very much higher concentrations of the antibiotic to inhibit cell-wall-mucopeptide formation.

3. The composition of the cell walls of the sensitive and resistant cells is the same.

4. The inhibitory effects upon mucopeptide synthesis by sensitive cells of sodium 6-(2:6-dimethoxybenzamido)penicillin (Methicillin, Celbenin, AB 1241), 6-(α-phenoxypropionamido)penicillanic acid (Broxil) and 6-aminopenicillanic acid are as predicted from their relative potencies tested as antibiotics.

5. Cells made resistant to benzylpenicillin do not require increased concentrations of sodium 6-(2:6-dimethoxybenzamido)penicillin to inhibit mucopeptide synthesis.

6. Sodium 6-(2:6-dimethoxybenzamido)penicillin, like benzylpenicillin, does not inhibit the formation of either proteins or nucleic acids by *Staphylococcus aureus*.

7. Sodium 6-(2:6-dimethoxybenzamido)penicillin inhibits almost completely mucopeptide synthesis by the penicillinase-producing strain *Staphylococcus aureus* 524/SC. The concentration required is similar to that needed by the sensitive strain Oxford.

We wish to thank Mr I. Mathison for continuous technical assistance during this work, and Dr H. R. Perkins for the chemical analyses of the cell-wall preparations.

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Biochem. J. (1961) **81**, 584

The Effects of Corn Oil on the Amounts of Cholesterol and the Excretion of Sterol in the Rat

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(*Received* 1 *May* 1961)

Using chicks as experimental animals, Peterson (1951) discovered that the addition of plant sterols to a cholesterol-containing diet decreased the concentration of cholesterol in the plasma. This effect was attributed by Peterson, Shneour, Peek & Gaffey (1953) to an inhibition of the formation of cholesterol esters in the mucosa by plant sterols, including β-sitosterol, present in soya-bean oil. Another mechanism proposed by Glover & Green (1955) for the inhibition of dietary-cholesterol absorption involved a blockage of the sites of absorption of cholesterol in the intestinal mucosa by β-sitosterol and other plant sterols. However, the results of Beveridge, Connell, Mayer & Haust (1958), showing that the concentration of cholesterol in human plasma was lowered by adding corn oil and β-sitosterol to a cholesterol-free diet, cannot be explained fully on the basis of either of the mechanisms cited above. If the limiting factor was ester formation, the absorption of dietary and biliary cholesterol should result in an increase of free cholesterol relative to ester cholesterol in the

intestine when plant sterols are present in the diet. Such an increase has not been reported. If, on the other hand, blockage of the sites of absorption of cholesterol was the limiting factor, the results of Beveridge *et al.* (1958) could be explained only if it was assumed that normally the absorption of biliary cholesterol was considerable. However, only minor amounts of biliary cholesterol appear to be absorbed (cf. Mead & Howton, 1960; Gould & Cook, 1958). Gould (1955) considered that, although the absorption in the intestine of β-sitosterol was limited, nevertheless this sterol might reduce hepatic cholesterol synthesis. However, no confirmation for this hypothesis was given.

In the present work the effect of corn oil on the metabolism of cholesterol was studied by determining the amounts of this constituent in the tissues and organs of rats of different ages. As Haust & Beveridge (1958) found in humans that corn-oil supplementation to a fat-free diet increased the excretion of bile acids, an overall study of the changes in cholesterol metabolism was not considered possible without determining also the amount of faecal sterols.

EXPERIMENTAL AND RESULTS

Two experiments were carried out with male Wistar rats. In the first experiment the object was to observe the effect of corn oil on the cholesterol concentration of the whole animal and of the serum of some of the rats. In the second experiment the concentrations of cholesterol in a number of organs and tissues and in the serum were determined. The excretion of sterols was studied in both experiments.

