

The Journal of
ALLERGY

VOL 47, NO. 2

FEBRUARY, 1971

Antigenicity of fractions from extracts of *Candida albicans*

The immediate and delayed-type respiratory responses
in guinea pigs

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*Fifteen guinea pigs were sensitized with *Candida albicans* and exposed to the aerosol of the polysaccharide fraction (PS) or protein fraction (Prot) from the extracts of the organisms. A definite decrease in respiratory conductance was seen immediately after the inhalation of PS. When the pigs were exposed to Prot or crude extracts, two types of reactions in the respiratory tract were observed in each. The first was an immediate decrease in conductance following inhalation, and the second was a delayed tachypnea occurring at 24 hours after exposure. Our previous study on guinea pigs immunized with egg albumin or killed tubercle bacilli showed that exposure to the former provoked an immediate decrease in conductance and exposure to the latter caused a delayed tachypnea. The transferability of the former reaction with serum and the latter via cells was demonstrated. Taking this into consideration, it may be suggested from the present investigation that PS has antigenic activity provoking an allergic reaction of the immediate type and Prot has the ability to elicit dual reactions of the immediate and delayed type in the respiratory tract of guinea pigs immunized with the organism. Histologic studies support this view.*

Asthma is a classic example of an immediate anaphylactic reaction in the respiratory tract of man. Evidence¹⁻⁵ has accumulated, however, that late bronchial reactions to provocative challenge with aerosolized extracts of *Candida albicans* is not infrequent in patients with asthma. Itkin and Dennis¹ suggested that the bronchial reaction, either immediate or delayed, was more likely an allergic one than a toxic one. The question arose that if the late bronchial reaction was an allergic one, then immediate and delayed hypersensitivity to *C. albicans* might be due to different components of the extract. We felt that the answer to this question might give us further information on the mechanism

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Received for publication April 16, 1970.

TABLE I. Chemical properties of the fractions from the extract of *C. albicans*

	Crude polysaccharide (K ₂ -S) (%)	Purified polysaccharide (PS) (%)	Crude protein (K ₁ -P) (%)	Purified protein (Prot) (%)
Protein	12.4	0.2	68.5	90.2
Carbohydrate	83.3	96.4	9.2	2.2

of delayed allergy in the respiratory tract in man by providing an experimental model of a delayed hypersensitivity reaction taking place against a defined antigen. It is the purpose of this communication to study the antigenicity of the fractions from extracts of *C. albicans* on the respiratory tract of guinea pigs sensitive to the organism to obtain a better understanding of the immunologic reactions involved in asthmatic patients who had provocative challenges with such extracts.

MATERIALS AND METHODS

Antigens

Details of the methods used for the purification of fractions from extracts of *C. albicans* have been reported previously.^{6,7} Antigens used in this experiment, however, were newly prepared by a slightly modified method (Fig. 1). Briefly, organisms of *C. albicans*, Ca 13 strain, were repeatedly extracted with 0.1N KOH solution and then with 1N KOH solution. The crude extracts (K₁ and K₂) were precipitated at pH 4.0 by the addition of HCl. These crude protein fractions were designated as K₁-P and K₂-P, respectively. The supernatant was deproteinized with trichloroacetic acid (TCA) at a concentration of 5 per cent, adjusted to pH 7.0 with dilute alkali and precipitated with ethanol. These crude polysaccharide fractions were designated as K₁-S and K₂-S, respectively. K₁-P and K₂-S were used for starting materials in this experiment. K₁-P contained 68.5 per cent protein and 9.2 per cent sugar. K₂-S contained 12.4 per cent protein and 83.3 per cent sugar (Table I).

The crude polysaccharide fraction, K₂-S, was further purified by the treatment with pronase and then chromatographed through a Dowex 50 column to obtain a purified polysaccharide fraction (PS-dw). PS-dw contained 0.2 per cent protein and 96.4 per cent sugar. This purified polysaccharide was analyzed by gas liquid chromatography and found to contain mannose with a small amount of glucose (10:0.6). The crude protein fraction, K₁-P, was further purified by zone electrophoresis giving a purified protein fraction (Z₆-4). This protein fraction contained 90.2 per cent protein and 2.2 per cent sugar. These purified polysaccharide and protein fractions were called PS and Prot, respectively. All extracts, and fractions thereof, of *C. albicans* were lyophilized.

