therefore should be utilized with specimens taken after the sixth month. Previous studies in our laboratory have shown that the great majority of children with congenital rubella no longer have detectable CF antibody at the ages 5 to 22 years; however, they continue to have neutralizing antibody(6).

The decrease or loss of detectable antibody in rubella syndrome infants during the period one to 5 months indicates that in spite of the chronic congenital infection of these children, the antibody response as determined with these methods is not rapid and does not reach maximum levels until after the sixth month. These findings are consistent with the reports that the development of 7-S rubella neutralizing antibody in congenitally infected children takes place primarily after the sixth month (7,8). It is of interest that at this same time most children cease having detectable virus in the nasopharynx(9).

Summary. Comparative antibody tests for rubella were conducted with the neutralization, indirect fluorescent, and complement fixation methods. All of these techniques demonstrated slightly different patterns of antibody response. Sero-conversion with infection was detected in almost all cases with the 3 methods. Antibodies detectable by fluorescent antibody and complement fixation tests decreased after 15 months following infection. By 10 to 20 years following rubella half of the CF tests were negative. Congenitally infected children showed a decrease in antibody between the first and fifth month of age which was most marked in the CF test. Their full antibody response in these cases did not occur until after the sixth month.

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A Method of Sensitizing Guinea Pigs to Fungus Antigens Without Infection. (31177)

COY D. SMITH AND SHERRIE L. NOEL (Introduced by M. L. Furcolow)
Department of Community Medicine, University of Kentucky Medical Center, Lexington

For the purpose of titrating and comparing antigens it would be desirable to sensitize the animals without infecting them with the organisms, providing the degree of sensitivity produced would be satisfactory for the titration. This method would be valuable particularly when dealing with those organisms that are hazardous to handle or that produce fatal disease in the animals. Although guinea pigs infected with *Histoplasma capsulatum* rarely die, the degree of sensitization produced is often irregular for titrating the skin antigens.

Freund and Gottschall have demonstrated previously that guinea pigs could be sensitized by subcutaneous injections with killed bacilli suspended in liquid petrolatum(1). Recently a single intraperitoneal injection of coccidioidin also was shown to induce delayed hypersensitivity in guinea pigs(2).

Our experience using various injection methods such as subcutaneous, or intraperitoneal to sensitize animals has not regularly produced the high level of sensitization necessary to standardize histoplasmin or blastomycin. This criticism is based on the irregu-

lar results obtained on large groups of guinea pigs using these methods.

Uhr *et al*(3) demonstrated previously that injection of guinea pigs in the footpads with various antigenic substances resulted in good sensitivity. We have shown previously that guinea pigs could be sensitized with undiluted histoplasmin and blastomycin plus an equal portion of Freund's complete or incomplete adjuvant after 3 consecutive injections in the footpad(4).

The present experiment demonstrates that a degree of sensitization satisfactory for use in titrating new fungal skin test antigens in guinea pigs can be obtained after a single injection in the footpad of the antigen when concentrated 10×, plus Freund's incomplete adjuvant. In the second experiment the degree of sensitization obtained by the footpad method was compared to that obtained by infection of the animals by intraperitoneal injection of living organisms.

Methods. The medium used for production of the new histoplasmin was an asparagine medium similar to that described by Smith for preparation of coccidioidin(5). Eleven different isolates of *Histoplasma capsulatum* were used to make 66 liters of histoplasmin. The inoculation and harvest techniques were similar to those used by Emmons (6). Each liter of antigen was treated as a separate lot. After harvesting, the antigens were screened for their initial potency by complement fixation tests of each lot against pooled patients' serum containing high antibody titers to histoplasmin. All antigen lots giving high titers were pooled to form one master lot of histoplasmin designated as lot HKC-43.

A mixture containing histoplasmin (lot HKC-30) (concentrated 10× by pervaporation techniques in dialyses tubing) plus an equal portion of Freund's incomplete adjuvant was emulsified by shaking for 30 minutes on an Eberbach shaker. One-tenth ml of the emulsion was injected into each hind footpad of 21 albino guinea pigs. The animals were skin tested with 0.1 ml of a 1:100 dilution of histoplasmin (lot HKC-5) prior to sensitization and found to be skin test negative. A skin reaction of 5 mm of induration or more, read after 24 hours, was considered positive.

Sixty-seven days after injection with the emulsion of histoplasmin and adjuvant, the 21 guinea pigs were skin tested simultaneously with 0.1 ml of histoplasmin (lot HKC-5) diluted 1:500 as the reference antigen, and the new antigen lot HKC-43 diluted 1:500, 1:1,000, and 1:1,500.

In the second experiment 10 guinea pigs were sensitized without infection by the method used in the previous experiment except that the dosage used was 0.2 ml of antigen-adjuvant emulsion injected into each hind footpad rather than 0.1 ml. In addition 10 guinea pigs were injected intraperitoneally with 1.0 ml of a saline suspension of *Histoplasma capsulatum.* The suspension was prepared from a mycelial phase culture grown on yeast extract medium(7) in Erlenmeyer flasks for 3 weeks at 28°C. This suspension contained 180,000 total particles per ml as determined by hemocytometer count of which 135,000 were spores. The suspension was found to contain 75% viable particles using colony plate count techniques; therefore each animal received a dose containing approximately 135,000 viable particles of *Histoplasma capsulatum.*

Sixty-five days following the injections of histoplasmin with adjuvant and viable particles the 2 groups of animals were skin tested with 0.1 ml of a 1:500 dilution of histoplasmin (lot HKC-5).

Results. It can be seen from Table I that 18 out of 21, or 85.7% of the animals showed positive skin reactions with a standard dose of the reference antigen (HKC-5). Ninety-five percent of the animals gave a positive reaction to a 1:500 dilution of HKC-43 with an average induration of 8.5 mm. Ninety percent of the animals were positive with the 1:1,000 dilution and 71% at a 1:1,500 dilution.

