OBSERVATIONS ON ACACIA AS AN IMMUNIZING, SENSITIZING, ANAPHYLACTOGENIC, AND DESENSITIZING ANTIGEN

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INTRODUCTION

A SERIES of studies of desensitization with haptens, as contrasted with whole antigens, is in progress in this laboratory. A cheap source of abundant polysaccharide hapten is needed to continue these studies efficiently. The report of Partridge and Morgan[4] suggested that acacia might be such a source. Acacia is cheap, readily available, and widely used commercially. Over 6,800 tons were imported from the Sudan in 1952.[1] It has been shown to be antigenic in rabbits and guinea pigs[2-4] and allergenic in man.[5-7]

Partridge and Morgan[4] reported that treatment of acacia with formamide yielded a polysaccharide which was not antigenic but was haptenic. This non-antigenic acacia derivative caused anaphylactic shock in guinea pigs sensitized passively with antiserum from rabbits immunized with a bacterial protein-formamide-acacia complex. This derivative also precipitated the antiserum, but did not produce antibodies by the immunizing schedule used by them.

Acacia was subjected to a series of screening procedures which are the subject of this report. There were several interesting observations, although the search for a suitable polysaccharide hapten with which to continue the desensitization studies was not successful.

Four different lots of acacia have been investigated—two so-called "bleached white sorts" and two "amber sorts," differing in that the former had been exposed to sunlight to bleach. All four samples yielded positive biuret tests, a pH of 5.0 in 1 per cent aqueous solution, and positive Molisch reactions in dilutions of 1:8,000,000. The nitrogen content was 0.37 per cent in two, 0.35 per cent in one, and 0.36 per cent in the other. These lots were labeled A, B, C, and D, arbitrarily.

Toxicity.—No untoward reactions followed intravenous, intra-abdominal, or subcutaneous injections of 2.0 and 10.0 ml. of 6 per cent solutions of acacia prepared at room temperature and sterilized by Seitz filtration. The pH of such solutions was about 4.5. Maytum and Magath,[2] however, reported peritonitis in guinea pigs unless the acacia solution was buffered to pH 6.6.

Active Sensitization of Guinea Pigs.—Twelve guinea pigs, weighing ±300 grams, were sensitized by single intra-abdominal injections of 600 mg. acacia

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(10.0 ml. of 6 per cent solution). One month later they were challenged intravenously with 60 mg. of the sensitizing or of other samples of the gum. Anaphylactic shock, fatal in ten, resulted in every case.

Two other guinea pigs were sensitized by the intra-abdominal injection of 160 mg. acacia-B with Freund's adjuvant (2.0 ml. of an emulsion of 2 parts 20 per cent acacia, 2 parts Bayol-F, 1 part Falba). One month later they were challenged with 60 mg. acacia-B intravenously. Typical anaphylactic death resulted in both.

Active Immunization of Rabbits.—Seventeen albino rabbits, weighing 2.0 to 2.5 kilograms initially, were immunized intravenously with arbitrarily selected doses of 5.0 ml. of 6 per cent acacia solution on three successive days of three successive weeks. Each animal thus received a total of 2,700 mg. acacia. Larger and smaller immunizing doses were not tried. The animals were bled for immune serum five to seven days after the ninth and last injection. Precipitin titers were highest (1:4,000) about five days after the last injection. Precipitins persisted in variable but decreasing titers for six to seven weeks.

In contrast, two rabbits injected subcutaneously with a single 2.0 ml. dose of the acacia-adjuvant mixture described previously (equivalent to 160 mg. of the gum) did not develop precipitins until four to five weeks after injection. The precipitin titers of these animals were lower (1:1,000) than in those immunized intravenously, but persisted for at least three months.

Attempts were made to demonstrate anamnestic responses in four immunized rabbits in which precipitins had disappeared. These were injected with 120 mg. of acacia subcutaneously, followed on two successive days by 300 mg. and 600 mg. intravenously. In one animal the 600 mg. injection caused anaphylactic death. In another, the precipitin titer rose to only 1:200, a very low level. In two others, no return of precipitin was observed seven days after the intravenous injections.

Although no, or very weak, anamnestic responses to three doses of antigen were demonstrated, a second full course of intravenous injections (three weekly for three weeks) reproduced precipitins of the original titers.