In Expt. 1 two series of animals were used: series 1, weanling rats (28 days; av. wt. 75 g.); series 2, 5-month-old rats (av. wt. 334 g.). In Expt. 2, 1-year-old rats (series 3; av. wt. 402 g.) were used. The basal low-fat diet (fat content less than 1·4 %) was based on that described by Longenecker (1939) and consisted of (%): casein 20, corn starch 60, sucrose 9, salt mixture 4, agar-agar 2, and dried yeast 5. In addition 2·6 mg. of α-tocopherol acetate, 85 i.u. of calciferol and 600 i.u. of vitamin A acetate, dissolved in paraffin oil, were administered orally once per week to each rat.

In both experiments all animals were first given the low-fat diet for 3 weeks. After this, the experimental groups received supplements of corn oil mixed into the low-fat diet, whereas the control groups continued to receive the low-fat diet alone. In both series 1 and 2 there were four experimental groups, each of five rats, receiving respectively 2 or 10 % supplements of crude or refined corn oil for a period of 5 weeks. In series 3 there was one experimental group of 12 animals which received the basal diet supplemented with 10 % of crude corn oil for 3½ weeks, whereas the control group of 10 rats continued on the basal diet. Although food and water were available *ad libitum* throughout, the food intake as measured in series 3 showed the diets to be nearly isocaloric, as the corn-oil-fed rats ate less food than the control animals (12·2 g. as compared with 13·3 g./rat/day). This difference in intake amounted to 2–3 kcal. out of a total of approximately 45 kcal./day.

At the end of the feeding period food was withheld for 12 hr. The animals of series 1 were then decapitated and those of series 2 and 3 were anaesthetized with ether and then exsanguinated by heart puncture.

The body-cholesterol concentrations of all groups are collected together in Table 1, and Table 2 shows concentrations of cholesterol in a number of organs and tissues of the 1-year-old rats of series 3.

Table 1. *Effect of corn-oil supplements on the body cholesterol of rats at different ages*

Age	Corn-oil supplement	Body cholesterol (mg./100 g. of rat)			Change in cholesterol (mg./100 g. of rat)	
		Free	Total	Ester (total – free)	Free	Ester
Weanling (28 days) (series 1)	None	110	138	28	—	—
	2% crude	135	160	25	+25	−3
	2% refined	134	162	28	+24	0
	10% crude	143	168	25	+33	−3
	10% refined	146	172	26	+36	−2
5 months (series 2)	None	96	127	31	—	—
	2% crude	109	138	29	+13	−2
	2% refined	108	139	31	+12	0
	10% crude	114	146	32	+18	+1
	10% refined	114	144	30	+18	−1
1 year* (series 3)	None	111	137	26	—	—
	10% crude	128	156	28	+17	+2

* Calculated from the individual organs and tissues.

Table 2. *Cholesterol in the tissues of 1-year-old rats (series 3) with and without a 10% crude corn-oil supplement*

Organ or tissue	Corn-oil supplement	Tissue cholesterol (mg./100 g. of tissue) Free	Total	Ester (total – free)	Change in cholesterol (mg./100 g. of tissue) Free	Ester
Aortas	–	120	164	44	—	—
	+	159	198	39	+39	–5
Hearts	–	112	150	38	—	—
	+	127	163	36	+15	–3
Livers	–	202	209	7	—	—
	+	200	227	27	–2	+20
Muscle (rectus femoris)	–	56	65	9	—	—
	+	66	74	8	+10	–2
Adrenals	–	166	1312	1146	—	—
	+	164	1308	1144	–2	–2
Testes	–	92	173	81	—	—
	+	109	176	67	+17	–14
Adipose (perinephric)	–	135	169	34	—	—
	+	144	178	34	+9	0
Intestines	–	116	201	85	—	—
	+	147	252	105	+31	+20
Pelts	–	119	195	76	—	—
	+	114	227	113	–5	+37
Remaining tissues	–	102	113	11	—	—
	+	118	134	16	+16	+5

Table 3. *Serum lipid and cholesterol of rats varying in age and on different corn-oil supplements*

