# The absorption of plant sterols by the fowl*

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1. A gas–liquid chromatographic method for the detection of phytosterols in tissues and excreta of birds was developed. The limitations of this method are discussed. 2. $\beta$- and $\gamma$-Sitosterol were detected in the plasma of adult cockerels and in the plasma, intestinal wall, and liver of 4-week-old cockerels. The proportion of sitosterols in the total sterol absorbed appeared to be affected by the nature of the dietary fat. 3. $\gamma$-Sitosterol was more effectively absorbed than $\beta$-sitosterol. The ratio of $\beta$ to $\gamma$ was altered by the nature of the dietary fat. 4. Using a diet containing 10% triolein and 1% cholic acid, and increasing the dietary plant sterol level up to 2%, increased the proportion of sitosterols in the intestinal wall of 4-week-old cocks. Further increases in dietary plant sterols did not produce additional increases in the proportion of sitosterols among intestinal wall sterols. 5. $\gamma$-Sitosterol and traces of $\beta$-sitosterol were found in eggs from hens given a diet supplemented with maize sterols.

The observation by Peterson (1951) that dietary phytosterols possess anti-hypercholesterolaemic activity in the fowl, and similar findings for the rat (Swell, Boiter, Field & Treadwell, 1954), for the rabbit (Beher, Baker & Anthony, 1957; Hermann, 1959), and for man (Beveridge, Connell, Mayer & Haust, 1958; Berge, Achor, Barker & Power, 1959), have stimulated renewed interest in the absorption and metabolism of plant sterols.

Schönheimer's (1929) report that plant sterols are not absorbed by animals caused investigators to consider sites of interference with cholesterol metabolism by phytosterols previous to the entry of cholesterol into the blood stream. Though it has been satisfactorily demonstrated that such interference occurs at the site of cholesterol absorption (Hernandez, Peterson, Chaikoff & Dauben, 1953), the possibility that other areas of interference exist has not been ruled out. Fisher, Weiss & Griminger (1963) could not show increased sterol excretion in fowls on a diet that included only a low level of maize sterols (0·05%) which influenced cholesterol metabolism. Gerson, Shorland & Adams (1961) observed an anti-hypercholesterolaemic effect upon injecting plant sterols into rats and speculated on whether plant sterols might also interfere with cholesterol metabolism after absorption.

Since Schönheimer's observation that plant sterols were not absorbed, considerable evidence, most of it indirect, has accumulated to indicate that they can be absorbed, if only to a limited extent. The balance studies of Ivy, Tsung-Min & Karvinen (1954) and Swell, Boiter, Field & Treadwell (1956) suggested a considerable absorption of plant sterols. Interpretation of these findings is, however, difficult, since the effect of

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the microflora on faecal sterol recoveries was not determined. The evaluation of these findings is further complicated because the extent of plant sterol absorption is described as a percentage of the amount administered; when amounts of sterol administered increase, the percentage that is absorbed progressively decreases. Ivy *et al.* (1954) and Curran & Costello (1956) have also reported the detection of plant sterol absorption using spectrophotometric methods. Gould (1954, 1955–6), Gould, Lotz & Lilly (1955), Swell, Trout, Field & Treadwell (1959) and Dunham, Fortner, Moore, Culp & Rice (1959) have each used tritium and $^{14}C$-labelled sitosterol and recovered some of the label in animal tissues.

Though it would be dogmatic to deny the validity of the evidence for plant sterol absorption provided by the cited reports, it is, nevertheless, not completely definitive. The conversion of sitosterol into cholesterol or its precursors in the intestine has been demonstrated by Werbin, Chaikoff & Jones (1960), whose work emphasizes the need for a method of identification that depends on chemical structure. At the present time, gas–liquid chromatography (GLC) provides the best available method for such an identification. Kuksis & Huang (1962) have used GLC to show the occurrence of $\beta$- and $\gamma$-sitosterol in the lymph of dogs after introducing a concentrated solution of sitosterol directly into the stomach. In the present investigation the absorption of phytosterols from maize and soya was investigated in the fowl, a species particularly susceptible to hypercholesterolaemia and atherosclerosis.