Two rabbits initially immunized to acacia-A, when reimmunized with acacia-D, produced sera of equal antiacacia-A and antiacacia-D titers. Similarly, two rabbits initially immunized with acacia-D, and reimmunized with acacia-A, produced sera of equal antiacacia-D and antiacacia-A titers.

Precipitins.—The precipitin rections were interesting in several respects. For example:

When the antiacacia sera were overlaid with 1:20 to 1:360 dilutions of acacia, a ring formed in a few minutes. It reached a maximum in about one hour at room or incubator temperature.

When the antisera were overlaid with 1:500 to 1:8,000 acacia dilutions, the appearance time of the ring was delayed to thirty minutes or longer in the lower dilutions, reaching maximum intensity and titer in two to four hours. The rings diffused on standing at room or refrigerator temperature. In the stronger concentrations (1:20, 1:40) the rings diffused downward. In the weaker dilutions diffusion occurred both up and down.

Mixing the contents of the precipitin tubes yielded no sediment, although the mixtures became hazy. Attempts to obtain some precipitate by varying the proportions of antigen and antibody, the time and temperature of incubation, and the speed of centrifugation were unsuccessful.

The titers of cross precipitin reactions with A, B, and D antiacacia rabbit sera using acacia-A, -B, -C, and -D as precipitating antigens were quite uniform. The two-hour (maximum) readings varied from 1:1,000 to 1:4,000. Direct titers were no higher than the cross-tests.

Occasionally, sera from normal rabbits gave slight ring tests with strong (1:20 to 1:100) acacia solutions; 1:400 and weaker acacia dilutions gave negative ring tests with normal rabbit serum. To test the specificity of these rings, several guinea pigs were given 3 to 5 ml. of the normal rabbit serum intra-abdominally. The next day they were challenged with 60 mg. of acacia intravenously. None showed anaphylaxis.

Acacia-A and -B, 1:80 to 1:100, gave occasional transient positive ring tests with Types II and VIII antipneumococcal rabbit serum but not in weaker acacia dilutions. Other acacia samples were not tested. However, Type II pneumococcal polysaccharide (1:1,000) did not precipitate antiacacia rabbit serum, in agreement with Partridge and Morgan.[4]

No precipitins were demonstrated in sera from guinea pigs which had been actively sensitized with acacia, although these same sera passively sensitized other guinea pigs readily.

Arthus Type Reactions.—Two-tenths milliliter of 6 per cent acacia was injected intracutaneously into four immunized rabbits with antiacacia precipitin titers of 1:4,000 to 1:8,000. The reactions were negligible the first day. On the second and third days the test sites were erythematous and palpably indurated in an area about 1.0 cm. in diameter with no central necrosis. The skins looked and felt normal in seventy-two to ninety-six hours.

In two other rabbits there was severe necrosis at seventy-two hours, although the diameter of the reaction was only 1.0 cm. and there was little surrounding erythema.

None of the rabbits immunized with acacia plus adjuvant gave definitely positive delayed skin reactions.

The flanks of two other rabbits were injected intracutaneously with 0.2 ml. of each of the following solutions of acacia: 0.1 per cent, 0.3 per cent, 0.6 per cent, 1.0 per cent, 3.0 per cent, 6.0 per cent and 20 per cent. The initial precipitin titers were 1:1,000 to 1:2,000. Small wheals appeared at all sites within about forty minutes and persisted for about twenty-four hours. The outlines were slightly irregular, but there was no central pallor or peripheral flare. The reactions were uniformly pink and slightly swollen. At forty-eight hours all the reactions had subsided except at the 6 per cent and 20 per cent sites. The 6 per cent sites were graded 2+ in one and 3+ in the other, and the 20 per cent sites were graded 3+ in one and 1+ in the other (1+ = firm, erythematous area, ±1.0 cm. diameter; 2+ = like 1+ with slight central necrosis; 3+ = central 1.0 cm. firm nodule with extreme central necrosis). At five days there was a small pea-sized lump at each of these sites.

Desensitization Against Arthus Type Reactions.—The last two rabbits just described were given a preliminary subcutaneous dose of 2.5 ml. (150 mg.) of 6 per cent acacia, followed by 2.0 ml., 5.0 ml., and 5.0 ml. intravenously within two days. The precipitin titer the next day had not changed in one, but had increased from 1:2,000 to 1:8,000 in the other. On that same day they were given 0.2 ml. of 6 per cent acacia intracutaneously. The Arthus reactions were essentially unchanged (2+) forty-eight hours later.

