STUDIES ON THE REGULATION OF THE SERUM-CHOLESTEROL LEVEL IN MAN

SERIAL SERUM-CHOLESTEROL DETERMINATIONS IN ACTIVE MEN ON THEIR ORDINARY DIETS AND THE LONG-TERM EFFECT OF CERTAIN SATURATED- AND UNSATURATED-FAT SUPPLEMENTS*

H. GORDON, B.Sc., M.B., Ch.B.

Clinical Research Bursar

and

J. F. BROCK, M.A., D.M., F.R.C.P.

Professor of Medicine in the University of Cape Town

The influence of dietary fats on the serum cholesterol is being studied intensively and it is now well established that the quality of a fat determines its effect on the human serum-cholesterol level. It has been repeatedly confirmed that while certain saturated fats increase the serum-cholesterol level, some highly unsaturated fats decrease it. The effects of these fats, however, have only been demonstrated in artificial circumstances, viz. in acute dietary experiments,[1, 2] or with the use of drastically modified or unnatural 'formula' diets,[3-5] or in patients confined to hospital on standardized diets.[6, 7]

Hence we have set out to investigate the effects of dietary fats for extended periods in men consuming their accustomed diets and continuing with their usual business and recreational pursuits. Before such a study could be undertaken it was necessary to assess the 'spontaneous' fluctuations in the serum-cholesterol level of our subjects under ordinary living conditions. This communication deals with a group of 30 men in whom week-to-week fluctuations in the serum-cholesterol level have been studied and it presents a preliminary account of the influence of dietary fat supplements and other factors on the serum-cholesterol level of some of these men.

PLAN OF INVESTIGATION

The 30 subjects (Table II) were all European men and included clerical workers, professional men and business executives. Their ages ranged from 25 to 59 years with a mean of 43·1 years. Six of the men had previously sustained a cardiac infarction and, of these, 4 remained with mild angina pectoris but were free from cardiac failure. One man had peripheral arterial disease with slight intermittent claudication and another had xanthelasma palpebrarum with hypercholesterolaemia. The remaining 22 men were healthy as judged by clinical examination, urine analysis, blood count, blood-urea level and electrocardiograph.

The men were asked not to modify their diets or their activity throughout the trial. They attended a special clinic held in the early morning once a week at the Groote Schuur Hospital. Their regular attendance was only occasionally broken by illness or by business or holiday trips away from the Cape. During the early stages of the investigation, the subjects took no breakfast before attending the clinic; later, a light breakfast was permitted, as single meals are known not to influence the serum-cholesterol level.[8, 9]

At each attendance the subject's weight and blood pressure were recorded and a sample of blood was taken for serum-cholesterol determination. Variations in the diet, physical activity, bowel habits and general health were noted. The initial control period lasted, on the average, 13 weeks, during which an average of 10 serum-cholesterol determinations were made. At the end of this period, various fats were given to the subjects to take as supplements to their regular diet.

The fats used, their dosage and the length of time they were consumed are indicated in Tables IV, V and VI. The fats were usually taken in 2 doses daily, at the subject's convenience in relation to meals. The oils were drunk raw; the solid fats were melted and drunk or combined with the food. The oils were generally taken for long periods, averaging 6 months; in a few cases, when the subject had to leave home for long business or holiday trips, the oils were given for shorter periods. The subjects who consumed the saturated fats were generally younger men and the period of administration was much shorter.

Subject 15 consumed 240 g. of dehulled sunflower seeds daily in addition to his regular diet. These contain about 45% of sunflower-seed oil. Subject 21 was not given any dietary supplement; instead he went onto a strict low-calorie diet. This diet was not rigorously controlled; it provided about 1,200 calories daily and was low in both fat and carbohydrate.

At the end of the fat-supplement period, the weekly observations continued during a second control period until the serum cholesterol level reached a steady state.

The iodine values of the fats and their sources are shown in Table I.

The analytical methods used were the following:

1. *Serum cholesterol:* Method of Abell *et al.*,[10] as modified by Anderson and Keys.[11] The reproducibility of the method was checked in our laboratory 3 times during the last year, on batches of 75-100 duplicate samples. The standard error of the method on each of these occasions was 3·9 mg.%, 4·2 mg.% and 3·8 mg.% respectively.

2. *Iodine value:* Macro-Wijs technique (A.O.C.S. official method Cd. 1-2).

Statistical Methods. To assess the spontaneous fluctuations of the serum cholesterol for each subject under the conditions of this study all the readings during the first control period were utilized. In subject 2, whose first control period was inadequate,

* From the Department of Medicine, University of Cape Town and the Groote Schuur Hospital, and the C.S.I.R./U.C.T. Clinical Nutrition Research Unit. Presented in part at the South African Medical Congress, Durban, September 1957, and in part at the Research Forum of the University of Cape Town, January 1958.

TABLE I. THE FATS USED IN THIS INVESTIGATION

Fat	*Iodine Value*	*Remarks*
Pilchard oil	180	Derived from the South African pilchard (*Sardina ocellata* Jenyns) and supplied by Marine Oil Refiners, Simonstown, Cape.
'Lipidex'	135-140	A blend of vegetable oil extracts, emulsified and flavoured, supplied by Grey Pharmaceutical Co. Inc., Mass., U.S.A.
Sunflower-seed oil ..	132-136	This oil has been refined, winterized and deodorized. A number of batches of fairly constant iodine values have been used; these were supplied by Cape Oil Products, Maitland, Cape.
Maize oil	120	This refined oil was supplied by Lever Bros., Durban.
Hydrogenated sunflower-seed oil.	74	Supplied by Lever Bros., Durban.
Butter	42-46	Several batches of commercial butter were used.
Hydrogenated coconut fat	6	Supplied by Marine Oil Refiners, Simonstown, Cape.

the readings of a later control period were utilized. For assessing the effects of the fat supplements, in subjects 1-10 and 16-19, the mean serum-cholesterol level of the first control period was compared with the mean of the last 4 readings of the fat-supplement period, i.e. when the serum-cholesterol level has become more or less stabilized on the new regime. These data for the fat-supplement period were again compared with similar data derived from the last 4 readings of the second control period.

The following formulae were used:

1. S^2 = variance of a subject's serum-cholesterol level

$$= \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

where n = number of readings of the serum cholesterol
x = individual values for serum cholesterol.

2. S = standard deviation of a subject's serum-cholestreol level.

3. Pooled standard deviation of the serum cholesterol for all the subjects studied

$$= \sqrt{\frac{\Sigma(n-1)S^2}{\Sigma(n-1)}}$$

4. Coefficient of variation for each subject $= \frac{S}{\bar{x}} \times 100$ where $\bar{x}$ = mean serum-cholesterol level.

5. For each group of individuals, the significance of the difference between the means of the serum-cholesterol level for each of the two control periods and the fat-supplement period was calculated according to this formula

$$t_{(n-1)} = \frac{\Sigma\triangle}{\sqrt{nS_{\triangle}^2}}$$

where $\triangle$ = difference between the means for each subject
$S_{\triangle}$ = variance of the difference
n = number of subjects.

