The effect of saponin, sterols and linoleic acid on the weight increase of growing rats

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The incorporation of lucerne meal in the diet of chicks prevents normal gains in weight (Alder, 1946; Draper, 1948; Cooney, Butts & Bacon, 1948; Lepkovsky, Shaeleff, Peterson & Perry, 1950; Heywang, 1950; Kodras, Cooney & Butts, 1951 *a*, *b*). This effect is attributed to the saponin fraction of lucerne (Peterson, 1950 *a*, *b*; Heywang & Bird, 1954), which has been shown to be a complex mixture of related substances (Coulson, 1958). However, the sapogenins derived from lucerne saponins have no effect upon the growth of chicks (Potter & Kummerow, 1954).

The mechanism whereby mixed lucerne saponins prevent normal gains in weight by chicks is unknown, but the effect can be partly reversed by the concurrent feeding of cholesterol or phytosterols, principally β-sitosterol (Peterson, 1950 *a*, *b*). The growth-depressing effect of lucerne meal is completely overcome by the inclusion of both cholesterol and cottonseed oil in the diet.

The commercially available saponin from *Quillaja saponaria* Molina reduces weight increase in chicks, much as do mixed lucerne saponins (Peterson, 1950 *b*), and because of its availability has been selected for more detailed study of the effect of triterpenoidal saponins on growth. It has been shown that the normal rate of weight increase in the growing rat is depressed by the addition of *Quillaja* saponin to the diet, but in rats higher levels of saponin are required to produce the effect than in chicks (Coulson, 1957).

This paper describes in more detail the effect, and also its reversal by the addition of cholesterol to the diet.

The reversal of the saponin effect by cholesterol may be due to the formation of insoluble unabsorbed complexes of saponins with 3-β-hydroxysteroids, similar to those formed in vitro with cholesterol (Kobert, 1933). The generality of this hypothesis was tested by substitution of β-sitosterol for cholesterol in the diet of rats, and also by observing whether saponin in the diet interfered with the intestinal absorption of ergocalciferol, which is derived from a structurally related 3-β-hydroxysteroid.

On several occasions during this investigation cholesterol itself was found to depress the normal gain in weight of rats. The choline content of the diet was increased to show whether even at normal levels this lipotropic factor was limiting the utilization of the diet; it was found to be without effect. The essential fatty acids, especially linoleic acid, are considered to be closely involved in cholesterol metabolism (Kritchevsky, Moyer, Tesar, Logan, Brown, Davies & Cox, 1954; Shapiro & Freedman,

1955; Aftergood, Deuel & Alfin-Slater, 1957; Wood & Migicovsky, 1958; Rona, Chappel & Gaudry, 1959). Peterson (1950*b*) had found that cottonseed oil, which is rich in linoleic-acid esters, has a potentiating effect upon the reversal by cholesterol of the growth depression caused by saponin, and for this reason we also studied the effect of adding to the diets linoleic acid or oil rich in its esters. Because linoleic acid is easily oxidized, the protective action of anti-oxidants (Remenschneider, 1955), such as α-tocopherol or nordihydroguiaretic acid, was also investigated.

EXPERIMENTAL

Animals and plan of experiments

Month-old male hooded Wistar rats, weighing between 50 and 65 g, were transferred from the stock colony to individual glass metabolism jars. They were fed on the basal diet for 2 days, then allocated at random to diets in such a manner that the total initial weight of each diet group was about the same. They were then fed on the experimental diets until each rat had consumed about 200–300 g. The amount given to each rat daily was restricted to the smallest amount eaten on the previous day by any rat except in Expt 2 when feeding was *ad lib*. Throughout the experiments, which lasted from 4 to 6 weeks, access to water was unrestricted. The temperature of the animal house was controlled at $18{\cdot}5 \pm 1°$.

The rats were weighed individually at 3-day intervals. In each experiment the weight increases of the rats were examined statistically.

Statistical analysis

Regression analysis was used to determine the relation between the weight increase of the rats and the dietary level of *Quillaja* saponin (Expt 1). Two- or three-factor experimental designs, with each factor at two levels, were used in the remainder of the investigations; the main treatment effects and their interactions were isolated by standard methods, due account being taken of missing values caused by deaths. Differences in mortality rate on various diets were tested for significance by calculations of their confidence intervals. The methods are described by Snedecor (1956) and Cochrane & Cox (1957).

Diets

The composition of the basal diets is given in Table 1. The experimental diets contained the test substances thoroughly mixed with the basal diets at the concentrations shown in the tables.

