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Received April 13, 1965. P.S.E.B.M., 1965, v119.

Coccidioidin Induction of Skin Sensitivity and Positive Serology.* (30335)

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In a study designed to compare skin test activity of coccidioidins, guinea pigs were rendered skin sensitive by a single intraperitoneal (ip) injection of coccidioidin. Sera, however, studied by micro-complement fixation and agar gel double diffusion techniques, both plate and tube, were negative (1). Further studies using an Ouchterlony technique devised to demonstrate blocking reactions in the guinea pig sera gave equivocal results.

Rabbits are also skin sensitized by a single subcutaneous (sc) injection. Sera from rabbits skin test positive after one and multiple sc injections of coccidioidin with and without adjuvants have been studied by agar gel techniques.

It is the purpose of this report to elucidate the relationship between skin sensitization and acquisition of demonstrable humoral antibodies in rabbits given parenteral injections of coccidioidin.

Materials and methods. Male New Zealand white rabbits, purchased locally, were maintained on the usual pelleted food. They were reference bled from the marginal ear vein, pre-injection, and weekly or bi-weekly thereafter. Merthiolate was added to the sera in a final dilution of 1:10,000. Sera were stored at —20°C until tested.

All rabbits were skin test negative to undiluted coccidioidin at the initiation of the study. They were skin tested at varied intervals with 2 coccidioidins, Smith's Lot 64D4 and Huppert's Lot XVB52F, received through the courtesy of Dr. Milton Huppert of the San Fernando VAH. No two skin tests were given in the same area. Test coccidioidins were injected intradermally (id) into a cleanly shaven site. Disposable tuberculin sy-

* These studies were supported by the V A Medical Research Program Scientific Information Exchange (SIE) Project No. ZO 17379C1.

ringes graduated to .05 ml and 26-gauge disposable needles were used. Skin reaction areas were measured at 24 and 48 hours and those exceeding 25 mm^2 considered positive.

In one experimental series, 7 rabbits were injected with single or multiple 0.5 ml aliquots of Huppert's coccidioidin in an equal volume of Freund's complete adjuvant given 2 to 8 weeks apart and 2 were injected with the same coccidioidin in a suspension of 1.3 μ diameter latex particles. In a second experimental series, 16 rabbits were divided into groups of 4. Group C received Freund's complete adjuvant and served as controls. All other animals were injected sc with 1 ml of Huppert's Lot XVB52F coccidioidin distributed to 4 sites, 2 in the nuchal area and 2 in the anterior fold of the hind legs. Group A received coccidioidin alone; Group B, coccidioidin in an equal volume of Freund's adjuvant; and Group D, coccidioidin in an equal volume of a buffered saline suspension of 1.3 μ diameter latex particles. Groups B and D received booster injections identical to their first injections after 12 and 23 weeks respectively.

The sera were tested by an Ouchterlony technique using Huppert's immunodiffusion coccidioidin Lot XVB52FC. A micro-modification of Huppert and Bailey's macroimmunodiffusion test(2) was employed and a 2-hour serum diffusion period allowed before antigen was added. Serum wells were recharged at 24 hours. Plates were incubated in a moist chamber at 25°C and developed bands recorded 3 days after the antigen addition. Faint bands were accentuated by application of 2% acetic acid for several minutes after washing the plates by immersion in 20 to 30 volumes of 0.15 M saline daily for 3 days to remove non-precipitated serum proteins.

Results and discussion. In the first experimental series, multiple serum specimens taken one to 3 weeks after 1, 2, 3, or 4 sc injections of coccidioidin in Freund's complete adjuvant were consistently negative after one injection when tested by the immunodiffusion technique described. However, serum specimens taken as early as one week after a second such injection showed definite positive results. Fig. 1 and 2 illustrate typical findings. A positive human reference serum was used in the top and bottom peripheral wells.

In Fig. 1, the pre-injection serum and the serum after one injection are negative. The serum after the fourth injection shows lines of increasing intensity over that of the third injection.

In Fig. 2, the serum after the second injection is positive but lines are more faint than those of sera collected after 3 and 4 injections. Note the spurs which reflect differences between rabbit serum and human serum with high complement fixation titer. Comparable multiple serum specimens from 2 rabbits after 1, 2, 3, and 4 sc injections of the same amount of coccidioidin suspended in a buffered saline suspension of 1.3 μ diameter latex particles were consistently negative.

