

CASTOR BEAN ALLERGY

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SUMMARY

In Ourinhos, city located in the "Estado de São Paulo, Brasil", in 1964, an epidemic of asthma, due to collective sensitization to castor bean dust, was investigated. Allergic manifestations occurred predominantly in atopic persons, living near a castor oil extracting industry. During the season of castor bean processing, symptoms were related to dry weather, wind and dust from the factory. Six out of 21 workers interviewed had allergic and toxic-irritative symptoms related to castor bean dust. The immunologic investigation included "in vivo" and "in vitro" studies of two c. b. extracts and specific antibodies.

Castor bean extract I, a crude extract, gave delayed intradermic reactions, due to ricin. Titration by passive transfer of castor bean extract II, ricin free, gave positive reactions at dilutions up to 1:100,000,000.

Escarification tests were positive in 26 out of 65 patients and passive transfer of reaginic activity was demonstrated in 18 out of 26 sera. Antibody titulation, by the method of passive transfer, gave titers between 1:60 and 1:1,800. Heating at 56°C for 1 hour destroyed skin-sensitizing activity. Positive passive hemagglutination reactions could be inhibited by previous incubation of serum with castor bean extract II. By radioimmuno-electrophoresis, using castor bean extract labelled with ¹³¹I, radioactive precipitin lines were identified in the alpha₂- and gamma-globulin zones of 4 sera.

INTRODUCTION

In 1964, approximately 100 persons were affected by allergic manifestation in Ourinhos, São Paulo. As symptoms started in August, during the season of industrial extraction of castor oil by the only big local industry, air pollution by highly allergenic castor bean dust was suspected as cause and confirmed by the following investigation, made in collaboration with the "Departamento de Saúde Pública e de Assistência Social do Estado de São Paulo".

MATERIAL AND METHODS

a) Clinical Study

At the "Posto de Saúde da cidade de Ourinhos", 65 patients were interviewed and submitted to skin tests. Sera of patients allergic to castor bean were separated, Seitz-filtered and kept at -20°C until used.

b) Immunological Study

1) Antigens

All extracts were prepared in the "Serviço de Alergia" (Dr. E. Mendes), "Departamento de Saúde Pública e de Assistência Social do Estado de São Paulo".

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Castor bean (*Ricinus communis*) extract I was prepared by routine method in Coca's solution. This extract was used for scratch testing.

Castor bean (*Ricinus communis*) extract II was prepared by boiling and alcohol treatment of castor bean pomace (GRABAR & KOUTSEFF⁶), thus destroying ricin, the castor bean toxin. This extract was used for passive transfer and "in vitro" testing. Analysis: pH 5.8; glucose: 1.05 mg/ml; protides: 5.7 mg/ml.

Common inhalant extracts (house dust, fungi, pyrethrum, grass, animal danders) were prepared in Coca's solution, by routine method (SHELDON et al.²²).

2) Antibody

Skin-sensitizing antibodies were tested by scratch and intradermal tests and passive transfer, following routine procedures (SHELDON et al.²²).

The following "in vitro" methods were also applied in the study of castor bean hypersensitivity:

1) Passive hemagglutination test (BOYDEN¹): tanned human group O erythrocytes, coated with castor bean extract II, 1:10, were added to progressive serial dilutions.

2) Immuno-diffusion in gel by HARTMANN & TOILLIEZ's modification⁹ of Ouchterlony's technique¹⁸.

3) Immunoelectrophoresis by GRABAR & WILLIAMS' method⁷ adapted to microscopic slides by SCHEIDECKER²¹, modified by FERRI & COSSERMELLI⁸.

4) Radioimmuno-electrophoresis, following YAGI et al. technique²⁵ of co-precipitation, by which labelled antigen/antibody complexes are precipitated in gel by anti-human serum or one of its fractions. Castor bean extract labelled with ¹³¹I was prepared by MacFARLANE's technique¹³ in the "Instituto de Energia Atômica" (Dr. Júlio Kieffer). Radioactivity varied between 560 and 900 mc/ml of castor bean extract.

RESULTS

a) Clinical Study

In 65 patients interviewed the most common symptoms were asthma and rhinitis, less often occurred conjunctival, cutaneous and general manifestations (Table I). Personal and family data of allergy and positive skin tests with common inhalants were obtained in many patients, indicating constitutional atopic predisposition.

