TABLE I. Effect of Varying the Concentration of Chymotrypsin Solution on Analysis of Serum Chymotrypsin Inhibitors.

| Chymotrypsin sol'n, mg/ml | Incubation time, min | Klett readings: Serum substrate control (S) | Klett readings: Enzyme control (C) | Klett readings: Serum assay tube (A) | % inhibition |
|---|---|---|---|---|---|
| .25 | 120 | 248 | 180 | 244 | 94.1 |
| .50 | " | " | 135 | 208 | 64.6 |
| .75 | " | " | 98 | 161 | 41.5 |
| 1 | " | " | 83 | 129 | 27.9 |
| 1.50 | " | " | 52 | 90 | 19.4 |
| 2 | " | " | 40 | 63 | 11.1 |

TABLE II. Effect of Varying the Concentration of Substrate Solution on Analysis of Serum Chymotrypsin Inhibitors.

| Substrate* sol'n, mg/ml | Incubation time, min | Klett readings: Serum substrate control (S) | Klett readings: Enzyme control (C) | Klett readings: Serum assay tube (A) | % inhibition |
|---|---|---|---|---|---|
| .25 | 105 | 86 | 32 | 49 | 27.5 |
| .50 | " | 137 | 54 | 77 | 27.7 |
| 1 | " | 250 | 83 | 145 | 37.1 |
| 1.50 | " | 362 | 120 | 203 | 34.1 |
| 2 | " | 490 | 165 | 264 | 30.5 |

* l-tyrosinehydroxamide.

tions on timed reactions have obviated the need for subjective end point determinations.

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## Inhibition of Rapid Production of Antibody by Cortisone. Study of Secondary Response.* (19872)

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(Introduced by Robert F. Loeb.)

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The administration of cortisone or ACTH to rabbits results in a diminution of circulating antibody, as has been demonstrated by independent quantitative immunochemical studies(1,2). The diminished level of antibody resulted if the hormone was given during the period of primary immunization, or after antibody production was well established. Since the disappearance rate of rabbit antibody pas-

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sively administered to rabbits was not altered by the administration of cortisone(2,3), it appeared that the lower amounts of antibody found in actively immunized animals could not be attributed to increased catabolism of antibody protein and that synthesis of antibody was inhibited. To study the effect of cortisone on the synthesis of antibody protein, it appeared desirable to employ a system in which the rate of synthesis is so very rapid that the rate of degradation may be considered negligible. Such a system obtains in the specific anamnestic or secondary response, in which the reexposure of an animal to a previously injected antigen is usually followed by an abrupt increase in circulating antibody to several times its former or basal level.

This study indicates that pretreatment with cortisone markedly inhibits the synthesis of antibody protein during the elicitation of the secondary response.

*Experimental.* The rabbits used in this experiment were from a group which, several months previously had been simultaneously immunized with crystallized egg albumin by 3 different procedures for other purposes. The rabbits labelled AV had been immunized with alum precipitated egg albumin administered intravenously; those labelled ES, with a saline solution of egg albumin intravenously; and those labelled AC, with alum precipitated egg albumin injected intracutaneously. After a rest period of 2 months, the animals were distributed into 2 groups, I and II, comparable according to method of prior immunization and antibody levels previously achieved. Equal distribution was further demonstrated by comparison of the antibody levels at 2 baseline periods (see *Results* and Table I). At the indicated intervals, all of the rabbits were subjected to the following sequence of procedures: 1) bleeding for first baseline levels (Day —12), 2) bleeding for second baseline level (Day —6), 3) intravenous injection of 1 ml of alum precipitated egg albumin containing 1 mg crystallized egg albumin nitrogen (Day 0), 4) first post-stimulus bleeding for antibody determination (Day +5), and 5) second post-stimulus bleeding for antibody determination (Day +13). Eight animals comprised Group I and received cortisone; 7 animals comprised Group II and served as controls. Beginning on Day —4 and continuing for the remainder of the experiment, cortisone‡ was administered to the rabbits of Group I in daily dosage of 25 mg intramuscularly. Antibody analyses were carried out in duplicate by the quantitative precipitin method of Heidelberger and Kendall (4), employing the Heidelberger and MacPherson micromodification(5,6) when indicated by preliminary precipitin tests. When supernate tests or the ratio of antigen nitrogen to antibody nitrogen indicated that the desired degree of slight antigen excess was not present, repeated duplicate analyses were made to achieve optimal precipitation of antibody. The amount of anti-egg albumin is expressed as micrograms of antibody nitrogen per ml of serum (μg AbN/ml).