Determination of concentration of nitrogen, protein, and sugar

The nitrogen content of the various fractions of the *C. albicans* extracts was determined by micro-Kjeldahl methods. The Folin phenol reagent, with modified analytic procedure by Lowry and associates,⁸ was used for the measurement of the protein content, and the anthrone reagent, according to Morris⁹ method, was used for the determination of carbohydrates. Egg albumin and mannose were used as standards for protein and carbohydrate, respectively.

Immunization

Fifteen young guinea pigs weighing 250 to 300 grams were injected intraperitoneally with 1.0 ml. of saline containing 10 mg. of *C. albicans* twice at 2 month intervals. Seven days after the second immunization, animals were tested with intradermal injections of 10 µg of PS and 3 µg of Prot in 0.02 ml. of saline, and the reactions were observed at 1 and 24 hours.

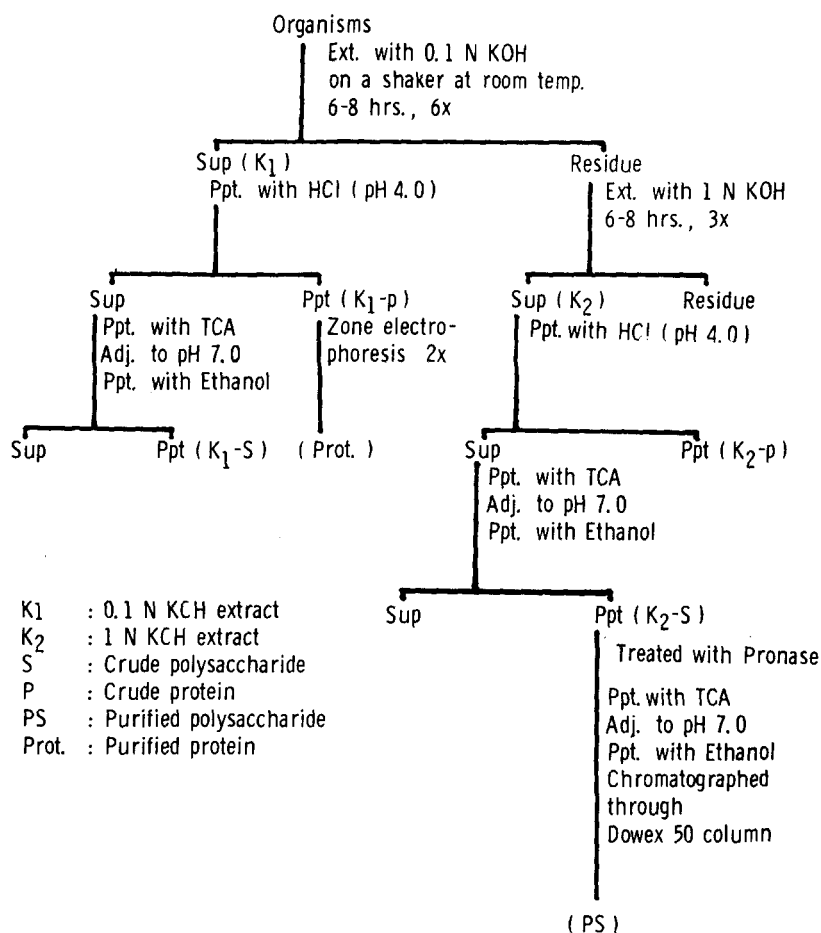


FIG. 1. Fractionation of immunologically active substances from *Candida albicans*.

One immunized animal was excluded from the inhalation study for the intravenous injection test.

Intravenous injection challenge

Seven milligrams of PS or 3 mg. of crude extract (K_1) was injected intravenously into each of two sensitized guinea pigs.

Inhalation test

Two, 3, 15, or 30 mg. of PS, 7, 7.5, or 10 mg. of Prot, and 8, 11, or 15 mg. of crude extract (K_1) in 1.0 ml. of saline were used as antigens for inhalation. The antigen solutions were aerosolized with a Vaponefrin No. 300 nebulizer at a flow rate of 3.5 L. of oxygen per minute. The length of the period of aerosolization varied from 10 to 15 minutes. Animals were exposed to inhalation of aerosolized antigen solution with face masks attached to their noses. The oscillation method of Mead,¹⁰ modified by Murphy and associates¹¹ and Swan and associates,¹² was used for the measurement of respiratory resistance and respiratory rate (RR) of the animals (Fig. 2). These values were recorded following the inhalation of saline and then 3, 5, 7, 10, 15, and 30 minutes and 1, 3, 4, 6, 24, and 48 hours after exposure to the



FIG. 2. Apparatus for measuring respiratory resistance in guinea pigs.

antigens. The average and the standard deviation of the airway resistance in 21 guinea pigs during the control state in 23 tests were 0.23 ± 0.06 cm. H_2O per milliliter per second. Respiratory resistance was converted to respiratory conductance (G_R). The values of G_R and RR were presented as per cent of those of the control state (following saline inhalation).