Scratch tests with castor bean extract were positive in 40% of cases. In the group of 26 patients allergic to castor bean, showing positive scratch tests, when compared with 39 patients with negative scratch tests, the following data were statistically¹⁴ significant (Table II): a) High incidence of symptoms during the months of August to January, which is the season of industrial extraction of castor bean oil; b) High incidence of symptoms during dry weather; c) Influence of wind and dust, coming from the castor oil processing plant; d) Appearance of symptoms also in other localities with castor oil extracting industries (Salto Grande, Santa Cruz, Bauru).

Sixty out of 65 individuals interviewed lived in the immediate neighbourhood of the castor bean industry, within a radius of 1 km. Industrial extraction of castor bean oil was done by compression and supplemental extraction of castor bean pomace by solvent, leaving a dry, very dispersible and highly antigenic residue.

Study of workers at the castor bean processing plant

Six out of 21 workers interviewed observed transitory manifestations of intolerance to castor bean in August, during the first weeks of contact with castor bean. These 6 workers suffered from atopic cutaneous and respiratory symptoms, as well as toxic-irritative digestive and general manifestations (Table III). Negative scratch tests with castor bean in all workers tested were related to lack of constitutional atopic predisposition, short time of employment at the castor bean factory and homes distant, generally outside the city limits.

TABLE I
Symptoms and history of allergy observed in 65 individuals* interviewed
at the Health Post, City of Ourinhos

Symptoms	no. cases	%
Respiratory		
Asthma	53	81.5
Rhinitis	30	46.1
Bronchitis	7	10.7
Cough	7	10.7
Mucoid sputum	11	16.9
Asphyxiation	2	3.0
Ophthalmic		
Conjunctivitis	5	7.6
Cutaneous		
Urticaria	6	9.1
Itching	5	7.6
Erythema	1	1.5
Prurigo	1	1.5
Angioneurotic edema	2	3.0
Gastrointestinal		
Vomiting	1	1.5
Fever	15	23.0
Profuse sweating	2	3.0
Headache	1	1.5
History of allergy:		
Personal	17	26.1
Family	32	49.2

* 33 masculine, 32 feminine. Age, from 8 months to 77 years

TABLE II
Factors influencing symptoms of patients allergic to castor bean dust

	% of patients interviewed
1. Season of industrial processing of castor bean (August to January)	65.3
2. Wind or dust from castor bean factory	46.1
3. Improvement of symptoms when leaving town	65.3
4. Worsening with return to Ourinhos	23.0
5. Symptoms also in other towns with castor bean industries (Salto Grande, Santa Cruz, Bauru)	15.3
6. Work with castor bean	3.8
7. Dry weather	26.9

b) *Immunological Investigation*

1) *Antigen study*

Castor bean extract I, a crude extract, prepared by routine procedure in Coca's solution, when injected intradermally, caused delayed inflammatory reactions, attributed to ricin, a termolabil toxin.

Castor bean extract II, ricin free, never caused delayed reactions. This extract possessed an extremely high allergenic potential, as evidenced by positive passive transfer with dilutions up to 1:100,000,000.

2) *Antibody study*

Scratch tests with castor bean extract were positive in 26 out of 65 persons tested (40%) in Ourinhos, contrasting with 2.8% of a control group of 69 individuals, 35 of whom were asthmatic, living in São Paulo.

Passive transfer tests were positive with 18 out of 26 reaginic sera from patients with castor bean allergy. Results of passive transfer tests were directly related to the degree of cutaneous hypersensitivity of the patient, whose serum served for passive transfer.

Titration of antibody, by passive transfer, resulted in titers between 1:60 and 1:1,800, in 6 reaginic sera.

Heating reaginic serum at 56°C for 1 or more hours caused complete destruction of skin-sensitizing activity.

Positive intradermal reactions with house dust, fungi, pyrethrum, animal danders and grass were observed in 40 out of 63 individuals, of whom 15 also had positive skin tests with castor bean extract.

Passive hemagglutination tests gave titers of 1:4, 1:10 and 1:64 in 3 out of 13 sera investigated. By preliminary incubation of allergic serum with castor bean extract, passive hemagglutination was inhibited, thus demonstrating specificity of this reaction.

