teeth are still brown. Depigmentation may therefore be the final outcome of different initial histological lesions.

The persistence of dental depigmentation in rats which have been deprived of vitamin A and subsequently treated with small doses of this vitamin affords an excellent demonstration of the reality of the conception of suboptimal nutrition. Rats in the group given 6 i.u. of vitamin A daily grew almost as rapidly as those given 16 i.u., but whereas 16 i.u. was sufficient to restore the brown colour of the teeth, 6 i.u. was inadequate. Ability to sustain rapid growth does not therefore necessarily imply that the animal is receiving the full supply of nutrients necessary for perfect health.

SUMMARY

1. Whitening of the teeth in rats was induced by deficiency of either vitamin A or vitamin E, and could be cured by restoration of whichever vitamin was missing.

2. A low dose of vitamin A, which was sufficient to restore rapid growth in rats previously deprived of this vitamin, was ineffective in restoring the normal brown colour of the teeth.

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The Microbiological Degradation of Steroids

1. THE STEROL CONTENT OF SOILS

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The wide distribution of free or esterified sterols in animal and vegetable tissues must result, during the decay of such organic material, in the deposition in soils of an appreciable quantity of various sterol substances. Schreiner & Shorey [1909; 1911] have reported the isolation from soils of two sterols. The first of these, agosterol, was present in the free condition, and was considered to be the sterol component of some plant tissue, or to have originated by structural alteration from some substance of entirely different composition present in the soil. The second, phytosterol, m.p. 135°, occurred only in the form of its esters, which were regarded as plant residues remaining unchanged in the particular peaty soil under examination. In general, however, the sterol content of soils seems to have received but little attention. The investigation of the various factors influencing the accumulation of sterols in, and their disappearance from, different types of soil forms the basis of the present work.

METHODS

The quantitative determination of soil sterols is attended by considerable difficulty. Humus material may be present in proportions of from less than 1% in the largely mineral soils to almost 100% in pure peat, and the purification of the sterols thus becomes lengthy and tedious. It has been found that a preliminary solvent treatment of the soil, followed by purification of the crude sterol extracts by means of the insoluble digitonides, yields solutions sufficiently pure for colorimetric estimation.

The soil sample is thoroughly dried in an air oven at 80°, and, after being ground in a mortar, is passed through a 30-mesh sieve to remove pebbles, plant fragments, etc. 1 kg. of soil obtained in this way is extracted continuously with acetone. After removal of the solvent by distillation, the brownish gummy residue is refluxed with a slight excess of alcoholic KOH for 30 min. The solvent is removed on a water bath, and the dark-coloured viscous liquid remaining taken up in a large excess of anhyd. K₂SO₄. This solid is

extracted for 4 hr. in a Soxhlet apparatus with petroleum, B.P. 40–50°, and the extract made up to known volume. The determination of sterol in this solution is based upon the method of Kelsey [1939]. A portion containing approximately 0.5 mg. sterol is evaporated to 0.5 ml. in a 15 ml. graduated centrifuge tube. 4.0 ml. of a 0.2% solution of digitonin in 95% alcohol are added, and the mixture evaporated to dryness. The solid residue is boiled with 12 ml. petroleum, B.P. 40–50°, the tube and contents centrifuged, and the supernatant yellowish liquid discarded. This is repeated until the extracts are no longer coloured. The residual solid is then gently refluxed with 7.0 ml. benzene for 45 min. to decompose the digitonide, and after being concentrated to 3 ml., the contents of the tube are finally diluted to 12 ml. with petroleum, B.P. 40–50°. Digitonin separates on thorough stirring, and is centrifuged off. The supernatant liquid is decanted, and the residue re-extracted three times with light petroleum. The combined extracts are evaporated to dryness, and the residue taken up in 5.0 ml. CHCl_3 . This solution is transferred to a 10 ml. glass-stoppered cylinder. 0.5 mg. cholesterol dissolved in 5.0 ml. CHCl_3 is measured into a second cylinder, and 1.0 ml. acetic anhydride and 0.1 ml. conc. H_2SO_4 are added to each. The solutions are thoroughly mixed, and are allowed to stand in a bath at 24° for 15 min.; the colours which have developed are then compared in a colorimeter.

Neither the digitonin precipitation used as the final purification in the above method, nor the Liebermann-Burchard colour reaction, is itself specific for sterols. Digitonin precipitation in the steroid group appears [Windaus, 1909] to depend upon a free —OH at C_3 in the cholane nucleus, and the *cis*-position of this —OH to the —Me at C_{10} . Thus, insoluble digitonides are also given by several bile acids, certain cholane cardiac poisons and their aglycones, and by various other compounds. The cardiac glycosides, in common with the saponins, many bile acids and other substances, also give the Liebermann-Burchard reaction for sterols. During the various purification stages described these compounds are eliminated, with the possible exception of certain cardiac aglycones which give both digitonin precipitates and positive colour reactions. These compounds, however, are probably far rarer in soils than the sterols, and no evidence of their presence in any of the final solutions has been obtained with the Legal nitroprusside test.

The H_2SO_4 -acetic anhydride colour may thus be regarded as indicative of the total sterol content of the soil, irrespective of whether the sterol was originally present in the free state, as its fatty acid esters, or in the form of its glycosides. No attempt has been made to identify individual sterols, the result in all cases being expressed in terms of cholesterol.

The accuracy of the method has been tested by determining on a number of soils the percentage recovery of cholesterol added to dried, sieved soil.