6. For each individual, the significance of the difference between the means of the serum-cholesterol level for each of the two control periods and the fat-supplement period was calculated as follows:

$$t_{(n+n'-2)} = \frac{\bar{x}-\bar{x}'}{\sqrt{\left(\frac{n+n'}{nn'}\right)\frac{(n-1)S^2+(n'-1)S'^2}{n+n'-2}}}$$

where $\bar{x}$ and $\bar{x}'$ are the mean serum-cholesterol levels for each period

n and n′ are the numbers of readings of the serum-cholesterol in each period

S and S′ are variances of the serum-cholesterol level in each period.

RESULTS

Fluctuations in the Serum Cholesterol during the Control Period

In the 30 subjects studied, from 6 to 18 determinations of the serum-cholesterol level have been made in each, with an average of 10·5 determinations per subject. These estimations were made during periods of 6 to 34 weeks

TABLE II. THE MATERIAL OF THIS INVESTIGATION AND THE INTRA-INDIVIDUAL FLUCTUATIONS IN THE SERUM-CHOLESTEROL LEVELS OF THE 30 SUBJECTS WHILE ON ORDINARY DIETS AND NORMAL ACTIVITY

Subject	*Age (years)*	*Clinical Status*	*Duration of Observation (weeks)*	*No. of Readings*	*Lowest and Highest Values (mg. %)*	*Range (mg. %)*	*Mean Level (mg. %)*	*Standard Deviation of Mean (mg. %)*	*Maximum Deviation from Mean (%)*	*Coefficient of Variation (%)*
1	56	C	16	11	225 258	33	244	9·8	7·8	4·1
2	41	C	13	11	306 347	41	321	13·0	7·9	4·0
3	49	N	34	10	187 230	43	215	14·2	13·0	6·6
4	55	N	9	7	213 274	61	244	20·4	12·3	8·4
5	53	N	18	14	220 262	42	239	12·8	9·6	5·3
6	33	N	9	8	185 238	53	220	16·0	15·9	7·3
7	47	C	15	13	207 257	50	239	14·7	14·4	6·1
8	46	N	23	18	230 301	71	260	17·9	15·8	6·9
9	43	N	15	13	224 262	38	246	12·0	9·0	4·9
10	59	C	13	9	211 240	29	220	8·2	9·1	3·7
11	35	N	13	10	139 173	34	157	10·2	11·5	6·5
12	51	N	19	14	193 264	71	216	19·0	22·2	8·8
13	45	N	14	12	193 233	40	213	13·9	9·4	6·5
14	57	C	10	10	244 340	96	299	29·6	18·4	9·9
15	25	N	12	10	259 298	39	283	13·1	8·5	4·6
16	27	N	10	10	150 185	35	169	11·4	11·3	6·7
17	27	N	10	10	130 194	64	175	19·4	25·7	11·1
18	35	N	10	10	166 209	43	187	13·7	11·7	7·3
19	30	N	10	10	182 208	26	196	9·4	7·1	4·8
20	44	N	24	21	179 229	50	210	12·4	14·8	5·9
21	46	N	10	11	202 259	57	228	16·5	13·6	7·2
22	45	X	6	6	360 398	38	373	13·7	6·7	3·7
23	46	C	6	7	356 395	39	371	14·5	6·5	3·9
24	55	N	15	11	243 286	43	267	13·9	9·0	5·2
25	42	N	6	6	194 229	35	216	12·0	10·2	5·5
26	48	N	12	9	197 231	34	215	9·4	8·4	4·4
27	48	P	8	8	225 274	49	256	18·2	12·1	7·1
28	41	N	7	7	239 272	33	255	10·4	6·7	4·1
29	38	N	8	8	182 204	22	193	7·7	5·7	3·9
30	26	N	14	12	181 215	34	197	11·0	9·1	5·6

N = normal. C = coronary heart disease. X = xanthelasma. P = peripheral arterial disease.

TABLE III. SUMMARY OF INTRA-INDIVIDUAL FLUCTUATIONS RECORDED IN TABLE II

	Minimum	*Maximum*	*Mean*
Age (years)	25	59	43·1
Duration of Observation (weeks)	6	34	13·0
Number of Readings	6	21	10·5
Mean Serum-cholesterol Level (mg. %) ..	157	321	237·4
Standard Deviation (mg. %)	7·7	29·6	14·7
Intra-individual Range (mg. %)	22	96	44·8
Maximum Deviation from Mean (per cent) ..	5·7	25·7	11·4
Coefficient of Variation	3·6	10·9	6·0

(average 13 weeks per subject). The data are summarized in Tables II and III. The intra-individual fluctuations have been analysed in a number of ways, as follows:

1. *The intra-individual range of the serum-cholesterol level*, i.e. the difference between each subject's highest and lowest values, varied from 26 to 96 mg. %, with an average for the group of 45 mg. %.

2. *The maximum deviation of the serum-cholesterol from the mean value* varied among the subjects from 6% to 22%, with a mean for the group of 11·9%.

3. *The standard deviation of the serum-cholesterol level* of each subject varied from 7·7 mg.% to 29·6 mg.%. Pooling of the standard deviations for all 30 subjects gave a value of 14·7 mg.%.

4. *The coefficients of variation* have been calculated to compare the fluctuations among the 30 subjects. These vary from 3·6% to 10·9% with an average for the group of 6·1%.

The Effect of Unsaturated-fat Supplements

These supplements have been studied in 18 men. In 4 of these (subjects 22-25) the trial is not yet complete and 4 others have withdrawn from the trial, 2 (subjects 11 and 12) because they gained too much weight and 2 (subjects 13 and 14) because they were unable to tolerate the oil. The results in the remaining 10 men are shown in Table IV. Figs. 1-5 are representative illustrations.

It will be seen that the mean serum cholesterol of this group fell from the first control level of 247 mg.% to 213 mg.% by the end of the fat-supplement period. The difference, 34 mg.%, is highly significant. Individual decreases in the serum-cholesterol level ranged from 2 to 87 mg.%. In 8 cases, the decrease was statistically significant, in subject 3 the change of 17 mg.% had no statistical significance, and in subject 10 the daily consumption of 50 g. of sunflower-seed oil for 29 weeks had no appreciable effect on the serum-cholesterol level.

The pattern of response varied in these subjects. In 6 of them there was a slowly progressive decrease in the serum-cholesterol level, which became stabilized after 2-3 months (Figs. 2 and 3). In the other two who responded there was an initial sharp fall, which was maintained throughout the fat-supplement period (Fig. 1). In none of these subjects was the fall in serum cholesterol followed by a secondary rise while the subject was still consuming the oil supplement.

Since the end of the second control period, subjects 1, 3, 4, 7 and 10 have completed a further period on an unsaturated-fat supplement, lasting about 6 months. The nature of the supplement and the changes in the serum-cholesterol level are indicated in Table V. Significant decreases in the serum-cholesterol level were again recorded by subjects 1, 3, 4 and 7 but in subject 10, once more, 50 g. of maize oil daily for 25 weeks failed to influence the serum-cholesterol level (Figs. 2 and 4).