The criteria determined by Howell for obtaining the critical titer of a skin test antigen is the greatest dilution that will give positive reactions in 80 to 90% of the sensitized animals(8). Therefore, applying this method on these tests, the 1:1,000 dilution of the new

TABLE I. Skin Test Results of Antigen Titrations in Artificially Sensitized Guinea Pigs.

Antigen lot and dilution	No. of animals sensitized	No. of animals positive	% Positive	Avg induration (mm)
HKC-5 1:500	21	18	85.7	7.2
HKC-43 1:500	21	20	95.0	8.5
HKC-43 1:1,000	21	19	90.5	6.9
HKC-43 1:1,500	21	15	71.0	6.4

TABLE II. Skin Test Results of Guinea Pigs Infected with *H. capsulatum* as Compared to Results of Guinea Pigs Artificially Sensitized to Histoplasmin.

Infected animals*

Antigen lot and dilution	No. of animals sensitized	No. of animals positive	% Positive	Avg induration (mm)
HKC-5 1:500	9‡	9	100	6.4

Artificially sensitized animals†

Skin test antigen lot and dilution	No. of animals sensitized	No. of animals positive	% Positive	Avg induration (mm)
HKC-5 1:500	10	10	100	6.5

* Each pig received 1.35×10^5 viable particles in saline, intraperitoneally.
† Each pig was injected in each hind footpad with 0.2 cc of 10× histoplasmin HKC-30 and Freund's incomplete adjuvant mixed in equal parts.
‡ One animal expired.

antigen in question would be the critical titer.

From the results of the second experiment (Table II), it can be seen that 100% of the animals sensitized to histoplasmin without infection had positive skin reactions when tested with the standard antigen. The average induration of this group was 6.5 mm. Also, 100% of the infected animals gave positive skin reactions with the same standard antigen. The average induration of the infected animals was 6.4 mm. It is evident from these data that there is essentially no difference in the degree of sensitivity produced in these 2 groups by the two methods of sensitization.

It is interesting to note that 100% of the animals showed positive skin tests in the second experiment in both infected and non-infected animals using the reference antigen whereas 85.7% reacted to this antigen in the first experiment. In the second experiment among the non-infected animals, the sensitizing dose used was double that used to sensitize the animals in the first experiment.

Other workers previously(8,9) did not use specifically measured doses of live organisms to sensitize animals by infection. *Histoplasma capsulatum* particles harvested from a standard medium such as Sabourauds normally have a low viability (3-20%)(10,11) compared to the medium used in the second experiment to grow the inoculum. Therefore, using an inoculum consisting of particles having 75% viability would increase the chances of giving a much higher infecting dose in Experiment 2 than that given by previous workers. Therefore, what appears to be an excessive dosage used in both infected and non-infected animals probably made the animals more sensitive in Exp. 2 compared to Exp. 1. The time lapse after the sensitizing injections and skin testing was approximately the same for both experiments.

Subsequently guinea pigs sensitized by this method using 10× concentrated blastomycin and coccidioidin have also proven to be of equal value for determining the potency of new lots of these antigens.

Summary. Guinea pigs were sensitized to histoplasmin using one dose of 10× concentrated antigen plus an equal amount of Freund's incomplete adjuvant. One-tenth ml of the mixture was injected into each hind footpad of the animals. The degree of sensitivity obtained was satisfactory for use in

titrating the potency of new skin test antigen lots. A second experiment was performed to compare the degree of sensitization obtained by the footpad method using non-infectious material to that obtained by injecting the guinea pigs with live *Histoplasma capsulatum*. There was essentially no difference in the degree of sensitivity obtained in the animals using the two methods.

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Influence of Nonessential L-Amino Acids on Growth of Rats Fed High Levels of Essential L-Amino Acids. (31178)

J. S. ADKINS, J. M. WERTZ, AND E. L. HOVE
Division of Nutrition, Bureau of Scientific Research, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D.C.

There has been renewed interest in the role of nonessential amino acids (NEAA) in diets containing mixtures of crystalline amino acids as the sole source of dietary nitrogen for young rats. The work in this field has been comprehensively reviewed(1,2). However, there have been few reports(3-7) of studies using only the natural (L) isomers of both the essential and nonessential amino acids.

Breuer and coworkers(7) recently reported that the diet of Rechcigl *et al*(8), containing a high level (15.68%) of essential amino acids (EAA) and no nonessential amino acids (except cystine and tyrosine), supported significantly better growth than did diets containing lower levels of EAA with NEAA to provide additional nitrogen. However, these workers did not study the effect of addition of NEAA to the high level EAA mixture. A casein diet was superior to any of the amino acid diets they tested. They later(9) reported that proline, asparagine, and glutamic acid were necessary for maximal growth of the rat using other amino acid mixtures.

Recently we reported* that weanling rats fed the L-amino acid mixture of Rechcigl *et al*(8) supplemented with NEAA grew at a significantly faster rate than rats fed only a mixture of high levels of EAA. The studies reported here were designed to investigate further the effects of addition of NEAA to this mixture on growth of weanling rats. In addition, observations were made on the effect of NEAA on liver composition and the plasma amino nitrogen levels.

Materials and methods. Weanling male Holtzman rats having initial weights of 45-50 g were housed in individual cages in a room maintained at 22 ± 1°C. Food intakes and body weights were recorded twice weekly. Rats were fed a complete diet, with 14% vitamin-free casein (General Biochemicals, Inc.) for a 10-day adaptation period except in Experiments III and IV. The rats were then provided with their assigned experimental diets and tap water *ad libitum* for 21 days.

The composition of the diets is given in

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