Age	Corn-oil supplement	Serum lipid (mg./100 ml. of serum)	Serum cholesterol (mg./100 ml. of serum) Free	Total	Ester (total – free)	Change in cholesterol (mg./100 ml. of serum) Free	Ester
5 months (series 2)	None	744	32	116	84	—	—
	2% crude	485	30	82	52	–2	–32
	2% refined	401	29	82	53	–3	–31
	10% crude	249	32	76	44	0	–40
	10% refined	197	25	60	35	–7	–49
1 year (series 3)	None	832	27	113	86	—	—
	10% crude	674	27	98	71	0	–15

Table 4. *Daily excretion of faecal cholesterol and coprosterol by rats varying in age and on different corn-oil supplements*

Age	Corn-oil supplement	Free cholesterol (mg./rat)	Free coprosterol (mg./rat)	Cholesterol plus coprosterol (mg./rat)	Change in cholesterol plus coprosterol	Ratio of cholesterol to coprosterol
Weanling (28 days) (series 1)	None	1·3	15·3	16·6	—	0·085
	2% crude	2·1	15·9	18·0	+ 1·4	0·132
	2% refined	1·7	14·9	16·6	0	0·114
	10% crude	3·5	25·6	29·1	+10·3	0·137
	10% refined	2·9	22·0	24·9	+ 4·9	0·132
5 months (series 2)	None	1·2	12·3	13·5	—	0·098
	2% crude	1·3	12·9	14·2	+ 0·7	0·101
	2% refined	1·2	13·2	14·4	+ 0·9	0·091
	10% crude	3·9	30·4	34·3	+20·8	0·128
	10% refined	3·6	27·6	31·2	+17·7	0·130
1 year (series 3)	None	1·2	11·6	12·8	—	0·103
	10% crude	3·0	27·6	30·6	+17·8	0·109

The serum-cholesterol concentrations of the rats of series 2 and 3 are shown in Table 3.

To determine the weight of faecal coprosterol and cholesterol excreted per day during corn-oil supplementation, the faeces of all animals within each group were collected daily into ethanol and stored in a refrigerator (see Table 4).

The fatty acid composition of the total lipids isolated from serum, tissues and faeces from animals of series 3, as well as that of the crude corn oil, are shown in Table 5. The annotations for the different fatty acids are those used by Farquhar, Insull, Rosen, Stoffel & Ahrens (1959).

Methods

As soon as possible after death, the complete animals (including heads) of series 1 and 2 were rapidly minced and heated in 2 vol. of 95% (v/v) ethanol for 2 hr. at 70° and sealed in jars for storage. The individual tissues and organs of series 3 were similarly treated in a Waring Blendor. The serum was freeze-dried and stored in sealed ampoules. The faeces were also preserved by heating in ethanol.

Within each group the materials thus preserved were combined and the ethanol and water removed on a water bath *in vacuo*. The residues were extracted as described by Gerson, Shorland, Adams & Bell (1959). The same procedure was used for the freeze-dried serum.

Cholesterol in the extracted lipids was determined by the method of Zak, Luz & Fisher (1957) with the ferric chloride–acetic acid–conc. sulphuric acid reagent. To render the method suitable for the determination of cholesterol in the presence of a very large excess of other lipids the solvent systems of the original method were modified and 1 ml. of 1% digitonin solution in ethanol–water (3:1, v/v) was used. The free cholesterol digitonide was washed with 2 ml. of acetone–ethanol (3:1, v/v). To determine total cholesterol a sample containing approximately 1 mg. of lipids was saponified by a modification of the procedure of Sperry & Webb (1950) before the method of Zak *et al.* (1957) was used as for free cholesterol. The samples were dissolved in 1 ml. of ethanol in 15 ml. centrifuge tubes and 1 ml. of ethanolic 0·5N-KOH was added. The tubes were immersed in a water bath at 45–50° for 30 min., and then neutralized (phenolphthalein) with aqueous 30% (v/v) acetic acid and evaporated to a small volume (about 0·1 ml.). The digitonide was precipitated as described above and washed with 2 ml. of ethanol–water (1:1, v/v). Faecal cholesterol and coprosterol were determined as described by Gerson (1960).