By immunodiffusion in agar gel and immunoelectrophoresis no precipitating antibody against castor bean could be demonstrated.

By radioimmunolectrophoresis, utilizing castor bean extract labelled with ^{131}I , radio-

active precipitin lines were observed in 4 sera in the zone of migration of alpha₂- and gammaglobulins (Fig. 1 A, B e C).

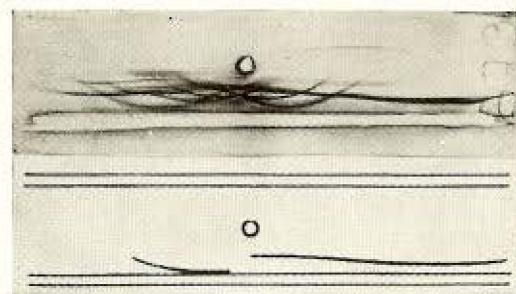
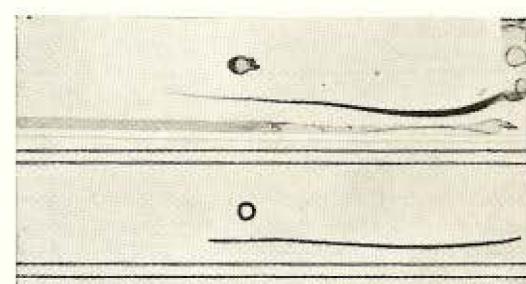
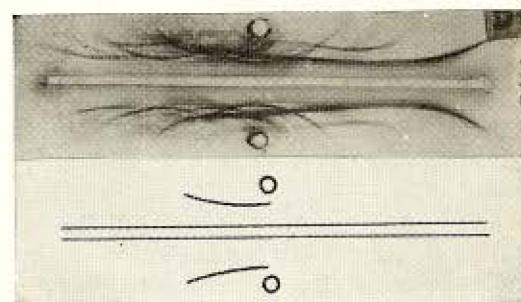


Fig. 1 — Radiolimmunolectrophoresis revealed after 4 days contact of film with immunoelectrophorogram

A) — Upper trough: Anti-IgM + ^{131}I castor bean
Lower trough: Anti-human serum + ^{131}I castor bean
Well: Serum of patient (S.5) allergic to castor bean



B) — Upper trough: Anti-IgA + ^{131}I castor bean
Lower trough: Anti-IgG + ^{131}I castor bean
Well: Serum of patient (S.5) allergic to castor bean



C) — Upper well: Serum of patient (S.1) allergic to castor bean
Lower well: Serum of patient (S.2) allergic to castor bean
Trough: Anti-human serum + ^{131}I castor bean

TABLE III

Symptoms due to castor bean dust, referred by 6 workers of the castor bean pressing mill

	Number of patients
Symptoms:	
Respiratory tract:	
Asthma	1
Bronchitis	1
Cough	1
Rhinitis	1
Cutaneous:	
Itching	5
Urticaria	3
Digestive tract:	
Bitter taste	2
Nausea	1
Dysentery	1
Illness	1
Fever	1
Generalized pains	1
Headache	1

DISCUSSION

Although considerable local economic benefit results from castor bean industrialization¹¹, air pollution by castor bean dust actually constitutes an ever increasing public health problem in the neighbourhood populations of castor bean processing industries. Collective outbreaks of asthma, due to industrial air pollution by castor bean dust have been reported in the past by FIGLEY & ELROD⁴ in Toledo, Ohio, GRIMM⁸ in Germany, ORDMANN¹⁷ in South Africa and PANZANI¹⁹ in Marseille. In Brasil, MENDES & CINTRA^{15, 16} studied an epidemic of collective asthma in Bauru, in 1954, affecting 150 individuals and causing 9 deaths in 4 days.

Both epidemics, in Ourinhos and Bauru, started in August, at the beginning of the industrial castor bean processing season. Influence of dry weather, wind and dust from the castor bean crushing mills was evident in both groups. Main symptoms were asthma and rhinitis, less often conjunctival and cutaneous symptoms were mentioned, all of which improved rapidly when leaving town.

In Ourinhos, all patients affected by castor bean allergy lived near the castor bean crushing mill, a fact also observed by FIGLEY & ELROD⁴.