Table 1. *Recovery of added cholesterol*

Initial sterol content mg./kg. soil	Cholesterol added mg.	Total sterols by analysis mg./kg. soil	Cholesterol recovered mg.	Recovery %
1.8	61.3	61.5	59.7	97.4
0.9	52.4	54.7	53.8	102.7
20.1	25.7	45.2	25.1	97.7
Nil	18.9	18.1	18.1	95.7

The difference in the figures obtained in four experiments with the original soil and that containing added cholesterol indicates the recovery of cholesterol (Table 1). The recoveries have been of a consistently high order.

DISCUSSION

Distribution of sterols in British soils

The soils examined in this survey have been collected from widely distributed areas throughout the British Isles, and include the majority of soil types found in this region. They have, in general, been taken from a depth of from 2 to 6 in., after removal of the surface layer of living or dead vegetation, but in certain instances where the profile showed deep peaty humus layers, sampling has been carried out at a depth of several feet.

Table 2. *Sterol contents of British soils*

Vegetative type	Source	Soil type	No. of samples	Sterol substances present
				mg./kg. soil
Forest land	Oak and oak-birch associations (Sherwood Forest)	Coarse acid sand	6	0.6–1.4
	Oak (Weald)	Light loam to heavy clay	2	0.1, 0.8
	Beech (Sherwood Forest, Epping Forest, Chiltern plateau)	Coarse acid sand	5	0.2–1.2
	Beech (South Downs)	Shallow, alkaline, light loam	2	0.8, 2.5
	Beech (Chiltern escarpment)	Strongly alkaline marl	1	3.2
Grass land	Acid grassland (southern Pennines, Malvern Hills)	Acid sand or loam	2	Nil, 0.8
	Neutral grassland (Trent and Thames valleys)	Chiefly loams	4	0.4–1.5
	Basic grassland (Chiltern escarpment)	Shallow, alkaline, marls	3	0.6–2.7
Fen land	Wicken Fen, Cambridge, Norfolk Broads	Black, slightly alkaline peat	4	2.3–8.5
Bog land	Peat moors, Athlone, Spring moors, New Forest, Dartmoor	Deep, acid peat	5	7.9–12.7
Heath land	Sherwood Forest, Pennine moors, Yorkshire moors, Central Scottish highlands	Coarse sand	6	Nil–1.1
Arable land	Various	Various	12	Nil–6.3

The results (Table 2) show clearly that definite relationships exist between sterol content and soil type. Deposition of sterols must depend primarily upon the nature and number of the animal and plant inhabitants, so that deposition will be at a maximum in soils favourable to the maintenance of a flourishing population. A characteristic of such soils, however, is the rapidity with which humus material undergoes disintegration. Since the sterol content of these soils shows no continued increase, this component of the humus is presumably decomposed or disposed of in some other manner. In soils of the opposite type there is a marked accumulation of sterols. Extreme acidity, lack of aeration, and excessive water content are all factors which, either individually or collectively, inhibit the decomposition of humus and its constituent sterols, and are thus directly responsible for the high sterol contents of bog and fen peats. In arable soils these adverse factors have been counteracted, and, whilst it is found that sterols are present in varying amount, the content of any particular soil shows a gradual decrease unless replenished by organic manuring.

Agencies responsible for sterol disappearance

In view of the insolubility of the sterols, and of their association with natural fats and oils, their disappearance from soils cannot be attributed to the leaching action of rain-water, but is probably the result of degradation of the sterol molecule by the co-ordinated activities of a variety of soil micro-organisms. Evidence in favour of this theory has been obtained experimentally in the following manner.

Six 0.05 g. quantities of cholesterol were accurately weighed, and each intimately mixed with 25 g. soil. The mixtures were placed in separate porous flower pots in which the drainage holes had been closed with watch-glasses, and the pots buried with their tops 1 in. below the surface of a well-worked loamy garden soil. One group of three pots was watered weekly with rainwater, whilst the second group was treated with a dilute solution of HgCl_2 .

Table 3. *Rate of disappearance of sterols from soil under different conditions*

Time months	Sterol content (mg.)	
	Group I (water)	Group II (HgCl_2)
0*	50.0	50.0
3	42.7	49.2
6	29.6	49.8
12	18.3	48.9

* The sterol content of the untreated 25 g. sample of soil is negligible.

At intervals of 3, 6 and 12 months respectively, one pot from each group was removed, and the sterol content of the soil determined (Table 3). In the first group sterol disappeared, but the sterilizing action of the HgCl_2 inhibited microbiological activity in the second group so that the sterol content altered little. Similar experiments, using 'organic' cholesterol in the form of brain tissue and egg yolk, gave comparable results although disappearance of sterol was here less rapid than with the crystalline material.

The nature of the biological agents responsible for sterol breakdown is being investigated, and there are indications that decomposition is initiated by certain acid-tolerant, aerobic bacteria.

SUMMARY

1. A method is described for the determination of soil sterols.
2. The sterol content of a wide range of British soils has been determined.
3. Accumulation of sterols in soils occurs under conditions of extreme acidity, waterlogging and lack of aeration.
4. Where these conditions are reversed, as in arable soils, the sterol content gradually decreases.
5. The disappearance of soil sterols has been demonstrated experimentally, and has been shown to result from the activities of microbiological agents.

The author wishes to express his thanks to colleagues who have assisted in obtaining soil samples.

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Liver Catalase

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Stern [1935-6] isolated biliverdin (dehydrobilirubin) from the split products of horse liver catalase; the pigment was identified by Lemberg [cf. Lemberg & Wyndham, 1936-7]. Biliverdin was not present in

the catalase preparation as such, but as a bile pigment haem compound bound to a protein similar to or identical with that characteristic of catalase [Lemberg & Wyndham, 1936-7]. After reduction