All determinations were carried out in quadruplicate and the mean values are quoted. The average standard deviation for quadruplicate sets of determinations was approximately $\pm 0{\cdot}0004$ mg. It was considered that any change involving less than 5 mg. of cholesterol/100 g. of tissue was not significant, owing to the limitation of the methods used and to the experimental design.

The fatty acid composition of the total lipids from tissues, serum and faeces was determined by gas–liquid chromatography in a column (0·5 cm. × 250 cm.) with diethylene glycol adipate polyester (20%, w/w) supported on Celite 545 as the liquid phase. The detector was based on that described by Lovelock, James & Piper (1959) with argon as carrier gas. The methyl esters were identified by comparison of R_F values with those of pure samples and with those given by Farquhar *et al.* (1959).

DISCUSSION

Table 1 shows that the amounts of free and esterified cholesterol of the whole animals of the control groups are not significantly affected by age. However, the increase in the tissue free-cholesterol concentration after the addition of corn oil in the mature animals is approximately half that of the weanlings. Table 1 also shows that the increase in the concentration of body free-cholesterol after a fivefold increase in the amount of corn oil administered is much less than might be expected if this increase was directly proportional to the amount of corn oil added. This result may be partly explained by the large increase in the rate of excretion of cholesterol and coprosterol with the higher amount of corn oil fed and is consistent with the existence of a homeostatic mechanism (cf. Tables 1 and 4).

The determination of coprosterol as well as cholesterol in the faeces was considered necessary since Snog-Kjaer, Prange & Dam (1956) and Coleman & Baumann (1957*a*, *b*) produced strong evidence that coprosterol is a bacterial hydrogenation product of cholesterol. The approximately constant ratio [$0{\cdot}113 \pm 0{\cdot}018$(S.D.):1·000] of cholesterol to coprosterol in the faecal sterols (see Table 4) indicates the attainment of equilibrium in the hydrogenation of cholesterol. No esterified sterols were found in the faeces.

The amounts of body cholesterol and of daily sterol excretion (Tables 1 and 4) show a considerable increase in cholesterol biosynthesis when corn oil is added to a low-fat diet. This increase is not balanced by the decrease in the amount of serum cholesterol ester (Table 3) in series 2 and 3.

Beveridge, Connell & Mayer (1957), who worked with human subjects on a diet containing fat, obtained similar results. Using a basal fat-free diet containing cholesterol, Klein (1958, 1959) found that the addition of corn oil raised the concentration of serum cholesterol in weanling rats, but in 6-month-old animals the concentration was lowered. Our work (Table 3) also indicates that age affects the changes in the concentration of serum cholesterol when corn oil is added to a low-fat diet. In our experiments the serum of weanling rats was not collected. In the 5-month-old animals the concentration of serum cholesterol was markedly lowered by the addition of corn oil to the diet, but in the 1-year-old rats this decrease was much smaller. Moreover, the age effect is also reflected in the decrease in total plasma lipids. Whereas the addition of 10% of crude corn oil to the basal diet

Table 5. *Fatty acid composition of serum tissue and faecal lipids of* 1*-year-old rats on a low-fat diet and on the same diet with the addition of* 10 % *of crude corn oil*

Tr., Trace.