The industrial process of oil extraction by compression and additional use of solvent, employed in Ourinhos and also in Bauru (MENDES & CINTRA¹⁵), and in South Africa (ORDMANN¹⁷), leaves a highly allergenic residue, containing ricinallergen, one of the most potent known allergens. With the use of solvent, turning the process of oil extraction much more profitable, danger of air pollution by the dry, highly dispersible residue has increased considerably.

Many patients seemed constitutionally predisposed to atopy, as may be deduced from frequent personal and family data of allergy and positive skin tests with common inhalants in 40% of patients with proven castor bean hypersensitivity. Sensibilization to ricinallergen may occur since early childhood, as was evident in Ourinhos, where the youngest patient was 13 months old, and 40% were less than 12 years old.

In spite of employee selection and industrial engineering techniques adopted by castor oil mills, symptoms of intolerance to castor bean, due to allergic and toxic-irritative mechanisms, may occur quite frequently in workers at castor bean processing mills, as was evident in Ourinhos, South Africa (ORDMANN¹⁷) and Toledo (FIGLEY & RAWLING⁵).

Castor bean extract I produced delayed inflammatory reactions, which were attributed to the toxin of castor bean, ricin. Boiling and alcohol treatment of castor bean extract II destroyed completely this toxin: no delayed reactions were observed with this very antigenic extract.

Scratch tests with castor bean extract were positive in 40% of the patients in Ourinhos; only 2.8% gave positive reactions in a control group of 69 patients, of whom 35 were asthmatics, all living in São Paulo.

By passive transfer, titration of skin-sensitizing antibodies was done in 6 sera and the destroying effect of heat on reaginic activity was demonstrated, confirming observations by COCA & GROVE², LOVELESS¹² and LAYTON et al.¹⁰.

By passive hemagglutination 3 sera gave positive reactions: all possessed also high reaginic titers.

Precipitins are only rarely demonstrated in sera of atopic patients. Thus we were not surprised by negative results by immunodiffusion in agar gel and immuno-electrophoresis. By the radioimmuno-electrophoresis technique, however, adapted to the study of castor bean allergy, positive radioautographs, with radioactive lines in the zones of migration of alpha₂- and gammaglobulins were obtained with 4 sera, thus constituting a new "in vitro" approach to the study of castor bean allergy.

RESUMO

Alergia à mamona

Foi investigada uma epidemia de asma na cidade de Ourinhos, em 1964, desencadeada por sensibilização coletiva a pó de semente de mamona. Foram acometidas, predominantemente, pessoas atópicas, residentes a menos de 1 km de distância da indústria de óleo de mamona. Durante o período de processamento da mamona, sintomas de alergia foram relacionados ao tempo seco, vento e fumaça da fábrica. Seis de 21 operários entrevistados apresentaram manifestações alérgicas e tóxico-irritativas, atribuídas à mamona. A investigação imunológica foi realizada por provas "in vivo" e "in vitro". de dois extratos de mamona e anticorpos específicos.

Extrato de mamona I deu reações intradérmicas do tipo tardio, atribuídas à ricina. Extrato de mamona II, livre de ricina, diluído até 1:100.000.000, determinou reações de transferência passiva positivas.

Foram positivas provas de escarificação em 26 de 65 pacientes e de transferência passiva com 18 de 26 soros reaginicos. O título de anticorpos reaginicos, pela técnica de transferência passiva, variou entre 1:60 e 1:1.800. Aquecimento a 56°C durante 1 hora destruiu toda atividade cuti-sensibilizadora. Reações de hemaglutinação passiva positivas foram inibidas por incubação prévia do sôro com extrato de mamona II. Por rádio-imuno-eletroforese, usando extrato de mamona marcado com ¹³¹I, foram identificados arcos radioativos nas zonas alfa₂- e gammaglobulina de 4 soros.