Side-effects of the Unsaturated-fat Supplements. Subjects 13 and 14 developed nausea, distension and anorexia when they took 50 g. of sunflower-seed oil daily. Emulsifying the oil in water (with powdered tragacanth) and embellishing it with synthetic ginger, strawberry or orange flavours did not improve its acceptability to these two men and they withdrew from the trial. None of the other subjects found the oils pleasant to take but they soon became accustomed to drinking them. The only symptom which could be attributed to the

TABLE IV. THE EFFECT OF A DAILY SUPPLEMENT OF UNSATURATED FAT ON THE SERUM-CHOLESTEROL LEVELS OF 10 MEN

First Control Period						*Fat-supplement Period*						*Second Control Period*			
Subject	*Age*	*Duration (weeks)*	*No. of Readings*	*Mean Serum Cholest. (A) mg.%*	*Standard Deviation of A*	*Fat*	*Dose (g.)*	*Duration (weeks)*	*Mean Serum Cholest. (B) mg.%*	*Difference (A-B) mg.%*	*Significance of Difference (p)*	*Duration (weeks)*	*Mean Serum Cholest. (C) mg.%*	*Difference (C-B) mg.%*	*Significance of Difference (p)*
1	56	16	11	244	9·8	SSO	50	33	217	−27	<0·001	13	232	+15	<0·01
2	41	3	3	340	11·5	PO or SSO	75	32	253	−87	<0·001	24	322	+69	<0·001
3	49	34	10	215	14·2	SSO	50	33	198	−17	<0·1	12	229	+31	<0·02
4	55	10	8	244	20·4	SSO	50	22	213	−31	<0·02	16	249	+36	<0·001
5	53	18	14	239	12·8	SSO	50	22	214	−25	<0·01	7	252	+38	<0·001
6	33	10	8	220	16·0	SSO	50	14	192	−28	<0·01	13	224	+32	<0·001
7	47	14	13	239	14·7	SSO	50	27	186	−53	<0·001	9	237	+51	<0·001
8	46	23	18	260	17·9	Lx	37	19	212	−48	<0·001	6	260	+48	<0·01
9	43	15	12	246	12·0	SSO	50	10	232	−14	<0·05	21	242	+10	<0·3
10	59	12	9	220	8·2	SSO	50	23	218	− 2	—	17	224	− 6	<0·4
Mean		15·5	10·5	247				23·5	213	−34	<0·01	13·8	246	+33	<0·001

SSO = Sunflower-seed oil. PO = Pilchard oil. Lx = 'Lipidex'.

TABLE V. THE EFFECT OF A SECOND SUPPLEMENT OF UNSATURATED FAT ON THE SERUM-CHOLESTEROL LEVELS AND BODY WEIGHT OF 5 MEN. (The effects of the first supplement are recorded in Tables IV and VI)

	Second Control Period		*Second Fat-supplement Period*						
Subject	*Mean Serum Cholest. (C) mg.%*	*Body Weight (lb.)*	*Fat*	*Dose (g.)*	*Duration (weeks)*	*Mean Serum Cholest. (D) mg.%*	*Difference (C-D) mg.%*	*Body Weight (lb.)*	*Change in Weight (lb.)*
1	232	188	Lx	37	30	215	−17	193	+5
3	229	166	MO	50	21	206	−23	170	+6
4	249	156	MO or Lx	50 / 37	21	195	−54	160	+4
7	237	158	MO	50	22	204	−33	168	+10
10	224	163	MO	50	25	230	+ 6	172	+9
Mean	234	166·2			24	210	−24	180·6	+6·4

Lx = Lipidex. MO = Maize oil.

TABLE VI. THE EFFECT OF A DAILY SUPPLEMENT OF SATURATED FAT ON THE SERUM-CHOLESTEROL LEVELS OF 4 MEN

		First Control Period				Fat-supplement Period						Second Control Period			
Subject	Age	Duration (weeks)	No. of Readings	Mean Serum Cholest. (A) mg.%	Standard Deviation of A	Fat	Dose (g.)	Duration (weeks)	Mean Serum Cholest. (B) mg.%	Difference (B-A) mg.%	Significance of Difference (p)	Duration (weeks)	Mean Serum Cholest. (C) mg.%	Difference (B-C) mg.%	Significance of Difference (p)
16	27	10	10	169	11·4	HCF	50	5	195	+26	<0·001	5	166	−29	<0·01
17	27	10	10	175	19·4	HCF	50	5	225	+50	<0·001	5	194	−31	<0·01
18	35	10	10	187	13·7	HCF	50	5	227	+40	<0·001	5	182	−45	<0·01
19	30	10	10	196	9·4	HCF	50	5	213	+17	<0·02	5	197	−16	<0·1
Mean		10	10	182				5	215	+33	<0·02	5	185	−30	<0·02

HCF = Hydrogenated coconut fat.

oil was variable mild constipation. The transient episodes of diarrhoea which we have previously reported in the metabolism ward[12] have not been encountered in the present series. Subject 15 was unable to tolerate sunflower-seed oil because of nausea and distension. Nevertheless he consumed with relish 240 g. of dehulled sunflower seeds daily and his serum-cholesterol level fell from 265 to 220 mg.% (Fig. 5).

Effect on Body Weight. Throughout the trial period the subjects claimed to be following the instruction to take the fats as supplements and not to be modifying their regular

TABLE VII. THE SAME SUBJECTS AS IN TABLE IV, SHOWING THE EFFECT OF THE UNSATURATED-FAT SUPPLEMENT ON THE BODY WEIGHT

Subject	First Control Period	Fat Supplement Period		Second Control Period	
	Final Weight	Final Weight	Change	Final Weight	Change
1	195	192	−3	188	−4
2	202	217	+15	213	−4
3	160	163	+3	166	+3
4	158	160	+2	156	−4
5	165	164	−1	162	−2
6	212	216	+4	214	−2
7	163	157	−6	158	+1
8	181	187	+6	184	−3
9	206	210	+4	208	−2
10	152	164	+12	163	−1
Mean	179·4	183·0	+3·6	181·2	−1·8

diet. A study of their weight changes (Table VII), however, shows that they were in fact not gaining weight as would have been expected. Thus, only subjects 2 and 10 gained much weight, while subject 7 actually lost 6 lb. Similarly, during the second control period, the fall in weight was slight. It will be recalled that subjects 11 and 12 gained weight so rapidly on starting the oil supplement that they withdrew from the trial.

The Effect of the Saturated Fats

The results in subjects 16-19, who consumed 50 g. of hydrogenated coconut fat daily for 5 weeks, are shown in Table VI and Fig. 6. It will be noted that this group of men were younger than the previous group (mean age: 30 years) and that their initial serum-cholesterol level was relatively low (mean 182 mg.%). During the fat-supplement period, all the subjects showed significant increases in the serum-cholesterol level and the mean increase for the group (33 mg.%) was highly significant. On stopping the fat, the serum-cholesterol level fell from a mean of 215 mg.% to 185 mg.%; this change of 30 mg.% is also statistically significant.

Subject 20 (Fig. 7) showed clear increases in his serum-cholesterol levels during two fat-supplement periods, in which he consumed 75 g. of hydrogenated sunflower-seed oil and 50 g. of butter, respectively. At the end of each fat-supplement period, the serum-cholesterol level returned towards the control level.

