Triparanol-effected accumulation of desmosterol in oestrone-induced hypocholesterolaemia

The mechanism for the hypocholesterolaemic effect in serum of oestrogens was postulated by Albers & Riggi (1966), Dill, Merola & Arnold (1966), Merola, Dill & Arnold (1968) to be a shift in serum cholesterol to the liver rather than inhibition of hepatic cholesterol synthesis. This conclusion was based on studies with rats whose cholesterol pools had been labelled with the obligatory cholesterol precursor [^{14}C] mevalonic acid. Under these conditions the oestrogen-induced decrease in serum cholesterol was not accompanied by a change in the specific activities of the serum or liver cholesterol. However, the decrease in serum cholesterol label was entirely accounted for by an increase in liver cholesterol label.

The findings with oestrogens were paralleled by the observations of Douglas, Ludwig & others (1966) who found that benzyl-*N*-benzyl carbethoxyhydroxamate induced hypocholesterolaemia by liver sequestration of cholesterol in rats and in rabbits on the basis of studies with [^{14}C] labelled chylomicra.

Evidence that oestrogens do not induce hypocholesterolaemia by inhibiting hepatic cholesterol synthesis may be adduced in another way. Bricker, Weis, & Siperstein (1972) have shown that rats, in whom cholesterol synthesis had been shut down by cholesterol feeding, did not give evidence of the presence of 24-dehydrocholesterol (desmosterol) when given triparanol, an agent previously shown (Avigan, Steinberg & others, 1960) to interrupt cholesterol synthesis at the desmosterol step in the biosynthetic pathway. Accordingly, we have adapted this approach to show that oestrone-induced hypocholesterolaemic rats given triparanol accumulate desmosterol to the same extent as do normo-cholesterolaemic ones given triparanol. This provides additional support for the view that oestrogens do not cause hypocholesterolaemia by shutting down hepatic cholesterol synthesis, especially early in the biosynthetic pathway.

Young male Wistar strain rats, $\sim$ 210 g each, in groups of 4 or 5 were assigned to control or oestrone-treated groups with lab chow and water provided *ad libitum*. Oestrone was given for 3 days at 5 mg kg^{-1} intragastrically (i.g.) before the administration of the triparanol (by courtesy of Richardson-Merrill) (A 3-day pre-treatment was shown previously by Merola & others, 1968, to be fully effective for inducing hypocholesterolaemia). Following this, half the rats were given triparanol-supplemented lab chow to provide the agent at 0·1 %, the level utilized by Bricker & others (1972). The oestrone-treated rats were given oestrone throughout the experiment. Seven days after triparanol treatment began, blood and liver were taken from all the rats for analysis for cholesterol and desmosterol. Cholesterol was estimated colorimetrically by the Turner & Eales (1957) modification of the Liebermann-Burchard procedure on an aliquot of the serum and on a light petroleum extract (b.p. 30–60°) of a sample of saponified liver. Additionally, desmosterol was estimated on an aliquot of a light petroleum extract of the saponified sera and livers by g.l.c. For g.l.c. estimation, a 0·25 cm $\times$ 1·8 m 3% OV-17 (100–120 mesh) column at 270° with the detector at 300°, similar to the procedure of Bricker & others (1972), was used. Reference cholesterol and desmosterol samples gave retention times of 13 and 15 min, respectively, with complete separation of their peaks.

It may be seen from the data summarized in Table 1 that triparanol exercised an adverse effect on the weight gains of the oestrone-treated rats which would explain their decreased liver weights. Desmosterol was not detected in the sera or livers of the control or oestrone-treated rats but invariably was present in the control and oestrone-treated rats given triparanol. Furthermore, the desmosterol contents

Table 1. *Triparanol- effected desmosterol accumulation in sera and livers of individual control and oestrone-induced hypocholesterolaemic Wistar strain male rats.*

	Wt Gain (g)	Serum Cholest.[b] (mg dl^{-1})	Serum Desmost.[c] (mg dl^{-1})	Liver Wt (g)	Liver Cholest[b,c] (mg)	Liver Desmost.[c] (mg)
Control	85	65	0	12	32	0
	60	74	0	14	30	0
	77	65	0	14	30	0
	76	62	0	13	28	0
Triparanol[a]	75	28	12	14	23	5
	56	37	3	15	25	10
	69	30	7	12	19	6
	81	30	2	14	29	9
Oestrone, 5 mg kg^{-1}, i.g.[d]	24	42	0	13	35	0
	36	36	0	13	24	0
	41	46	0	13	35	0
Oestrone, 5 mg kg^{-1}, i.g. +triparanol	−13	30	1	10	18	5
	−68	30	6	7	18	3
	−39	36	3	7	18	2
	−13	36	3	9	22	6

[a] At 0·1% along with ground lab chow.
[b] Not corrected for the reduced colour given by desmosterol.
[c] On a light petroleum extract of a saponified aliquot.
[d] Two rats lost during test for causes other than associated with medication.

of the sera and livers of the triparanol-, and the triparanol and oestrone-treated rats were clearly of the same order. Thus, there is no basis for considering that oestrogen pre-treatment materially modified desmosterol accumulation in the sera and livers of these rats.

The point to be made is that desmosterol accumulates to about the same extent in the triparanol-treated rats whether they were rendered hypocholesterolaemic by oestrone pre-treatment or not. Consequently, the data support the conclusion reached earlier (Albers & Riggi, 1966; Dill & others, 1966; Merola & others, 1968) that oestrogens do not materially effect hypocholesterolaemia by blocking hepatic cholesterol biosynthesis.

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