Six days after the last previous intravenous doses, the rabbits were given 5.0 ml. (300 mg.) intravenously twice daily for three days. The immediate skin reactions the day after the last dose were weakly positive. The delayed reactions were 1+ at twenty-four hours. The precipitin titer then was 1:2,000 in one and 1:4,000 in the other.

Four days after the last preceding intravenous injections, the rabbits were given 2.5 ml. of 6 per cent acacia subcutaneously in the morning and 5.0 ml. intravenously in the afternoon. They were given 10.0 ml. (600 mg.) of 6 per cent acacia intravenously twice daily for the next two days. The immediate reactions two days later were faint; the forty-eight-hour reactions were negative in both. The precipitin titer was 1:1,000 in one rabbit and 1:4,000 in the other. The next day the Arthus type response was still negative. The animals were considered desensitized at this point. In these two desensitized animals the precipitin titer in one was the same as at the beginning of the experiment. In the other the titer had risen slightly, from 1:2,000 to 1:4,000. It is of interest that so much antigen was required to desensitize. No effort was made to determine the duration of this desensitization. More study should be given to this problem.

Heterologous Passive Sensitization.—Twenty-nine guinea pigs were sensitized with 1.0 ml. antiacacia-A, -B, or -D rabbit sera, intra-abdominally, with precipitin titers of 1:2,000 to 1:4,000. The next day the animals were challenged with 60 mg. acacia-A, -B, -C, or -D intravenously. All the pigs reacted by anaphylactic death when challenged with either the specific or the other samples of acacia.

Sensitizing doses of antiacacia-A rabbit serum were titrated against shocking doses of acacia-A and acacia-C solutions in thirty-one pigs. Definite anaphylaxis was produced by as little as 0.1 to 0.2 ml. of antiserum and 2.0 to 3.0 mg. of antigen. However, the smallest combinations which regularly produced fatal or severe anaphylaxis were 0.2 and 0.33 ml. antiserum with 6.0 mg. of antigen. Larger doses of antigen, with 0.33 ml. antiserum, invariably caused fatal shock. Again crossed antigen-antibody systems were as effective as the direct ones. Antisera with lower precipitin titers (1:200) required 1.0 to 2.0 ml. for passive sensitization.

Duration of Heterologous Passive Sensitization.—Four guinea pigs were sensitized passively with 0.33 ml. of antiacacia-A rabbit serum. They were challenged with 60 mg. of acacia intravenously, one on the seventh, two on the tenth, and one on the fourteenth day. The reactions were: anaphylactic death in one on the seventh day; moderate anaphylaxis with prompt recovery in two on the tenth day; and an equivocal reaction in the fourth animal on the four-

teenth day. These are in accord with earlier observations[11] that anaphylactic reactions were obtained irregularly after ten days and not at all after thirteen days in guinea pigs sensitized passively with heterologous antiserum. The role of larger amounts of sensitizing antibody in prolonging passive sensitization was not studied.

Homologous Passive Sensitization.—One guinea pig was sensitized passively with 1.0 ml. of antiacacia guinea pig serum intra-abdominally. Moderately severe shock occurred when challenged with 60 mg. acacia intravenously the next day. Two other animals, sensitized with 3.0 ml. antiacacia guinea pig serum, had fatal anaphylaxis when challenged the next day.

The antiserum was obtained from guinea pigs which had been given a single intra-abdominal injection of 10.0 ml. of 6 per cent acacia twenty-one days before.

Heat-Treated Acacia.—A 20 per cent solution of acacia-B, heated in a water bath at 96° C. for six hours, was diluted to make a 6 per cent solution. Two rabbits were immunized with this heated acacia by the schedule described (5.0 ml. of 6 per cent solution intravenously for three successive days of three successive weeks). Both animals produced immune serum which passively sensitized to and gave precipitin reactions with the heated acacia and with all four samples of native acacia.

Two other rabbits were immunized with acacia-A which had been heated at 100° C. for two hours in a drying oven. This dry-heated acacia was also antigenic, producing precipitating and sensitizing antiserum specific for both heated and native acacia.