TABLE I. Effect of Cortisone on Antibody Levels of Rabbits before and after Induction of a Secondary Response with Crystalline Egg Albumin.

| Rabbit No. | Days before and after the antigen stimulus: −12 | −6 | +5 | +13 |
|---|---|---|---|---|
| | μg AbN/ml | | | |
| **Group I—Treated with 25 mg cortisone daily from 4th day before antigen** | | | | |
| AV 168 | 52 | 32 | 28 | 24 |
| 169 | 83 | 65 | 48 | 68 |
| 177 | 48 | 35 | 17 | — |
| ES 180 | 0 | 9 | 0 | 0 |
| 187 | 16 | 14 | 7 | 0 |
| AC 193 | 37 | 39 | 33 | — |
| 195 | 9 | 6 | 7 | — |
| 199 | 19 | — | 25 | — |
| **Group II—Controls** | | | | |
| AV 170 | 12 | 9 | 50 | 128 |
| 171 | 73 | 53 | 100 | 140 |
| ES 186 | 32 | 30 | 39 | 41 |
| 188 | 16 | 30 | 156 | 243 |
| AC 192 | 2 | 2 | 22 | 37 |
| 202 | 38 | 27 | 59 | 144 |
| 204 | 17 | 8 | 15 | 98 |

*Results.* Before the stimulating dose of antigen was given, the animals of Groups I and II were comparable in the distribution of their baseline antibody levels (Table I, Fig. 1). On Day —12 before the stimulating dose of antigen was administered, the range of titers in Group I was 0-83 μg AbN/ml with

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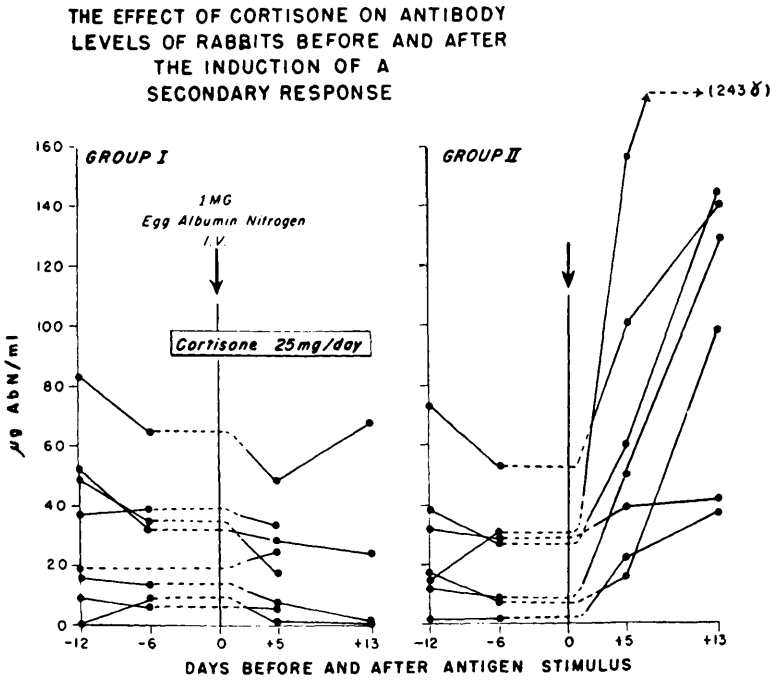


FIG. 1.

the median animals showing 19 and 38 μg AbN, while that of Group II was 2-73 μg AbN ml with a median of 17 μg AbN ml. Similarly, on Day —6, the range and the median of Group I were 6-65 μg AbN/ml and 32 μg AbN/ml, respectively, and those of Group II were 2-53 μg AbN/ml and 27 μg AbN/ml, respectively.

Five days after the administration of 1 ml alum precipitated egg albumin containing 1 mg egg albumin nitrogen, a precipitous rise occurred in the antibody levels of the control animals of Group II, as seen in Table I and Fig. 1, with increases of severalfold in some instances. The range of absolute values was 15-156 μg AbN/ml with a median value of 50 μg AbN/ml. The antibody levels of the cortisone-treated animals of Group I remained approximately the same, or were slightly reduced, with a range of 0-48 μg AbN/ml and median values of 17 and 25 μg AbN/ml. By the 13th day after the antigen stimulus, further marked increases occurred in the control group with little change in the few remaining cortisone-treated animals. There was a substantial incidence of diarrhea and death in rabbits treated with 25 mg cortisone for several days. From previous experience, it appears unlikely that the effects of cortisone on antibody production are related to debilitation. Some animals with marked loss of weight and in morbid states, as well as well-nourished healthy animals, ranked haphazardly among both the better and poorer antibody producing groups(1).