## EXPERIMENTAL

### *Chickens, diets and experimental procedures*

*Expt* 1. Six White Leghorn cocks weighing approximately 2 kg each were used. They were housed in individual cages with free access to water and food and were allotted, at random, in pairs to three dietary treatments: basal diet + 15% saturated, long-chain triglyceride, chain length twelve to eighteen carbons (generously supplied by Drew Chemical Corp., Boonton, New Jersey) (LCT); basal diet + 15% LCT + 5% maize sterols; basal diet + 15% triolein + 5% maize sterols. The composition of the basal diet is given in Table 1. A level of protein higher than that required for adult cocks was used because the same diet was to be given later to chicks. The relatively high level of fat and the inclusion of cholic acid were thought advisable to facilitate sterol absorption. The two different fats (LCT and triolein) were used to determine in a preliminary way whether the degree of saturation of the fat had any significant effect on plant sterol absorption. An absorbent (see Table 1) was added to prevent unpalatability of the feed due to the excess oiliness of the diet resulting from its high fat content (for fowl). The triolein, cholic acid, and maize sterols were mixed separately from the other dietary components in order to be able both to saturate the fat with sterol and then to add it as a single entity. It proved impossible to saturate LCT with the plant sterol, since this fat had to be melted and, on hardening, lost its original flaky characteristic and set in a solid mass that could not be readily mixed into the diet. The mixed diets were stored in a refrigerator from which they were given to the birds as required.

After 10 days on the experimental diet, 10 g of excreta were collected from the birds and pooled according to feeding group for GLC analysis of the sterols; 5 ml blood were removed from each bird through a brachial cannula for spectrophotometric and GLC analysis.

Table 1. *Expts* 1–4. *Composition of basal diets*

| Expts 1 and 2 | | Expts 3 and 4 | | |
|---|---|---|---|---|
| Ingredient | Amount (%) | Ingredient | Amount (%) Expt 3 | Expt 4 |
| Soya-bean meal (50% protein) | 40·0 | Isolated soya protein* | 20·0 | 20·0 |
| Fat absorbent† | 1·0–5·0 | Cellulose‡ | 3·0 | 3·0 |
| Dicalcium phosphate | 2·0 | Mineral mix§ | 4·9 | — |
| Limestone with trace minerals | 1·0 | Vitamin mix§ | 0·2 | 0·2 |
| Cholic acid | 1·0 | Choline chloride (70% solution) | 0·3 | 0·1 |
| Sodium chloride | 0·5 | DL-Methionine | 0·4 | — |
| Vitamin mix§ | 0·2 | Cholic acid | 1·0 | — |
| Choline chloride (70% solution) | 0·2 | Triolein | 10·0 | |
| DL-Methionine | 0·2 | Limestone with trace minerals | — | 3·0 |
| Glucose monohydrate | To 100 | Dicalcium phosphate | — | 4·0 |
| | | Fat absorbent† | — | 1·0 |
| | | M.C.T.‖ | — | 12·0 |
| | | Glucose monohydrate | To 100 | To 100 |

* Assay Protein C-1; Archer-Daniels-Midland Co., Minneapolis, Minnesota.
† Micro-cel E, a calcium silicate preparation, product of Johns-Manville Products Corp., Manville, New Jersey.
‡ Solka-Floc; Brown Co., Berlin, New Hampshire.
§ For composition see Summers & Fisher (1961).
‖ Saturated, medium-chain triglyceride, chain length six to twelve carbons, generously supplied by Drew Chemical Corp., Boonton, New Jersey.

*Expt* 2. Twenty-four male cross-bred chicks (New Hampshire ♂ × Columbian ♀) were given from day-old to 4-week-old a standard chick starter diet. The birds were then allotted at random to groups of six for each of the four following dietary treatments: basal + 12% LCT; basal + 12% LCT + 3% maize sterols; basal + 12% saturated, medium-chain triglyceride, chain length six to twelve carbons (generously supplied by Drew Chemical Corp., Boonton, New Jersey) (MCT) + 3% maize sterols; basal + 12% triolein + 3% maize sterols. In both Expts 2 and 3 the quantity of plant sterols available for addition to the diets was limited and thus relatively few chicks were used. All the chicks on one dietary treatment were therefore allotted to one cage. In the analysis of the results within-cage standard errors were used to test for the significance of the differences between tissue sterol concentrations produced by the different dietary treatments. In the use of this procedure it was assumed that the differences in tissue sterols were solely due to the different dietary treatments. At present there is no evidence to suggest that this assumption is wrong. The cages used were electrically heated and the chicks had free access to food and water. The basal diet used for Expt 2 was essentially the same as that used in Expt 1, except that the absorbent was decreased from 5 to 1% and the amount of fat in the diet in the various treatments was reduced from 15 to 12%. After the chicks had received this diet for 14 days, 2 ml blood were taken from each one by heart puncture and the

chicks were then killed by chloroform anaesthesia. The liver was removed and a 5 g sample taken. The small intestine of each chick was also removed and dissected open. The contents were rinsed out with tap water; a 5 g sample of the intestinal wall was taken from the distal end and stored in 0·9% (w/v) saline in the refrigerator until analysis. Spectrophotometric analysis was performed on individual samples and GLC analyses on samples pooled by treatment.

