fluorescence was not seen within any of the cells of the gland.

*Discussion.* Local concentrations of parathyroid hormone have been visualized for the first time in intercellular spaces lying between groups of chief cells. The validity of this finding rests on the specificity of the antiparathyroid hormone serum used in the fluorescent antibody studies. No evidence for immunochemical heterogeneity was detected by three independent criteria: double diffusion, complement fixation, and immunofluorescence (using bovine tissues other than parathyroid glands). Furthermore, the specificity of the immunofluorescent studies was shown by the lack of staining when (i) normal rabbit serum was employed instead of antiparathyroid hormone serum, when (ii) fluorescein-labeled antirabbit globulin was applied directly to the sections without prior treatment with rabbit antiparathyroid hormone serum, and when (iii) the antiparathyroid hormone serum had been absorbed with parathyroid extract.

Since no reaction with antiparathyroid hormone serum could be demonstrated within any of the cells of the parathyroid gland, it is possible that hormone in the cells is not in a form which can be demonstrated by the indirect fluorescent antibody technique, or that the technique may not be sensitive enough to visualize the quantities of hormone present in each cell.

*Summary.* The indirect fluorescent antibody technique revealed local concentrations of parathyroid hormone in intercellular tissue spaces between cords and within acini formed primarily of chief cells in bovine parathyroid glands. In this study, specific immunofluorescence was not seen within parenchymal cells of the parathyroid gland.

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## Cholesterol Solubilization by Solutions of Bile Salts and Bile Salts Plus Lecithin* (32983)

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Cholesterol, the principal component of most gallstones, is essentially insoluble in water. Bile salts alone will not solubilize the quantities of cholesterol present in bile (1), but, the addition of lecithin to bile salt solutions increases cholesterol solubilization to values observed in human gallbladder bile (2,3). It is generally accepted that the bile

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salts, lecithin and cholesterol are present in bile in the form of mixed micelles (4). Recently, Small *et al.* (5) provided phase diagrams of the solubilization of cholesterol by sodium cholate or a mixture of bile salts and lecithin. However, a complete study of the effectiveness of each of the bile salts present in human bile with and without lecithin is lacking.

*Materials and Methods.* The taurine and glycine conjugates of cholic, chenodeoxycholic, and deoxycholic acids (Calbiochem) were used without further purification. In each experiment, the bile acid content was determined by assaying the final solution after incubation as follows: the test solution of the bile salt was hydrolyzed in 2 *N* NaOH in an autoclave at 120°C for 3 hours, acidified to pH 2.0 with 6 *N* HCl, and the bile acids were extracted with diethyl ether. For a mixture, the individual bile acids were separated by thin-layer chromatography (6) prior to assay. The individual bile acids were quantitatively determined by ultraviolet absorption at 386 mμ in 65% sulfuric acid using the corresponding pure acids as standards. In the concentrations studied, neither cholesterol nor the hydrolysis products of lecithin gave significant absorption at 386 mμ.

Egg lecithin was prepared and assayed as previously described (7). Cholesterol (Nutritional Biochemicals) used in solubilization studies was recrystallized 3 times from 95% ethanol and was quantitatively assayed as in previous studies (7).

To prepare a given model solution, the bile salt was dissolved in 0.05 *M* phosphate buffer, pH 7.0. When necessary, the pH was readjusted to 7.0. When lecithin was to be incorporated into a model solution, an ethanolic solution of the phospholipid was added to a 10-ml Erlenmeyer flask and the ethanol was evaporated *in vacuo* with warming. One ml of the bile salt solution was then added to dissolve the lecithin residue.

To measure the solubilizing power of a given solution, excess crystalline cholesterol (20 mg), finely ground with a mortar and pestle, was added to each flask. The reaction mixtures were brought to a temperature of 37°C, flushed with nitrogen, and then sealed in an atmosphere of nitrogen. The flasks were then incubated at 37°C with continuous shaking for 4 days and without shaking for 2 additional days. After incubation, the contents were filtered through a 0.22 μ Millipore filter with the temperature maintained at 37°C. Materials which passed through the filter were considered to be dissolved as confirmed by the absence of turbidity and studies utilizing cholesterol $^{14}C$.