*Discussion.* The present study, in conjunction with other work(1-3,7) indicates that cortisone or ACTH administration results in lower antibody levels. Failure to consider factors such as species, dosage, antigen-antibody system and method of analysis has contributed to some of the discrepancies noted in the current literature, as has been discussed(12). The lower antibody level is in large part due to an inhibition of the synthetic phase of antibody protein metabolism by cortisone, much as that hormone appears to inhibit synthesis of many tissue proteins, although some proteins may be effected in other ways(8-11).

Other steps associated with the production of antibody appear to be suppressed by cortisone. "Assimilation" of antigen, a necessary prelude to antibody synthesis, is apparently

inhibited as suggested by the work of Fagraeus and Berglund(13), and Moeschlin *et al.*(14). Employing an ingenious splenic transplant method, Fagraeus and Berglund demonstrated that cortisone inhibited the secondary response of rabbits to typhoid bacilli if the hormone was present shortly after the antigen injection. Two or 3 days after antigen stimulation, the presence of cortisone did not appear to inhibit antibody synthesis, as measured by the double dilution method. Morphologically, the site of "assimilation" of antigen is strikingly disturbed by cortisone in studies on acute disseminated encephalomyelitis where an absence of granuloma formation at the site of antigen-adjuvant deposition was noted (15). In addition are the reports of visible persistence of injected heterologous erythrocytes within the cells of cortisone-treated animals(16) and the many observations on the persistence and proliferation of various microorganisms in experimental and clinical infections of cortisone-treated hosts(17).

The production of antibody may in part be dependent on the stimulation of an inflammatory response(29) and the inhibition of antibody production by cortisone may be considered as part of the non-specific anti-inflammatory activity of cortisone observed in studies on wound healing and inflammatory processes(18). The examples of failure to assimilate antigen cited above may also be classified with this general action of the hormone. Clinically, the increased serum globulins and fibrinogen associated with various inflammatory states are also diminished coincident with the diminished inflammation resulting from cortisone or ACTH administration(19). There are, therefore, at least 3 steps in the formation of antibody at which cortisone probably exerts an inhibitory effect: the "assimilation" of antigen, the inflammatory reaction that is associated with injection of antigen, and the synthesis of antibody globulin. Perhaps these steps, in turn, have a common biochemical denominator.

Since several similarities with respect to lymphoid tissue and antibody production exist among vit. $B_6$ deficiency, X-radiation and adrenal cortical hyperactivity(20), it appears pertinent to note that the specific anamnestic response has also been inhibited by vit. $B_6$ deficiency in rats(20). With X-ray, Dixon *et al.* observed some delay in the appearance of the specific anamnestic response(21), but whether X-ray inhibits the magnitude of the response is not known since the amounts of antibody were not reported.

There has been considerable speculation on the allegation that non-specific factors, particularly adrenal cortical hormones(22) may elicit an anamnestic response. Repeated independent observations with quantitative immunochemical methods have failed to confirm any augmentation of circulating antibody due to the hormones(7,23,24) or to other non-specific stimuli(25-27). Indeed, following adrenal cortical hormone, a slight decrease in circulating antibody may be observed(7,24). Despite the great stimulus afforded by the specific antigen in the present study, the adrenal cortical hormone is decidedly inhibitory and cannot itself be considered a stimulus. There is no reason to abandon the concepts of Landsteiner(28) and of Heidelberger and his coworkers(25,26) that immunological reactions including the anamnestic or secondary response are chemically specific.

*Summary.* Cortisone markedly inhibited the rapid production of antibody associated with the specific anamnestic or secondary response. The effect of cortisone on antibody protein metabolism is discussed.

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## Role of Ionizing Radiation in Eliciting Tumors of the Pituitary Gland in Mice.* (19873)

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The finding that large fatal growths of the pituitary gland form in mice about a year after injection of thyroid-destroying doses of radioiodine, $I^{131}$ (1), has been verified in several laboratories. These growths, as much as 200 times as large as the normal gland, are composed almost entirely of chromophobic cells, and their development is neither favored nor retarded by gonadectomy (4). Furth *et al.* (3,5) have found that transplants of the pituitary growths form large tumorous masses and metastasize in radiothyroidectomized hosts, and that later transplant generations grow even in normal hosts. More recently it has been shown that injection of thyroxine (2,6) or thyroid gland implants (6) inhibit the development of the tumors, suggesting that they are stimulated by the hypothyroidism induced by the radiothyroidectomy. However, radiothyroidectomy alone is insufficient to produce the tumors, since they do not develop in mice whose thyroids were destroyed by *small* quantities of $I^{131}$ while they were being fed a low-iodine diet (6). Thyroids of mice fed such diets absorb a much larger proportion of the administered $I^{131}$, making it possible to destroy the thyroid glands with much smaller quantities of $I^{131}$. This suggests that the radiant energy in high doses of $I^{131}$ may be a factor in precipitating the

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