Tissue or organ	Corn-oil supplement	Fatty acids (moles %) 10:0, 12:0	13:0	14:0	15:0 br.	15:0	16:0 iso	16:0	16:1	17:0 br.	17:0	18:0 iso	18:0	18:1	18:2	18:3	20:0	20:1	20:2	20:3	20:4
Serum	–	0·4	—	3·9	—	2·7	—	34·9	7·1	—	1·2	Tr.	13·7	25·5	5·5	Tr.	—	—	—	—	5·1
	+	0·2	—	1·8	—	0·8	—	36·5	1·5	—	1·2	Tr.	15·8	17·6	15·9	Tr.	—	—	—	—	8·7
Aorta	–	—	—	1·2	—	0·2	—	36·8	7·1	—	0·5	0·5	4·0	39·1	6·8	Tr.	Tr.	1·8	—	—	1·5
	+	—	—	1·1	—	Tr.	—	24·4	3·0	—	Tr.	Tr.	4·1	40·0	20·4	—	3·0	3·0	—	—	1·0
Heart	–	0·4	—	0·9	—	0·1	0·1	24·6	4·9	—	0·4	0·4	15·1	28·6	14·6	—	0·6	—	1·7	0·7	7·7
	+	0·4	—	1·5	—	0·2	0·3	24·9	2·9	—	0·8	0·6	9·4	33·7	20·7	—	1·0	—	0·3	0·1	3·2
Liver	–	Tr.	—	1·1	—	—	—	33·7	8·6	—	—	—	16·5	25·5	6·4	—	—	—	—	—	8·2
	+	0·7	—	1·4	—	Tr.	—	33·0	3·5	—	Tr.	Tr.	24·5	25·2	10·9	—	—	—	Tr.	—	1·8
Muscle (rectus femoris)	–	Tr.	—	2·2	—	0·7	—	31·0	10·7	—	0·7	0·7	5·7	35·1	12·1	—	—	1·1	—	—	—
	+	—	—	1·3	—	Tr.	—	22·1	5·4	—	Tr.	Tr.	3·3	43·8	21·5	—	—	2·6	—	—	—
Adrenals	–	—	—	1·6	—	0·5	—	16·7	2·5	—	0·8	—	15·1	57·6	4·1	—	0·9	—	—	—	0·2
	+	—	—	1·0	—	0·5	—	25·0	3·1	—	0·8	—	4·8	51·1	12·6	—	0·5	—	—	—	0·8
Testes	–	—	—	0·8	—	—	—	31·0	9·8	—	Tr.	Tr.	3·1	42·3	10·6	—	—	—	—	—	2·4
	+	—	—	1·3	—	Tr.	—	20·3	12·1	—	Tr.	0·7	2·1	50·0	12·4	—	—	—	—	—	1·3
Adipose (perinephric)	–	—	—	0·8	—	0·3	—	31·2	8·0	—	0·3	0·3	1·1	46·0	10·9	Tr.	0·3	0·7	—	—	—
	+	—	—	0·8	—	—	—	24·7	5·8	—	—	—	1·6	51·9	14·8	0·1	Tr.	0·3	—	—	—
Faeces	–	0·9	1·0	1·6	10·3	7·3	1·9	27·2	2·4	2·4	1·7	0·9	10·0	9·3	3·3	—	1·4	18·4*			
	+	Tr.	0·4	1·3	8·0	9·5	1·5	36·7	2·9	2·5	1·8	0·7	10·6	11·0	4·0	—	0·7	8·4*			
Crude corn oil		—	—	—	—	—	—	10·8	0·1	—	—	—	1·2	37·7	50·0	—	0·2	—	—	—	—

* Acids of unknown constitution with R_F values greater than those for 20:0 acids.

decreased the lipids of 5-month-old rats from 744 to 249 mg./100 ml. of serum, in the 1-year-old animals the lipids were decreased from 832 to only 674 mg./100 ml. of serum. It is remarkable that cholesterol accounts for only approximately 10% of these decreases. The mechanism involved in the lowering of serum-lipid and serum-cholesterol concentrations is at present unknown, as are the reasons for the effect of age.