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REFERENCES

1. BOYDEN, S. V. — The adsorption of protein on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. *J. Exp. Med.* 93:107-120, 1951.
2. COCA, A. F. & GROVE, E. F. — Studies in hypersensitivity. XIII — A study of the atopic reagins. *J. Immun.* 10:445-465, 1925.
3. FERRI, R. G. & COSSERMELLI, W. — Analyse immuno-électrophorétique: micro- et macro-méthodes. *Rev. Franc. Etud. Clin. Biol.* 9:134-138, 1964.
4. FIGLEY, K. D. & ELROD, R. H. — Endemic asthma due to castor bean dust. *J.A.M.A.* 90:79-82, 1928.
5. FIGLEY, K. D. & RAWLING, F. F. A. — Castor bean: an industrial hazard as a contaminant of green coffee dust and used burlap bags. *J. Allergy* 21:545, 1950.
6. GRABAR, P. & KOUTSEFF, A. — Différenciation, dans le ricin, de la toxine (ricine) et d'un allergène. *Compt. Rend. Soc. Biol.* 117:700-704, 1934.
7. GRABAR, P. & WILLIAMS Jr., C. A. — Méthode permettant l'étude conjugée des propriétés électrophorétiques et immunochimiques d'un mélange de protéines: application au serum sanguin. *Biochim. Biophys. Acta* 10:193-194, 1953.
8. GRIMM, V. — Veröffentl. a.d. Med. Verwalt. 26:5, 1928. Cit. em Hansel, K. — *Tratado de Alergia*. Madrid, Labor, 1946.
9. HARTMANN, L. & TOILLIEZ, M. — Micro-méthode d'étude en gélose de la réaction antigène-anticorps (variante du procédé d'Ouchterlony). *Rev. Franc. Etud. Clin. Biol.* 11:197-199, 1957.
10. LAYTON, L. L.; YAMANAKA, E.; GREENE, F. C. & PERLMAN, F. — Atopic reagins to penicillin, pollens and seeds: thermolability, titer and persistence in the skin of passively sensitized Macaque monkeys. *Int. Arch. Allergy* 23:87-94, 1963.

11. LINS, E. R. — Aspectos da produção e comercialização da mamona no Estado de São Paulo. *Agricultura em São Paulo. Bol. Div. Econ. Rural* 11:15-30, 1964.
12. LOVELESS, M. H. — Immunological studies of pollinosis. I — The presence of two antibodies related to the same pollen antigen in the serum of treated hay fever patients. *J. Immun.* 38:25, 1940.
13. MacFARLANE, A. S. — Efficient trace-labelling of proteins with iodine. *Nature* 182:53, 1958.
14. MAINLAND, D. — *Elementary Medical Statistics*. Philadelphia, Saunders, 1952, p.p. 93-146.
15. MENDES, E. & CINTRA, A. U. — Collective asthma, simulating an epidemic, provoked by castor bean dust. *J. Allergy* 25:253-259, 1954.
16. MENDES, E. & CINTRA, A. U. — Etiologia del "Asma epidémica de Bauru". *Alergologia* 21:1109-1126, 1954.
17. ORDMANN, D. — An outbreak of bronchial asthma in South Africa affecting more than 200 persons, caused by castor bean dust from an oil-processing factory. *Int. Arch. Allergy* 7:10-24, 1955.
18. OUCHTERLONY, O. — *In vitro* method for testing the toxin producing capacity of diphtheria bacteria. *Acta Path. Microbiol. Scand.* 25:186-191, 1948.
19. PANZANI, R. — Étude de l'allergie entre la graine de ricin et *Spondylocladium*. *Int. Arch. Allergy* 21:288-293, 1962.
20. PRAUSNITZ, C. & KÜSTNER, H. — Studien über die Überempfindlichkeit. *Zbl. Bakt. I. Abt. Orig.* 86:160, 1921. Cit. em *Referate* 72: 233-234, 1921.
21. SCHEIDEGGER, J. J. — Une micro-méthode de l'immuno-électrophorèse. *Int. Arch. Allergy* 7:103-110, 1955.
22. SHELDON, J. M.; LOVELL, R. G. & MATHEWS, K. P. — *A Manual of Clinical Allergy*. Philadelphia, Saunders, 1953.
23. SMALL, W. S. — Increasing castor bean allergy in Southern California due to fertilizer. *J. Allergy* 23:406-415, 1952.
24. STAVITSKY, A. B. — Micromethods for the study of proteins and antibodies. *J. Immun.* 72:360-367, 1954.
25. YAGI, Y.; MAIER, P. & PRESSMANN, D. — Immunoelectrophoretic identifications of guinea-pig anti-insulin antibodies. *J. Immun.* 89:736-744, 1962.

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