*Results. Cholesterol solubilization by bile salts alone.* To determine the relative solubilizing effects of the individual bile salts, the slopes or regression coefficients expressing the relation of the molar quantities of cholesterol solubilized to the concentrations of bile salt in solution (Fig. 1) were determined by the method of least squares (Table I) (8). The slopes of the relative solubilizing effect of the individual bile salts and a mixture of bile salts were compared utilizing the standard deviation of the slopes and the *Q* test for multiple comparisons (8). With 12 determinations, a difference in slope of .005 could be detected in 95% of such series. On the basis of the *Q* test, glycodeoxycholate solubilized significantly more cholesterol than any other bile salt tested. Taurodeoxycholate was the next most effective, solubilizing more cholesterol than taurochenodeoxycholate and taurocholate.

The amount of cholesterol solubilized by a mixture of bile salts was intermediate between the values observed for glycodeoxycholate and taurocholate; no more cholesterol was solubilized than would have been by the sum of the equivalent amounts of the individual bile salts (Fig. 1, Table I). The mixture used in these experiments contained glycine conjugates in a 3:1 ratio to taurine conjugates and cholic: chenodeoxycholic:deoxycholic in a 1.5:1.0:0.7 molar ratio.

In the range of bile salt concentrations studied, which corresponded to that found in human bile, 13–300 m*M* (9), the amount of cholesterol solubilized was proportional to the bile salt concentration irrespective of the type of bile salt or conjugate. The bile salts solubilized only about one third as much cholesterol as might be found in comparable

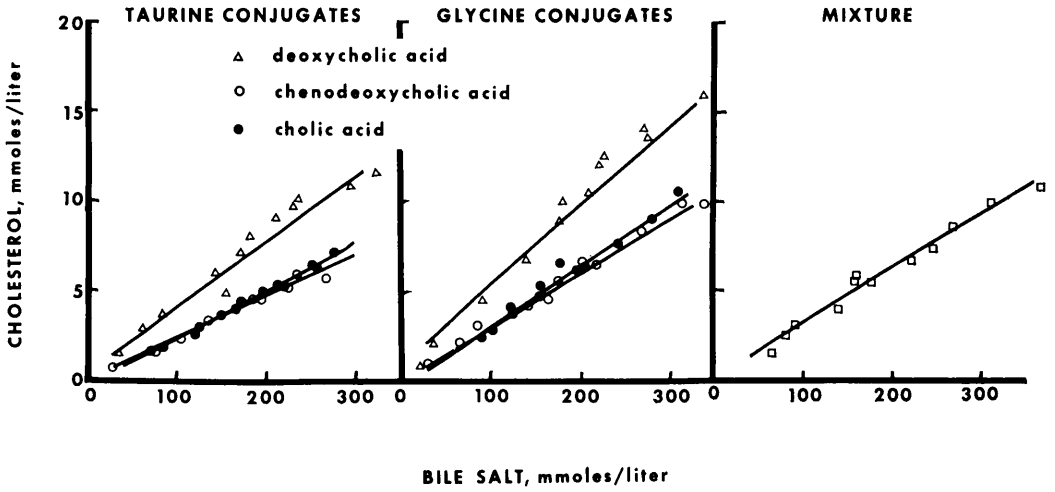


FIG. 1. Cholesterol solubilization by aqueous solutions of conjugated bile salts. Crystalline cholesterol was incubated for 6 days at 37°C as described in "Methods" with graded concentrations of the individual bile salts. Insoluble material was removed with a 0.22 $\mu$ Millipore filter. The straight lines were calculated by the method of least squares.

concentrated human gallbladder bile (Fig. 1).