*Expt* 3. Fifty cross-bred male chicks were given a standard chick starter ration from age 1 day to 3 weeks. After being on the experimental basal diet (see Table 1) for a 5-day period, the chicks were divided into five groups of similar weight of ten chicks each. The groups were allotted to the following dietary regimens: basal diet; basal diet + 0·5% soya sterols; basal diet + 1·0% soya sterols; basal diet + 2·0% soya sterols; and basal diet + 5·0% soya sterols. As in Expt 2, the birds were housed in heated community cages and had free access to food and water. Isolated soya protein (Assay protein C-1; Archer-Daniels-Midland Company, Minneapolis, Minnesota) was substituted in Expt 3 for the soya-bean meal, since this eliminated the presence of soya sterols from the basal diet (analysis had previously indicated that the soya-bean meal provided a certain amount of such sterols). The soya sterol concentrate used in Expt 3 was a mixture of equal weights of partially purified soya sterols, which contained considerable amounts of xanthophyll, and of purified soya sitosterols (soya sterols 100 and 115, generously supplied by General Mills, Kankakee, Illinois). The diets were mixed in the same way as described in Expt 1, that is the soya sterol mixture was separately mixed with the triolein before being added to the remainder of the diet. After 8 days on the experimental diets, the birds were weighed and 2 ml blood removed from each by heart puncture before killing by chloroform anaesthesia. A 10 g sample of the small intestinal wall was removed and prepared as in Expt 2. All samples were pooled in pairs and all steps for all analyses were carried out in arrays of five including one pooled sample from each treatment.

*Expt* 4. Six White Leghorn hens with good egg-laying records were housed in individual cages and fed on a high-fat diet (Table 1) for 1 week. All the eggs that were laid during the last 2 days of this 1st week were collected and served as controls. The four birds that continued to lay eggs at a good rate were then fed on the basal diet supplemented with 3% maize sterols. MCT was used in this diet because it had promoted better absorption of plant sterols in the other experiments, and isolated soya protein was used for the reasons stated previously. All eggs laid from the 4th to the 7th day of the experimental period were collected. Since one bird stopped laying, the eggs finally analysed came from only three hens. The whole eggs were analysed for phytosterols by GLC analysis.

### *Analytical procedures*

*Total lipid extraction.* The tissues for analysis were homogenized in a Waring Blendor with a small volume of 2:1 (v/v) chloroform–methanol. The homogenate was then washed into a screw-top bottle, with a Teflon gasket, containing sufficient additional chloroform–methanol to provide between seventeen and twenty times the weight of the tissue sample. Extraction was allowed to proceed by shaking for 24 h

at room temperature. The homogenate was filtered and washed with more solvent to ensure complete extraction. The bulk of the solvent was then evaporated slowly by gentle steaming.

*Saponification and separation of the non-saponifiable fraction (NSF).* For these procedures the methods of the Association of Official Agricultural Chemists (1960) were used.

Table 2. *Recovery of sterols and sterol mixtures from a florosil liquid–solid chromatographic system*

(Mean values for five samples)

| Sterol | Recovery (%) |
|---|---|
| Cholesterol | 91 |
| Maize sterols* | 89 |
| Soya sterols 100† | 83 |
| Soya sterols 115‡ | 92 |

* Purified preparation generously supplied by A. E. Staley Manufacturing Co., Decatur, Illinois. For composition see Table 5.

† A crude preparation generously supplied by General Mills, Kankakee, Illinois. For composition see Table 5.

‡ A refined preparation generously supplied by General Mills, Kankakee, Illinois. In Expt 3, a mixture of equal quantities, by weight, of soya sterols 100 and 115 was used; its composition is given in Table 5.

