agents of disease.

Summary. The yolk sac of the developing chick embryo has proved to be highly suitable for the propagation of the following pathogenic fungi: *Actinomyces bovis, Nocardia asteroides, Nocardia intracellularis, Sporotrichum Schenkii, Histoplasma capsulatum, Cryptococcus neoformans* and *Coccidioides immitis.* This method promises to be of great value for the experimental study of these mycotic infections. It is quite likely that many other of the pathogenic as well as non-pathogenic fungi may be studied with profit by infection of the yolk sac of chick embryos.

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Effect of Cortisone on Passively Induced Skin Hypersensitivity in Man. (18456)

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Beneficial effects following the use of ACTH and cortisone have been described in status asthmaticus, hay fever, serum sickness, atopic dermatitis and gastrointestinal allergy (1-7). The tuberculin test has also been inhibited by these two agents(8,9) and cortisone has prevented anaphylactic shock in sensitized mice(10). Germuth and Ottinger (11) demonstrated that the concomitant administration of a sensitizing antigen and either ACTH or cortisone to normal rabbits prevented the development of hypersensitivity and suppressed antibody formation. The same animals, however, manifested the Arthus phenomenon if passively sensitized. Although these reports suggest that cortisone and ACTH have an effect on immune responses, certain manifestations of hypersensitivity have not been suppressed by the administration of these hormones. ACTH or cortisone, given prior to the injection of a shocking dose of tetanus antitoxin horse serum in sensitized guinea pigs, failed to alter the subsequent course of anaphylactic shock(8,12,13). The administration of cortisone to sensitized rabbits has also been ineffective in altering the active or passive Arthus phenomenon (8,14).

The present study was undertaken to determine the effect of cortisone on passively

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induced hypersensitivity in man. Under the conditions of this experiment, cortisone did not effect the immediate wheal and erythema type of skin hypersensitivity in passively sensitized human beings.

Materials and methods. The method employed for the demonstration of passive skin sensitization was essentially that described by Prausnitz and Küstner(15). Two patients under treatment with cortisone served as donors. Serum of one patient suffering from severe serum sickness was obtained after 4 days of treatment with cortisone in a total dose of 600 mg. The other serum was drawn from a patient with an obscure type of diffuse vascular disease who had an incidental ragweed hypersensitivity manifested clinically by hay fever and a positive skin test to ragweed pollen extract. This serum was obtained after the patient had been treated for 13 days with a total dose of 1500 mg of cortisone. The recipients were volunteers selected from hospital patients who had no clinical history of serum sickness or hay fever and who were not sensitive by skin test to the materials employed in the experiment. Six volunteers who were not receiving cortisone served as controls. Fourteen volunteers were selected from patients under treatment with cortisone for a variety of illnesses in doses of at least 100 mg daily for from 3 to 37 days at the time the Prausnitz-Küstner test was performed. After passage through a Swinney filter, the donors' sera were diluted 1:5 and 1:10. These sera in dilutions of 1:100 produced positive Prausnitz-Küstner reactions inconsistently in the control group of volunteers. The experiments were therefore carried out with the lower serum dilutions. Dilution of all materials was performed with sterile isotonic saline. One tenth of a milliliter of the diluted sera was injected intradermally into each of 4 sites on the arm, thigh or back of the volunteers. The 4 passively sensitized sites were tested 24 to 48 hours later by the intradermal injection of approximately 0.025 ml of the test solutions as follows:

Site No. 1 was tested with the appropriate antigen, either tetanus antitoxin horse serum diluted 1:10, or ragweed pollen extract containing 500 total nitrogen units per ml. This site was prepared to determine whether a positive Prausnitz-Küstner reaction could be elicited and to serve as a control for the second site. Site No. 2 was tested with the appropriate antigen, to which cortisone had been added in a concentration of 2.5 mg per ml, in an attempt to increase the concentration of cortisone at the locus of antigen-antibody union. Local injection of higher concentrations of cortisone were found to be impractical for reasons discussed below. The cortisone was prepared from a suspension of cortisone acetate containing 25 mg per ml (Cortone, Merck). Site No. 3 was tested with the appropriate antigen to which was added, in a 1:10 dilution, the vehicle employed for suspending cortisone. This vehicle contained 1.5% benzyl alcohol, 0.4% polyoxyethylene sorbitan monooleate, 0.5% sodium carboxymethylcellulose, and 0.9% sodium chloride in distilled water. This test was done as a control in the event of inhibition of the reaction at site No. 2. Site No. 4 was tested with the suspension of cortisone alone in order to demonstrate that a wheal at site No. 2 represented a specific skin test for the antigen and not a skin reaction to cortisone. The cortisone was diluted in order to obviate a wheal and erythema reaction found to occur in a significant number of cases when the undiluted cortisone preparation was injected intradermally. This reaction was found to be due in part to the suspending agents and preservatives in the vehicle.

