related to emulsification of the substrate or the stabilization of the enzyme. The present findings on the interrelationships of cholesterol and bile salt concentration suggest some type of combination between cholesterol and bile salt. The data on the esterification system are especially significant. They indicate that 1 molecule of bile salt is associated with 1 molecule of cholesterol in the reaction with the fatty acid. It seems likely that the levels of activity observed were related to the concentration of a complex between cholesterol and bile salts rather than the concentration of either of the compounds, independently. In the hydrolytic system there was some effect of bile salt concentration above a 1:1 molar ratio with the cholesterol ester. However, the results are in accord with the hypothesis of complex formation between cholesterol or cholesterol ester and bile salt. If the mechanism of the bile salt effect is complex formation, the differences between the different bile salts could be due either to ease of complex formation or a specificity of cholesterol esterase related to the structure of the bile salt molecule.

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Cortisone Effect on Antibody Levels in Rabbits Simultaneously Immunized with Serum Albumin and Sheep Cells.* (20667)

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We have reported(1) that in a rabbit simultaneously immunized with bovine serum albumin and mumps vaccine, treatment with cortisone might markedly suppress the formation of antibody to the serum albumin while leaving the antibody to mumps apparently unaffected as compared with control animals. The antibody to mumps was measured by 3 different serological tests (hemagglutination inhibition, complement fixation and neutralization) which showed good agreement but all 3 tests are at best semi-quantitative and gave no indication of the actual amounts (weights) of the antibody involved. In order to establish whether cortisone might affect 2 different antigen-antibody systems differently in the same rabbit we have investigated the effect of cortisone on the sheep cell hemolysin system in rabbits, this being a system accessible to quantitative study and one in which preliminary experiments indicated no difference whatsoever in the hemolysin or agglutinin serum dilution titers of animals treated or not treated with cortisone.

Materials and methods. Antigens. Rabbits were immunized simultaneously with alum-precipitated bovine serum albumin and with

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either whole sheep red blood cells or stromata prepared by laking these cells. In order to give comparable doses of cells and stromata the following procedure was adopted. Approximately one liter of sheep blood was obtained in Alsever's solution and filtered to give a uniform suspension. The volume of packed cells (18%) in this suspension after centrifuging at a definite speed and time (2500 RPM, 10 min.) was noted and aliquots were withdrawn as needed, washed and diluted in saline to give a calculated 5% suspension of cells, the concentration used for immunization. Another aliquot of the cell suspension was then laked, thoroughly washed at high speed until the supernate contained only a trace of N, then suspended in a volume of saline equivalent to that which would have been used to dilute the original suspension aliquot to a 5% concentration of whole cells. *Immunization.* All antigen injections were intravenous and given over a period of 15 days. Eight animals received 7 injections of one mg each of alum-precipitated bovine serum albumin and 7 simultaneous injections of one ml of stromata suspension equivalent to a 5% cell suspension. Of these, 4 were treated with cortisone (Group A) and 4 not (Group B). Nine animals were immunized with BSA as above and with 5% cells as for stroma. Of these, 5 (Group C) were treated with cortisone and 4 were controls (Group D). Cortisone was started 3 days prior to the first antigen injection and continued at a dose of 4 mg daily 5 days per week until the 7th day after the last immunizing injection, at which time the animals were bled by cardiac puncture. During the course of the experiment there were 3 deaths; one each in Groups B, C, and D. *Antibody.* The volumes of sera obtained were estimated and a weight of sodium ethyl mercurithiosalicylate was added to each to give a 1:10000 concentration. Anti-albumin N was determined by the quantitative precipitin procedure(1). Serum precipitin titers were obtained by adding 2 μg of bovine serum albumin to one ml of serum dilution. Quantitative stroma agglutinin N was determined essentially by the method of Heidelberger and Treffers(2) except that the sera were not inactivated at 56°C. Hemag-

TABLE I. Summary of Antibody Levels in Cortisone Treated and Control Animals.