*Purification of sterols from NSF.* Initially attempts were made to analyse the crude NSF directly by GLC. This proved difficult, both because of the presence of non-saponifiable material other than sterols, and because these other non-saponifiable substances caused a steadily increasing background interference during GLC analysis. The NSF was, therefore, further purified by the liquid–solid chromatographic procedure of Eisner & Firestone (1963). Approximately 40 mg NSF dissolved in 0·5 ml chloroform were placed on the column (2 × 30 cm, packed with 30 g activated florosil) and diluted by passing the following solvents through the column at a rate of about 100 drops/min: (*a*) 50 ml hexane (fraction 1); (*b*) 120 ml 5% diethyl ether in hexane (fraction 2); (*c*) 120 ml 15% diethyl ether in hexane (fraction 3); (*d*) 175 ml 30% diethyl ether in hexane (fraction 4); (*e*) 175 ml 50% diethyl ether in hexane (fraction 5); and (*f*) 150 ml 2% methanol in diethyl ether (fraction 6). Fraction 4 contained most of the desired sterols. The other fractions contained few or no sterols; when sterols were present, the components of the mixture were in the same proportion as in fraction 4, provided no contamination of the column by water had occurred. Table 2 gives the recovery values for various sterols and sterol mixtures from the liquid–solid chromatographic system employed. The values show that the proportion of cholesterol to phytosterols in a mixture is not significantly changed by the system; this was confirmed by testing such mixtures. The soya sterol 100 sample subjected to liquid–solid chromatography contained a considerable quantity of xanthophyll, which was for the most part removed, accounting partly for the relatively low recovery value.

*GLC.* At the time these experiments began, the separation and quantitative determination of small amounts of sitosterols and of stigmasterol in the presence of large amounts of cholesterol had only been achieved in one laboratory (Kuksis & Huang,

1962). Kuksis & Huang effected the separation of cholesteryl and sitosteryl acetates by using a low percentage of the silicone SE-30 as liquid phase. In the present study, SE-52 (approximately 2·5%)+XE-60 (approximately 0·8%) were used as the liquid phase. Since all the columns prepared differed slightly in their characteristics, the conditions for operation are given in the form of ranges within which optimal performance was obtained:

Chromatograph: Jarrell Ash 700.
Column: 6 ft by $\frac{1}{4}$ in internal diameter, glass.
Packing: approximately 2·5% SE-52+0·8% XE-60 on 80/100 Gas Chrom Z.
Column temperature: 240–255°.
Argon flow rate: 40–60 ml/min.
Stream splitter: 30° above column temperature.
Injector: as for stream splitter.
Detector (argon ionization): 240°.
Recorder range: 1–10 mV.
Recorder response: $10^{-8}$ (cholesterol peak); $10^{-9}$ (phytosterol peaks).
Sample: 1–5 $\mu$l of a saturated solution of sterols in hexane or chloroform.
The cholesterol peak appeared at about 25 min and $\beta$-sitosterol at about 32 min after injection of sample.

To ensure positive identifications, standard mixtures of phytosterols (1–5%) and cholesterol (99–95%) were made. These mixtures were introduced into the chromatograph as saturated solutions in chloroform. The volume of saturated solution used was adjusted to provide an amount of cholesterol in the same range as that in the samples that were analysed. The quantitative determination of the relative amount of sterols in mixtures is not yet satisfactory. In the analyses performed in the present work, column overloading with respect to cholesterol added considerable uncertainty to an already difficult procedure. Each sterol in a mixture was expressed as a percentage of the total detectable twenty-seven or more carbon 3-$\beta$-hydroxy-steroids. Thus, the area under each peak on the chromatogram was found and expressed as a percentage of the sum of the areas of all peaks. Peak area was determined by multiplying the height by the width at half height, except where a peak deviated from the Gaussian curve, in which event triangulation was used. This method of quantitation gave reasonably accurate results if the standard mixtures of sterols used contained concentrations of the phytosterols and cholesterol within the same range. Under such conditions, column overloading was unnecessary. When column overloading was necessary, the analytical system did not give an accurate measurement of the proportions of sterols in a mixture. Under conditions of overloading, however, the method was reproducible, and gave proportional representation of the difference in the concentrations of separate sterols between samples, provided the total quantity of cholesterol introduced into the chromatograph was kept within the same narrow range for any given set of analyses. The results presented herein, therefore, are relative in respect to the samples; except in a general way, they cannot be related to the spectrophotometric analyses for total sterols.