Temperature measurement

A thermometer was inserted in the rectum of each guinea pig for 8 minutes following the inhalation of saline and then at 30 minutes, 3 hours, and 24 hours after antigen exposure.

Arterial blood gas analysis and cell counts

Arterial blood was anaerobically drawn from the carotid arteries of 3 sensitized and 3 control guinea pigs under local anesthesia at 24 hours after antigen exposure. The blood was analyzed for PO_2 , PCO_2 , and pH by a blood gas analyzer (model 113-S1, IL INC), and white blood cell counts were also recorded.

Histologic examination

Animals were killed by bleeding 15 minutes following intravenous challenge or at 48 hours after aerosol exposure for macroscopic and microscopic investigations.

RESULTS

Degree of immunization was evaluation by skin test and intravenous provocative challenge.

Skin test

A wheal with erythema measuring 7×7 mm. for immediate reactions and an induration measuring 7×7 mm. for delayed reactions were considered to

TABLE II. Skin and bronchopulmonary reactions to inhalation of extracts of *Candida albicans* in 14 guinea pigs inoculated with the organism and in 7 control guinea pigs

Guinea pig No.	Skin reaction (mm.)				Bronchopulmonary reaction (% of control)					
	1 hr.		24 hr.		Inhalation material	Amount (mg.)	0-30 min.		24 hr.	
							Conduc-tance*	Resp. fre-quency*	Conduc-tance	Resp. fre-quency
	PS	Prot	PS	Prot						
<i>Sensitized</i>										
1	0	0	0	12 × 12	Prot	7.5	93.7	110	67.5	152
2	10 × 10	0	6 × 6	12 × 12	Prot	7.5	105.0	82	119.0	159
3	12 × 12	9 × 9	0	12 × 14	Prot	7	111.0	84	86.5	186
4	9 × 9	8 × 8	0	12 × 12	Prot	10	84.3	79	80.0	221
5	8 × 8	8 × 8	0	13 × 13	Prot	10	60.0	69	57.5	115
12	13 × 14	8 × 8	3 × 3	10 × 10	PS	2	125.0	106	96.5	100
					PS	30	43.2	105	82.5	114
11	11 × 12	8 × 8	0	11 × 10	PS	3	100.0	116	89.0	100
					PS	15	86.3	77	148.5	96
10	12 × 12	6 × 6	6 × 6	9 × 8	PS	15	95.9	118	109.0	100
8	10 × 12	7 × 7	3 × 3	9 × 11	Crude	8	89.7	111	128.5	230
9	7 × 8	7 × 7	0	9 × 9	Crude	11	70.6	102	70.0	253
7	7 × 7	7 × 9	0	9 × 9	Crude	15	57.1	41	92.5	130
13	7 × 7	3 × 3	0	8 × 8	Crude	15	67.9	111	71.9	345
14	8 × 8	4 × 4	2 × 2	9 × 9	Crude	15	93.9	99	103.2	273
15	5 × 5	2 × 2	3 × 3	7 × 7	Crude	15	82.6	62	96.0	230
<i>Control</i>										
16					(Prot 5)		83.6	78	100.0	82
17					(PS 2)		82.9	97	79.0	105
18					(Crude 8)		80.6	95	84.0	122
19					Prot 10		114.5	110	100.0	140
20					PS 30		102.5	89	103.0	103
21					Crude 15		101.0	106	92.5	103
22					Crude 15		113.0	125	109.5	120

*Average values occurring in 30 minutes following exposure are presented.

represent positive reactions. Thirteen of 15 animals at one hour after PS injection and all animals at 24 hours following Prot injection were positive. The results of the skin tests are shown in Table II. The animals with positive reactions were exposed 10 days after the skin tests to the inhalation of an aerosol of the antigens.