Substances added to the basal diets. They were *Quillaja* saponin, saponin white (from *Gypsophila* spp.), cholesterol (British Drug Houses Ltd) and α-tocopherol (Roche Products Ltd), β-sitosterol and nordihydroguiaretic acid (L. Light and Co. Ltd), 'segregated' sunflower-seed oil (Sonnol 64) fractionated with liquid propane to increase the linoleic-ester content to 67 % of the total glycerides (kindly supplied by H. M. Faure and Co. Ltd and A. Boake Roberts Ltd) and linoleic acid. An initial attempt to produce a linoleic-acid concentrate by fractionally distilling technical

grade 'linseed oil acids (free)' (British Drug Houses Ltd) then centrifuging at 0° and decanting, produced an oil of the correct iodine value but with a very pungent odour. It was used in Expt 1 with diet A. A much purer sample of linoleic acid was prepared from 'segregated' sunflower-seed oil by the method of Parker, Koos & Swern (1955) and was stored under nitrogen at 4° until required for Expts 4, 5, 9, 10 and 11 in conjunction with diet B.

Table 1. *Composition of basal diets*

	Diet		
	A	B	C*
Major constituents (g/100 g)			
Rice starch (British Drug Houses Ltd)	37	—	—
Potato starch (British Drug Houses Ltd)	10	—	—
Sucrose (icing sugar, Tate and Lyle Ltd: containing not more than 1½% calcium phosphate)	27	75	—
Fat-free casein (British Drug Houses Ltd)	20	20	—
Mineral salt mixture (British Drug Houses Ltd)	5	5	—
Whole yellow maize, ground	—	—	76
Wheat gluten, ground (British Drug Houses Ltd)	—	—	20
$CaCO_3$ (B.P.)	—	—	3
NaCl (A.R.)	—	—	1
Vitamins (mg/100 g) added to diets			
Choline chloride	150	150	—
Nicotinic acid	10	10	—
Calcium D-pantothenate	10	10	—
Thiamine	1	1	—
Riboflavin phosphate	1	1	—
Pyridoxine hydrochloride	1	1	—
p-Aminobenzoic acid	1	1	—
Vitamins (i.u./rat/week) given by mouth			
Vitamin A†	3750	3750	—
Ergocalciferol†	750	750	—

* See below. † As Radiostoleum (British Drug Houses Ltd).

Purpose of experiments

Expt 1. The purpose of Expt 1 was threefold: (1) to examine the effect on weight gain of rats of increasing levels of *Quillaja* saponin in diet A, which contained starches as the principal energy source, (2) to examine the interaction of *Quillaja* saponin and cholesterol when fed to rats, and (3) to see if vitamin D deficiency was caused by high levels of *Quillaja* saponin.

Expt 2. A study was made of the effect of *Quillaja* saponin on the weight gain of initially rachitic rats and their utilization of ergocalciferol. *Ad lib.* feeding with a standard rachitogenic diet C (U.S. Pharmacopoeia, XIII (1947) rachitogenic diet no. 2) was continued through the experiment. *Quillaja* saponin was mixed with diet C and ergocalciferol (Radiostoleum, British Drug Houses Ltd) was given by mouth. X-ray photographs of the femurs of the rats were used to assess the presence or absence of rickets, and these findings were confirmed by the line test (cf. Hawk, Oser & Summerson, 1947).

Expts 3–9. In these experiments, a basal diet B was used in which sucrose replaced

starches as principal energy source to minimize impurities. The effects upon the weight gain of rats of *Quillaja* saponin and cholesterol and of a third dietary factor were simultaneously studied. The third factor was choline (Expt 3), linoleic acid (Expts 4, 5), 'segregated' sunflower-seed oil rich in linoleic esters (Expts 6, 7) or linoleic acid in the presence of an anti-oxidant (Expts 8, 9).

Expt 10. The results of Expts 8 and 9 led us to study further the effect of *Quillaja* saponin and of linoleic acid protected by an anti-oxidant upon weight gain of rats. In this experiment the linoleic acid was first mixed with nordihydroguiaretic acid and then with the diet B, so that only those rats receiving linoleic acid got nordihydroguiaretic acid as well.

Expt 11. For cholesterol, β-sitosterol was substituted to test whether the effect of *Quillaja* saponin could be reversed by sterols other than cholesterol.

Expt 12. Saponin white was substituted for *Quillaja* saponin to see if these two saponin preparations from different plants behaved in the same manner.

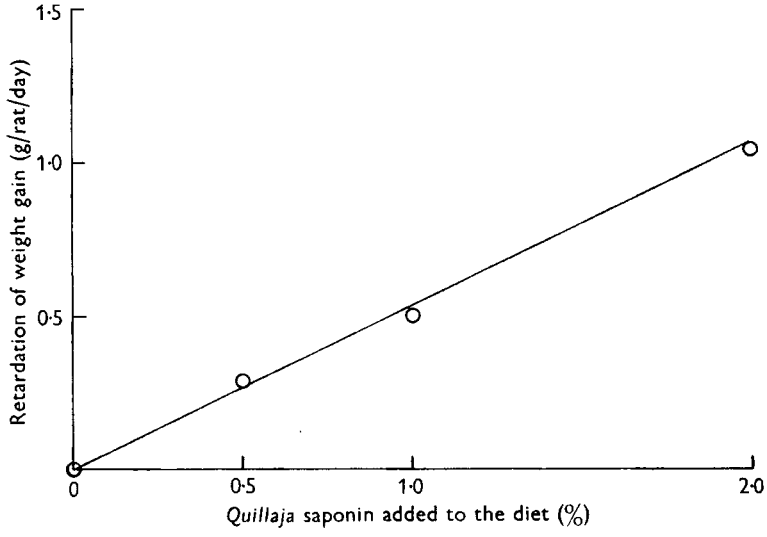

Fig. 1. Expt 1. Depression of weight gain of nineteen young rats on diets containing *Quillaja* saponin. Growth rate in absence of *Quillaja* saponin, 2·26 g/rat/day. ○, arithmetic mean. $y = 0{\cdot}53\ (\pm 0{\cdot}071)\ x$; $r_{17} = 0{\cdot}844$. Significance, $P = 0{\cdot}001$.