*Spectrophotometric determination of cholesterol.* The method used was a modified Liebermann–Burchard colour reaction. It was based on that of Zlatkis, Zak & Boyle (1953) as modified by Searcy & Bergquist (1960). This method produces equal colour intensity for cholesterol and cholesteryl esters. Phytosterols can also be determined but they have different chromogenicities. Table 3 shows the differing chromogenicities of samples of equal concentration for different sterols compared with cholesterol, which was arbitrarily given a value of 100%. $\beta$-Sitosterol had a much reduced chromogenicity, and the presence of either $\gamma$-sitosterol or stigmasterol in the mixture considerably increased colour development. Since the proportions of the phytosterols are altered during absorption by the animal, it is not sensible to apply any of these spectrophotometric readings to total sterol values obtained from animal tissues. The need for a source of pure $\gamma$-sitosterol is emphasized by these results. It may well be that this sterol has a chromogenicity in excess of that of cholesterol.

Table 3. *Chromogenicity of various sterols and sterol mixtures compared with cholesterol by the colorimetric method of Zlatkis* et al. (1953)

(Mean values for three determinations; for method see above)

| Sterol | Chromogenicity (%) |
|---|---|
| Cholesterol* | 100 |
| Maize sterols† | 94 |
| Soya sterols 100† | 92 |
| Soya sterols 115† | 86 |
| $\beta$-Sitosterol‡ | 44 |

* SCW grade (standard for clinical work), Nutritional Biochemicals Corp., Cleveland, Ohio.
† See footnotes, Table 2.
‡ Nutritional Biochemicals Corp., Cleveland, Ohio.

## RESULTS AND DISCUSSION

### *Expt* 1

The sterol patterns by GLC analysis of the excreta of birds given two different dietary fats, with and without added maize sterols, are shown in Table 4. No cholesterol was found in the excreta of birds receiving 15% triolein; this indicates complete reabsorption of any endogenous cholesterol, and also indicates no excretion of cholesterol in the urine, as had been suggested by Edwards, Marion & Driggers (1960). The excreta from birds given LCT contained cholesterol; the addition of maize sterols to the LCT-containing diet decreased cholesterol in the excreta. It is difficult to reconcile these results with the idea that plant sterols inhibit cholesterol absorption. The excreta of birds receiving no added maize sterols in the diet did contain plant sterols, indicating that the basal diet must have contributed plant sterols. The source of these sterols was found to be the soya-bean meal. Table 5 shows the GLC sterol pattern for the maize sterols and for soya sterols. These patterns show that the excreta phytosterol pattern of the birds in Expt 1 corresponded more closely to the sterol pattern of soya sterol than to that of maize sterols. For birds receiving the dietary maize sterols, the reverse was true.

Table 4. *Expt* 1. *Excreta sterol patterns* and plasma sterol concentrations and patterns* of adult cocks given diets with or without maize sterols*

| Sterol | LCT,† 15% | | Triolein, 15% + 5% maize sterols |
|---|---|---|---|
| | Without maize sterols | + 5% maize sterols | |
| | Excreta | | |
| Cholesterol (%) | 3·5 | 0·6 | 0 |
| γ-Sitosterol (%) | 10·6 | 13·8 | 12·9 |
| Stigmasterol (%) | 9·3 | 2·2 | 1·9 |
| β-Sitosterol (%) | 76·8 | 83·5 | 85·3 |
| | Plasma | | |
| Cholesterol (%) | 97·5 | 96·2 | 96·7 |
| γ-Sitosterol (%) | 0·8 | 1·9 | 2·1 |
| Stigmasterol (%) | 0 | 0 | Trace |
| β-Sitosterol (%) | 1·7 | 1·8 | 1·3 |
| Total phytosterol (%) | 2·5 | 3·7 | 3·4 |
| Total sterol‡ (mg/100 ml) | 116 | 132 | 126 |

* Each pattern represents the means of duplicate analyses on samples pooled by treatments. Each GLC component is represented as a percentage of total detectable twenty-seven or more carbons, 3-β-hydroxy-steroids.

† Saturated, long-chain triglyceride, chain length twelve to eighteen carbons, generously supplied by Drew Chemical Corp., Boonton, New Jersey.

‡ Determined spectrophotometrically as described on p. 695, and measured as cholesterol.

Table 5. *Analysis by gas–liquid chromatography of maize and soya sterol mixtures*

(Mean values for five determinations)

| Sterol detected | Maize sterol* (%) | Soya sterol 100* (%) | Soya sterol mixture† (%) |
|---|---|---|---|
| γ-Sitosterol | 9·6 | 14·5 | 15·3 |
| Stigmasterol | 1·3 | 9·8 | 4·9 |
| β-Sitosterol | 89·0 | 75·5 | 79·7 |

* See footnotes to Table 2.