Formamide-Treated Acacia.—Acacia-A and -B were each treated by the formamide method of Partridge and Morgan.[4] No difference could be established between the A and B samples so treated. This formamide-treated acacia contained 0.28 per cent N, compared (1) to 0.24 per cent N as reported by Partridge and Morgan and (2) to 0.35 to 0.37 per cent N of the native gum. When the immunizing schedule of these authors[4] was used in two rabbits (3 × 0.5 mg. intravenously; one week's rest; 3 × 0.5 mg. intravenously), neither precipitins nor sensitizing antibodies could be demonstrated, in agreement with Partridge and Morgan.[4]

However, when three rabbits were immunized with formamide-treated acacia by the schedule used with the native gum (3 × 300 mg.; four days' rest; 3 × 300 mg.; four days' rest; 3 × 300 mg.), potent immune sera, comparable to those from whole acacia, were obtained from each animal. Guinea pigs passively sensitized with such rabbit antisera developed fatal anaphylactic shock when challenged with formamide-treated, heat-treated, or native acacia.

Formamide-treated acacia proved less anaphylactogenic than the native gum. The minimal fatal shocking dose of formamide-treated acacia observed in twelve pigs sensitized with 0.33 ml. antiacacia-A rabbit serum was about five times (30 mg.) that of the native gum. Sixty milligrams, or ten times the fatal challenging dose of whole acacia, killed only one of three pigs.

Formamide-treated acacia was highly reactive chemically (see discussion).

Unsuccessful Attempts to Extract a Polysaccharide Hapten from Acacia.—Since the formamide treatment of acacia by the method of Partridge and Morgan[4] resulted in a material which was antigenic and highly reactive chemically, and therefore unsatisfactory, five other methods were used to attempt to extract a polysaccharide hapten. None of the polysaccharides obtained by these methods retained their immunologic specificity and, therefore, they were not the desired haptens. Because of this, only brief outlines of the methods employed are given:

(a) Acacia, made acid with hydrochloric acid at pH 1 and 3.5, and with acetic acid at pH 4 and 6, was heated in the water bath for two hours. The polysaccharides were then precipitated with alcohol. Abundant yields of polysaccharide were obtained at pH 3.5 and 4.0.

(b) Acacia was heated with 8 per cent sodium hydroxide plus 8 per cent sodium acetate in the Arnold sterilizer, then adjusted to pH 6 with hydrochloric acid and precipitated with alcohol.

(c) The method of Heidelberger, Avery, and Goebel,[9] which consists of treating with strong hydrochloric acid at room temperature, precipitation by alcohol, and purification by successive treatments with acetone, sodium nitrite, and acetic acid, was tried.

(d) The method of Campbell[12] for extracting an antigenic polysaccharide from ascaris was applied to acacia. This consists essentially of treatment with sodium hydroxide and ether at pH 8, followed by treatment with trichloroacetic, phosphotungstic and sulphuric acids with repeated alcohol precipitation.

(e) The Myrvik[14] method for extracting polysaccharide from tubercle bacilli was also tried. This consisted of heating acacia with 4 per cent sodium acetate and 8 per cent sodium hydroxide and precipitating from the alkaline solution with alcohol. This was purified by repeated precipitation with alcohol from a pH of 8.0.

All these gave positive Molisch reactions in dilutions of 1:1,000,000 or more. None gave specific precipitin tests with antiacacia rabbit serum, although several gave nonspecific ring tests with normal and with immune sera. None caused dyspnea or anaphylaxis when used to challenge by aerosol or intravenous injection, although the animals reacted subsequently to challenges with whole acacia by aerosol and by vein.

The search for a polysaccharide hapten from acacia has been abandoned, for the present at least.

Desensitization.—Six guinea pigs were passively sensitized with 0.33 ml. antiacacia-A rabbit serum intra-abdominally. Two of them served as controls. Another pair received six desensitizing intra-abdominal injections of gradually increasing amounts (15, 30, 45, 60, and 150 mg.) of acacia-A during the succeeding two days. The remaining two guinea pigs were given similar desensitizing doses of formamide-treated acacia. No anaphylactic or other untoward symptoms were observed from the intra-abdominal desensitizing injections (large as they were in comparison with the intravenous shocking doses). Twenty-four

hours after the final desensitizing injection, the animals desensitized with the acacia-A were challenged intravenously with 120 mg. acacia-A (twenty times the usual lethal dose). The animals desensitized with formamide-treated acacia were challenged with 60 mg. of it intravenously. No anaphylactic or other symptoms were observed, while control animals were fatally shocked by smaller doses of the respective antigens.

