XLV. A NOTE ON THE PRODUCTION BY IRRADIATION WITH ULTRA-VIOLET LIGHT OF ANTI-RACHITIC PROPERTIES IN STEROLS DERIVED FROM THE SMALL SIAK ILLIPE NUT (*PALAQUIUM BURCKII*).

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It has been shown separately by Drummond [1925] and by Hess [1925], together with their co-workers, that cholesterol, when irradiated with ultra-violet light, becomes activated, so that it is able to promote calcification and temporary growth in young rats fed on a diet deficient in fat-soluble vitamins. The same observation was made by Hess for phytosterol, prepared from cotton-seed oil.

A supply of mixed sterols, derived from quite a different source, *i.e.* the fat of the small Siak Illipe nut (*Palaquium Burckii*), was placed at the writers' disposal by the kindness of Dr C. W. Moore, and it seemed of interest to test whether these sterols possessed the capacity for activation, which has already been demonstrated for cholesterol and phytosterol from other sources.

Preparation of the sterols.

The sterols were prepared in the following manner. The unsaponifiable matter was separated from a quantity of the fat and formed a semicrystalline mass, most of which dissolved on extraction with boiling acetone and was redeposited in the cold, in the form of small colourless needles, melting indefinitely from 170–180°. The recrystallisation was carried out three times, but the substance so obtained still remained a mixture. It is upon this mixture that the tests about to be described were made. Further experiment showed that from the mixture an acetyl derivative could be obtained, which yielded one of the higher sterols in a pure condition. This sterol, which was designated *palasterol*, resembles in all respects androsterol, isolated from *Apocynum androsaemifolium* [Moore, 1909], and lupeol, derived from *Zanthoxylum macrophyllum* [Goodson, 1921]. Androsterol and lupeol are believed to be identical.

Technique.

For the test young rats were used which had been placed on a diet deficient in fat-soluble vitamins at a weight of about 40 g., and which had been on the diet for 33 days and were only growing very slowly. When an anti-rachitic stimulus of any kind, whether light or food, is applied to such rats, they begin to grow for a time at a much faster rate, though they finally decline and die. Subsequent investigation of their bones shows that they are more highly calcified than would be the case with untreated controls.

The diet used was as follows:

Inactivated caseinogen	300 g.	Marmite	75 g.
Starch	750 ,,	Lemon juice	75 cc.
Hardened cotton-seed oil	225 ,,	Distilled water	300 ,,
Salt mixture	75 ,,		

The salt mixture was as follows:

Sodium chloride ...	51·9 parts.	Calcium phosphate	162·0 parts.
Magnesium sulphate ...	164·0 ,,	Calcium lactate ...	390·0 ,,
Sodium dihydrogen phosphate	104·1 ,,	Ferric citrate ...	35·4 ,,
Dipotassium hydrogen phosphate	286·2 ,,		

Black and white rats bred in the Lister Institute were used.

The dose of sterols given was 0·005 g. daily, administered six times a week. The dose was fed embedded in a very small pellet of white bread and hardened cotton-seed oil. Control rats which did not receive the dose received the pellet.

The mixture of sterols, in the form of a white powder, was irradiated by weighing out the dose for two days, spreading the powder as thinly as possible, dry, upon a glass plate covered with paper, and exposing it for 30 minutes, at a distance of 14 inches, to a mercury vapour quartz lamp. The lamp was a powerful one, having only been in use for a few months. It was an Ulviarc burner, type Y2, of the Hewittic Electric Co.; on a circuit of 140 volts it was using a current of 3·5 amperes.

For the direct irradiation of rats to be used as controls a similar lamp was used, which had been in use for over 18 months and which was therefore proportionately less powerful. The rats were exposed for 10 minutes daily, six times a week, at a distance of 50 cm.

At the end of the experiment the leg bones of each rat were dissected out, the water content was determined, the fat was extracted with boiling ether and alcohol and the bones were ashed.

Experimental.

Three groups of animals were used for the experiment, one group of rats receiving irradiated sterols, one of rats receiving non-irradiated sterols and one of control rats irradiated directly. Each group contained three animals, and all belonged to the same litter. The accompanying Table I shows the result. The rats receiving the irradiated sterols increased in weight by 22–31 g. in the period of the experiment; those irradiated directly by 20–27 g., while

Table I. *Data of the increase in weight and composition of the bones of rats on a diet deficient in fat-soluble vitamins.*

Treatment	No. of Rat	Sex	Weight at beginning of treatment g.	Max. weight during treatment g.	Increase in weight g.	Duration of treatment days	% H_2O in fresh bone	% fat in fresh bone	% ash in wet bone	% ash in dry bone	% ash in fat ext. bone
Direct irradiation	213	♀	51	78	27	37	42·6	8·1	27·6	48·2	56·2
from 33rd day	215	♀	50	71	21	34	45·4	10·4	23·8	43·6	53·9
	216	♀	51	71	20	37	47·0	5·5	25·2	47·5	52·9
0·005 g. irradiated	217	♀	47	78	31	36	41·8	12·0	25·4	43·6	55·0
sterols daily from	219	♀	49	71	22	34	46·0	9·8	23·4	43·4	53·1
33rd day	220	♂	63	92	29	35	43·8	11·1	24·4	43·5	54·3
0·005 g. non-irradi-	214	♀	54	60	6	24	51·9	5·2	21·5	44·8	50·2
ated sterols daily	218	♀	51	57	6	37	45·6	12·4	21·4	39·4	51·2
from 33rd day	221	♂	57	69	12	34	59·0	0·7	20·4	50·0	50·9

Rat 221 lost 25 % of weight before the end of the experiment and rat 214 about 15 %, with the result that the fat in the bones was largely replaced by water, an occurrence which makes the percentage of ash in the dry bones inordinately high. Rat 214 succumbed to a vitamin A deficiency after 57 days on the deficient diet.

those which received non-irradiated sterols only increased by 6–12 g. The ash percentages corresponded; those of the rats irradiated directly and of the rats which received irradiated sterols were considerably higher than those of the rats which received the non-irradiated material. The mixed sterols derived from the Siak Illipe nut are, therefore, capable of becoming potent as an anti-rachitic when they are irradiated with ultra-violet light, in the same way as cholesterol and phytosterol.

All the rats in the experiment showed marked symptoms of xerophthalmia and signs of decline, due to deprivation of vitamin A, at about the 50th day on the deficient diet. The onset of these symptoms was entirely comparable in all the three groups, indicating that the sterols contained no appreciable quantity of vitamin A, either before or after irradiation.

SUMMARY.

1. A mixture of sterols, from the fat of the small Siak Illipe nut, was irradiated with ultra-violet light, whereby the power of promoting growth and calcification in young rats, on a diet deficient in fat-soluble vitamins, was imparted to the sterols.

2. The mixture of sterols contained no appreciable quantity of vitamin A, either before or after irradiation.

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REFERENCES.

Drummond, Rosenheim and Coward (1925). *J. Soc. Chem. Ind.* **44**, 123 T.
Goodson (1921). *Biochem. J.* **15**, 127.
Hess, Weinstock and Helman (1925). *J. Biol. Chem.* **63**, 305.
Moore (1909). *J. Chem. Soc.* **95**, 739.