† A mixture of equal amounts, by weight, of soya sterols 100 and 115 (see footnotes to Table 2).

Table 4 also gives the results of the spectrophotometric as well as of the GLC sterol analyses of blood plasma. No significance can be attached to the differences in spectrophotometric values expressed as cholesterol, in view of the small number of birds used. From the results of the GLC analyses, however, there is no question that the sitosterols were absorbed by the birds even when no concentrated source of phytosterols was included in the diet. The exact amount of phytosterol contributed by soya-bean meal was not determined. It is certain, however, that, compared with the 5% of added maize sterols, it was insignificant. The γ-sitosterol content of the diets was appreciably smaller than that of β-sitosterol. Yet, in the blood, γ-sitosterol tended to be the predominant sitosterol in two of the three treatments, an observation confirmed in later studies. This result is, therefore, similar to the finding of Kuksis & Huang (1962) that γ-sitosterol is more efficiently absorbed than β-sitosterol by the

dog. No good evidence for stigmasterol absorption was found, and only one sample contained a trace of this phytosterol. Stigmasterol, however, is only a minor component of maize sterols, and demonstration of its absorption, if it occurs, would presumably require increasing its level in the diet.

Table 6. *Expt 2. Plasma and tissue sterol concentrations and patterns* of young cocks given maize sterols in combination with different dietary fats*

| Sterol | LCT, 12% | | MCT, 12%+3% maize sterols | Triolein, 12%+3% maize sterols |
|---|---|---|---|---|
| | Without maize sterols | +3% maize sterols | | |
| | Plasma | | | |
| Total sterols† (mg/100 ml) | 199±18 | 208±15 | 224±9 | 237±10 |
| Cholesterol‡ (%) | 99·2 | 97·8 | 96·9 | 96·7 |
| γ-Sitosterol (%) | 0·7 | 1·6 | 2·2 | 2·6 |
| β-Sitosterol (%) | 0·1 | 0·7 | 0·9 | 0·7 |
| Total phytosterol (%) | 0·8 | 2·3 | 3·1 | 3·3 |
| | Liver | | | |
| Total sterols† (mg/g fresh weight) | 5·97±0·12 | 5·12±0·15 | 5·33±0·14 | 5·41±0·24 |
| Cholesterol‡ (%) | 100 | 98·8 | 98·9 | 98·7 |
| γ-Sitosterol (%) | 0 | 0·9 | 0·8 | 1·0 |
| β-Sitosterol (%) | 0 | 0·3 | 0·2 | 0·2 |
| Total phytosterol (%) | 0 | 1·2 | 1·0 | 1·2 |
| | Intestinal wall | | | |
| Total sterols† (mg/g fresh weight) | 5·67±0·30 | 5·42±0·25 | 5·65±0·34 | 5·93±0·23 |
| Cholesterol‡ (%) | 99·5 | 94·2 | 91·8 | 98·7 |
| γ-Sitosterol (%) | 0·3 | 4·5 | 6·3 | 6·4 |
| β-Sitosterol (%) | 0·3 | 1·3 | 2·0 | 1·1 |
| Total phytosterol (%) | 0·6 | 5·8 | 8·3 | 7·5 |

LCT, saturated, long-chain triglyceride; MCT, saturated, medium-chain triglyceride.

* Each component of the GLC pattern is represented as a percentage of total detectable twenty-seven or more carbons, 3-β-hydroxy-steroids.

† Mean values for six samples with standard errors; determined spectrophotometrically and measured as cholesterol.

‡ Mean of duplicate GLC determinations on samples pooled by treatment.

## *Expt 2*

Table 6 shows the sterol concentrations and patterns in the plasma, liver and intestinal wall of young cocks given diets containing maize sterols in combination with various fats. The total sterol content of plasma appears to have been increased by the addition of plant sterols to the diet. There was also a trend for higher plasma sterol concentrations when birds received triolein and MCT as the dietary fats. These increases probably largely reflected an increased absorption of endogenous cholesterol, or other changes in the metabolism of cholesterol, since Fisher & Kaunitz (1964) reported higher blood cholesterol levels in chickens given MCT than in those given LCT. The plasma sterol pattern, however, indicates that increased absorption of maize sterols might also have contributed to the increased plasma sterol concentration.