RESULTS

Expt 1. When *Quillaja* saponin, mixed with diet A, was given to growing rats, the retardation in weight gain was shown to be proportional to the dose of saponin over the range 0–2% (Fig. 1). At the 3% level, mortality was high and the surviving rats were considered to be non-representative: their weight increases were not used in the regression analysis. The effect of adding cholesterol to the diets containing 1% saponin was tested factorially in this experiment, and the results are given in Table 2. No sign of vitamin D deficiency could be detected by a study of X-ray photographs of the femurs of rats on diets with high levels of saponin.

Expt 2. The results given in Table 3 show that *Quillaja* saponin added to the diet at a level high enough to depress the weight gain did not interfere with the absorption of ergocalciferol or its curative effect upon rickets.

Table 2. *Expt* 1. *Mean values with their standard errors for weight increase of rats fed for* 28 *days on diet A, supplemented with* 1 *g linoleic-acid concentrate/*100 *g diet, with* Quillaja *saponin and cholesterol*

(Groups of five; number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	(1)	2·26 ± 0·17 (0)
1·0	0	(*s*)	1·76 ± 0·17 (0)
0	1·0	(*c*)	1·41 ± 0·19 (1)
1·0	1·0	(*sc*)	1·51 ± 0·17 (0)

Mean food consumption: 9·50 g/rat/day

Effect or interaction (g/rat/day)

S	−0·20 ± 0·18
C	−0·55 ± 0·18**
SC	+0·30 ± 0·18

** 0·01 > *P* > 0·001.

Table 3. *Expt* 2. *Mean values with their standard errors for weight increase of initially rachitic rats fed* ad lib. *for* 28 *days on diet C, supplemented with* 1 *g linoleic-acid concentrate/*100 *g diet, with* Quillaja *saponin and ergocalciferol*

(Groups of three)

Saponin (*S*) (%)	Ergocalciferol (*D*) (i.u./rat/day)	Treatment combination	Mean weight increase (g/rat/day)	Degree of rickets
0	0	(1)	0·61 ± 0·056	+
2·0	0	(*s*)	0·44 ± 0·056	+
0	0·5	(*d*)	0·58 ± 0·056	0
2·0	0·5	(*sd*)	0·39 ± 0·056	0

Effect or interaction (g/rat/day)

S	−0·18 ± 0·06*
D	−0·04 ± 0·06
SD	−0·01 ± 0·06

* 0·05 > *P* > 0·01.

Expts 3–9. In these experiments, *Quillaja* saponin consistently depressed the weight gain of rats, an effect specifically reversed by cholesterol (Tables 4–10). The addition of choline (Table 4) or linoleic acid (Tables 5, 6) did not affect the rate of gain, whereas 'segregated' sunflower-seed oil rich in linoleic esters (Tables 7, 8) or linoleic acid protected by an anti-oxidant (Tables 9, 10) were beneficial.

Expt 10. The normal action of *Quillaja* saponin was completely reversed by the addition to the diet of linoleic acid containing nordihydroguiaretic acid (Table 11).

Expt 11. The replacement of cholesterol by β-sitosterol in this experiment showed

that, unlike cholesterol, β-sitosterol did not reverse the effect of *Quillaja* saponin on the weight gain of rats (Table 12).

Expt 12. Saponin white was similar to *Quillaja* saponin in its adverse effect on weight gain, but this effect was not reversed by cholesterol (Table 13).

Table 4. *Expt* 3. *Mean values with their standard errors for weight increase of rats fed for* 25 *days on diet B, supplemented with* 0·5 *g linoleic acid*/100 *g diet, with* Quillaja *saponin, cholesterol and choline*

(Groups of four; number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	Choline chloride (*X*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0·15	(1)	2·52 ± 0·139 (1)
3·0	0	0·15	(*s*)	2·01 ± 0·139 (1)
0	1·0	0·15	(*c*)	2·31 ± 0·120 (0)
3·0	1·0	0·15	(*sc*)	2·39 ± 0·120 (0)
0	0	1·2	(*x*)	2·43 ± 0·120 (0)
3·0	0	1·2	(*sx*)	1·88 ± 0·120 (0)
0	1·0	1·2	(*cx*)	2·44 ± 0·120 (0)
3·0	1·0	1·2	(*scx*)	2·27 ± 0·120 (0)

Mean food consumption: 9·64 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	−0·53 ± 0·13**	*SC*	+0·24 ± 0·09*
$(+C)$	−0·04 ± 0·12	*SX*	−0·07 ± 0·09
$C(-S)$	−0·10 ± 0·13	*CX*	+0·06 ± 0·09
$(+S)$	+0·38 ± 0·13*	*SCX*	−0·05 ± 0·09
X	−0·05 ± 0·09		

* 0·05 > *P* > 0·01. ** 0·01 > *P* > 0·001.

