in the DSc group. Clofibrate and diosgenin with the supplement caused further small but significant reductions in hepatic cholesterol concentrations but diosgenin with vitamin C (DAASc) did not reduce plasma cholesterol more than diosgenin alone (DSc). Clofibrate and diosgenin therefore require ascorbic acid for their hypocholesterolemic actions and are potentiated by supplementary vitamin C. Their combination with vitamin C could have therapeutic implications in man.

References

GINTER, E. & ZIOCH. Z. (1972). Raised ascorbic acid consumption in cholesterol-fed guinea-pigs. *Int. J. Vit. Nutr. Res.*, **42**, 72–79.

ODUMOSU, A. & WILSON, C.W.M. (1973). Metabolic availability of Vitamin C in guinea-pig. *Nature, Lond.* **242**, 519–521.

ODUMOSU, A. & WILSON, C.W.M. (1978). Vitamin C and the cholesterol-lowering effect of clofibrate. *Br. J. Pharmac.*, **64**(3), 477P.

The effect of vitamin C deficiency and supplementation on the weight pattern and skin potential of the guinea-pig

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Some female scorbutic guinea-pigs are able to synthesize ascorbic acid (Odumosu & Wilson, 1971). Since females only were able to do this, the sexes might respond differently if similarly tested when availability of vitamin C is the determining factor. Ten male and 10 female mongrel guinea-pigs were housed separately and fed with rabbit pellets containing 25 mg vitamin C/100 g, with free access to drinking water containing vitamin C, 53 mg/100 ml. After 7 days, 5 males and 5 females were transferred to a scorbutogenic diet and Vitamin C free drinking water. The other 2 groups continued on the supplemented diet. For 29 days weight changes, plasma ascorbic acid levels, and skin potential values were recorded. The group diets were then alternated and measurements continued. Plasma and dietary ascorbic acid were measured with 2,4-dinitro-phenylhydrazine (Roe & Kuether, 1943). Skin potential was measured with a millivoltmeter and AgCl electrodes (Edmonds & Cronquist, 1970) using depilated skin areas.

Females maintained higher plasma ascorbic acid and skin potential values in the scorbutic state (Table 1). Body weights initially increased more rapidly in the scorbutic than in the supplemented guinea-pigs. They began to fall in the scorbutic groups before the diet change was introduced. Skin potential values rose in the supplemented groups and fell in the scorbutic groups together with plasma ascorbic acid changes. When the diets were alternated, plasma ascorbic acid values moved in the opposite directions. Skin potential values also immediately reversed their directions of change. These results indicate the changes produced by the essential electron donor in the semi-dehydroascorbate-ascorbic acid system (Ghirretti & Ghirretti-Magendie, 1977) and suggest it may be essential for maintaining basal body energy.

Table 1 Mean values for plasma ascorbic acid (AA, mg/100 ml), body weights (g) and skin potentials (mV) of guinea-pigs receiving vitamin C supplemented or scorbutogenic diets before and after alternation of their diets on day 29.

	Males			*Females*			*Males*			*Females*		
Day of Diet	*Plasma AA*	*Body Wt*	*Skin Pot*	*Plasma AA*	*Body Wt*	*Skin Pot*	*Plasma AA*	*Body Wt*	*Skin Pot*	*Plasma AA*	*Body Wt*	*Skin Pot*
	Supplemented Diet						Scorbutogenic Diet					
0	0.58	532	—	0.47	532	—	0.53	556	—	0.66	550	—
7	0.59	546	7.4	0.50	545	7.4	0.48	569	6.4	0.59	562	8
15	0.64	558	9.2	0.58	650	9.8	0.21	565	6.2	0.64	571	7.2
29	0.67	582	12	0.75	595	12	0.04	541	0.2	0.45	565	3.6
	Scorbutogenic Diet						Supplemented Diet					
7	0.72	593	9.8	0.72	607	10.6	0.12	554	2.6	0.54	576	4.1
14	0.48	566	7.8	0.73	607	9.4	0.2	562	5	0.72	602	6.6
28	0.12	522	2.4	0.36	609	7	0.45	582	10	0.93	621	10

References

EDMONDS, C.J. & CRONQUIST, A. (1970). A simple millivoltmetre and electrodes for measurement of rectal electrical potential in man. *Med. and Biol. Engng.*, **8**, 409–410.