The liver sterol levels were significantly decreased ($P < 0.05$) by the addition of plant sterols to the diet. This effect was observed also when dietary MCT and triolein were given. The dietary maize sterols did not produce any significant change in sterol concentrations in the intestinal wall. Sitosterols were found in the plasma, intestinal wall and liver sterols of birds receiving 3% dietary maize sterols. The plasma and intestinal tissues of birds not receiving dietary maize sterols also contained sitosterols. As in Expt 1, these must have been derived from the soya-bean meal in the basal diet. The highest proportion of sitosterols was found in the intestinal wall of birds receiving dietary maize sterols. There was an indication that the liquid fats (MCT and triolein) promoted better absorption of plant sterols than did LCT. This might possibly have been due to the methods used in incorporating the plant sterols into the diet. The difficulty in producing an intimate mixture of fat, bile salt, and sterol with LCT has been previously mentioned. The results of Expt 2 confirm the observation previously made that $\gamma$-sitosterol was more efficiently absorbed than $\beta$-sitosterol. Stigmasterol was not detected in any sample.

Table 7. *Expt* 3. *Food intake, weight gain and plasma and intestinal wall sterol concentrations and patterns* of young cocks given diets containing* 10% *triolein and graded levels of soya sterols*†

| | Dietary soya sterols (%) | | | | |
|---|---|---|---|---|---|
| Measurement | 0 | 0·5 | 1·0 | 2·0 | 5·0 |
| Weight gain (g/8 days) | 102 | 111 | 109 | 113 | 117 |
| Food intake (g/8 days per bird) | 225 | 240 | 238 | 248 | 252 |
| | | Plasma | | | |
| Total sterols‡ (mg/100 ml) | 155±8 | 158±7 | 158±10 | 157±8 | 154±6 |
| Cholesterol§ (%) | 100 | 97·4 | 98·7 | 97·2 | 98·8 |
| $\gamma$-Sitosterol (%) | 0 | 2·2 | 1·2 | 2·6 | 1·1 |
| $\beta$-Sitosterol (%) | 0 | 0·3 | 0·1 | 0·2 | 0·1 |
| Total phytosterol (%) | 0 | 2·5 | 1·3 | 2·8 | 1·2 |
| | | Intestinal wall | | | |
| Total sterols‡ (mg/g fresh weight) | 4·6±0·2 | 4·7±0·2 | 4·1±0·1 | 4·6±0·2 | 4·3±0·2 |
| Cholesterol§ | 100§ | 95·8±0·9§ | 95·9±0·2 | 94·6±0·9 | 94·5±0·1 |
| $\gamma$-Sitosterol (%) | 0 | 3·5±0·5 | 3·5±0·3 | 4·3±0·7 | 4·3±0·1 |
| $\beta$-Sitosterol (%) | 0 | 0·7±0·2 | 0·6±0·1 | 1·0±0·2 | 1·2±0·1 |
| Total phytosterol | 0 | 4·2 | 4·1 | 5·3 | 5·5 |

* Each component of the GLC pattern is represented as a percentage of total detectable twenty-seven or more carbons, 3-$\beta$-hydroxy-steroids.

† A mixture of equal amounts, by weight, of soya sterols 100 and 115; for composition see Table 5.

‡ Mean values with standard errors for five pair-pooled samples; determined spectrophotometically and measured as cholesterol.

§ Mean of duplicate GLC determinations on samples pooled by treatment.

## *Expt* 3

In this experiment, isolated soya protein was used instead of soya-bean meal since the former is essentially free of sterols. The level of fat (triolein) was slightly lowered, and since our supply of purified maize sterols had run out soya sterols were used. The sterol concentrations and patterns of plasma and intestinal wall (Table 7) did not follow

the patterns observed in Expt 2. In Expt 3 sterol concentrations in both the plasma and the intestinal wall were lower than in Expt 2, suggesting that perhaps the lower level of dietary fat might have decreased cholesterol reabsorption. The use of isolated soya protein appeared to have completely eliminated phytosterols from the basal diet, since none was detected in tissue from birds receiving no added plant sterols. The elimination of plant sterols from the basal diet established with certainty the identity of the plant sterols detected in all of these studies by the methods employed. The differences in sterol pattern of the intestinal wall for birds given 2 and 5% of soya sterols compared with those given respectively 0·5 and 1% bordered on statistical significance ($P < 0{\cdot}1$). This suggests that the intestinal wall became saturated with phytosterols at approximately the 2% level in the diet (an intake of approximately 0·5 g/day). The level of phytosterol necessary to produce this saturation, and the extent of saturation of intestinal wall sterols by phytosterol, would probably change if the quantity or the type of dietary fat, or both, were changed.