Skin tests with the above preparations were performed at distant unsensitized sites as controls. The skin reaction of a wheal and erythema was observed after 15 to 20 minutes and graded from 1 to 4+. Reactions showing wheals more than 2.0 cm in diameter were recorded as 4+, 1.5 to 2.0 cm as 3+, 1.0 to 1.5 cm as 2+, and distinct wheals less than 1.0 cm as 1+. In 4 volunteers the experiment was performed both before and during treatment with cortisone to determine whether a partial, if not complete, inhibition

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TABLE I. Passive Transfer of Skin-Sensitizing Antibodies from Patients Receiving Cortisone to Volunteers Also Receiving Cortisone.

Volunteer	Skin tests at passive transfer and distant control sites with: Horse serum diluted 1:10	Control	Horse serum diluted 1:10 and cortisone 2.5 mg/ml	Control	Ragweed extr. 500 N units/ml	Control	Ragweed extr. 500 N units/ml and cortisone 2.5 mg/ml	Control
E.*	++	0	+++	0	++	0	++	0
Y.	++++	0	++++	0	+++	0	+++	0
M.	++	0	+++	0	++	0	++	0
C.	+++	0	+++	0	++	0	++	0
A.	+++	±	+++	±				
A.Y.	++	±	++	±	+	0	+	0
M.C.	++	0	++	0	++	0	++	0
I.	+++	0	+++	0				
B.	++	0	++	0				
M.O.	++	0	++	0	++	0	+++	0
C.M.	++	±	++	0				
I.	++	±	++	±				
I.G.	+	0	+	±				
J.	++	±	++	0				

* Volunteer received 300 mg of cortisone per day.

of the Prausnitz-Küstner reaction by cortisone could be noted. It was also considered of interest to determine simultaneously the effect of cortisone on the tuberculin test. Three volunteers, who were found to have a positive Mantoux test with a 1:100 dilution of old tuberculin prior to treatment with cortisone, were retested while receiving cortisone. The tuberculin tests were read after 48 hours and the area of induration measured.

Results. Positive skin tests were consistently demonstrated in all of the 5 volunteers, not receiving cortisone, who were passively sensitized with serum from a patient receiving cortisone for 4 days in a total dose of 600 mg for the treatment of severe serum sickness. Similarly, 5 volunteers showed a positive reaction when skin tested after passive sensitization with the serum of a patient with ragweed hay fever receiving a total dose of 1500 mg of cortisone in 13 days. In none of these volunteers did the addition of cortisone locally, combined with the antigen employed in performing the skin test, reverse the reaction.

Positive skin test reactions to horse serum were demonstrated in all of the 14 volunteers receiving cortisone treatment who had been passively sensitized with the serum of the patient with serum sickness under treatment with cortisone. All 7 of the same group of volunteers who were passively sensitized with the serum of the patient with ragweed hay fever receiving cortisone treatment, also showed a positive skin test to ragweed pollen extract. In no instance did the local addition of cortisone reverse or partially inhibit the positive skin test. The results of these experiments are summarized in Table I.

In 4 cases the Prausnitz-Küstner reaction, elicited with both antigen-antibody systems, was compared before and during treatment of the volunteer with cortisone. No significant quantitative difference in the reactions was noted and therefore, partial inhibition of the reaction by treatment with cortisone was not demonstrated. The results of this experiment appear in Table II.

In one volunteer (E.), the dose of cortisone was raised from 100 mg to 300 mg per day. Passive sensitization was carried out on the third day of treatment and skin tests with the appropriate antigens were performed on the fourth day. Despite this relatively large dose of cortisone, no inhibition of the Prausnitz-Küstner reaction was noted when compared with the same reactions elicited before cortisone therapy was instituted.

TABLE II. Comparison of Prausnitz-Küstner Reaction Before and During Treatment of Volunteers with Cortisone.

Volunteer	Antigen preparation	Skin tests at sites passively sensitized with serum from patients with serum sickness and hay fever treated with cortisone: Before cortisone	During cortisone
C.	Horse serum diluted 1:10	+	+++
	Horse serum dil. 1:10 and cortisone 2.5 mg/ml	+	+++
	Ragweed extr. 500 N units/ml	++	++
	Ragweed extr. 500 N units and cortisone 2.5 mg/ml	++	++
M.	Horse serum diluted 1:10	+++	++
	Horse serum dil. 1:10 and cortisone 2.5 mg/ml	+++	+++
	Ragweed extr. 500 N units/ml	++	++
	Ragweed extr. 500 N units and cortisone 2.5 mg/ml	++	++
Y.	Horse serum diluted 1:10	+++	++++
	Horse serum dil. 1:10 and cortisone 2.5 mg/ml	+++	++++
	Ragweed extr. 500 N units/ml	+++	+++
	Ragweed extr. 500 N units and cortisone 2.5 mg/ml	+++	+++
E.*	Horse serum diluted 1:10	+++	++
	Horse serum dil. 1:10 and cortisone 2.5 mg/ml	+++	+++
	Ragweed extr. 500 N units/ml	++	++
	Ragweed extr. 500 N units and cortisone 2.5 mg/ml	++	++

* Volunteer received 300 mg of cortisone per day.