Table 5. *Expt* 4. *Mean values with their standard errors for weight increase of rats fed for* 26 *days on diet B with* Quillaja *saponin, cholesterol and linoleic acid*

(Groups of four)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	Linoleic acid (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	2·43 ± 0·114
3·0	0	0	(*s*)	1·80 ± 0·114
0	1·0	0	(*c*)	2·23 ± 0·114
3·0	1·0	0	(*sc*)	1·95 ± 0·114
0	0	0·5	(*l*)	2·48 ± 0·114
3·0	0	0·5	(*sl*)	1·79 ± 0·114
0	1·0	0·5	(*cl*)	2·38 ± 0·114
3·0	1·0	0·5	(*scl*)	2·25 ± 0·114

Mean food consumption: 10·2 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	−0·66 ± 0·11***	*SC*	+0·23 ± 0·08**
$(+C)$	−0·20 ± 0·11	*SL*	+0·02 ± 0·08
$C(-S)$	−0·15 ± 0·11	*CL*	+0·10 ± 0·08
$(+S)$	−0·31 ± 0·11*	*SCL*	+0·05 ± 0·08
L	+0·12 ± 0·08		

* 0·05 > *P* > 0·01. ** 0·01 > *P* > 0·001. *** 0·001 > *P*.

Table 6. *Expt* 5. *Mean values with their standard errors for weight increase of rats fed for* 37 *days on diet B with* Quillaja *saponin, cholesterol and linoleic acid*

(Groups of four; number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	Linoleic acid (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·50 ± 0·082 (0)
3·0	0	0	(*s*)	0·61 ± 0·082 (0)
0	3·0	0	(*c*)	1·16 ± 0·082 (0)
3·0	3·0	0	(*sc*)	0·67 ± 0·082 (0)
0	0	1·0	(*l*)	1·45 ± 0·082 (0)
3·0	0	1·0	(*sl*)	0·76 ± 0·082 (0)
0	3·0	1·0	(*cl*)	0·87 ± 0·095 (1)
3·0	3·0	1·0	(*scl*)	0·48 ± 0·095 (1)

Mean food consumption: 8·19 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	$-0{\cdot}79 \pm 0{\cdot}08^{***}$	$L(-C)$	$+0{\cdot}05 \pm 0{\cdot}08$
$(+C)$	$-0{\cdot}44 \pm 0{\cdot}09^{**}$	$(+C)$	$-0{\cdot}24 \pm 0{\cdot}09^{*}$
$C(-S)$	$-0{\cdot}46 \pm 0{\cdot}09^{***}$	SC	$+0{\cdot}17 \pm 0{\cdot}06^{**}$
$(+S)$	$-0{\cdot}11 \pm 0{\cdot}09$	SL	$+0{\cdot}08 \pm 0{\cdot}06$
$(-L)$	$-0{\cdot}14 \pm 0{\cdot}08$	CL	$-0{\cdot}15 \pm 0{\cdot}06^{*}$
$(+L)$	$-0{\cdot}43 \pm 0{\cdot}09^{**}$	SCL	$-0{\cdot}02 \pm 0{\cdot}06$

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 7. *Expt* 6. *Mean values with their standard errors for weight increase of rats fed for* 38 *days on diet B with* Quillaja *saponin, cholesterol and 'segregated' sunflower-seed oil*

(Groups of four; number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	'Segregated' sunflower-seed oil (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·90 ± 0·078 (0)
3·0	0	0	(*s*)	1·18 ± 0·086 (1)
0	3·0	0	(*c*)	1·53 ± 0·078 (0)
3·0	3·0	0	(*sc*)	1·21 ± 0·078 (0)
0	0	1·5	(*l*)	2·14 ± 0·078 (0)
3·0	0	1·5	(*sl*)	1·60 ± 0·078 (0)
0	3·0	1·5	(*cl*)	1·70 ± 0·078 (0)
3·0	3·0	1·5	(*scl*)	1·45 ± 0·078 (0)

(1·5 % 'segregated' sunflower-seed oil is equivalent to 1·0 % linoleic esters)

Mean food consumption: 8·00 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	$-0{\cdot}62 \pm 0{\cdot}08^{***}$	SC	$+0{\cdot}17 \pm 0{\cdot}06^{**}$
$(+C)$	$-0{\cdot}29 \pm 0{\cdot}08^{**}$	SL	$+0{\cdot}06 \pm 0{\cdot}06$
$C(-S)$	$-0{\cdot}40 \pm 0{\cdot}08^{***}$	CL	$-0{\cdot}06 \pm 0{\cdot}06$
$(+S)$	$-0{\cdot}07 \pm 0{\cdot}08$	SCL	$-0{\cdot}03 \pm 0{\cdot}06$
L	$+0{\cdot}27 \pm 0{\cdot}06^{***}$		

** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 8. *Expt 7. Mean values with their standard errors for weight increase of rats fed for 25 days on diet B with* Quillaja *saponin, cholesterol and 'segregated' sunflower-seed oil*

(Groups of four; the number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	'Segregated' sunflower-seed oil (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·85 ± 0·061 (0)
3·0	0	0	(*s*)	0·90 ± 0·086 (2)
0	3·0	0	(*c*)	1·74 ± 0·061 (0)
3·0	3·0	0	(*sc*)	1·49 ± 0·061 (0)
0	0	4·5	(*l*)	2·18 ± 0·061 (0)
3·0	0	4·5	(*sl*)	1·78 ± 0·061 (0)
0	3·0	4·5	(*cl*)	1·97 ± 0·061 (0)
3·0	3·0	4·5	(*scl*)	1·80 ± 0·061 (0)

(4·5 % 'segregated' sunflower-seed oil is equivalent to 3·0 % linoleic esters)

Mean food consumption: 7·24 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	$-0{\cdot}68 \pm 0{\cdot}07^{***}$	$L(-S)$	$+0{\cdot}28 \pm 0{\cdot}06^{***}$
$(+C)$	$-0{\cdot}21 \pm 0{\cdot}06^{**}$	$(+S)$	$+0{\cdot}60 \pm 0{\cdot}07^{***}$
$(-L)$	$-0{\cdot}60 \pm 0{\cdot}07^{***}$	$(-C)$	$+0{\cdot}60 \pm 0{\cdot}07^{***}$
$(+L)$	$-0{\cdot}28 \pm 0{\cdot}06^{***}$	$(+C)$	$+0{\cdot}27 \pm 0{\cdot}06^{***}$
$C(-S)$	$-0{\cdot}16 \pm 0{\cdot}06^{*}$	*SC*	$+0{\cdot}23 \pm 0{\cdot}05^{***}$
$(+S)$	$+0{\cdot}30 \pm 0{\cdot}07^{**}$	*SL*	$+0{\cdot}16 \pm 0{\cdot}05^{**}$
$(-L)$	$+0{\cdot}24 \pm 0{\cdot}07^{**}$	*CL*	$-0{\cdot}17 \pm 0{\cdot}05^{**}$
$(+L)$	$-0{\cdot}10 \pm 0{\cdot}06$	*SCL*	$-0{\cdot}12 \pm 0{\cdot}05^{*}$

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 9. *Expt 8. Mean values with their standard errors for weight increase of rats fed for 33 days on diet B, with 50 mg α-tocopherol/100 g diet, with* Quillaja *saponin, cholesterol, and linoleic acid*

(Groups of four; number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	Linoleic acid (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·85 ± 0·052 (0)
2·0	0	0	(*s*)	1·03 ± 0·060 (1)
0	2·0	0	(*c*)	1·66 ± 0·052 (0)
2·0	2·0	0	(*sc*)	1·27 ± 0·052 (0)
0	0	2·0	(*l*)	2·31 ± 0·052 (0)
2·0	0	2·0	(*sl*)	1·49 ± 0·060 (1)
0	2·0	2·0	(*cl*)	1·82 ± 0·052 (0)
2·0	2·0	2·0	(*scl*)	1·51 ± 0·052 (0)

Mean food consumption: 8·39 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	$-0{\cdot}82 \pm 0{\cdot}06^{***}$	$L(-C)$	$+0{\cdot}46 \pm 0{\cdot}06^{***}$
$(+C)$	$-0{\cdot}35 \pm 0{\cdot}05^{***}$	$(+C)$	$+0{\cdot}20 \pm 0{\cdot}05^{**}$
$C(-S)$	$-0{\cdot}34 \pm 0{\cdot}05^{***}$	*SC*	$+0{\cdot}24 \pm 0{\cdot}04^{***}$
$(+S)$	$+0{\cdot}13 \pm 0{\cdot}06$	*SL*	$+0{\cdot}02 \pm 0{\cdot}04$
$(-L)$	$+0{\cdot}03 \pm 0{\cdot}05$	*CL*	$-0{\cdot}13 \pm 0{\cdot}04^{**}$
$(+L)$	$-0{\cdot}24 \pm 0{\cdot}05^{**}$	*SCL*	$+0{\cdot}02 \pm 0{\cdot}04$

** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 10. *Expt* 9. *Mean values with their standard errors for weight increase of rats fed for* 34 *days on diet B, with* 0·4 *mg nordihydroguiaretic acid/*100 *g diet and with* Quillaja *saponin, cholesterol and linoleic acid*

(Groups of four; the number that died in each group in parentheses)

