This is also true of the 3 antibacterial agents previously found to be inactivated by bile salts, namely polymyxin, ristocetin and vancomycin(1). In the light of the findings herein reported, it may very well be that diminished or absent absorption of certain antifungal and antibacterial agents when administered orally is due to their inactivation by the high concentration of bile salts normally present in the intestinal tract rather than to their poor diffusion through the intestinal wall because of their large molecular size. Studies leading to reversal of bile inactivation of these antimicrobial agents could lead to their increased absorption and hence clinical usefulness.

Summary. One per cent bile salts inactivates amphotericin B, chlorquinaldol, gentian violet and nystatin to a considerable but variable degree depending upon the antimycotic agent. A number of factors possibly involved in the mechanism of this inactivation have been studied and are discussed.

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Serum Cholesterol Determinations as Affected by Vitamin A. (24309)

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It was observed in this laboratory that Vit. A administration resulted in an increase in total serum cholesterol and that as the serum Vit. A level increased, serum total cholesterol increased proportionately. It was then necessary to determine if the elevation of serum Vit. A was associated with an apparent or real increase in total serum cholesterol.

The method used(1) for determining serum cholesterol was one widely used in clinical laboratories. Since the reagent ferric chloride is used both in this method and in measurement of Vit. A, it was felt that perhaps Vit. A was interfering with determination of cholesterol. It was therefore believed worthwhile to investigate the effect of Vit. A on the ferric chloride method of determining serum cholesterol.

Methods and materials. Serums were taken from a group of 5 patients, having the following Vit. A concentration as determined by the Carr-Price method(2); patient 1, 55; patient 2, 28; patient 3, 56, patient 4, 37, and patient 5, 40 μg% respectively. Of this group of serums, 3 had elevated cholesterol levels and 2 had normal cholesterol levels. Varying amounts of Vit. A alcohol equivalent to 15-150 μg per 100 ml serum were added to each serum (Tables I and II). In each case, a control tube was set up containing 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 ml respectively of Vit. A alcohol (3 μg per ml in Mallinckrodt chloroform) were added and the volume made up to 0.6 ml with chloroform.

A total cholesterol determination was then done on each of the 6 tubes from the 5 different serums by the method of Zak(3).

The same group of 5 serums was set up

TABLE I. Effect of Varying Concentration of Vit. A on Determination of Total Serum Cholesterol by the Zak Procedure(3).

	mg % total cholesterol						
	Control	15 μ %*	30 μ %*	60 μ %*	90 μ %*	120 μ %*	150 μ %*
Patient 1	403	417	425	455	480	510	545
2	363	363	380	415	445	470	500
3	321	326	330	360	390	420	445
4	249	253	260	295	325	360	390
5	205	208	210	230	255	280	300

* Vit. A added.

TABLE II. Effect of Varying Concentration of Vit. A on Determination of Total Serum Cholesterol by the Schoenheimer-Sperry Procedure(4).

	mg % total cholesterol						
	Control	15 μ %*	30 μ %*	60 μ %*	90 μ %*	120 μ %*	150 μ %*
Patient 1	411	412	414	415	417	420	420
2	367	366	366	363	369	370	372
3	319	320	323	325	330	328	330
4	250	253	252	254	255	255	256
5	201	202	201	203	204	205	207

* Vit. A added.

as before, and a total cholesterol determination made on each by the Schoenheimer-Sperry saponification-extraction method(4) with a slight modification.*

Results. The results, in mg% total cholesterol, shown in Tables I and II represent an average of tests run in triplicate.

With addition of 30 μg of Vit. A per 100 ml serum, and using the Zak method, there was an increase of 10-15 mg% total cholesterol. However, with the addition of greater amounts of Vit. A, 120-150 μg per 100 ml, there was an increase of 100-200 mg% total cholesterol.

The results with the Schoenheimer-Sperry saponification-extraction method, showed no appreciable elevation of total serum cholesterol, even with addition of 150 μg Vit. A per 100 ml serum. This was probably due to the fact that the Vit. A had been separated from the total cholesterol by precipitation of the latter with digitonin.

It is of interest that the control samples, with no Vit. A added, show very similar values when determined by either the Zak or Schoenheimer-Sperry methods.

Discussion. The results of this investigation demonstrate the fact that excessively high serum Vit. A levels will cause interference with cholesterol determination when the Zak procedure is used. Such interference is not noted when Vit. A is separated from the serum before cholesterol determinations are made. This observation may account for the elevations in serum cholesterols following Vit. A therapy as reported by Lasch(5).

It should also be pointed out that care must be taken by the clinical laboratory in choosing a method for determination of total serum cholesterol when high serum levels of Vit. A are suspected.

Summary. 1. A study was made of the effects of various levels of Vit. A in the serum on total cholesterol as determined by a method using a ferric chloride color reagent and a saponification-extraction method. 2. Comparison between the 2 methods showed that the Vit. A must be separated from the serum before making total cholesterol determinations by the ferric chloride color reagent method.

* The precipitate was dissolved in glacial acetic acid and ferric chloride color reagent added instead of the Libermann-Burchard reaction.

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