Table 2 shows the changes in cholesterol of various organs and tissues when a 10% corn-oil supplement is given to 1-year-old rats. As expected in the rats of series 3 the increase in the cholesterol ester of the livers is accompanied by a decrease in the serum cholesterol ester. These results are in harmony with the findings of other workers (cf. Alfin-Slater, Aftergood, Wells & Deuel, 1954; Klein, 1958).

Of particular significance in connexion with studies on atherosclerosis is the observation that in 1-year-old rats whereas 10% corn-oil supplementation produces a 17% decrease of the serum ester cholesterol (Table 3), the free cholesterol of the aortas and hearts increases by 33 and 13% respectively (Table 2). If cholesterol metabolism is linked with the incidence of atherosclerosis then the work now reported indicates that serum cholesterol bears no direct relationship to the amounts of this constituent present in the arteries or in other tissues. Similar results were reported by Merrill (1958), using rat livers. Howard & Gresham (1960) also concluded from their work that 'plasma cholesterol is not necessarily an index of the severity of atherosclerosis and that experiments involving only the determination of plasma cholesterol are of limited value'. The use of serum-cholesterol concentrations may not only give a false picture of the cholesterol concentrations of the tissues, but further obscures the relationships, if any, between cholesterol and the incidence of atherosclerosis. The elevation of sterol excretion, however, affords some indication of major changes in cholesterol biosynthesis in the tissues.

When corn oil is added to the diet the amounts of cholesterol in the tissues and organs are generally raised. In the light of the work of Swell, Trout, Field & Treadwell (1958), the increases in the free and ester cholesterol in the intestinal tissues indicate increased biosynthesis of these constituents. An increase due to the reabsorption of biliary and intestinal cholesterol from the intestinal lumen cannot be entirely ruled out, but since the proportion of free cholesterol in the intestine remains constant at 58% of the total cholesterol (Table 2), the relative increase of free cholesterol, which would be expected if β-sitosterol inhibited esterification as suggested by Peterson *et al.* (1953), does not occur. Further, the blocking of intestinal absorption of cholesterol by β-sitosterol, as suggested by Glover & Green (1955), cannot account for the changes in serum cholesterol described in this paper, since no cholesterol was included in the diet. The increased amounts of cholesterol in the tissues of the rats, together with the increased excretion of sterols in the faeces with corn-oil supplementation found in the present investigation, are consistent with the promotion of biosynthesis of cholesterol but do not exclude the possibility of lowered cholesterol catabolism. The probability that the feeding of corn oil promotes the biosynthesis of cholesterol rather than the decrease of cholesterol catabolism is supported by the recent work of Hill, Webster, Linazasoro & Chaikoff (1960), who have shown that there is increased formation of cholesterol in the liver of rats after the addition of corn oil to a fat-free diet.

Fig. 1 shows a linear increase in free cholesterol with increasing amounts of lipid in the body of the rat from the onset of maturity. On the other hand, the amount of esterified cholesterol is not related to that of the body lipids. The graphs also clearly show the difference in the amounts of free cholesterol between the controls and rats receiving 10% of corn oil.

Table 5 shows that the organ and tissue lipids in the corn oil fed, as compared with the control group, contain more linoleic acid (18:2) in response

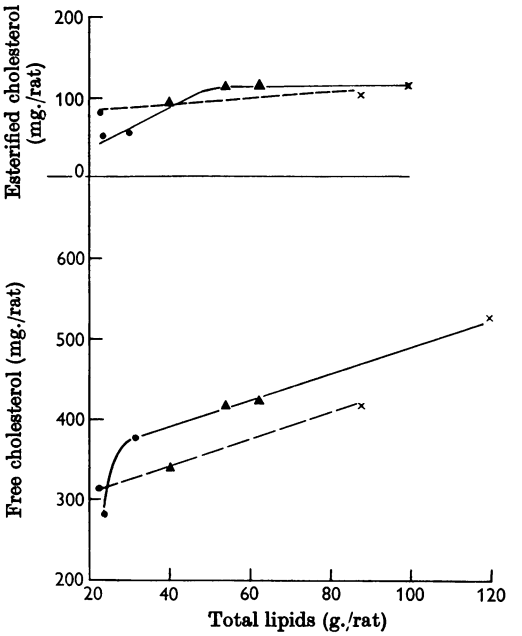

Fig. 1. Relationship of total body lipids to total body cholesterol in rats. —, 10% corn oil; - - -, low fat; ●, series 1; ▲, series 2; ×, series 3.