Saponin (*S*) (%)	Cholesterol (*C*) (%)	Linoleic acid (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·39 ± 0·068 (0)
2·0	0	0	(*s*)	1·35 ± 0·079 (1)
0	2·0	0	(*c*)	1·45 ± 0·068 (0)
2·0	2·0	0	(*sc*)	1·86 ± 0·068 (0)
0	0	2·0	(*l*)	1·55 ± 0·068 (0)
2·0	0	2·0	(*sl*)	1·49 ± 0·068 (0)
0	2·0	2·0	(*cl*)	1·49 ± 0·068 (0)
2·0	2·0	2·0	(*scl*)	2·08 ± 0·068 (0)

Mean food consumption: 8·12 g/rat/day

Effect or interaction (g/rat/day)

$S(-C)$	$-0{\cdot}05 \pm 0{\cdot}07$	*SC*	$+0{\cdot}27 \pm 0{\cdot}05^{***}$
$(+C)$	$+0{\cdot}50 \pm 0{\cdot}07^{***}$	*SL*	$+0{\cdot}04 \pm 0{\cdot}05$
$C(-S)$	$0{\cdot}00 \pm 0{\cdot}07$	*SL*	$-0{\cdot}01 \pm 0{\cdot}05$
$(+S)$	$+0{\cdot}55 \pm 0{\cdot}07^{***}$	*SCL*	$+0{\cdot}05 \pm 0{\cdot}05$
L	$+0{\cdot}14 \pm 0{\cdot}05^{**}$		

** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 11. *Expt* 10. *Mean values with their standard errors for weight increase of rats fed for* 29 *days on diet B with* Quillaja *saponin and linoleic acid, and with* 20 *mg nordihydroguiaretic acid/*100 *g linoleic acid* (*equivalent to* 0·4 *mg nordihydroguiaretic acid/*100 *g diet*)

(Groups of eight; number that died in each group in parentheses)

Saponin (*S*) (%)	Linoleic acid (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	(1)	0·84 ± 0·042 (0)
3·0	0	(*s*)	0·61 ± 0·059 (4)
0	2·0	(*l*)	1·07 ± 0·042 (0)
3·0	2·0	(*sl*)	1·29 ± 0·042 (0)

Mean food consumption: 6·52 g/rat/day

Effect or interaction (g/rat/day)

$S(-L)$	$-0{\cdot}23 \pm 0{\cdot}07^{*}$
$(+L)$	$+0{\cdot}22 \pm 0{\cdot}06^{**}$
$L(-S)$	$+0{\cdot}23 \pm 0{\cdot}06^{**}$
$(+S)$	$+0{\cdot}68 \pm 0{\cdot}07^{***}$
SL	$+0{\cdot}23 \pm 0{\cdot}05^{***}$

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 12. *Expt* 11. *Mean values with their standard errors for weight increase of rats fed for* 39 *days on diet B with* Quillaja *saponin, β-sitosterol and 'segregated' sunflower-seed oil*

(Groups of four; number that died in each group in parentheses)

Saponin (*S*) (%)	β-sitosterol (*C*) (%)	'Segregated' sunflower-seed oil (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·10 ± 0·058 (0)
3·0	0	0	(*s*)	0·58 ± 0·067 (1)
0	3·0	0	(*c*)	1·15 ± 0·058 (0)
3·0	3·0	0	(*sc*)	0·67 ± 0·058 (0)
0	0	1·5	(*l*)	1·44 ± 0·058 (0)
3·0	0	1·5	(*sl*)	1·02 ± 0·058 (0)
0	3·0	1·5	(*cl*)	1·17 ± 0·058 (0)
3·0	3·0	1·5	(*scl*)	0·85 ± 0·058 (0)

(1·5 % 'segregated' sunflower-seed oil is equivalent to 1·0 % linoleic esters)

Mean food consumption: 6·36 g/rat/day

Effect or interaction (g/rat/day)

S	−0·43 ± 0·04***	*SC*	+0·04 ± 0·04
C(−*L*)	+0·07 ± 0·06	*SL*	+0·06 ± 0·04
(+*L*)	−0·22 ± 0·06**	*CL*	−0·14 ± 0·04**
L(−*C*)	+0·38 ± 0·06***	*SCL*	+0·01 ± 0·04
(+*C*)	+0·10 ± 0·06		

** 0·01 > *P* > 0·001. *** 0·001 > *P*.

Table 13. *Expt* 12. *Mean values with their standard errors for weight increase of rats fed for* 33 *days on diet B with saponin white, cholesterol and 'segregated' sunflower-seed oil*

(Groups of four; number that died in each group in parentheses)

Saponin white (*S*) (%)	Cholesterol (*C*) (%)	'Segregated' sunflower-seed oil (*L*) (%)	Treatment combination	Mean weight increase (g/rat/day)
0	0	0	(1)	1·47 ± 0·064 (0)
3·0	0	0	(*s*)	0·67 ± 0·129 (3)
0	3·0	0	(*c*)	1·38 ± 0·064 (0)
3·0	3·0	0	(*sc*)	0·70 ± 0·064 (0)
0	0	1·5	(*l*)	1·69 ± 0·064 (0)
3·0	0	1·5	(*sl*)	1·26 ± 0·074 (1)
0	3·0	1·5	(*cl*)	1·59 ± 0·064 (0)
3·0	3·0	1·5	(*scl*)	1·53 ± 0·064 (0)