None of these subjects experienced any untoward effect from the fat supplements. As with subjects consuming

TABLE VIII. THE SAME SUBJECTS AS IN TABLE VII, SHOWING THE EFFECT OF THE SATURATED-FAT SUPPLEMENT ON BODY WEIGHT

Subject	First Control Period	Fat Supplement Period		Second Control Period	
	Final Weight	Final Weight	Change	Final Weight	Change
16	139	141	+2	137	−4
17	130	134	+4	134	0
18	157	162	+5	159	−3
19	173	173	0	173	0
Mean	149·7	152·5	+2·8	150·7	−1·8

unsaturated fats, the gains in weight during the supplement periods were less than had been anticipated, again suggesting an unconscious restriction of intake at this stage (Table VIII).

The Relation of Changes in Body Weight to Changes in the Serum-cholesterol Level

The effect of weight reduction in subject 21, who was obese, is recorded in Fig. 8. It will be noted that on two occasions drastic caloric restriction produced marked and sustained falls in weight. With each episode of rapid weight loss, there was only a transient fall in the serum-cholesterol level. In fact, despite a loss of 30 lb. of body weight, at the

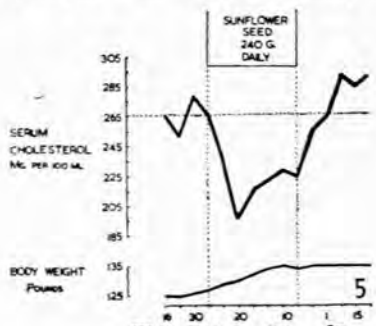

Fig. 5. Subject 15. A sharp fall in the serum-cholesterol level and a substantial gain in weight occurred when sunflower seeds were consumed.

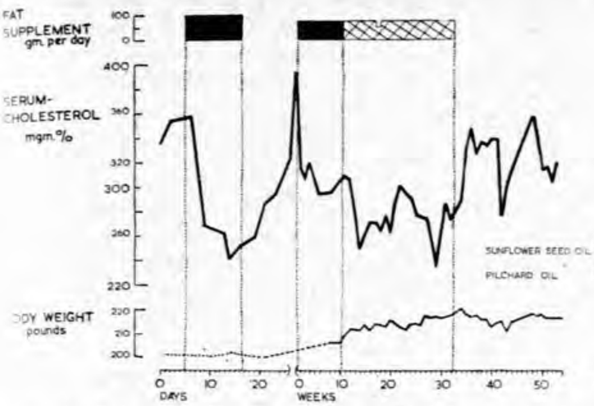

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Fig. 1. Subject 2. During the first part of the investigation, the subject was in hospital; 100 g. of sunflower-seed oil daily for 12 days produced a prompt fall in his serum-cholesterol level. On discharge from hospital, his serum-cholesterol level was recorded weekly and its reduction was maintained for 32 weeks by the use of 75 g. supplements of sunflower-seed oil or pilchard oil. The reduction in serum-cholesterol level was accompanied by a marked increase in weight.

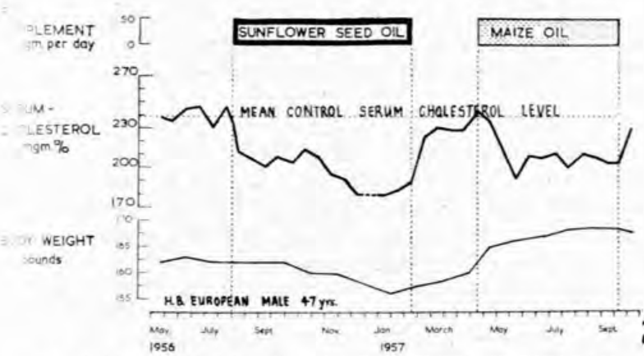

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Fig. 2. Subject 7. During the sunflower-seed-oil period the fall in serum-cholesterol level was accompanied by a loss of weight; during the maize-oil period, it was accompanied by an increase in weight. (Each point on the serum-cholesterol graph represents the mean of 2 consecutive readings of the serum-cholesterol level.)

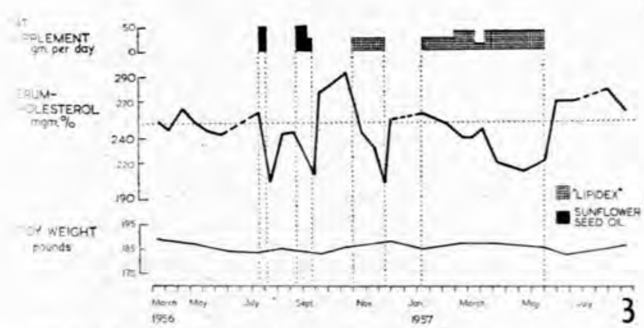

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Fig. 3. Subject 8. Short courses of sunflower-seed oil and 'lipidex' produced prompt decreases in the serum-cholesterol level. The decrease was sustained when a 'lipidex' supplement was taken for 19 weeks. Serum cholesterol recorded as in Fig. 2.

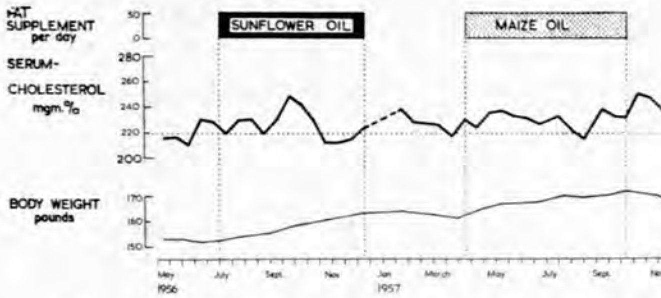

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Fig. 4. Subject 10. Neither sunflower-seed-oil nor maize-oil supplements produced a significant change in the serum-cholesterol recorded as in Fig. 2.

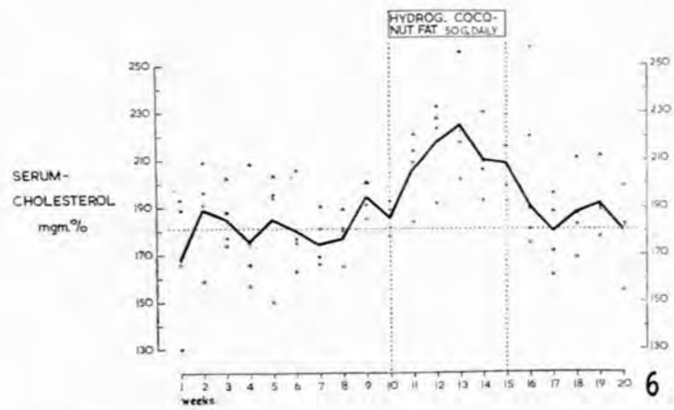

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Fig. 6. The weekly changes in the serum cholesterol of subjects 16-19 are recorded. The continuous line represents the mean level for these 4 men. Note that during the hydrogenated-coconut-fat-supplement period, all the serum-cholesterol readings were above the mean level of the first control period.