Five days later, the two guinea pigs desensitized with acacia-A were re-challenged with 60 mg. acacia-A intracardially, again with negative reactions, showing persistence of desensitization for at least six days.

A marked increase in tolerance, but not complete desensitization, followed sublethal anaphylactic reactions to small intravenous doses of acacia in four animals.

Aerosol Experiments.—Seventeen guinea pigs, passively sensitized with antiacacia rabbit serum, were exposed in a glass box to 3 per cent or to 6 per cent acacia aerosol fog. Twelve developed moderate to severe dyspnea in three to nine minutes. Normal pigs are not affected by acacia aerosols.

Aerosol Desensitization.—Ten of the passively sensitized pigs that reacted were left in the fog for from ten to eighty-five minutes. The dyspnea gradually lessened and finally disappeared. When these ten animals were re-exposed to acacia aerosol after intervals ranging from ten minutes to several days, nine showed no dyspnea. The tenth pig, after an initial aerosol exposure of ten minutes, developed moderate dyspnea on re-exposure. This pig and the two others which had been removed from the fog when their initial reactions began were desensitized by several short aerosol re-exposures.

So far, fatal or even severe anaphylactic shock has not been produced in actively or passively sensitized pigs by the inhalation of acacia fog. This is in contrast to (1) the occasional rapid anaphylactic death of guinea pigs sensitized with antipneumococcal rabbit serum when exposed to aerosol of the type-specific polysaccharide,[13] and (2) the frequent anaphylactic death from specific aerosols in congenitally sensitive pigs.

Seventeen actively sensitized pigs were desensitized locally by exposures to 3 per cent acacia aerosol. Desensitization time was much longer than for passively sensitized pigs. Specifically, three took from one to two hours, eight took from two to three hours, two took from four to five hours, three took from seven to nine hours, and one took eleven hours. The average was three and three-tenths hours, compared with an average of less than one hour for the passively sensitized group. This difference has been observed with other antigen-antibody systems also.

Active local sensitization by inhalation of an aerosol of whole acacia was attempted unsuccessfully. Six guinea pigs were exposed to an aerosol of 3 per cent acacia for one hour daily, five days each week for three weeks. This was repeated three weeks later and again five weeks later. They did not react to an aerosol of 6 per cent acacia when challenged before the second and third or after the third series of aerosol exposures. The conclusion is obvious that active sensitization by inhalation sufficient to cause reactions to an aerosol of

whole acacia is not readily produced. Perhaps other schedules of aerosol exposures would be successful.[10] Through an oversight, the animals were not challenged intravenously to see if systemic sensitization had been produced.

Congenital Sensitization to Acacia.—Nineteen guinea pigs, born of mothers actively sensitized to acacia, were challenged with 3 per cent acacia aerosol. Fifteen were challenged three weeks after birth. There were three prompt anaphylactic deaths, plus two severe, three moderate, and two slight-moderate reactions and one slight reaction. Four of the pigs did not react to the aerosol. The aerosol exposure of those that reacted by anaphylactic death was less than three minutes. The others reacted in less than five minutes in the fog.

Four pigs were challenged when they were 3 days old. All four reacted moderatedly to a thirty-second exposure to the aerosol. When they were 6 weeks old, they were rechallenged. There was one anaphylactic death, plus one slight-moderate and two moderate reactions.

The anaphylactic deaths were in sharp contrast to the failure to elicit fatal reactions in pigs that had been actively or passively sensitized directly. Pigs congenitally sensitive to rabbit serum were also highly reactive to aerosols. The reactions were specific, that is, they did not react to aerosols of saline or of pneumococcal polysaccharides. Nor do normal newborn pigs react to acacia aerosols.

DISCUSSION

No demonstrable chemical or immunologic differences between the four lots of acacia were found in these limited experiments. It seems evident that the antigenicity of acacia is inherent in the gum and is not due to contaminants.

Although the chief reason for this study—the search for a polysaccharide hapten from acacia—was not successful, a number of interesting observations were made. Examples are: the atypical anamnestic, precipitin, and Arthus reactions; the persistence of precipitin titers when the Arthus reactions seemed suppressed by desensitizing procedures; the marked chemical reactivity of the formamide-treated acacia; the difference between aerosol desensitization times of actively and passively sensitized pigs; and the high incidence of anaphylactic death from acacia aerosol in congenitally sensitive pigs.