to the relatively high linoleic acid content (50 %) of the dietary fat. As Mead & Howton (1957) demonstrated that linoleic acid (18:2) is converted in the rat into arachidonic acid (20:4), it could be expected that the addition of corn oil to a low-fat diet would result in an increase in the arachidonic acid content of the lipids in the tissues and organs of the rat, such as has been observed by Rieckehoff, Holman & Burr (1949). However, with the exception of the adrenal glands, the addition of corn oil to the diet in the present work resulted in a decrease in arachidonic acid in the lipids of those organs where it was found, i.e. in the aortas, hearts, livers and testes. Apart from the livers this is accompanied by an increase in free cholesterol. In the adrenal glands neither cholesterol nor arachidonic acid changed significantly. On the other hand, in the serum lipids the amount of arachidonic acid was increased and the serum cholesterol diminished by the addition of the dietary corn oil. Our results are consistent with an inverse relationship between the amounts of cholesterol in the tissues and arachidonic acid in the tissue lipids. Klein (1959) has also shown that the elevation of liver cholesterol in rats on feeding cholesterol is accompanied by a decrease in arachidonic acid in the lipids of this organ.

The fatty acid composition of the faecal fat is very unlike that of the tissue and serum lipids, differing largely in the presence of substantial amounts of C_{15} and C_{17} branched and normal fatty acids, as well as higher (C_{20} and above) acids of unknown constitution. The unusual fatty acid composition is perhaps related to the bacterial origin of these lipids. Corn-oil supplementation does not substantially affect the composition of faecal fatty acids, apart from the elevation of the palmitic acid content at the expense of fatty acids, whose methyl esters under the experimental conditions used had retention times greater than that of methyl arachidate.

SUMMARY

1. Supplementation of a low-fat diet with 2 or 10 % of corn oil led to an increase in the cholesterol concentrations of the tissues and organs of rats, particularly the hearts, aortas, livers, intestines and muscle.

2. The increases in the cholesterol of the tissues and organs were accompanied by a large decrease in the concentration of serum lipids and a considerable decrease in the concentration of serum cholesterol, these effects being relatively greater in young animals. The decrease in serum cholesterol which accompanied an increase in the overall cholesterol concentrations of the tissues indicates that the serum cholesterol is not a suitable measure of the changes in cholesterol concentration in the animal as a whole. However, some indication of changes in the body cholesterol of rats can be gained by measuring the rate of sterol excretion.

3. There appears to be a definite relationship between body cholesterol and total body lipids in mature rats, the ratio being lower in rats fed on a low-fat diet than those receiving a corn-oil supplement.

4. The fatty acid composition of the tissue and serum lipids reveals an inverse relationship between the concentrations of cholesterol in the tissues and that of arachidonic acid in the lipids, the reason for which is not understood.

We wish to acknowledge Dr Muriel E. Bell, C.B.E., for her interest and encouragement, and the staff, especially Mr G. G. Dunkley, Nutrition Research Department, Medical School, University of Otago, Dunedin, N.Z., for their assistance.