Intravenous injection challenge

Both sensitized animals which received PS (No. 10) or crude extract (No. 6) intravenously fell into anaphylactic shock and died in 10 minutes. Animal No. 10 had received previous exposure to 15 mg. of PS by aerosol a month prior to the injection.

Inhalation test

Sixteen inhalations in 14 immunized guinea pigs and 7 inhalations in 7 control guinea pigs were performed. The results are summarized in Tables II, III, and IV and Figs. 3, 4, 5, and 6.

TABLE III. Changes in respiratory frequency of guinea pigs as per cent of the control values by inhalation of extracts of *Candida albicans*

Inhalation material	No. pigs	Average change in respiratory frequency (%)	
		0-30 min.*	24 hr.
<i>Sensitized</i>			
PS	3	101	103
Prot	5	85	167
Crude	6	87	243
<i>Control</i>			
PS, Prot, crude mixture	3	90	103
PS	1	89	103
Prot	1	110	140
Crude	2	115	112

*Average values occurring in 30 minutes following exposure are presented.

TABLE IV. Changes in conductance of guinea pigs as per cent of the control values produced by inhalation of extracts of *Candida albicans*

Inhalation material	No. pigs	Average change in conductance (%)	
		0-30 min.*	24 hr.
<i>Sensitized</i>			
PS	3	75.1	113.1
Prot	5	90.8	82.1
Crude	6	76.6	93.7
<i>Control</i>			
PS, Prot, crude mixture	3	87.0	87.7
PS	1	102.5	103.0
Prot	1	114.5	100.0
Crude	2	107.0	101.0

*Average values occurring in 30 minutes following exposure are presented.

Changes after PS inhalation (Fig. 3)

No remarkable changes in either G_R or RR were seen after inhalation of 2 or 3 mg. of PS by two immunized pigs (Nos. 11 and 12). Four weeks later, 15 mg. or 30 mg. of PS was given to each of them and 15 mg. of PS to another pig (No. 10*). G_R decreased immediately after the exposure, and this effect lasted for 30 minutes. A slight decrease in RR was also seen. The pig which inhaled 30 mg. of PS fell into a state of anaphylactic shock. G_R and RR returned to normal values in 1 to 3 hours after inhalation in all animals.

Changes after Prot inhalation (Fig. 4)

Five sensitized guinea pigs (Nos. 1 to 5) inhaled 7, 5, or 10 mg. of Prot. G_R decreased immediately after the exposure, as was seen in the animals after PS inhalation. Twenty-four hours later, a marked increase in RR was seen in each of them, the average being 167 per cent of the control values.

*No. 10 guinea pig was used for the intravenous challenge test, as described above, 4 weeks after the inhalation.

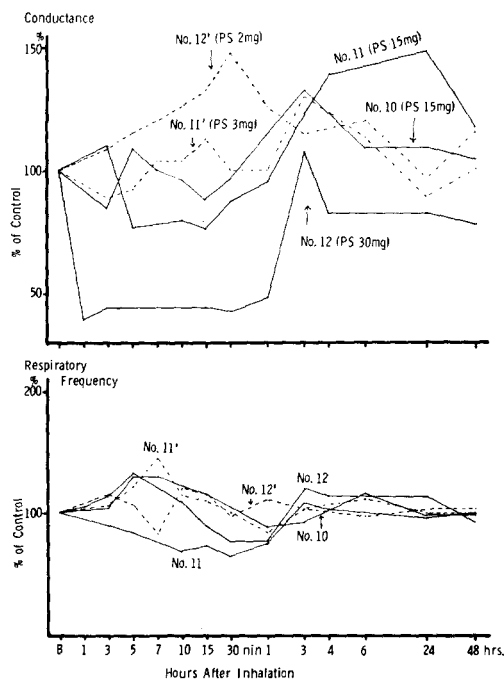


FIG. 3. Reactions due to inhalation of polysaccharide fraction of *Candida albicans* in 5 guinea pigs inoculated with the organism.

Changes after the inhalation of crude extract (Fig. 5)

Six immunized guinea pigs (Nos. 7 to 9 and 13 to 15) inhaled 8, 11, or 15 mg. of crude extract (K_1). Conductance decreased following the exposure, as was seen with PS or Prot. The decreased G_R in Pig No. 7 improved at 30 minutes, again fell at 1 to 3 hours after the exposure, and then returned to normal. A marked tachypnea at 24 hours (with an average value of 243 per cent of control RR) was observed in all animals, as was seen in the inhalation of Prot.

