5 ml. of $N H_2SO_4$. After 3–4 days, the white needles which had formed were collected and dried *in vacuo*. Yield, 0.13 g., m.p. 83° , giving a yellow colour with concentrated HCl and in the Liebermann-Burchard test, and a red colour with concentrated H_2SO_4 . There was difficulty in removing solvent from this substance for analysis. (Found: C, 70.8; H, 9.5%. M (titration), 395. $C_{24}H_{38}O_5$ requires C, 70.9; H, 9.4%; M, 406.) The acid could be recrystallized by acidification of a hot alkaline solution as described, but did not form crystals from organic solvents. It dissolved easily in alcohol, acetone, and acetic acid, and to some extent in ether, but appeared to be insoluble in benzene and light petroleum.

SUMMARY

1. By a simple three-stage process (oxidation, purification, reduction), cholic acid has been con-

verted into deoxycholic acid, with an overall yield of 40–50 % of theoretical.

2. Preferential oxidation of cholic acid at C_7 was performed by adding measured amounts of concentrated K_2CrO_4 solution to cholic acid dissolved in acetic acid buffered with Na acetate.

3. The partially purified product was then reduced with Na ethylate-hydrazine, yielding deoxycholic acid. Deoxycholic acid was also prepared from an impure semicarbazone, the preparation and properties of which were used to follow and define the conditions of the oxidation. The intermediate 7-keto-3:12-dihydroxycholanolic acid and its ethyl ester have been isolated and examined.

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Dental Depigmentation in the Rat

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In normal rats the anterior surfaces of the incisor teeth are a rich brown or orange. According to Marshall [1921] the pigment is confined to the outer fibrous layer. The whitening of the teeth which occurs in vitamin A deficiency has been described by Wolbach & Howe [1925; 1933], Smith & Lantz [1933], Schour, Smith & Hoffman [1938], Schour, Hoffman & Smith [1941], Irving & Richards [1939] and H. Mellanby [1939]. In recent experiments on the interaction of vitamin A and vitamin E, Davies & Moore [1941] found that under certain circumstances whitening of the teeth may result from the exclusion of vitamin E from the diet. It was suggested that this lesion might be caused by deficiency of either vitamin. Evidence in favour of this view is given in the present communication.

EXPERIMENTAL

Grading of depigmentation. To assess the degree of depigmentation in individual rats each tooth was awarded marks according to the following arbitrary scale.

Description	Marks given
White	25
Faint brown mottling on white	20
Cream	15
Mottled brown and white	10
Deep cream	10
Light brown	5
Brown	0

Thus the dental depigmentation of a rat with four completely white teeth is expressed by 100 marks, while four completely brown teeth are indicated by 0 marks.

The cure of white teeth in rats deficient in vitamin A by the administration of carotene

In routine tests for vitamin A in samples of butter and margarine, male albino and piebald rats were given a basal diet of caseinogen (Glaxo, alcohol extracted) 20 %, sugar 60 %, arachis oil 15 %, salt mixture 5 % and dried yeast 10 %. Vitamin E was supplied as 1 mg. weekly of *dl*- α -tocopherol acetate dissolved in arachis oil, and vitamin D as 1 drop of 'Radiostol' (B.D.H.). In addition small doses of

the butter or margarine were given to supply enough vitamin A to allow fairly rapid growth. At the end of the test 12 rats with completely white teeth were nevertheless available for use in the present investigation. These were kept on the same basal diet and divided into 4 groups, which were dosed with a solution of carotene in arachis oil at levels of 2, 6, 16 and 44 i.u. per rat daily.

The changes in weight during the next 59 days are shown in Table 1.

of caseinogen ('light white') 25 %, sugar 50 %, lard 10 %, dried yeast 10 % and salt mixture 5 %. Vitamin A was supplied at the level of about 1000 i.u./week in the form of halibut liver oil. The animals, most of which showed marked dental depigmentation, were then divided into two groups, each comprising three rats which had received the deficient diet for 4 months, and four rats which had received the same diet for 7 months. One group was now given 1 mg. of tocopherol acetate weekly,

Table 1. Growth of rats receiving different doses of carotene

Daily dose of carotene i.u./day	Group 1 2			Group 2 6			Group 3 16			Group 4 44		
Rat no.	1	2	3	4	5	6	7	8	9	10	11	12
Wt. (g.) on day 1	178	173	210	160	154	143	166	148	182	126	112	187
Wt. (g.) on day 60	174	130	Died	210	197	180	219	220	222	194	193	267
Mean increase in wt. (g.)	-23			+43			+55			+76		

Signs of the return of brown colour to the teeth were seen in some rats of groups 3 and 4 after 30 days, and after 60 days most of the animals had almost normal teeth. The teeth in groups 1 and 2 remained almost completely white. The marks given are shown in Table 2.

the other received no vitamin E. In connexion with another experiment each group received occasional injections of lard, a treatment which would not be expected to influence the present results.

The weights of the rats at the time of division into groups, when they were already nearly full

Table 2. Dental depigmentation in rats receiving different doses of carotene

Daily dose of carotene i.u./day	Group 1 2			Group 2 6			Group 3 16			Group 4 44		
Rat no.	1	2	3	4	5	6	7	8	9	10	11	12
Depigmentation of teeth (arbitrary marks)	Day 1	100	100	100	100	100	100	100	100	100	100	100
	Day 60	75	90	Dead	90	90	80	20	20	10	10	45
Mean mark at end of exp.		83			87			17			22	

Examination of the unerupted portions of the teeth gave similar results.

grown, and after further periods of 84 and 203 days, are given in Table 3.