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Biochem. J. (1961) **81**, 591

Deacetylcephalosporin C

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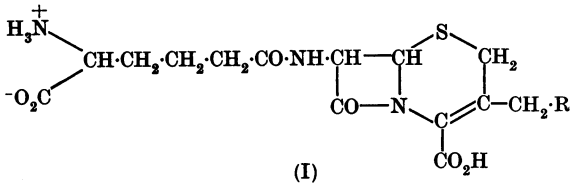
(*Received* 15 *May* 1961)

Structure I (R = $CH_3 \cdot CO \cdot O$) has been proposed for cephalosporin C (Abraham & Newton, 1961; Hodgkin & Maslen, 1961). This paper describes the formation and some of the properties of deacetylcephalosporin C (I; R = $\cdot OH$). Deacetylcephalosporin C appeared to be one of the products formed when cephalosporin C was treated with dilute alkali at room temperature. However, under these conditions hydrolysis of the acetoxyl group was accompanied, to some extent, by opening of the β-lactam ring and by other changes in the molecule. The conversion of cephalosporin C into deacetylcephalosporin C in neutral aqueous solution was accomplished, in good yield, by use of an acetyl esterase. Enzymes which hydrolyse esters of acetic acid, but which differ from acetylcholinesterases in that they are not inhibited by low concentrations of eserine, have been found to occur in plants, fungi and animal tissues (Hofstee, 1960). The acetyl esterase used here was that from citrus fruits described by Jansen, Jang & Macdonnell (1947). They reported that acetyl esters were hydrolysed by this enzyme much more readily than esters of other acids, but that *N*-acetyl groups were not hydrolysed.



(I)

METHODS AND EXPERIMENTAL

Elementary analyses were by Weiler and Strauss. Infrared spectra were measured with a Perkin–Elmer double-beam photometer model 21.

Electrophoresis and chromatography on paper. Electrophoresis on Whatman no. 1 paper (14 v/cm. for 2·5 hr.) was carried out as described by Newton & Abraham (1954) in collidine–acetate buffer (0·05 M to acetate), pH 7·0, in pyridine–acetate buffer (0·05 M to acetate), pH 4·5, and in 10% (v/v) acetic acid, pH 2·2. The apparatus used was similar to that of Flynn & de Mayo (1951). Paper chromatograms were run on Whatman no. 1 paper in butan-1-ol–acetic acid–water (4:1:4, by vol.) (Woiwod, 1949), and in propan-1-ol–water (7:3, v/v). Samples of 50–200 μg. were applied to the paper. After chromatography, or after electrophoresis in 10% (v/v) acetic acid, the paper was placed between the viewer and a source of ultraviolet light (230–400 mμ; Corning 9863 filter). Compounds with the cephalosporin C chromophore appeared as dark, light-absorbing, spots.

Bioautographs. After paper electrophoresis or chromatography the paper was dried in air and applied to the surface of a plate of nutrient agar seeded with *Staphylococcus aureus* (Oxford strain, NCTC 6571) or with *Salmonella typhi*, strain 'Mrs S' (Felix & Pitt, 1935). After 20 min. the paper was peeled off and the plate incubated at 37° overnight. Clear zones with no bacterial growth marked the positions of substances active against the test organisms.

Antibacterial activities. These were measured by the hole-plate method, cephalosporin C being used as a standard (Abraham, Newton & Hale, 1954). *Staph. aureus* (NCTC 6571) and *Salm. typhi*, strain 'Mrs S' (Felix & Pitt, 1935) were the test organisms. One unit of activity is contained in 0·1 mg. of cephalosporin C sodium salt dihydrate.

Electrometric titrations. These were carried out at 20° in the manner described by Newton & Abraham (1953).

Citrus acetyl esterase. Partially purified preparations of acetyl esterase were prepared from orange peel by the method of Jansen *et al.* (1947), except that $Na_2S_2O_4$ (0·5 mg./ml.) was added to the press juice, before precipitation with $(NH_4)_2SO_4$, to minimize oxidation. The precipitate obtained with $(NH_4)_2SO_4$ (Jansen *et al.* 1947) was mixed with 0·1 M-sodium oxalate to form a slurry and the latter dialysed against a large volume of 0·1 M-sodium oxalate (adjusted to pH 5·5) at 2° for 60 hr. Most of the solid went into solution and the remaining insoluble material was removed by centrifuging.