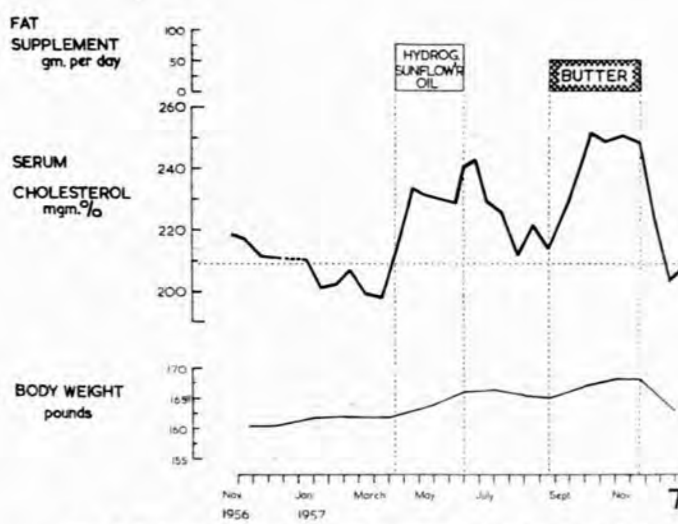

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Fig. 7. Subject 20. The increase in serum-cholesterol level produced by a supplement of 75 g. of hydrogenated sunflower-seed oil (iodine value 74) is less than that produced by 50 g. of butter (iodine value 42-46) serum cholesterol recorded as in Fig. 2.

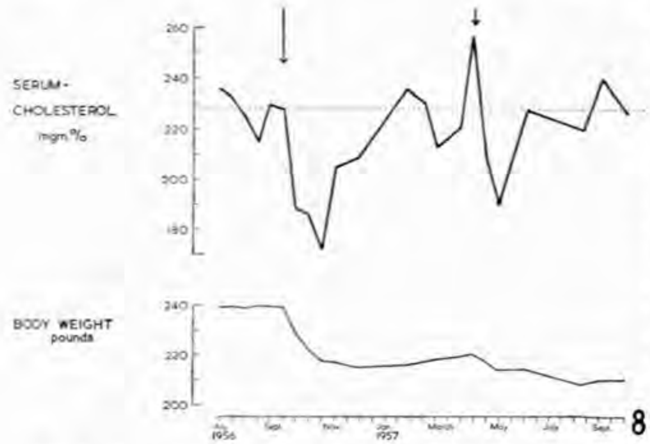

Fig. 8. Subject 21. The arrow indicates when large reductions in daily caloric intake were instituted. These reductions produced sustained decreases in body weight, but only a temporary fall in the serum-cholesterol level. Serum cholesterol recorded as in Fig. 2.

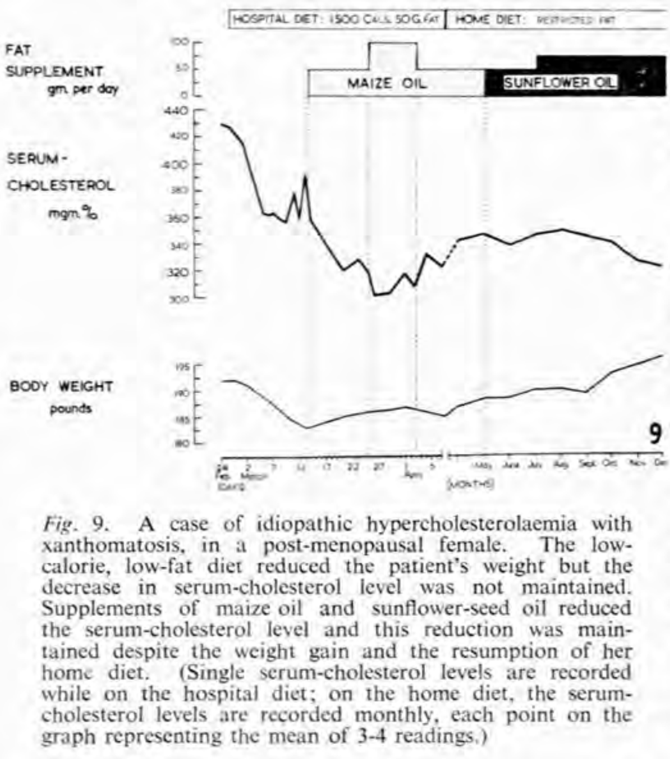

Fig. 9. A case of idiopathic hypercholesterolaemia with xanthomatosis, in a post-menopausal female. The low-calorie, low-fat diet reduced the patient's weight but the decrease in serum-cholesterol level was not maintained. Supplements of maize oil and sunflower-seed oil reduced the serum-cholesterol level and this reduction was maintained despite the weight gain and the resumption of her home diet. (Single serum-cholesterol levels are recorded while on the hospital diet; on the home diet, the serum-cholesterol levels are recorded monthly, each point on the graph representing the mean of 3-4 readings.)

end of the 64 weeks of observation the serum cholesterol was almost exactly at its control level of 218 mg.%.

It will be seen from the data in Tables IV, V and VII that there is no constant relationship between the change in weight during the fat-supplement period and the change in the serum-cholesterol level. Subject 2 (Fig. 1), who had the greatest fall in serum-cholesterol level (87 mg.%), gained the most weight (15 lb.). Similarly, subject 15 gained 10 lb. during the 6 weeks in which he consumed sunflower seeds, while his serum-cholesterol level was substantially decreased (Fig. 5). The dissociation between change in weight and change in serum-cholesterol level is clearly seen in subject 7 (Fig. 2): during the sunflower-seed oil period the subject lost weight, while his serum-cholesterol level fell. During the maize-oil period, the fall in serum-cholesterol level was accompanied by a gain in weight.

Fig. 9 illustrates the observations made in an overweight female subject with idiopathic hypercholesterolaemia. The low-calorie, low-fat diet produced rapid loss of weight and a fall in the serum-cholesterol level which was not sustained. Maize-oil supplements produced a marked fall in the serum-cholesterol level while the subject gained weight. Further unsaturated-fat supplements maintained a reduced serum-cholesterol level despite a more liberal home diet; the body weight, however, returned to its former level.

DISCUSSION

In 1937, Sperry[13] stated that 'in most, if not all, persons in health the concentration of cholesterol in the blood serum is maintained at a constitutional level which is characteristic for the individual and from which large deviations do not ordinarily occur'. Sperry arrived at this conclusion after a study of the changes in the serum-cholesterol level in 25 men and women who, like our subjects, were continuing with their customary activity and diets. In each subject at least 2 and usually 3 estimations of the serum cholesterol were made, at intervals varying from 2 weeks to 28 months. The intra-individual ranges in this group varied from 0 to 49 mg.% with a mean of 20 mg.%. The average standard deviation was 12·1 mg.%.

In our series a comparison of the individual mean serum-cholesterol levels in the two control periods (Tables IV and VI) confirms that each individual has a 'characteristic' serum-cholesterol level which under normal living conditions is fairly constant. But when repeated estimations are made in the same person at frequent intervals and under the same circumstances, marked fluctuations occur about this 'characteristic' level. The data in Table I (summarized in Table II) show that when many readings of the serum cholesterol are made, far greater fluctuations occur than Sperry noted with his comparatively few data. Our findings are best stated in terms of the pooled standard deviation of 14·7 mg.%, which indicates that, when repeated estimations of an 'average' subject's serum cholesterol are made, 95% of the values obtained will be within 29·4 mg.% of his mean level.