In the second experimental series, an amount of coccidioidin equal to that of 2 injections in the first series was chosen to test the hypothesis that ability to produce demonstrable rabbit antibody might be dose dependent as our studies have indicated is the case with skin test activity in rabbits(3). The adjuvant effect of latex particles was studied because of the implications of phagocytosis on induction of antibody response.

Multiple serum specimens from Group A (coccidioidin only) and Group C (Freund's adjuvant only) tested by immunodiffusion at weekly intervals following the injection, were consistently negative. Sera from Group B (coccidioidin in Freund's) obtained 12 days post injection were also negative, but those of 3 weeks post injection were immunodiffusion positive. Bands became increasingly distinct and multiple with sera obtained between the 3rd week and 3 months post injection. Fig. 3 illustrates results obtained with sera from a Group B rabbit. Sera obtained one week after the booster injection produced strong bands which were demonstrable in successive serum specimens obtained up to 3 months later when the animals were sacrificed.

Multiple serum specimens from Group D (coccidioidin in latex) rabbits were immunodiffusion negative if obtained before the booster injection. Fig. 4 illustrates positive

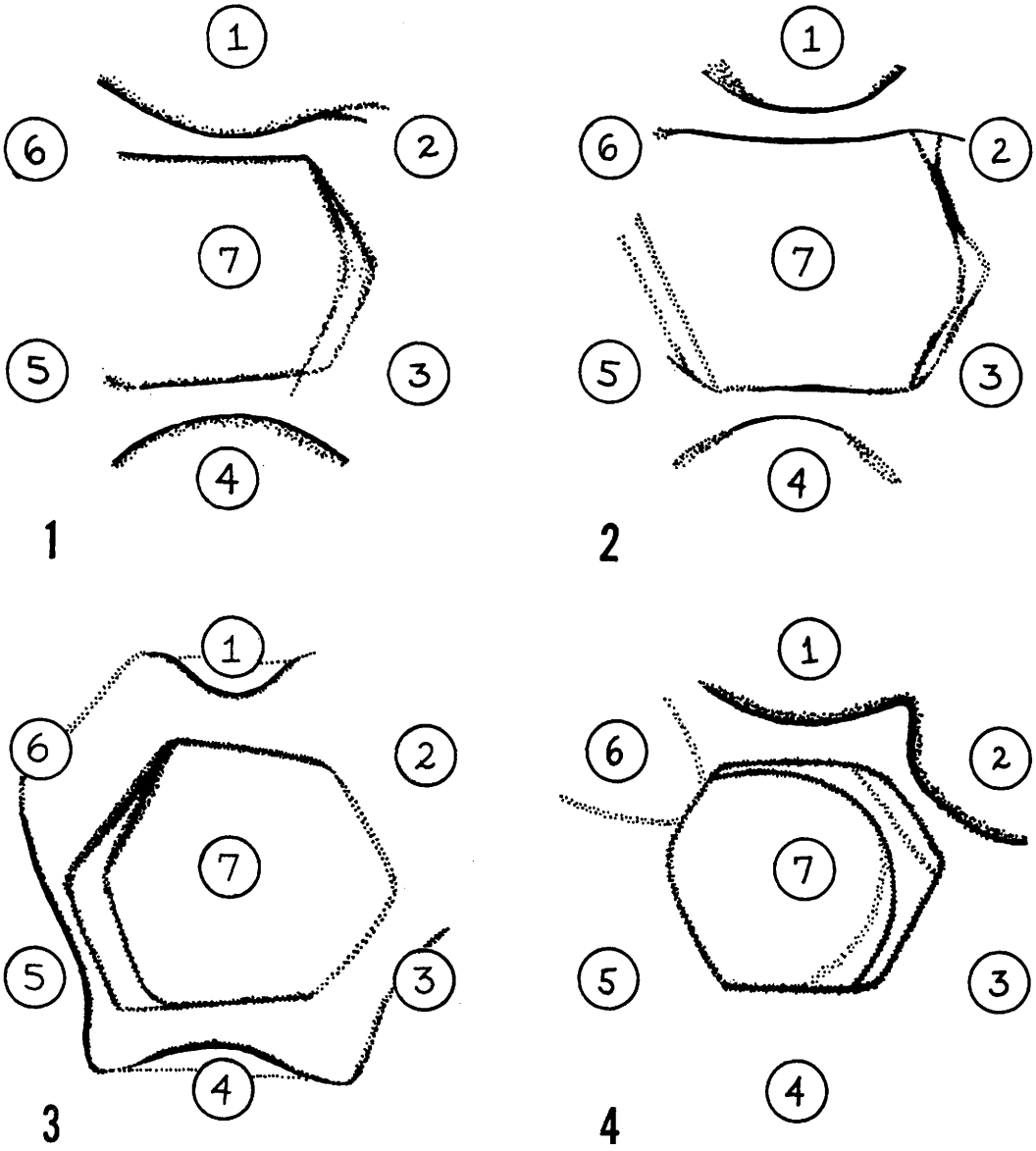

FIG. 1. Effect of 0-4 injections of coccidioidin in Freund's complete adjuvant on successive serum specimens. Exp. Series I, Rabbit #1. Wells: (7) coccidioidin; (1) and (4) human positive serum; (6) pre-injection serum; (5) after 1st injection; (3) after 3rd injection; (2) after 4th injection.

FIG. 2. Effect of 0-4 injections of coccidioidin in Freund's complete adjuvant on successive serum specimens. Exp. Series I, Rabbit #11. Wells: (7) coccidioidin; (1) and (4) human positive serum; (6) pre-injection serum; (5) after 2nd injection; (3) after 3rd injection; (2) after 4th injection.

