Studies of Serum Desmosterol Levels in Hypercholesteremic Subjects Treated with Triparanol (MER-29)

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THE new approach to the treatment of hypercholesteremic states, i.e., inhibition of cholesterol biosynthesis, has certain real or potential disadvantages. Thus, when the inhibitor acts early in the cholesterol metabolic pathway (as with benzmalecene), there is an accumulation of triglycerides probably due to a diversion of available acetate from cholesterol synthesis to triglyceride synthesis.[1] When the inhibitor acts late in the pathway, i.e., after cyclization of squalene to form the steroid nucleus, an accumulation of a steroid precursor of cholesterol may result. Such a "late" inhibitor is triparanol (MER-29), and indeed, the cholesterol precursor, desmosterol (24-dehydrocholesterol) has been demonstrated in the liver and serum of rats[2] and in the serum of patients[3–5] taking this compound.

We, as others, have been concerned about the appearance and accumulation of desmosterol, which structurally so closely resembles cholesterol, because of the potential danger of atherogenesis from this precursor sterol. Therefore, we have followed the serum level of desmosterol in a prolonged investigation of the effects of triparanol on lipids and lipoproteins. This report will be concerned only with the problem of desmosterol accumulation.

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METHODS

Most of the available chemical methods for the determination of cholesterol determine other sterols as well. If the usual colorimetric analysis for "cholesterol" (which measures both desmosterol and cholesterol) is utilized during triparanol therapy, the specific progress of the serum *cholesterol* concentration cannot be followed. It has been shown that the Zak method,[6] which uses ferric chloride for cholesterol color development measures both total cholesterol and total desmosterol, and that the Abell method,[7] which uses a modified Liebermann-Burchard reagent for color development measures cholesterol plus 52 per cent of the desmosterol.[4] We have found that in our laboratory the Zak method measures total cholesterol and 80 per cent of desmosterol, whereas the Abell procedure measures total cholesterol plus 56 per cent of the desmosterol. These values have been obtained using both desmosterol‡ and cholesterol standard solutions, individually and in combination. Thus, by the following algebraic formula§ it is possible to determine the desmosterol content of serum:

$$\begin{aligned} Z &= C + 0.80D \\ -(A &= C + 0.56D) \\ \hline Z - A &= 0.24D \end{aligned}$$

where

Z = mg. % sterol as determined by the Zak method,
A = mg. % sterol as determined by the Abell method,
C = cholesterol, and D = desmosterol.

We have used these technics in studying the serum of ten patients with disorders of lipid metab-

‡ The desmosterol was kindly supplied by Dr. T. R. Blohm of Wm. S. Merrell Co.

§ The formula represents a modification of relationships proposed by Frantz et al.[4]

TABLE I

Effect of Triparanol (MER-29) Administration on Serum Cholesterol and Total Sterol Concentrations

Patient	Diagnosis	Control Cholesterol Average* (mg./100 ml.)	Triparanol Therapy							
			Cholesterol				Total Sterol			
			Average†		Minimal Value		Average		Minimal Value‡	
			mg./100 ml.	% Fall	mg./100 ml.	% Fall	mg./100 ml.	% Fall	mg./100 ml.	% Fall
M. K.	Xanthoma tendinosum	547	415	24	386	30	469	14	465	15
B. T.	Familial hypercholesteremia	600	308	49	244	59	413	31	428	29
S. T.	Familial hypercholesteremia	415	216	48	188	55	318	23	326	22
G. B.	Familial hypercholesteremia	484	267	45	171	65	392	19	427	12
I. F.	Familial hypercholesteremia	399	300	25	230	42	370	7	358	10
M. H.	Xanthoma tuberosum	614	293	52	230	63	338	45	284	54
R. J.	Nephrotic syndrome	654	393	40	304	54	630	4	624	5
E. F.	Coronary artery disease	444	336	24	261	41	437	2	395	11
E. W.	Xanthoma tuberosum	447	318	29	285	36	412	8	393	12
S. R.	Idiopathic hypercholesteremia	354	244	31	202	43	287	19	272	23
Average		496	309	37	250	49	407	17	397	19

* Average of three values taken at weekly intervals.

† Average of seven values taken over three month period, three months after MER-29 therapy started except for E. W. whose average represents a two-month period.

‡ Total sterol (cholesterol plus desmosterol) on same serum which demonstrated maximum per cent fall in cholesterol.

olism, including xanthoma tendinosum and tuberosum (three), familial hypercholesteremia (four), idiopathic hypercholesteremia (two) and nephrotic syndrome (one). The subjects received triparanol,* 1,000 or 1,500 mg. daily in divided doses. These subjects were studied for periods of five to thirteen months and are still under observation.

RESULTS

All subjects demonstrated significant falls in serum cholesterol levels averaging 37 per cent ($p = <0.001$) (Table I). When desmosterol plus cholesterol (hereafter referred to as total sterol) was measured on these same serum samples the apparent fall in "cholesterol" was less (17 per cent) but still significant ($p = <0.01$). The *maximum* fall in true cholesterol levels averaged 49 per cent, and in total sterol concentrations, 19 per cent.

Desmosterol was not demonstrable in the serum obtained before treatment. During the administration of triparanol, the maximum increase of desmosterol averaged 167 mg. per 100 ml. and ranged from 84 mg. per 100 ml. to 320 mg. per 100 ml. This represented an average of 37 per cent of the total sterol.