Similar data have been reported by Peters and Man.[14] They studied 17 normal men and women but in only 7 of these were 5 or more readings made; in these, the intra-individual ranges varied from 25 to 123 mg.% with an average of 52 mg.%.

Several other studies testify to the intra-individual variability of the serum-cholesterol level. None of these is directly comparable with our own; usually too few readings of the serum cholesterol were made, sometimes with an unacceptable analytical technique, and in most cases the subjects were in hospital on standardized diets. Even under the latter circumstances marked fluctuations may occur. Thus, Hatch *et al.*[15] made repeated estimations of the serum-cholesterol level in 48 hypertensive subjects during a 6-week period in hospital on a controlled diet (calories 2,200, fat 85 g.). The intra-individual range averaged 47 mg.%, which is remarkably close to our figure of 45 mg.% for normotensive subjects on free diets.

Steiner and Domanski[16] have stated that in cases of coronary heart disease far greater fluctuations in the serum-cholesterol level occur than in controls. In their 15 cases of coronary heart disease, who were apparently ambulant and on ordinary diets, an average of 38 estimations of the serum cholesterol were made over periods varying from 2 to 26 months. The mean level for this group was 355 mg.%, the intra-individual range averaged 99 mg.%, and the individual standard deviation averaged 24·8 mg.%. In a control group, these authors found a mean level of 255 mg.%, with an intra-individual range averaging 32 mg.% and an average individual standard deviation of 8·7 mg.%. The control group, however, included cases of peptic ulcer, diabetes mellitus and others on controlled diets in hospital[8] and cannot be compared with the 'coronary' group. In our series, among the few cases of coronary heart disease only subject 14 showed an intra-individual fluctuation which was substantially greater than the mean for the whole group.

It is clear, therefore, that several readings of an individual's serum cholesterol should be made before deciding whether his level is above, below, or within the average limits for his age, sex and race. Serial determinations are also necessary in studying the effect of dietary or other factors on the serum-cholesterol level. In this respect, the 'internally controlled' trial with base-line observations both before and after the experimental period is particularly valuable. If such a procedure were more generally adopted much of the present confusion and contradiction in cholesterol research might be avoided.

FACTORS INFLUENCING THE SERUM-CHOLESTEROL LEVEL

Much of the present interest in cholesterol arises out of its occurrence in atheromatous lesions and the possibility that there may be a relationship between elevated serum-cholesterol levels and coronary heart disease. Cholesterol, however, is present in all animal tissues and it has considerable physiological, pathological and clinical importance. It is chemically related to several biologically significant substances; these include the hormones, progesterone, aldosterone and hydrocortisone; vitamin D; the bile acids; and the benzpyrene group of carcinogenic hydrocarbons. It is an important physiological constituent of myelin and of the red-cell membrane. Pathologically, it is prominent in gall-stones, in xanthomata, and in some chronic granulomatous lesions. Thus, there are many incentives for studying the factors which determine the characteristic level of an individual's serum cholesterol and the deviations which occur from it. The already voluminous literature on the subject is being rapidly expanded. This is a suitable time, therefore, to pause and to assess the present status of our knowledge of these factors.

A. Endogenous Factors

1. *Genetic*

Although the hereditary influences which are so important in cases of hypercholesterolaemia have been very fully studied,[17, 18] their role in determining the serum-cholesterol level in the general population is not yet known. The serum cholesterol is affected by so many environmental factors that the clear separation of the genetic influence from 'family habits' in related persons is extremely difficult. On the other hand, some differences in serum-cholesterol level which at first sight seem to be of environmental origin may in fact be genetically conditioned (e.g. the differences between smokers and non-smokers—see below). Similarly, a genetic factor may account for the variability of individual responses to the same environmental agent, e.g. the failure in subject 10 of both sunflower-seed-oil and maize-oil supplements to decrease the serum-cholesterol level.

An attempt at assessing the importance of genetic factors was made by Gildea *et al.* in 1936;[19] they found that in 12 men of pyknic habitus the mean serum-cholesterol level was 230 mg.%; in 18 leptosomic men the corresponding value was 168 mg.% and in 8 men of intermediate body build it was 203 mg.%. Unfortunately, the sample studied was small and there is no indication that these authors age-matched the groups. More recently, however, in 97 young men with coronary heart disease and in an age-matched control series, Gertler and White[20] found that the ectomorphs (roughly corresponding to the leptosomic habitus) had substantially lower serum-cholesterol levels than the endomorphs and mesomorphs.

2. *Age*

It is generally accepted that the serum-cholesterol level increases with age, but opinions vary about the age when the peak level is reached. For men, it has been variously put at 33 years,[21] 45 years,[22] and 55 years.[23] An increasing serum-cholesterol level is not an inevitable manifestation of the aging process. In Johannesburg, Bersohn and Wayburne[24] found that while European and Bantu babies at birth had similar serum levels (mean of 75 mg.% and 72 mg.% respectively), the European mothers had much higher levels than the Bantu mothers (means of 258 mg.% and 190 mg.% respectively). Furthermore, Pomeranze *et al.*[25] have recently shown that babies fed with an artificial soy-milk formula do not develop the rapid increase in serum-cholesterol level which usually occurs in the first few months of life. Clearly then environmental and genetic factors may be partly responsible for the age-increase in the serum-cholesterol level. The annual increment in an individual's serum cholesterol, however, is small, and is not important in relation to the week-to-week or month-to-month fluctuations.

3. *Sex*

The serum-cholesterol level of men or women may be lowered by the administration of oestrogens.[26, 27] In women, cyclical fluctuations of circulating-cholesterol level have been correlated with fluctuating oestrogenic activity.[28] Thus in 12 healthy young women marked falls in the plasma-cholesterol level occurred at the time of ovulation and just before menstruation, i.e. at the peaks of oestrogenic activity. In 6 healthy men no cyclical changes were observed. It is presumably the activity of oestrogenic hormone which accounts for the fact that women often have lower serum-cholesterol levels than men during the reproductive years of their lives.[21, 29]

B. Exogenous Factors

1. *Season*

In 1924, Currie[30] reported that in Glasgow the plasma cholesterol of normal individuals varied with the season, the highest levels occurring in summer (mean, 280 mg.%) and the lowest in winter (mean, 110 mg.%). He suggested that these changes might be due to seasonal variations in the food supply or to a stimulating effect of sunshine on endogenous cholesterol synthesis. Currie did not explain how he obtained his data and it appears that different groups of subjects were analysed in the different seasons. Furthermore, his technique for measuring the plasma cholesterol is not acceptable. In 1932 in Canada, McEachern and Gilmour[31] refuted Currie's findings, but their data too are quite inadequate. It must be concluded that the effect of seasonal changes on the serum-cholesterol level are unknown.

2. *Exercise*

The low serum-cholesterol levels of underprivileged races have been attributed by Mann *et al.*[32] to the greater physical activity of these peoples. In the Cape Peninsula inter-racial survey,[33] it was found that the Bantu heavy labourers had lower serum-cholesterol levels than the Bantu light workers; in the European and Cape Coloured groups, however, there was no relationship at all between degree of physical activity and the serum-cholesterol level. In a wider survey, including data from Italy, Sweden, the USA and South Africa, Keys *et al.*[34] concluded that the activity of work played only a minor role in regulating the serum-cholesterol level.

