S.D.G. Organon,
Organon Laboratories Limited,
Newhouse,
Lanarkshire ML1 5SH, U.K.

M. F. SUGRUE†
W. R. BUCKETT**

Scientific Development Group,
Organon,
Oss, The Netherlands

H. VAN RIEZEN

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** Present Address: Novo A/S, 2880-Bagsvaerd, Copenhagen, Denmark.
† To whom correspondence and reprint requests should be addressed.

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Experimental atherosclerosis in the rat: biochemical evaluation

The rat has been generally considered to be resistant towards the development of atheromatous arterial lesions, induced by a cholesterol-rich diet (Fillios, Andrus & others, 1956). However, in resistant species, such as baboon or rat, factors such as mechanical injury (Gutstein, Lazzarini-Robertson & La-Taillade, 1963), vitamin D_2 administration (Bajwa, Morrison & Ershoff, 1971) or immunization with foreign proteins (Howard, Patelski & others 1971) are often used in addition to the cholesterol diet. Altman (1973) found that rats treated for five consecutive days with an olive oil solution containing vitamin D_2 and cholesterol, exhibited extensive atherosclerotic lesions in the aorta, e.g. calcification as well as lipidic plaque formation.

To characterize these histological observations biochemically, male and female Sprague-Dawley rats, 310 $\pm$ 10 g, were randomized, divided into 4 different groups of 10 rats each and treated orally (dose kg^{-1} day^{-1}) for 5 days as follows: I, olive oil, 1·5 ml; II, vitamin D_2, 8 mg in olive oil, 1·5 ml; III, cholesterol, 40 mg in olive oil, 1·5 ml; IV, vitamin D_2, 8 mg + cholesterol, 40 mg in olive oil, 1·5 ml. At the end of the treatment the animals were fasted for 24 h and killed by a blow on the head.

Plasma and aorta samples were collected for estimation of lipids. The tissue was homogenized in physiological saline and then lyophilized. Total cholesterol, triglycerides and phospholipids were estimated respectively by slightly modified methods of Bloor (1916), Van Handel, Zilversmit & Bowman (1957), and Svanborg & Svennezholm (1961), after lipid extraction by the method of Carlson (1963). Vitamin D had no effect on cholesterol concentration.

The effect of treatments is shown in Table 1. In plasma the treatment with vitamin D_2, alone and with cholesterol, increased the fat concentrations, while cholesterol alone did not change the plasma lipid pattern. No significant interaction between D_2 and cholesterol was observed.

In aorta the combination of cholesterol with vitamin D_2 significantly increased the lipid concentrations but the individual treatments had no effect, thus the administration of cholesterol with vitamin D_2 affected significant changes in the triglyceride and

Table 1. *Effect of oral administration for 5 consecutive days of the olive oil solution of cholesterol and vitamin D_2 on the lipids concentration of plasma and aorta in Sprague Dawley rats.*

		Plasma			Aorta		
Treatment	Dose (kg^{-1} day^{-1})	Chol. mg $100\ ml^{-1}$ ± s.e.	Trigl. mg $100\ ml^{-1}$ ± s.e.	Phosphol. mg $100ml^{-1}$ ± s.e.	Chol. mg g^{-1} ± s.e.	Trigl. mg g^{-1} ± s.e.	Phosphol. mg g^{-1} ± s.e.
Olive oil	1·5 ml	70·4 ±3·7	95·8 ±11·4	115·2 ±3·8	2·7 ±0·1	18·0 ±2·2	8·4 ±0·4
Olive oil+ vitamin D_2	1·5 ml 8 mg	114·8* ±9·2	124·7 ±19·1	141·5* ±7·0	2·9 ±0·1	15·5 ±3·7	8·3 ±0·6
Olive oil+ cholesterol	1·5 ml 40 mg	65·1 ±4·1	70·2 ±9·1	129·0 ±8·2	3·0 ±0·3	13·7 ±2·0	8·1 ±0·4
Olive oil+ cholesterol+ vitamin D_2	1·5 ml 40 mg 8 mg	99·1* ±5·3	120·2 ±11·2	151·6* ±7·0	3·6* ±0·1	32·4*†‡ ±4·5	11·6*† ±0·6

Analysis of variance:
*$P<0.01$ versus olive oil treated group.
†$P<0.05$, ‡$P<0.01$ } significance of the interaction: Cholesterol × Vitamin D_2.

phospholipid concentrations as assessed by variance analysis with a 2 × 2 factorial design.

The arterial content of triglycerides and phospholipids was similar to that obtained by feeding rats with fat and cholesterol-rich diet for three months according to Vijayagopal & Kurup (1973).

Unpublished observations suggest that the age of the rats seems to be a critical factor in inducing the atherosclerotic lesions; the treatment described does not significantly affect the lipid concentration either in plasma or in aorta in rats of 150–250 g.

This procedure would seem to be suitable for the induction of atheromatous-like lesions in rats.

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Instituto Franco Tosi S.p.A.
Laboratorio Ricerche Farmacologiche
Via L. di Breme, 26
20156 *Milan, Italy.*

RODOLFO TESTA
CESIRA CANESTRINI
CLAUDIA OLDANI

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