The demonstrated antigenicity of the formamide-treated acacia was disappointing but not surprising, since the nitrogen content was reduced only from 0.36 in whole acacia to 0.28 per cent by the formamide treatment. Some unexpected observations on formamide-treated acacia have compensated, in part, for the failure to find the hapten. For example: formamide-treated acacia is quite active chemically, as demonstrated by its striking ability to precipitate gamma globulin from serum promptly. This should be studied further elsewhere, since this laboratory is not equipped to pursue this interesting and potentially useful observation.

The formamide-treated acacia is a weaker and more unpredictable anaphylactogen than acacia, although it readily produces precipitins and sensitizing antibodies in good titer. When several guinea pigs were sensitized passively and divided into two groups, the first group reacted anaphylactically when challenged

intravenously with whole acacia. Reactions in the other group, challenged with the same dose of formamide-treated acacia, showed either very slight or fatal anaphylaxis, quite unaccountably. It is conjectured that nonantibody globulins of the recipients' plasma combined with the chemically active formamide-acacia sufficiently to neutralize the amount of available antigen necessary to produce anaphylaxis.

Several of the polysaccharides obtained by acid or alkali treatment of acacia gave nonspecific ring precipitin tests with normal guinea pig and rabbit sera. Several others were inert when overlaid on or mixed with specific and nonspecific antisera. None retained the immunologic specificity of acacia. Whereas the search for a specific polysaccharide hapten in acacia has been abandoned, at least temporarily, this does not mean that a hapten may not be extracted from acacia eventually.

The Arthus experiments were interesting in several respects, namely: (1) The small area of skin involved in the reaction, even when necrosis was observed; (2) the negligible effect of the large desensitizing doses of acacia on the precipitin titers; (3) the large amount of desensitizing acacia necessary to suppress the Arthus reactions; and (4) inability to obtain sedimented precipitates from antigen-antibody mixtures prevented quantitative studies of the amount of antigen necessary to neutralize precipitin in vivo or in vitro. If these observations can be repeated and extended, a re-examination of the apparently established role of precipitin in the Arthus reaction would seem in order.

The poor anamnestic response to a short course of reinjections of acacia after precipitins had disappeared was unexpected and difficult to explain in the face of the good precipitin response to a subsequent full immunizing course of acacia.

The aerosol experiments are presented in summary form, as more detailed studies and discussions have been reported.[13] Others are in progress.

SUMMARY

Four different lots of gum acacia were found to be equally effective as immunizing, sensitizing, anaphylactogenic, and desensitizing antigens, as shown by cross-precipitin tests and cross-anaphylaxis experiments.

The antigenicity of acacia is considered to be a property of the native substance, and not due to a contaminant or impurity.

Rabbits were readily immunized with, and guinea pigs actively sensitized to, acacia.

Antiacacia rabbit serum in small doses passively sensitized guinea pigs. They reacted to intravenous and aerosol challenges with acacia. This sensitization lasted about thirteen days.

Homologous antiacacia serum also passively sensitized guinea pigs, but none of these pigs has reacted, so far, to an aerosol challenge with acacia. The duration of passive sensitization with homologous serum was not studied.

Precipitins persisted in rabbits for six to seven weeks after intravenous immunization, and for three months after a single subcutaneous injection of acacia with Freund's adjuvant.

Precipitin behavior was bizarre in that precipitates could not be collected by centrifugation, although specific ring tests were obtained with ease.

Passively sensitized guinea pigs were effectively desensitized by intra-abdominal injections of acacia.

Exposure to acacia aerosol produced dyspnea in a high percentage of actively, passively, and congenitally sensitized animals.

Continued or repeated aerosol exposure produced local desensitization readily in passively sensitized guinea pigs. Desensitization of actively sensitized pigs took much longer.

Arthus type reactions to intracutaneous injections of acacia in immunized rabbits were obtained in varying degree. Desensitization against this type of reaction followed intravenous injections of several large doses of acacia.

Formamide-treated acacia had a lower nitrogen content than the native gum, but was still antigenic, although its anaphylactogenic and precipitating powers seemed to be reduced. Formamide-treated acacia precipitated globulin (chiefly gamma, by crude tests) from normal and immune guinea pig and rabbit serum.

Heating the acacia did not appreciably affect its specificity or immunizing properties.

Treatment of acacia by several methods, using acid or alkali with and without heat, failed to yield a haptenic polysaccharide, although nonhaptenic polysaccharides which had lost their immunologic specificity were obtained readily.

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