Nevertheless, other lines of investigation have indicated the importance of exercise. In their classical studies of the extraneous factors influencing the serum-cholesterol level, Groen *et al.*[3] found that in their subjects increased physical activity often had a marked lowering effect. Taylor *et al.*[35] demonstrated the effect of exercise in 9 healthy young men; when they increased their fat intake by 46 g. daily and at the same time increased their calorie expenditure by walking on a treadmill for 2 hours, no increase in serum cholesterol occurred. Presumably the increased exercise prevented the anticipated rise in the serum-cholesterol level, but the value of the experiment would have been enhanced had the effect of the fat increment also been studied in the same subjects without exercise.

The evidence, then, is fragmentary and not conclusive but it is sufficient to suggest that varying activity may partly account for otherwise unexplained serum-cholesterol fluctuations.

3. *Smoking*

Gofman *et al.*[36] have found higher levels of serum cholesterol in those smoking 20 or more cigarettes daily than in non-smokers. The differences, however, were not striking; they were maximal in men aged 20-29, among whom the serum cholesterol of the smokers averaged 226·4 mg.% as opposed to 205·2 among the non-smokers. Bronte-Stewart noted a similar relationship among the European and Cape Coloured men under 40 years of age in the Cape Peninsula.[37] He suggested that the heavy smoker dulls his palate and therefore seeks out spicier and more savoury foods; the latter are rich in fat and it is this factor which increases the serum-cholesterol level rather than smoking *per se*.[38] This suggestion is interesting, but the evidence relating smoking and the serum-cholesterol level is scanty and requires further study.

4. *Physical Stress*

Acute physical stress has been shown to decrease the serum-cholesterol level. Thus Kyle *et al.*,[39] in 10 cases, observed a mean fall of 16% 8-72 hours after a variety of major surgical operations. Similarly, Biorck *et al.*[40] have reported that the serum-cholesterol level may fall after acute cardiac infarction. In some of their patients it appears that this fall was transient, but in most cases the studies were incomplete. Neither of these two groups of authors make mention of their patients' food consumption and it is possible that the falls in the serum-cholesterol level may have been partly due to dietary restriction in the early post-operative or post-infarction period. McQuarrie and Stoesser[41] studied the effect of acute infections in 27 children. They found that at the height of the illness the serum cholesterol was low in comparison with its level on the 8-9th day of convalescence; in general, the serum-cholesterol level varied inversely with the white blood count. Some of their patients were on a constant diet throughout the period of investigation, and it was shown that in these the serum-cholesterol changes were independent of the food intake. In 9 children with afebrile disorders, the production of artificial fever with TAB etc. was without effect on the serum cholesterol, indicating that fever as such is not responsible for the low levels encountered in infections.

In adult subjects on various dietary regimes, Groen *et al.*[3] found that reductions in the serum-cholesterol level occurred with upper-respiratory-tract infections and with gastro-intestinal disorders. We have noted similar effects; in Fig. 10 some examples are shown of the changes in serum cholesterol apparently resulting from accompanying influenza,

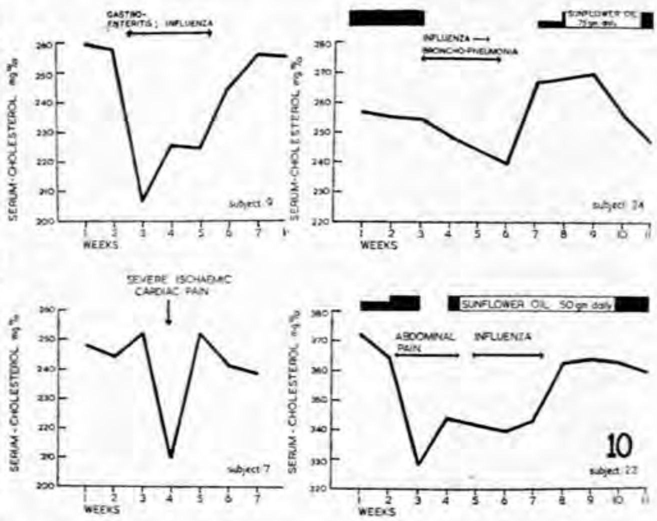

Fig. 10. The week-to-week changes in the serum-cholesterol level accompanying infection, bowel disorders and coronary insufficiency.

pneumonia, gastro-enteritis and coronary insufficiency in subjects on their usual home diets with and without fat supplements. It must be emphasized, however, that both in our experience and from a perusal of the records published by Groen *et al.* it is not always possible to distinguish the changes due to physical stress from apparently 'spontaneous' fluctuations in the serum-cholesterol level.

The mechanism of these changes is not known. Stoesser and McQuarrie[42] in 1935 suggested that cholesterol combines with bacterial toxins and its circulating level falls. More recently, the role of the pituitary-adrenal axis has been invoked. In normal subjects ACTH produces a fall in the serum-cholesterol level.[43] It is postulated that stress increases the production of ACTH, which in turn stimulates adreno-cortical activity; thus the conversion of cholesterol to adrenal hormone is enhanced and the level of circulating cholesterol falls. It may be noted that in normal persons cortisone, which does not promote cholesterol-corticosteroid turn-over, has little effect on the serum-cholesterol level.[43] Studies of the action of ACTH and cortisone on the blood lipids in normal men are scanty and further investigation is necessary.

5. *Emotional Stress*

The quantitative study of emotional stress is almost impossible and an assessment of its effect on the serum-cholesterol level is fraught with difficulty. Nevertheless, Groen *et al.*[3] gained the impression that domestic disharmony and business difficulties increased the serum-cholesterol level of some of their subjects. Very recently, Hammerstein *et al.*[44] have studied 12 men who had survived cardiac infarction. Weekly estimates of the serum-cholesterol level were made under conditions which apparently resembled those of our study except that the diets were more standardized. They report that 'on 20 occasions the serum cholesterol rose higher than the mean value for that individual by more than 15%. It was striking that 19 of the 20 occasions of high cholesterol corresponded with periods that had been separately judged as particularly stressful for the individuals concerned'. A similar result has been reported by Rosenman and Friedman,[45] who measured the serum cholesterol of 42 accountants twice weekly during the first half of 1957. They found that, with the increased pressure of work preceding the business 'dead-line' periods, the serum-cholesterol level was substantially higher than during less turbulent times. Both these investigations have only been reported in abstract and their detailed publication is awaited with interest.

6. *Diet*

We have recently reviewed the role of various dietetic factors in determining the serum-cholesterol level[46] and this discussion will be restricted to those aspects which are relevant to the present investigation.

(*a*) *Caloric Balance*. Walker *et al.*[47] have stated that 'caloric balance appears to play a major role in controlling . . . serum-lipid levels'. In their study of 39 subjects, however, weight reduction had little significant effect on the serum-cholesterol level. On a diet containing 1,250 calories, of which 36% was derived from fat, their subjects lost on the average 19 lb. in 106 days (i.e. about 1·6 lb. per week). During this period, the mean reduction in the serum-cholesterol level for the group was 17 mg.%, which is not statistically significant. In fact, those subjects who lost most weight actually showed an increase in the serum-cholesterol level.