Effect of control inhalation of the antigens (Fig. 6)

Each of three control animals (Nos. 16 to 18) inhaled a mixed solution containing 2 mg. of PS, 5 mg. of Prot, and 8 mg. of crude extract (K_1). One normal guinea pig (No. 20) received control exposure to 30 mg. of PS, one (No. 19) inhaled 15 mg. of Prot, and 2 (Nos. 21 and 22) received 15 mg. of crude extract. No remarkable change in G_R or RR was seen except a slight unstable decrease in G_R following the exposure in some and a slight increase in RR at 24 hours in one pig.

Arterial blood gas tensions and white blood cell counts

Marked hypoxia was seen in arterial blood of 3 sensitized animals 24 hours after exposure to crude extract, while P_{CO_2} and pH were normal. Leukocytosis

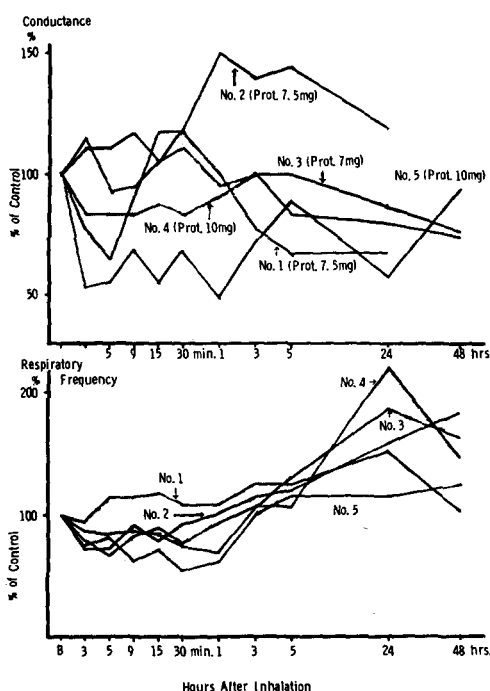


FIG. 4. Reactions due to inhalation of protein fraction of the extract of *Candida albicans* in 5 guinea pigs inoculated with the organism.

also was noted (Table V). For comparison, results of analysis of arterial blood from three untreated normal guinea pigs are shown in Table VI.

Temperature measurement

An increase in body temperature in 3 sensitized guinea pigs was seen following exposure to crude extract. This was not observed in normal guinea pigs exposed to the same antigens (Table V).

Histologic examination

Distinct hyperinflation was noted in the lungs of both guinea pigs which died in anaphylactic shock following the intravenous injection of 7 mg. of PS or 3 mg. of crude extracts. Histologic findings consisted of marked hyperinflation, constriction of bronchi and bronchioles, and venous congestion (Fig. 7, A). Similar but less conspicuous changes of acute hyperinflation were seen in the lungs at 48 hours after PS inhalation (Fig. 7, B). The sensitized lungs exposed by inhalation to Prot or crude extract showed obvious macroscopic alterations consisting of multiple red-brownish areas with irregular limits (Fig. 8, A and B). Histologic examination revealed intensive infiltrations consisting of polymorphonuclear cells, moderate numbers of eosinophils, and mononuclear cells (mostly lymphocytes with some macrophages) in peribronchial and perivascular areas and extending into interstitial tissues. Hemorrhages were seen in

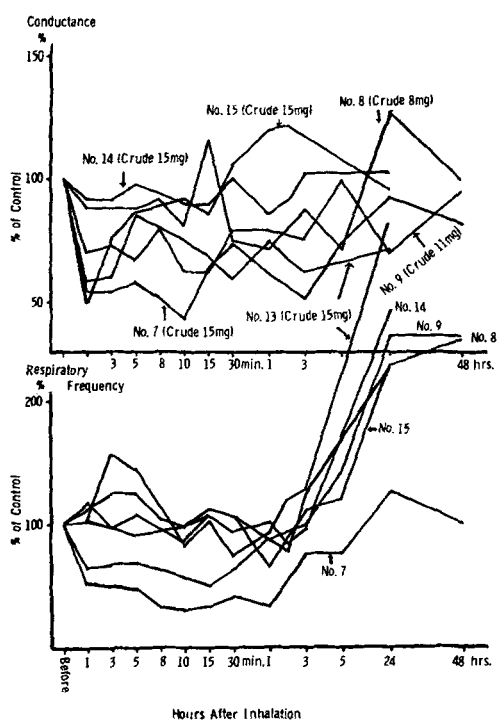


FIG. 5. Reactions due to inhalation of crude extract of *Candida albicans* in 6 guinea pigs inoculated with the organism.

