and a lowered urinary citrate output. Winberg and Zetterström (1956) observed these changes in an infant aged 11 months who over a period of five months had received 8,000,000 units of vitamin D and whose serum calcium level had risen to 16 mg./100 ml. It may be that citrate metabolism in infancy and disorders of it conditioned by vitamin D and vitamin D-like substances show a different pattern from that seen at an older age.

Summary

Observations have been made on the normal serum citrate level in infancy (1.2–3.5 mg./100 ml.) and on the serum citrate level and urinary citrate output in idiopathic hypercalcaemia of infancy.

It is concluded that in idiopathic hypercalcaemia the serum citrate is low in the acute phase of the disease and rises up to or beyond the upper limit on recovery with a subsequent fall to average levels.

There is a statistically significant negative correlation between the serum calcium level and the serum citrate level in idiopathic hypercalcaemia.

The urinary output of citrate is low in the acute

phase of the disease rising to normal levels with recovery.

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