Group	Weight: At start	Weight: At end	mg N anti-BSA*/ml serum	Anti-BSA precipitin tit.	AbN absorbed/ml serum by S.S.† 1st absorp.	AbN absorbed/ml serum by S.S.† 2nd absorp.	AbN absorbed/ml serum by S.S.† Total	S.C.‡ agg. tit.	S.C. hemolysin tit.	Agg. for S.C. in supernate, 3rd absorp.
A	2680	2735	<.010	2	.17	0	.17	320	3200	0
	2630	2680	<.010	4	.16	0	.16	320	1600	+
	2660	2810	.028	4	.15	.05	.20	320	1600	0
	2565	2850	.040	8	.12	.01	.13	320	800	0 .17
B	2325	2545	.122	16	.14	0	.14	320	800	+++
	2300	2550	.088	16	.12	.02	.14	320	3200	
	2175	2800	.044	8	.14	0	.14	320	1600	.14
C	2430	2600	.050	8	.19	0	.19	2560	3200	++++
	2400	2700	.044	8	.20	.02	.22	1280	6400	
	2405	2625	.050	8	.24	0	.24	2560	6400	++++
	2350	2650	.068	16	.28	.05	.33	1280	6400	.25
D	2350	2550	.122	16	.22	.07	.29	1280	6400	+++
	1975	2360	.236	32	.30	.07	.37	2560	6400	
	2500	2975	.184	32	.27	.04	.31	2560	1600	.32

Group A—Immunized with BSA and stroma, cortisone-treated. B—Immunized with BSA and stroma, control. C—Immunized with BSA and cells, cortisone-treated. D—Immunized with BSA and cells, control. A third cell absorption is not included in the table since significant amounts of N were not removed from any of the sera. The second absorption was done on 2/3 of the supernate of the first absorption and the values for agglutinin N have been adjusted accordingly.

* Bovine serum albumin (BSA). † Sheep cell stromata (SS). ‡ Sheep cells (S.C.).

glutinin and hemolysin serum dilution titers were done in the usual way using 0.5 ml of serum dilution plus 0.5 ml of 0.25% red cells for the agglutinin titrations and 0.4 ml serum dilution, 0.2 ml of 0.5% cells and 0.2 ml of 1:20 guinea pig complement for hemolysin.

Results. The results obtained are summarized in Table I. All animals were immunized and bled at the same time and each procedure was done on all 14 sera at the same time. The effect of cortisone on anti-albumin N levels is readily apparent and is also reflected in the precipitin titers. The anti-albumin N levels in the cortisone treated animals average only 1/3 of that in the corresponding control group. Groups C and D appear to have higher anti-albumin levels than A and B. While these groups all ran concurrently, they were housed in 2 separate rooms, the room containing Groups C and D being air-conditioned and the other not, while the weather was warm. Whether this accounts for the differences observed or whether the fact that one pair of groups received whole sheep red cells and the other red cell stromata has not been determined.

The agglutinin N presents a different picture. Some uncertainty exists as to the absolute amounts of agglutinin N since, as was found by Heidelberger and Treffers(2), there is a loss of stromata N in the stroma-saline blanks. The values given in Table I are maximum agglutinin N levels obtained by subtracting the N of the stroma-saline blanks from the total N obtained with each serum. The minimum agglutinin N values, obtained by subtracting the total N contained in the aliquot of SS suspension used for absorption† would be lower by the same amount for each serum, approximately 0.1 mg N. In any case the values given are strictly comparable.

Comparing the agglutinin N values of groups A and B it can be seen that the cortisone had no effect on antibody produced in response to stroma injection. In fact, the cortisone-treated group A appears to have higher antibody levels than the Group B controls. The agglutinin titers are uniform in both groups and do not reflect the differences in antibody N levels, which is not surprising since these differences are much less than 2-fold. The hemolysin titers on the other hand show a great variation (although no significant difference between the 2 groups) again without reflecting the differences in antibody N among the individual animals of Groups A and B.