Several other studies testify to the inconsistent effects of weight reduction. Poindexter and Bruger,[48] using very low-calorie diets (600-885 calories, with about 30% from fat) produced substantial reductions in weight but the serum-cholesterol level increased in some subjects and decreased in others. Both Young *et al.*[49] and Moore *et al.*[50] in studies on overweight women found that reducing diets (1,400 to 1,500 calories with 48-50% from fat) generally raised the serum cholesterol while the weight fell.

The amount of fat in the reducing diet is important in determining its effect on the serum-cholesterol level. Thus, Anderson *et al.*,[51] using isocaloric reducing diets (1,200 calories), have shown that with 18% fat-calories, the serum-cholesterol level is lower than with 58% fat-calories.

Passing from reducing diets, we need to consider the effects of chronic malnutrition. Keys *et al.*[52] in their monumental work *The Biology of Human Starvation* have summarized the literature on the effects of complete and partial starvation on the blood lipids in man, and draw attention to the inadequacy of the data and the inconsistency of the results. In their own experiment on 36 conscientious objectors who subsisted on semi-starvation rations for 6 months, the fall in the serum-cholesterol level, although statistically significant, was quite small. The mean level on the control diet (total calories 3,492, fat-calories 32%) was 169 mg.% and by the end of the semi-starvation regime (total calories 1,570, fat-calories 17%) it had fallen to 152 mg.%. This fall in the serum-cholesterol level was accompanied by an average loss of 18·5% of body weight.

Our colleagues Schendel and Hansen[53] have recently reviewed the literature and reported their own observations on the changes in the serum cholesterol which occur in protein malnutrition of infants (kwashiorkor). During the acute stage, the serum-cholesterol level is very low (mean, 93 mg.%); in those cases in which a satisfactory initiation of cure accompanies the administration of a fat-free diet, it rises promptly to normal levels (mean, 179 mg.%). Most cases of kwashiorkor, however, are complicated by respiratory or intestinal infection, and this may be at least partly responsible for the low serum-cholesterol levels.

Finally, we note that the effect of caloric increase has been inadequately studied. Walker *et al.*[47] have claimed that in two cases the supplementing of the basal diet with 2,000 calories derived from carbohydrate increased the serum-cholesterol level. From the data which they present, however, we are unable to draw the same conclusion. Our own limited investigations (unpublished) have shown that the increase in the serum-cholesterol level which accompanied large carbohydrate supplements is small and slow when compared with calorically equal or lesser supplements of saturated fat.

(*b*) *Low-fat Diets*. Low-fat diets are often recommended for decreasing the serum-cholesterol level and, while such diets are generally effective in this respect, it must be realized that very severe fat restriction is necessary to produce an adequate response. For example, Anderson and Keys[54] achieved a fall of only 21 mg.% by changing the fat content of the diet from 140 to 70 g. (i.e. from 37% to 18·5% fat-calories). In two large series of hypertensive cases treated with the notorious rice diet (which is almost fat-free), Kempner[55] and Starke[56] reported decreases in the serum-cholesterol level of 57 mg.% and 71 mg.% respectively. It must be noted that in these studies there was no control period and only single estimations of the serum cholesterol were made before and after institution of the diet. On the other hand, Hatch *et al.*[15] have conducted a careful investigation into this type of diet; several estimations of the serum-cholesterol level were made both during a control period and during the various dietary regimes, all of which were carried out in hypertensive subjects in hospital. The control diet (2,200 calories) contained 85 g. of fat (35% fat-calories). Changing to diets containing 55 and 20-40 g. of fat respectively produced insignificant falls in the serum-cholesterol level. Changing to the rice diet (3 g. of fat) for 4-20 weeks produced a mean fall of 33 mg.% in a group of 44 patients; i.e. a fall which is almost the same as that produced in our 10 subjects simply by supplementing their regular diets with 50 g. of unsaturated fat. Most patients will rebel against the continued use of very low-fat diets and it is unlikely that they will be widely used in practice for reducing high-serum-cholesterol levels.

(*c*) *Unsaturated Fats.* The present investigation has shown that the changes produced by unsaturated fats in acute metabolic experiments and with artificial diets can be reproduced under normal living conditions by feeding the fats as supplements to the diet. It also appears that the effect of these fats can be maintained for as long as they are consumed and that their activity is independent of changes in body weight.

The sustained reduction in the serum-cholesterol level is consistent with the epidemiological evidence. The association in the Eskimo of relatively low serum-cholesterol levels with high intakes of unsaturated marine fats has been repeatedly observed.[57] In Yugoslavia, Brozek *et al.*[58] compared two populations; those living on the mainland in the North consume large amounts of animal fat and have significantly higher serum-cholesterol levels (238 mg.%) than their compatriots living on islands off the Adriatic coast (198 mg.%), who have a quantitatively similar fat intake which is, however, almost entirely olive oil. Similarly, in a survey amongst clothing workers in New York, Epstein *et al.*[59] found that Jews had higher serum-cholesterol levels (248 mg.%) than Italians (226 mg.%); the total fat intake was the same for both groups, but 32% of the Italians' fat intake was from vegetable sources while the Jews obtained only 20% of their fat from this source. Finally, it will be recalled that vegetarians may consume substantial amounts of fats most of which are unsaturated but they maintain relatively low serum-cholesterol levels.[60-62]

The routine use of unsaturated-fat supplements as a practical measure for lowering serum-cholesterol levels is probably undesirable. Many individuals will find the consumption of about 4 tablespoonsful of oil daily irksome or unpleasant. Moreover, there may be a marked gain in weight. In practice, it is likely that the most satisfactory cholesterol-decreasing regime will be one in which the foods containing saturated fats are moderately restricted and partly replaced by foods rich in unsaturated fats.[46]

It is, however, of interest in this connection that several of our subjects did not gain weight during the fat-supplement periods and the total weight change for the groups was far less than was expected from a caloric increment of 450 daily. There was no evidence of malabsorption in any of these subjects, so it is likely that they must unwittingly have been restricting their food intake. This can probably be attributed to the well-known 'satiety effect' of dietary fat, but an internal metabolic effect of the fat can at present not be excluded.

SUMMARY

1. Repeated estimations of the serum-cholesterol level have been made in 30 men during an observation period averaging 13 weeks. During this period the men continued with their customary activities and consumed their usual home diets. In these men, it was found that while there appears to be a characteristic level of serum cholesterol for the individual, there may be marked fluctuations around this level from week to week. The causes of these fluctuations are not always apparent.

2. In 10 of these men, the long-term effects of a dietary supplement of unsaturated fat have been investigated. In 8 of them a significant decrease in the serum-cholesterol level was produced; the mean decrease for the whole group was 34 mg.%. On withdrawing the unsaturated-fat supplement, the serum-cholesterol usually returned to its former level. In 5 of these men, a second trial, with a different unsaturated fat, produced a similar effect.

3. In another 5 men it was shown that, under similar circumstances, saturated-fat supplements raised the serum cholesterol significantly.

4. The effects of the fat supplements were independent of changes in body weight.

5. The endogenous and exogenous factors which regulate the serum-cholesterol level are reviewed. It is concluded that the most practical cholesterol-decreasing regime is one in which saturated dietary fats are moderately decreased and partly replaced by unsaturated fats.

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