GHIRRETTI, F. & GHIRRETTI-MAGENDIE, A. (1977). The effect of Vitamin C on the intracellular oxygen transport. *Int. J. Vit. Nutr. Res.* **16**, 41–52.

ODUMOSU, A. & WILSON, C.W.M. (1971). Metabolic availability of ascorbic acid in female guinea-pigs. *Br. J. Pharmac.*, **42**, 637–638.

ROE, J.H. & KUETHER, C.A. (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of ascorbic acid. *J. Biol. Chem.* **147**, 399.

The effects of oestradiol-17β and tamoxifen on the development of mouse embryos cultured over collagen

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The late preimplantation mouse blastocyst becomes noticeably sticky after its escape from the zona pellucida. Around this time there is a change in the staining reaction of its trophoblastic surface coat to colloidal iron-Prussian blue. Whereas the surface coat of the 80–86 h *post-coitum* (h p.c.) blastocyst stains red that of the 96–100 h p.c. blastocyst, i.e. after the surface coat change (SCC), stains blue. Implantation in the mouse is an oestrogen-dependent phenomenon and so is the surface coat change (Holmes & Dickson, 1973). Tamoxifen, a non-steroidal antioestrogen and a drug which prevents implantation in the mouse, prevents the SCC *in vivo* (Bloxham, Pugh & Sharma, 1975). It is also known that blastocysts can be induced to undergo the SCC *in vitro* if to the Whittingham's (1971) medium in which they are incubated is added oestradiol-17β at a concentration greater than 1.5×10^{-10} M and that this effect of oestradiol-17β is antagonized by tamoxifen in a concentration dependent manner (Bloxham & Pugh, 1977).

The importance of oestradiol to maturation and implantation had been called into doubt, however, when Jenkinson & Wilson (1973) showed that mouse blastocysts in Whittingham's medium would develop normally and even go on to mimic the phases of attachment and invasion if incubated over a layer of reconstituted collagen. It was therefore of interest to investigate 80 h p.c. embryos grown in the Jenkinson & Wilson system (groups of 19–26 embryos in 2 ml Whittingham's medium) for the occurrence of the SCC.

The change had occurred in all 8 morphologically normal embryos removed at 36 h of culture and by 68 h all morphologically normal (24 out of 32) embryos had attached to the collagen. In the absence of collagen all embryos remained free-floating and none of the 15 stained at 16 h or 36 h had undergone the SCC. In the absence of collagen but presence of oestradiol (10^{-8} M), all 9 blastocysts examined had undergone the SCC after 16 h but none of the 14 remaining became attached.

The addition of oestradiol (10^{-8} M) to the collagen containing culture system allowed the percentage of blastocysts which attached to rise from 74.28% to 90.47% ($P < 0.05$) and attachment occurred 10 h earlier. The further addition of tamoxifen (2.8×10^{-10} M) caused the proportion of blastocysts which became attached to fall to 39.13% at 68 h of culture. In the absence of oestradiol, SCC and implantation were totally prevented by tamoxifen (2.8×10^{-10} M).

It can be concluded that the simultaneous presence of oestrogen and an appropriate surface increase the rate of development and the frequency of attachment of mouse embryos in culture, that the anti-implantation action of tamoxifen *in vitro* is independent of exogenous oestradiol and that the surface coat change precedes implantation *in vitro*.

References

BLOXHAM, P. & PUGH, D.M. (1977). Tamoxifen inhibition of an *in vitro* oestradiol-induced surface coat change on mouse blastocysts. *Br. J. Pharmac.*, **60**, 517–20.

BLOXHAM, P., PUGH, D.M. & SHARMA, S.C. (1975). An effect of tamoxifen (ICI 46.474) on the surface coat of the late preimplantation mouse blastocyst. *J. Reprod. Fert.* **45**, 181–83.

HOLMES, P.V. & DICKSON, A.D. (1973). Estrogen-induced surface coat and enzyme changes in the implanting mouse blastocyst. *J. Embryol. exp. Morph.* **29**, 639–45.

JENKINSON, E.J. & WILSON, I.B. (1973). *In vitro* studies on the control of trophoblast outgrowth in the mouse. *J. Embryol. exp. Morph.*, **30**, 21–30.

WHITTINGHAM, D.G. (1971). Culture of mouse ova. *J. Reprod. Fert. Suppl.*, **14**, 7–21.