Unfortunately, even with the pooling of samples in pairs, there was insufficient material in the plasma samples to detect, and determine meaningfully, the sitosterols. The samples, therefore, had to be pooled by treatment. The results showed that the relationship between diet and sterol patterns observed for the intestinal wall sterols did not occur in the blood. The slight increase in dietary stigmasterol resulting from the switch to soya sterols, as compared with previous trials when maize sterols were used, did not lead to sufficient absorption to make this sterol detectable. The better absorption of $\gamma$-sitosterol, compared with that of $\beta$-sitosterol noted in the previous experiments, was substantiated in this experiment.

### *Expt* 4

The eggs collected during the period when the high-fat diet containing no maize sterols was given contained no detectable phytosterols. All eggs obtained from the three hens given the maize sterol supplement contained $\gamma$-sitosterol (Table 8). Only one egg showed definite proof of the presence of $\beta$-sitosterol. In a few other eggs detector noise suggested the presence of $\beta$-sitosterol but definite or measurable evidence was not obtained on the corresponding chromatograms.

### *Methodology*

The primary problem in the GLC analyses carried out in this study was the large quantity of cholesterol present in all tissue samples. Completely accurate determination of the sitosterols could probably only be achieved upon removal of part or all of the cholesterol from the samples. The numerical results presented are perhaps best interpreted as indicating that there was more of a particular component in one sample than in another. Since in the present work no attempt was made to determine the total weight of tissue used, it was not possible to determine the total quantity of sterol in a tissue from the concentration of sterol in that tissue. It could not be concluded, therefore, that a decrease in the percentage of sitosterols in the plasma compared with that in the intestinal wall represented an absolute decrease in the sitosterols. The apparent

decrease might have been due to an increase in the amount of cholesterol in which the sitosterols were distributed.

*Absorption of plant sterols*

The detection of sitosterols in the blood of cockerels fed on soya-bean meal as the only sterol source indicated that the normal diet can provide sufficient quantities of sitosterols to allow absorption. The extent to which absorption occurs, however, is probably dependent, among other factors, on the fat content of the diet. Since phytosterols interfere with cholesterol absorption, it is probable that, conversely, cholesterol interferes with phytosterol absorption. Although in the present studies none of the diets contained cholesterol, the high levels of dietary fat used probably enhanced the reabsorption of endogenous cholesterol. The small proportion of cholesterol in the sterol patterns of excreta (Expt 1) supports this suggestion.

Table 8. *Expt* 4. *Sterol pattern* of eggs from hens given* 3% *dietary maize sterols*†

| | Egg sterol (%) | | |
|---|---|---|---|
| Egg no.‡ | Cholesterol | γ-Sitosterol | β-Sitosterol |
| 1A | 99·6 | 0·4 | Trace |
| 1B | 99·4 | 0·6 | 0 |
| 2A | 99·3 | 0·6 | 0·2 |
| 2B | 99·6 | 0·4 | 0 |
| 3 | 99·5 | 0·5 | 0 |

* Each component of the GLC pattern is represented as a percentage of total detectable twenty-seven or more carbons, 3-β-hydroxy-steroids.

† Eggs from hens not receiving dietary maize sterols contained no detectable phytosterols.

‡ Eggs referred to by the same number were from the same hen.

Even though the phytosterols and cholesterol have a very similar chemical structure, cholesterol is preferentially absorbed, and γ-sitosterol is better absorbed than β-sitosterol. The fact that better absorption is responsible for the higher tissue levels of γ-sitosterol rather than slower elimination is suggested by the finding of a higher level of γ-sitosterol than of β-sitosterol in the intestinal wall. In the results from Expt 2 there are indications that the absorbability of the two isomers of sitosterol was differently affected by different dietary fats. The ratio of γ to β was consistently greater in all tissues examined with triolein as the dietary fat than in those with MCT.

*Note added April* 1966. In the above report, the name 'γ-sitosterol' was used in accordance with the terminology used by Eisner & Firestone (1963) and Kuksis & Huang (1962). Recently, however, some confusion has arisen over the identity of the material formerly named 'γ-sitosterol' and it now appears that this material is, in fact, campesterol (Copius-Peereboom, J. W. (1965), *J. Gas Chromat.* **3**, 325). It seems, therefore, that the name 'campesterol' should have been used in place of 'γ-sitosterol'.

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