In seven of the ten subjects, desmosterol was demonstrable in the serum within two weeks. In the others, desmosterol was not found in the serum until after three, four and eleven weeks of triparanol administration. The time of appearance of desmosterol was not related to dose.

In two subjects there was no desmosterol

* Triparanol was generously supplied by Dr. J. S. Scanlan and Dr. R. H. McMaster of the Wm. S. Merrell Co.

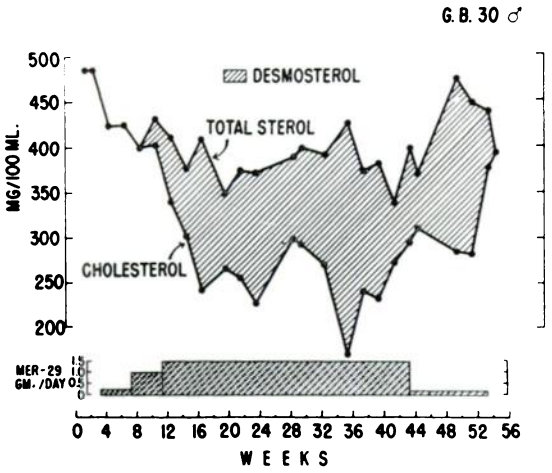

FIG. 1. Appearance of desmosterol in serum of patient with familial hypercholesterolemia during triparanol therapy.

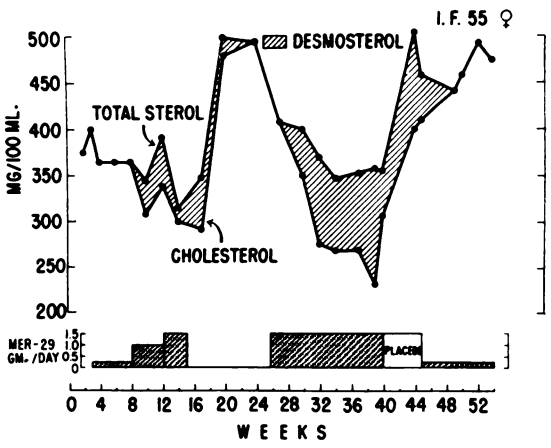

FIG. 2. Effect of interdiction of triparanol therapy on serum desmosterol levels of patient with familial hypercholesterolemia.

in the serum during initial four and eight week periods of 250 mg. triparanol daily, but desmosterol promptly appeared when they were switched to the larger doses (Fig. 1). Aside from these, there appeared to be no correlation between the presence of desmosterol and triparanol dosage. Nor was there a relationship between the duration of the study and desmosterol level, i.e., once a level was established there appeared to be fluctuations around that level rather than progressive accumulation of the sterol.

Following cessation of triparanol therapy (by switching to placebo) in three of the subjects, desmosterol continued to be demonstrable in the serum for five to six weeks (Fig. 2).

COMMENTS

It would appear from these studies that progressive accumulation of desmosterol in the serum of patients on triparanol therapy does not represent an inherent potential danger to the continuation of this mode of therapy. Although Frantz et al.[4] described one patient in whom the total sterol rose during therapy, in our series the rise of serum desmosterol was always associated with a fall in total sterol. If one postulates that desmosterol is as atherogenic as cholesterol, one still would not expect *increased* atherosclerosis as the result of triparanol therapy because the total sterol (desmosterol plus cholesterol) levels never exceeded the initial levels of cholesterol. It is not yet known whether the presence of desmosterol in serum in amounts reported herein can produce harmful effects. Long-term biologic effects of desmosterol are not known, but it would appear that desmosterol is converted to and loses its identity in bile acids in a manner indistinguishable from that of cholesterol.[8]

Of interest is the fact that the concentration of serum desmosterol did not progressively increase. Once desmosterol was demonstrated in the serum, the concentration fluctuated from week to week. It might be expected that a compound which completely inhibits the last step of cholesterol biosynthesis would produce a progressive accumulation of the immediate precursor. Since our data indicate that this does not occur, they suggest that desmosterol itself might act as an effective inhibitor in the chemical negative feed-back system regulating cholesterol biosynthesis.[9] The fact that the serum cholesterol concentration does not fall to zero indicates that inhibition of biosynthesis is incomplete or that an alternative pathway for cholesterol biosynthesis is present and, indeed, one has recently been suggested by Steinberg and Avigan.[10]

It is clearly recognized that the preceding comments must be tempered by the fact that

serum desmosterol is not determined directly by the method presented. The desmosterol values represent the difference between two methods, each controlled with appropriate crystalline desmosterol and cholesterol standards.

SUMMARY

The concentration of desmosterol, the immediate precursor of cholesterol in the synthetic chain, was followed in the serum of ten patients with disorders of lipid metabolism and hypercholesteremia given triparanol (MER-29) for a prolonged period.

All subjects demonstrated falls in total sterol (desmosterol plus cholesterol) levels which averaged 17 per cent. This was significant, but less than the average fall in serum cholesterol of 37 per cent. During triparanol administration, the maximum increase of desmosterol averaged 167 mg. per 100 ml., which represented 37 per cent of the total sterol. Progressive increases in serum desmosterol levels as triparanol therapy continued did not occur. It is not yet known whether the presence of desmosterol in serum in these amounts can produce harmful effects.

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ADDENDUM

After this paper was submitted for publication, similar findings were reported in nine normo- or mildly hypercholesteremic subjects.[11]

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