*Cholesterol solubilization by bile salts and lecithin.* In order to study the effect of lecithin on cholesterol solubilization by a bile salt solution, we first added varying concentrations of lecithin to fixed concentrations of sodium glycodeoxycholate (Fig. 2, Table II). Three concentrations, 87, 178, and 265 mmoles per liter, were used thus sampling the range of total bile salt concentrations observed in human bile (9). Over the range studied, the maximum quantity of lecithin that would dissolve was limited to approximately 1 mmole per 2 mmoles of bile salt. For this series of experiments, the following equation was found by the method of least squares to express the relationship between the amount of cholesterol solubilized, bile salt concentration and lecithin concentration (8):

$$y = 0.044x_1 + 0.34x_2$$

where, $y$ = the amount of cholesterol solubilized in mmoles; $x_1$ = concentration of glycodeoxycholate in mmoles; $x_2$ = concentration of lecithin in mmoles. Thus, for every 3 mmoles of lecithin added, 1 additional mmole of cholesterol was solubilized. More importantly, solubilization by lecithin ap-

TABLE I. Cholesterol Solubilization by Bile Salts.[a]

| Bile salt | Cholesterol solubilized (m$M$/m$M$[b]) | Significantly higher solubilization per mmole of bile salt than:[c] |
|---|---|---|
| Glycodeoxycholate (GD) | .046 ± .003 | TCh, TC, M, GCh, GC, TD |
| Taurodeoxycholate (TD) | .037 ± .004 | TCh, TC |
| Glycocholate (GC) | .036 ± .002 | TCh |
| Glycochenodeoxycholate (GCh) | .029 ± .001 | None |
| Mixture (M) | .029 ± .002 | None |
| Taurocholate (TC) | .027 ± .002 | None |
| Taurochenodeoxycholate (TCh) | .023 ± .001 | None |

[a] This table presents a statistical analysis of the data plotted in Fig. 1. There are 12 samples for each bile salt.

[b] Regression coefficient ± SD of the regression coefficient.

[c] Differences are considered significant at the 5% level when $(b_i - b_j)/S_p > Q.05$; See Ref. (8).

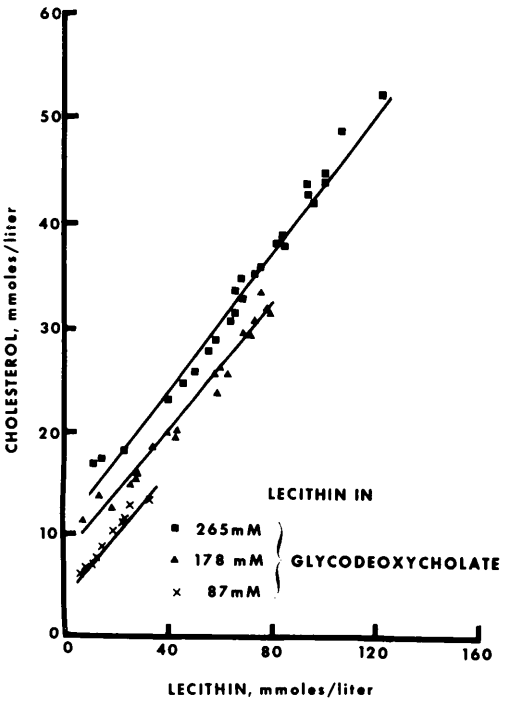


FIG. 2. The effect of added lecithin on cholesterol solubilization by aqueous solutions of sodium glycodeoxycholate. Duration and conditions of incubation and analysis of data are as in Fig. 1.

peared to be independent of the concentration of bile salt. The partial correlation coefficient (8) for cholesterol solubilization and concentration of lecithin with bile salt concentration held constant was 0.98.

To test whether the effect of lecithin on cholesterol solubilization was equivalent for each of the bile salts, varying amounts of lecithin were added to solutions containing 180 mmoles per liter of the individual bile salts and a mixture. The slopes expressing the molar quantities of cholesterol solubilized by concentrations of lecithin in solution were determined by the method of least squares. The slopes were computed for each of the bile salts and the mixture and then compared by analysis of covariance (8) (Table III). There was no significant difference between them. When the data from all the samples of all of the bile salts were pooled, the slope was 0.36.

TABLE II. Cholesterol Solubilization by Lecithin and Glycodeoxycholate.[a]

| | No. of samples | Cholesterol solubilized (m$M$/m$M$[b]) |
|---|---|---|
| Lecithin | 59 | .341 ± .009 |
| Glycodeoxycholate | 59 | .044 ± .004 |

[a] Analysis of variance in the data from Fig. 2 by a multiple regression model (8) shows a statistically significant relationship between the quantity of cholesterol solubilized and the quantities of lecithin and of glycodeoxycholate in solution, $F = 1770.46$ with degrees of freedom 2 and 56 which is highly significant.