The cure of white teeth in rats deficient in vitamin E by the administration of dl-α-tocopherol

Female albino rats were kept for periods of 4 or 7 months on a diet deficient in vitamin E, consisting

In group 1 two rats died after receiving tocopherol for about 3 months. With rat no. 8 death was probably due to sepsis following the injection of lard. Rat no. 7 was not autopsied, but it was emaciated and had overgrown teeth which may have caused difficulty in eating. The data in Table 3

Table 3. Weights of rats receiving vitamin E-deficient diet

Group 1. Given 1 mg. of tocopherol acetate per rat, each week								
Rat no.	...	13	14	15	16	17	18	19
Wt. (g.) on day 1		202	250	196	187	169	182	186
Wt. (g.) on day 84		227	227	214	245	192	203	171
Wt. (g.) on day 203		237	239	237	Dead	203	217	Dead
Mean increase of the five rats surviving 203 days = 27 g.								
Group 2. Given no added tocopherol								
Rat no.	...	20	21	22	23	24	25	26
Wt. (g.) on day 1		165	176	182	202	165	167	192
Wt. (g.) on day 84		187	192	197	211	177	185	197
Wt. (g.) on day 203		170	192	188	204	173	177	182
Mean increase of five rats showing greatest growth = 9 g.								

show that little change in weight occurred in either group.

The degree of dental pigmentation at the beginning of the test period was less uniform in both groups than in the preceding experiment in which deficiency of vitamin A had been involved. The individual marks after various periods are shown in Table 4.

The mineral content of the teeth in avitaminosis E

Estimations of ash content and of fluorine in white and normal brown teeth were kindly made by Dr H. H. Green. The teeth examined were taken from rats which had been used in previous experiments [Davies & Moore, 1941]. The results are given in Table 5. There would therefore appear to

Table 4. *Dental depigmentation of rats receiving vitamin E-deficient diet*

Group 1. Given 1 mg. tocopherol acetate per rat, each week									
	Rat no. ...	13	14	15	16	17	18	19	Mean
Depigmentation of teeth (arbitrary marks)	Day 1	100	0	100	70	70	100	100	76
	Day 84	0	0	0	0	0	0	100	15
	Day 203	0	0	0	Dead	0	0	Dead*	0
Group 2. Given no added tocopherol									
	Rat no. ...	20	21	22	23	24	25	26	Mean
Depigmentation of teeth (arbitrary marks)	Day 1	100	90	100	90	90	90	90	93
	Day 84	100	70	90	60	70	70	65	75
	Day 203	100	50	90	90	95	90	95	87

* Teeth still white at death, but malformed and overgrown.

If we omit from consideration rat no. 14, which had brown teeth at the beginning, and also no. 19, which was diseased, the remaining five rats in group 1 all showed complete cure as the result of treatment with tocopherol. Of the seven rats in group 2 only no. 21 showed slight improvement, the teeth of the other six remained almost completely white.

At the end of the experiment the five surviving rats of group 1, and five rats of group 2, were killed and the vitamin A reserves in their livers estimated by the SbCl_3 method. For group 1 the mean total reserve was 14,000 i.u. For group 2, in confirmation of previous observations on the effect of vitamin E deficiency in lowering the vitamin A reserve [Moore, 1940], the mean value was only 4700 i.u., which, however, is still about twice the reserve found in a wild rat shot near this laboratory [Davies & Moore, 1934].

Table 5. *Mineral analysis of teeth from normal rats and from rats deficient in vitamin E*

Rat no.	Wt. of lower incisors g.	Dry matter %	Ash content %	Fluorine p.p.m.* original tooth
(a) Normal rats (brown teeth)				
1	0.198	86.5	64.5	50
2	0.129	87.5	64.0	115
3	0.107	89.5	65.0	140
4	0.140	89.5	62.5	140
Mean	0.144	88.2	64.0	111
(b) Deficient rats (white teeth)				
1	0.141	89.0	67.5	35
2	0.189	90.5	71.5	80
3	0.166	89.5	66.5	180
4	0.125	90.5	72.0	80
Mean	0.155	89.9	69.4	94

* p.p.m. = parts per million.

be a marked increase of the ash content in whitened teeth. The values for F were within the normal range in both groups.

DISCUSSION

The above experiments suggest that whitening of the teeth in the rat results from deficiency of either vitamin A or of vitamin E, and may be cured by the restoration of whichever of these vitamins is missing. Since vitamin E influences the efficiency of storage and utilization of vitamin A [Moore, 1940; Bacharach, 1940; Davies & Moore, 1941] the occurrence of a common lesion in deficiency may imply a further aspect of their interaction. Although deficiency of vitamin E may under some circumstances cause a secondary deficiency of vitamin A, indicated by the premature depletion of the vitamin A reserves in the liver, whitening of the teeth in vitamin E deficiency may occur even when large amounts of vitamin A are given and the reserves in the liver are high. While therefore the dental lesion may possibly result from a disturbance in the function of vitamin A as a result of the absence of vitamin E, a material deficiency of vitamin A is not an essential aetiological factor.

Dental depigmentation also occurs in fluorosis [McCollum, Simmonds, Becker & Bunting, 1925] and in Mg deficiency [Duckworth, 1942]. The lesion may therefore result from various dietary abnormalities, which may or may not be interrelated. Moreover, Irving [1942] has detected lesions in the enamel-forming organs of rats deficient in vitamin E, distinct from those observed in avitaminosis A, at a stage of the deficiency when the

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