Three volunteers showed a moderately positive tuberculin reaction to a dilution of 1:100 of old tuberculin before treatment with cortisone. After receiving cortisone for three days in a total dose of at least 500 mg, only one of these volunteers showed a significant partial reversal of the tuberculin reaction. This was demonstrated simultaneously with the failure of the same subject to show any inhibition of the Prausnitz-Küstner reaction. This case therefore demonstrated a difference in the effect of cortisone on the two classical types of skin hypersensitivity.

Discussion. Under the conditions of the above experiments, cortisone does not interfere with the passive transfer of skin sensitizing antibodies, nor does it inhibit the wheal and erythema type of hypersensitivity resulting from the union of antigen and sensitizing antibody in the skin of human beings. These results are in agreement with reports of similar studies performed in experimental animals(8,11-14).

The experimental data do not preclude the possibility that larger doses of cortisone may inhibit or reverse the Prausnitz-Küstner reaction. However, the dosage employed was usually adequate to achieve a clinical response of the diseases for which the volunteers were treated. Beneficial effects with similar doses of cortisone have been reported in various allergic diseases usually associated with the immediate type of skin hypersensitivity (3,4). The fact that only one of 3 of our volunteers showed definite inhibition of the tuberculin test does not imply that the dose of cortisone was inadequate since one volunteer in whom such inhibition was not manifest was receiving 300 mg of cortisone per day while the test was performed. It has also been noted by other observers(9,14) that the reversal of the tuberculin reaction is not a consistent phenomenon in human beings treated with cortisone. However, in the volunteer in which reversal occurred, it was not associated with a similar reversal of the Prausnitz-Küstner which was simultaneously elicited.

The mechanism by which cortisone may favorably influence the clinical course of hypersensitive states is not known. The evidence presented favors the hypothesis that such improvement as may be noted during treatment with cortisone operates through mechanisms other than the inhibition of the union of antigen and sensitizing antibody(14). In this respect it is interesting to note that direct skin tests with the offending antigen were not suppressed in three cases of bronchial asthma who showed marked clinical im-

provement on ACTH(1). It is also of interest that some patients receiving ACTH have been observed to develop urticaria as a manifestation of hypersensitivity to the ACTH preparation itself(16).

Summary. (1) Positive Prausnitz-Küstner reactions were consistently demonstrated in volunteers passively sensitized with sera from 2 hypersensitive donors treated with cortisone. (2) Positive Prausnitz-Küstner reactions were similarly demonstrated in *cortisone treated* volunteers passively sensitized with sera of the cortisone treated donors. (3) There was no significant difference in the Prausnitz-Küstner reactions of those volunteers tested before and during treatment with cortisone. (4) It may be concluded that under the conditions of the experiments described, cortisone does not influence passively induced skin hypersensitivity in man.

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Diet of Mother and Vitamin B_{12} Content of Tissues of Infant Rats. (18457)

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The vit. B_{12} content of various organs and tissues of the rat was determined by a rat growth assay method and the kidney contained the largest amount(1). The vit. B_{12} content of the liver, spleen, heart and kidney of 28-day-old rats from mothers receiving the basal diet and the basal diet supplemented with vit. B_{12} concentrates from different sources is reported.

Experimental methods. The composition of the basal diet of the mothers is given in Table I. The females in Group 1 received the basal diet. Those in Group 2 received a supplement of 2.0 g of Merck's APF concentrate No. 3 per kilo of diet. Those in Group 3 received 1.0% of Lederle APF concentrate and those in Group 4 received 3.0% of liver "L." The mothers received the experimental diets from 28 days of age until they had an opportunity to produce at least 4 litters. The young in a few litters from each group were killed at 28 days and the tissues of 2 to 4 rats in each litter were combined into one sample. There were 1 to 3 samples of tissues per litter, depending upon the number of young in the litter. For example, if there were 10 young in a litter the tissues from 4, 3 and 3 rats respectively were combined to give 3 different samples. Vit. B_{12} was liberated from the tissues by the procedure described by Couch *et al.*(3) and

TABLE I. Composition of the Basal Diet.

Basal mixture	%
Cerelose	58
Casein	30
Woodpulp	3
Mineral mixture(2)	5
Wesson oil	4
Vit. supplement per 100 g basal mixture	
Vit. A	3000 I.U.*
Vit. D	425 I.U.
	mg
Menadione	2.5
Alpha tocopherol	2.5
Thiamine hydrochloride	1
Riboflavin	1
Pyridoxine hydrochloride	1
Ca pantothenate	4
Niacin	5
Choline chloride	100
Biotin	.02
Inositol	10
Para amino benzoic acid	50

* Vit. A and D were supplied by Mead Johnson's oleum percomorphum.

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