[b] Regression coefficient ± SD of the regression coefficient.

TABLE III. Cholesterol Solubilization by Lecithin in Bile Salt Solutions.[a]

| Sample | No. of samples | Cholesterol solubilized[b] |
|---|---|---|
| Lecithin in 180 m$M$ of | | |
| glycodeoxycholate | 13 | .36 ± .02 |
| taurodeoxycholate | 12 | .33 ± .02 |
| glycocholate | 14 | .36 ± .02 |
| glycochenodeoxycholate | 10 | .34 ± .01 |
| mixture | 15 | .39 ± .05 |
| taurocholate | 15 | .37 ± .05 |
| taurochenodeoxycholate | 13 | .34 ± .02 |
| Pool | 92 | .36 ± .01 |

[a] From analysis of covariance (8), $F = .550$ ($D_f$ 6,78) Not significant. The quantity of cholesterol solubilized per mmole of lecithin is not significantly different for different bile salts.

[b] Regression coefficient ± SD of the regression coefficient.

*Discussion.* When tested *in vitro*, there appeared to be small differences in the ability of individual bile salts to solubilize cholesterol. Solubilization was not enhanced by combining bile salts in the proportions found in human gallbladder bile.

When lecithin was added to a bile salt solution, cholesterol solubilization was markedly increased; approximately 1 mmole of cholesterol was dissolved for every 3 mmoles of lecithin added regardless of the nature of the original bile salt. This ratio of lecithin to cholesterol is similar to that observed in native bile (9). The exact nature of the micellar interaction between bile salts, lecithin, and cholesterol in bile is not completely understood. Our own studies (7) with

fractions of bile and whole gallbladder bile suggest that the lecithin and cholesterol are closely associated and remain so even when the bile salts are removed. Whatever the manner of combination within the micelle, both fatty acids of the lecithin molecule probably contribute to the solubilization of the cholesterol since preliminary studies from this laboratory have shown that lysolecithin solubilized only about one half the amount of cholesterol solubilized by lecithin (10).

*Summary.* Model synthetic solutions were used to study cholesterol solubilization by the 6 conjugated bile salts found in human bile individually and as a mixture, and by lecithin in combination with bile salts. In the range of concentrations found in bile, bile salts alone solubilized only about one third as much cholesterol as might be found in comparable concentrated human gallbladder bile. Between 0.023 and 0.046 mmoles of cholesterol were solubilized per mmole of bile salts. Addition of lecithin to a bile salt solution increased the quantity of cholesterol dissolved to values observed in bile; 0.36 mmoles of cholesterol was solubilized per mmole of added lecithin. Cholesterol solubilization by lecithin was independent of the concentration and nature of the bile salt.

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## Inhibition of Heat-Induced Denaturation of Serum Proteins by Mixtures of Nonsteroidal Anti-Inflammatory Agents and Amino Acids (32984)

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Recent studies by Nielsen *et al.*(1) have shown that 1-day-old chicks given diets deficient in zinc developed bone and joint deformities similar to those found in patients suffering from rheumatoid arthritis (RA). The addition of zinc, histidine, or histamine reversed the condition(1), as did antiarthritic agents such as aspirin, cortisone, indomethacin, and phenylbutazone.[1] Recent findings by Gerber and Gerber(2) show that patients suffering from RA have lower than normal blood serum concentrations of histidine (1.90 ± 0.03 mg/100 ml, $n$= 215 vs 1.37 ± 0.04 mg/100, ml $n$ = 80). Feeding a high protein diet exerts a protective action against adjuvant-induced arthritis in rats(3). Moreover, it is known that certain tryptophan metabolites are found in excess in the urine of patients with RA(4). The excess, however, is not specific for RA and is seen in other conditions including bladder cancer, porphyria, scleroderma, systemic lupus erythematosus, and pregnancy(5). These findings indi-

[1] Chem. Eng. News **45**, 14 (1967).