FIG. 3. Effect of injection of coccidioidin in Freund's complete adjuvant on successive serum specimens. Exp. Series II, Rabbit #30. Wells: (7) coccidioidin; (1) and (4) rabbit positive reference serum; (2) post injection, 3 wk; (3) post injection, 4 wk; (5) post injection, 3 mo; (6) after booster, 1 wk.

FIG. 4. Immunodiffusion results of post booster sera. Exp. Series II. Rabbits injected with: Coccidioidin in Freund's, Group B. Coccidioidin in latex, Group D. Wells: (7) coccidioidin; (1), (2), and (3), Group B sera; (4), (5), and (6), Group D sera; (1) rabbit #30, 2 wk post booster; (2) and (3) rabbits #30 and #20, 3 mo post booster; (4), (5), and (6) rabbits #35, #36, and #25, 2½ wk post booster.

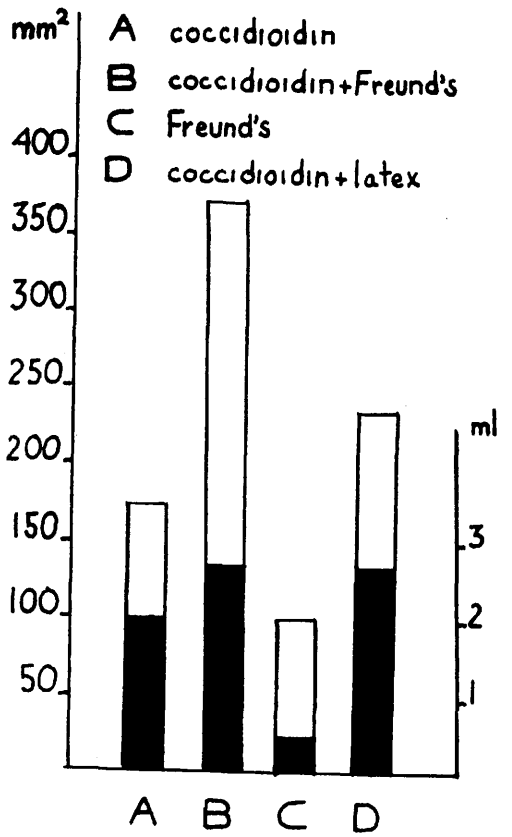

FIG. 5. Rabbit skin sensitivity to Huppert's undiluted coccidioidin in relation to amount of injected coccidioidins. Groups A, B, and D received Huppert's antigen sc. All groups received both Smith's and Huppert's coccidioidin id. □ = Average skin reaction area. ■ = Total injected coccidioidin (sc and id).

results obtained from 3 Group D rabbits when serum specimens taken after booster injections were tested.

Definite bands were discernible in Group D serum specimens obtained one week after the booster injection had been given. Post booster serum specimens from Group D rabbits, however, never produced bands of such intensity or multiplicity as were obtainable from those of Group B rabbits.

Induction of skin sensitivity. Skin tests were positive (reaction areas 25 mm² or greater) in all groups except C as early as 8 days after injection. Skin sensitization was demonstrable after one sc injection and persisted up to 8 months. Skin sensitivity was evident even though sera taken before, on the day of, or a few days to months after the skin test gave strong positive immunodiffusion results.