(1·5 % 'segregated' sunflower-seed oil is equivalent to 1·0 % linoleic esters)

Mean food consumption: 7·45 g/rat/day

Effect or interaction (g/rat/day)

S(−*L*)	−0·74 ± 0·08***	*SC*	+0·07 ± 0·05
(+*L*)	−0·34 ± 0·07***	*SL*	+0·20 ± 0·05***
C	−0·02 ± 0·05	*CL*	+0·01 ± 0·05
L(+*S*)	+0·22 ± 0·06**	*SCL*	+0·01 ± 0·05
(+*S*)	+0·61 ± 0·08***		

** 0·01 > *P* > 0·001. *** 0·001 > *P*.

DISCUSSION

The S effect. Peterson (1950*b*) showed that the weight gain of chicks is depressed by the addition of *Quillaja* saponin to the diet. This negative *S* effect, due to *Quillaja* saponin, has now been shown to occur with growing rats (Fig. 1 and Tables 3–9 and 12). An alternative source of saponin, saponin white, also had the *S* effect (Table 13), although these two mixed saponins are chromatographically different (Coulson, 1958) and are obtained from different plants (saponin white from *Gypsophila* spp.).

Peterson also showed that the adverse effect of *Quillaja* saponin on the weight gain of chicks could be reversed by the addition of cholesterol to the diets containing saponin. This positive *SC* interaction also occurred with growing rats (Tables 4–10). The *SC* interaction did not occur in an experiment in which saponin white was substituted for *Quillaja* saponin (Table 13), or when β-sitosterol was used in place of cholesterol (Table 12). The presence of *Quillaja* saponin in the diet of rachitic rats did not prevent absorption of curative doses of ergocalciferol (Table 2). The interaction of *Quillaja* saponin would appear to be specifically with cholesterol and not with β-hydroxysteroids in general when tested upon growing rats. This finding contrasts with those of Peterson (1950*b*) that some phytosterols (e.g. sitosterol, stigmasterol), as well as cholesterol, can reverse the inhibitory effect of lucerne saponins upon the growth of chicks.

The C effect. In several experiments, it was noticed that cholesterol itself could exert an inhibitory negative *C* effect upon the weight gain of rats (Tables 2, 6, 7, 9); a similar effect was noticed by Sperry & Stoyanoff (1935) when feeding rats on synthetic diets. This inhibition was particularly evident in the experiment summarized in Table 2, in which the rats were all fed on diets prepared from diet A. The *C* effect here may, in fact, have been measuring the toxic action of the mixture of free linoleic-acid concentrate and cholesterol (Raulin, 1954; Raulin & Jacquot, 1954). That it was so is suggested by the fact that the presence of linoleic acid, but not of its esters, resulted in a greater inhibition by cholesterol (Tables 6, 9 and cf. 7) ($C(-L)$ and $C(+L)$ effects). The method of preparing the linoleic-acid concentrate used in the experiment illustrated in Table 2 could have led to a partially peroxidized product. It is relevant to note that in this experiment, in which the diets contained this linoleic-acid concentrate, the standard errors of estimates appeared much greater than in other experiments.

In consequence, the action of various factors associated with cholesterol metabolism was investigated.

The X effect. The usual dietary level of choline was shown to be adequate, by increasing it from 0·15 to 1·2% (as choline chloride); no improvement in performance was found at the higher level, nor did it influence the *S* effect or *SC* interaction when 1% cholesterol was given (Table 4). The lower level of choline is presumably high enough to prevent the toxic effect of a mixture of cholesterol and the free fatty acids derived from sunflower-seed oil described by Raulin (1954).

The L effect. When free linoleic acid unprotected by anti-oxidants was incorporated in the diet, there was no appreciable effect (Tables 5, 6), but when segregated

sunflower-seed oil was used in the diets as a source of linoleic esters, there was a marked increase in the rate of weight gain, i.e. a positive *L* effect (Tables 7, 8, 12, 13). When free linoleic acid in the diet was protected from oxidation by the addition of anti-oxidants, such as α-tocopherol or nordihydroguiaretic acid, the *L* effect became significant (Tables 9–11).

The SL interaction. Although linoleic acid showed a general growth-promoting *L* effect when esterified or protected by anti-oxidants, a specific *SL* interaction leading to a diminution of the *S* effect was only seen when the linoleic-ester level was exceptionally high (Table 9) or when saponin white was substituted for *Quillaja* saponin (Table 13). The apparently significant *SL* interaction shown in Table 11 is unavoidably confounded with the effect of the anti-oxidant nordihydroguiaretic acid given only to those rats receiving linoleic acid and should not be interpreted as a specific reversal of the *S* effect by linoleic acid itself.