A comparison of the agglutinin N values in Groups C and D shows still another picture. There is little difference between the 2 groups insofar as the first absorption is concerned, but the second absorption brings the agglutinin N values for Group D to a slightly higher level than those in Group C. The difference between these groups may or may not be significant—the average of cortisone treated Group C is 0.25 mg N/ml, average of control Group D is 0.32 mg N/ml—but is not at all comparable to the difference in anti-albumin N. The agglutinin and hemolysin titers are not different in the 2 groups. Again these titers do not correspond well with the agglutinin N values in the same animals, but a comparison between the stromata-immunized groups and the cell-immunized groups does show a broad correlation between agglutinin N and titer.

Such a comparison also brings out 2 other points and these are that an average increase of less than 2-fold in agglutinin N values in the cell immunized groups vs. the stroma immunized groups resulted in a 4- to 8-fold increase in agglutinin titer and that the hemolysin titer did not increase to the same extent as the agglutinin titer. The statistical significance of these observations is open to question but they have a reasonable explanation in that the cell immunized animals might be expected to have antibody to antigens not given the stroma immunized animals. This could conceivably lead to a higher agglutinin titer in animals immunized with cells, whereas the agglutinin N value being obtained with stroma would not be affected as much. Specu-

† For the first, second and third absorptions these values are 0.730, 0.366 and 0.382 mg N respectively. Each value being the average of 4 samples taken at intervals during the pipetting of the stromata suspension into the 14 tubes. The maximum deviation from the average was less than ± 2.0% indicating that the stroma suspensions could be pipetted quite accurately.

lation along these lines leads to the interesting question of whether more than one sheep cell antigen can give rise to hemolytic antibody and if so, does the hemolysin titer reflect the sum of these antibodies or does it represent only that antibody present in highest concentration.

Discussion. The data indicate that in a rabbit simultaneously immunized with bovine serum albumin N and sheep red cells or stroma, cortisone may suppress antibody to the soluble protein antigen while leaving unaffected the development of antibody to the particulate antigen. Since the effect of cortisone on mumps antibody(1) seems to be intermediate between its striking effect on anti-albumin on the one hand and its complete lack of effect on anti-sheep cell stroma on the other, it is suggested that with increasing particle size of the antigen there is less effect of cortisone on antibody production. This hypothesis is consistent with the fact that while anti-stroma was unaffected there did appear to be a slight effect on antibody to whole sheep cells since an *in vivo* release of soluble antigen from cells could lead to suppression of antibody to this soluble antigen(s) by cortisone.

The fact that Bjørneboe *et al.*(3) have found that antibody to pneumococcal vaccine is suppressed by cortisone would seem to be inconsistent with the particle size idea, but an examination of their data can show otherwise. The route of antigen inoculation used was intravenous, as in this paper, and the rabbits of comparable size. Using a dose of 10 mg of cortisone daily the difference in the average antibody levels of their treated and control groups is less than the difference found above and previously(1) in the anti-albumin levels of animals not treated or receiving 4 mg of cortisone daily. With a dose of cortisone more nearly comparable to that used here, 2.5 mg daily, Bjørneboe *et al.* found much less suppression of antibody than is characteristic for anti-albumin and considerable overlapping of individual treated and control levels, a situation similar to that described above for antibody to whole sheep cells. It is entirely possible that whatever suppressive effect cortisone exerts on antibody to pneumococcal vaccine is directed against antibody to soluble constituents (*in vivo*) of the pneumococcus. Moreover, instances may be found in the literature where immunization with bacterial products is affected by cortisone or ACTH, whereas immunization with whole bacteria is not (*e.g.*, 4, pp. 20, 52, and 87).

The most basic objection to the antigen size idea comes from the fact that albumin, mumps virus and red cells differ not only in size, but also of course chemically and that while bovine serum albumin may be regarded as a single antigen the red cell and possibly the mumps virus are in reality multiple antigens. What is needed is a single antigen in very different particle sizes. An approach to this might be made by conjugating bovine serum albumin and red cells with some chemical grouping (*e.g.*, phenylisocynate) which destroys the original specificity of the antigen and results in antibody directed primarily against the conjugated group(5). This study is in progress.

Summary. In rabbits simultaneously immunized with bovine serum albumin and sheep red cells or stroma, cortisone suppressed the formation of antibody to bovine serum albumin but had little or no effect on the production of hemolysin or agglutinin as measured by serum dilution titers and quantitative agglutinin N.

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