Fig. 5 indicates a comparison of skin sensitivities demonstrable in the 4 groups of rabbits. Positive skin reactions to Huppert's antigen were elicited even in Group C rabbits but not before they had received four 0.1 ml injections of undiluted coccidioidins intradermally for the purpose of skin testing. In all groups, when individual rabbit skin reactions to Smith's and Huppert's coccidioidins were compared, Huppert's antigen usually elicited a larger skin reaction. Of 23 such skin test reaction area comparisons, 15 were larger and 8 were smaller. These observations may reflect antigenic differences between the two coccidioidins which are prepared by entirely different methods.

It should be emphasized that all *subcutaneous* injections in both experimental series were of Huppert's coccidioidal antigen. Further studies are in progress to determine if Smith's coccidioidin injected sc will produce comparable results.

The demonstration of skin sensitization and humoral antibody production resulting from parenteral injection of Huppert's coccidioidal antigen, also suggests the possibility of evaluating such injections for their protective effect against challenge with live arthrospores of *Coccidioides immitis*. Pappagianis *et al*(4) described a protective effect in mice given sc injections of lysates of the Silveira strain of *C. immitis*. Huppert's coccidioidal antigen is prepared by a modification of the Pappagianis method(2).

Summary. Serological responses demonstrable by an Ouchterlony technique, and skin sensitizations were produced in rabbits injected subcutaneously with coccidioidal antigen in either Freund's complete adjuvant or latex particles. Negative immunodiffusion results, but positive skin tests were produced by rabbits injected subcutaneously with antigen alone. The possibility of producing skin sensitivity by repeated intradermal injections of coccidioidin is discussed.

The advice and suggestions of Dr. Milan Bier and the technical assistance of Mr. Archie Wood are gratefully acknowledged.

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Received April 14, 1965. P.S.E.B.M., 1965, v119.

Physical and Chemical Properties of H-1 Virus. II. Partial Purification.* (30336)

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H-1 virus is one of a group of small agents that produces a specific deformity when they are injected into newborn hamsters(1,2). A previous paper(3) noted that the hemagglutinating property of this virus was stable over a wide range of heat and pH changes. It was observed during these studies, which were done with virus prepared from filtrates of infected hamster livers, that precipitates formed at pH 4. This finding is the basis for the method used for partial purification of H-1 virus described in this paper.

Methods and materials. Virus. Two sources of H-1 virus were used for these experiments: 1) H-1 (ham) was derived from livers of baby hamsters injected 5 days previously with H-1 virus that had had 6 passages in baby hamsters after its original isolation from the transplantable human tumor HEp #1(1, 4). This material was prepared as a 10% homogenate of pooled livers in distilled water and then filtered through a 0.03 Selas filter. It was kept at 4°C until used. 2) H-1 (tc) was also originally derived from HEp #1, but was passed in newborn hamsters for 8 passages and then grown in a tissue culture of Salk monkey heart for 13 days by Dr. H. W. Toolan.

Virus hemagglutination activity was assayed by hemagglutination of guinea pig red blood cells and reported in hemagglutination (HA) units as the reciprocal of the highest dilution that completely agglutinated all the red cells.

Virus infectivity studies employed newborn hamsters injected within 48 hours after birth. Virus was detected in these animals by death, deformity or the production of antibodies, depending on whether large or small amounts of infectious virus were present.

Anti H-1 globulin was prepared by combining equal amounts of cold saturated ammonium sulfate and serum from adult hamsters injected at birth and deformed by the H-1 virus. The resulting precipitate was dissolved in distilled water, reprecipitated with saturated ammonium sulfate and dialyzed overnight against saline.

Protein was determined by the Folin-Ciocolteau method(5).

Ultracentrifugation was performed in a Beckman-Spinco model L preparative ultracentrifuge using either a 40 rotor or a SW-39, as noted.

Electron microscope examinations. A small drop of suspension of the isolated fraction was placed on the carbon film on a 400-mesh grid. Before the thin layer of fluid remaining on the grid had dried, a drop of 2% phosphotungstic acid, adjusted to pH 7.0 with NaOH, was applied. Pictures were taken with a JEM-7 electron microscope (high tension 80 KV, double condensor, 50 μ objective aperture, initial magnifications $\times$ 40,000 and $\times$ 80,000).

Results. Table I shows some typical data. As had been noted previously, when the H-1

* Aided by Grant E-343 from Am. Cancer Soc., and Grant CA-07826-01 from Nat. Cancer Inst., N.I.H., U.S.P.H.S.