The CL interaction. The negative *CL* interaction (Tables 6, 8, 9, 12) ($L(-C)$ and $L(+C)$ effects) showed that the beneficial *L* effects of linoleic acid or its esters is reduced in the presence of cholesterol or β-sitosterol more than would be expected from the main *C* and *L* effects. Since β-sitosterol is not absorbed by rats (Schoenheimer, 1932; Breusch, 1938; Rosenheim & Webster, 1941) the site of the *CL* interaction must with this sterol be in the gut.

The SCL interaction. The *SCL* interaction measures the effect upon the *SC* interaction of the absence or presence of linoleic acid or esters. It was found to be significant in only one experiment (Table 8), in which a high level of 'segregated' sunflower-seed oil was added to the diet to supply linoleic esters. In the presence of linoleic esters the *SC* interaction was reduced ($SC(-L) = +0{\cdot}35 \pm 0{\cdot}07^{***}$, $SC(+L) = +0{\cdot}11 \pm 0{\cdot}06$). This finding is consistent with the view that the site of this interaction is the liver, since linoleic esters are known to cause a mobilization and reduction of cholesterol and lipids in this organ (Aftergood *et al.* 1957).

Peterson (1950*b*) failed to produce a reversal of the *S* effect by implantation of stilboestrol in his chicks, although it resulted in a tenfold increase in the total plasma cholesterol. As this increase in total plasma cholesterol may well have arisen from reduction in the cholesterol content of the liver, Peterson's findings again would be consistent with the liver's being the site of the *SC* interaction. It is further known that eating certain saponins, including *Quillaja* saponin, damages the liver (Kobert, 1933; Ender, 1955; Enslin & Wells, 1956; de Kock & Enslin, 1958).

The effect of anti-oxidants. The addition of anti-oxidants to the diet showed a difference in the actions of the two substances chosen for study. When α-tocopherol was used, its only noticeable effect was to make linoleic acid act in the same manner as its esters (*L* effect, Table 9). This is the effect that would be expected if the free unsaturated fatty acid is more easily oxidized than the ester and if α-tocopherol was acting purely in an anti-oxidant capacity.

When nordihydroguiaretic acid was added to the diet (Table 10), the *L* effect of linoleic acid was again significant, like that with α-tocopherol. However, the *S* effect of *Quillaja* saponin was completely reversed in the presence of nordihydroguiaretic acid; i.e. there was an increase in weight gain instead of the usual decrease caused by

dietary saponin. The *C* effect of cholesterol was also reversed in sign: cholesterol in the presence of the anti-oxidant increased the rate of weight gain. In the experiment illustrated in Table 11, *Quillaja* saponin in the absence of both linoleic acid and nordihydroguiaretic acid depressed the rate of weight gain (Table 11, $S(-L)$ effect). However, in the presence of both linoleic acid and nordihydroguiaretic acid the $S(+L)$ effect was positive, which confirmed the results of the previous experiment (Table 10). There would appear to be a function of nordihydroguiaretic acid separate from its anti-oxidant role, in that its presence in catalytic amounts leads to faster rate of gain by growing rats on diets containing either *Quillaja* saponin or cholesterol.

Toxicity of saponin. During these experiments, it was noticed that the mortality of rats on diets containing saponin was much higher than that of rats on diets that either contained no saponin or contained saponin and certain additional dietary factors (Table 14). Which particular dietary factor was responsible for reducing the toxicity is unknown.

Efficiency of food conversion. The rates of weight gain discussed were all obtained under conditions of restricted feeding: they are therefore an indirect measure of the efficiency of food conversion under these conditions.

Table 14. *Number of rats on various diets, and number that died*

Group	Diet	No. of rats: At risk	No. of rats: Died
A	Basal diets A and B alone or containing cholesterol (or β-sitosterol) or linoleic acid (or 'segregated' sunflower-seed oil) or both	154	2
B	Basal diets A and B containing *Quillaja* saponin (or saponin white)	60	17
C	Basal diets A and B containing *Quillaja* saponin (or saponin white) and cholesterol (or β-sitosterol) or linoleic acid (or 'segregated' sunflower-seed oil) or both	109	3

Mortality A = mortality C ≪ mortality B**

** 0·01 > P > 0·001.

SUMMARY

1. Month-old male Wistar hooded rats were given in restricted quantities for 4–6 weeks a basal diet to which various supplements were added.

2. *Quillaja* saponin (2–3 g/100 g diet) and saponin white (3 g/100 g diet) depressed the rate of weight gain.

3. *Quillaja* saponin (2 g/100 g diet) did not affect utilization of ergocalciferol.

4. The depression of weight gain caused by *Quillaja* saponin was specifically reversed by cholesterol (1–3 g/100 g diet) but not by β-sitosterol (3 g/100 g diet).

5. Cholesterol (1–3 g/100 g diet) also retarded weight gain.

6. Linoleic acid when esterified or protected by an anti-oxidant increased the rate of gain whereas free linoleic acid had no effect.

7. Choline in the diets tested did not affect weight gain.

8. Nordihydroguiaretic acid appeared to have a stimulatory effect, separate from its anti-oxidant role, but α-tocopherol did not.

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