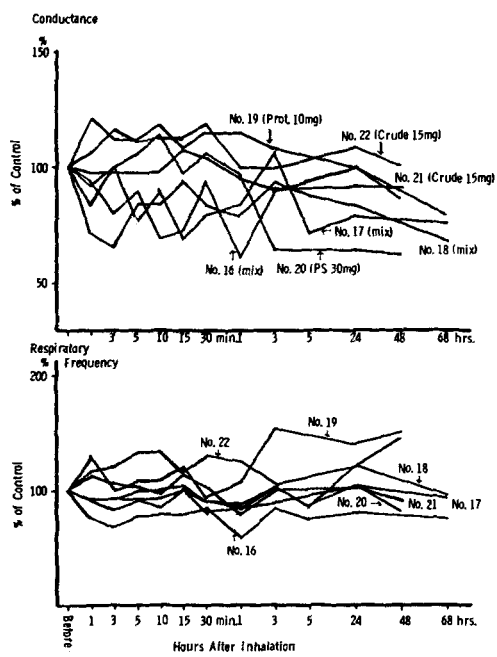


FIG. 6. Reactions due to inhalation of extracts of *Candida albicans* in 7 control guinea pigs.

TABLE V. Changes in body temperature, gas tensions, and white blood cell counts in arterial blood in guinea pigs exposed to the extracts of *Candida albicans*

Guinea pig No.	Inhalation material	Temperature (°C.)				Arterial blood (24 hr.)				
		Before	30 min.	3 hr.	24 hr.	Po ₂ (mm. Hg)	Pco ₂ (mm. Hg)	pH	WBC (/mm. ³)	Eosino- phils (%)
<i>Sensitized</i>										
13	Crude	38.6	39.1	39.2	39.6	56.0	29.9	7.43	6,300	5
14	Crude	38.4	39.4	39.4	39.2	33.5	32.5	7.34	12,800	1
15	Crude	38.7	39.4	39.5	39.7	46.5	40.5	7.22	8,600	1
<i>Control</i>										
19	Prot	39.0	39.2	38.8	38.1					
20	PS	38.0	38.0	37.0	38.1					
21	Crude	37.7	38.4	37.5	38.3					
22	Crude	38.0	38.1	38.2	38.2					

Table VI. Gas tensions and white blood cell counts in arterial blood in untreated normal guinea pigs

Animal No.	Po ₂ (mm. Hg)	Pco ₂ (mm. Hg)	pH	WBC (/mm. ³)	Eosinophils (%)
23	78.0	24.3	7.43	6,800	2
24	79.0	24.7	7.31	4,500	2
25	84.0	24.3	7.24	4,800	2

some animals. The interstitial and peribronchial tissues and alveolar spaces were edematous (Figs. 9, A and B, and 10). No such reaction was seen in the lungs of nonsensitized control animals exposed to the antigen mixtures (Fig. 10, B), although much smaller peribronchial infiltrations of lymphocytes were seen not infrequently.

DISCUSSION

Previous studies from our laboratory have shown that delayed skin reactions could be elicited in guinea pigs with a protein fraction from extracts of *C. albicans*. Using the same materials as in the present investigation, Aoki and associates^{6, 7} reported that crude polysaccharide and crude protein fractions (K₂-S and K₁-P), when injected into guinea pigs previously exposed to living *C. albicans* organisms, were capable of provoking delayed reactions, but their activity was markedly reduced by digestion with proteolytic enzymes. The activity of the purified protein fraction (Prot) was more than 100 times that of the purified polysaccharide fraction (PS). Evidence also has been obtained from study of extracts of *C. albicans* that the polysaccharide fraction elicits immediate reactions, while the protein fraction evokes dual skin reactions, consisting of weak immediate and strong delayed responses, in guinea pigs as well as in men.^{5, 17} Prot and the crude polysaccharide fraction, when mixed with Freund's complete adjuvant and injected into the foot pads of guinea pigs, were able to induce delayed skin hypersensitivity. PS and pronase-treated crude antigens failed to sensitize animals. However, PS was capable of reacting with sera of

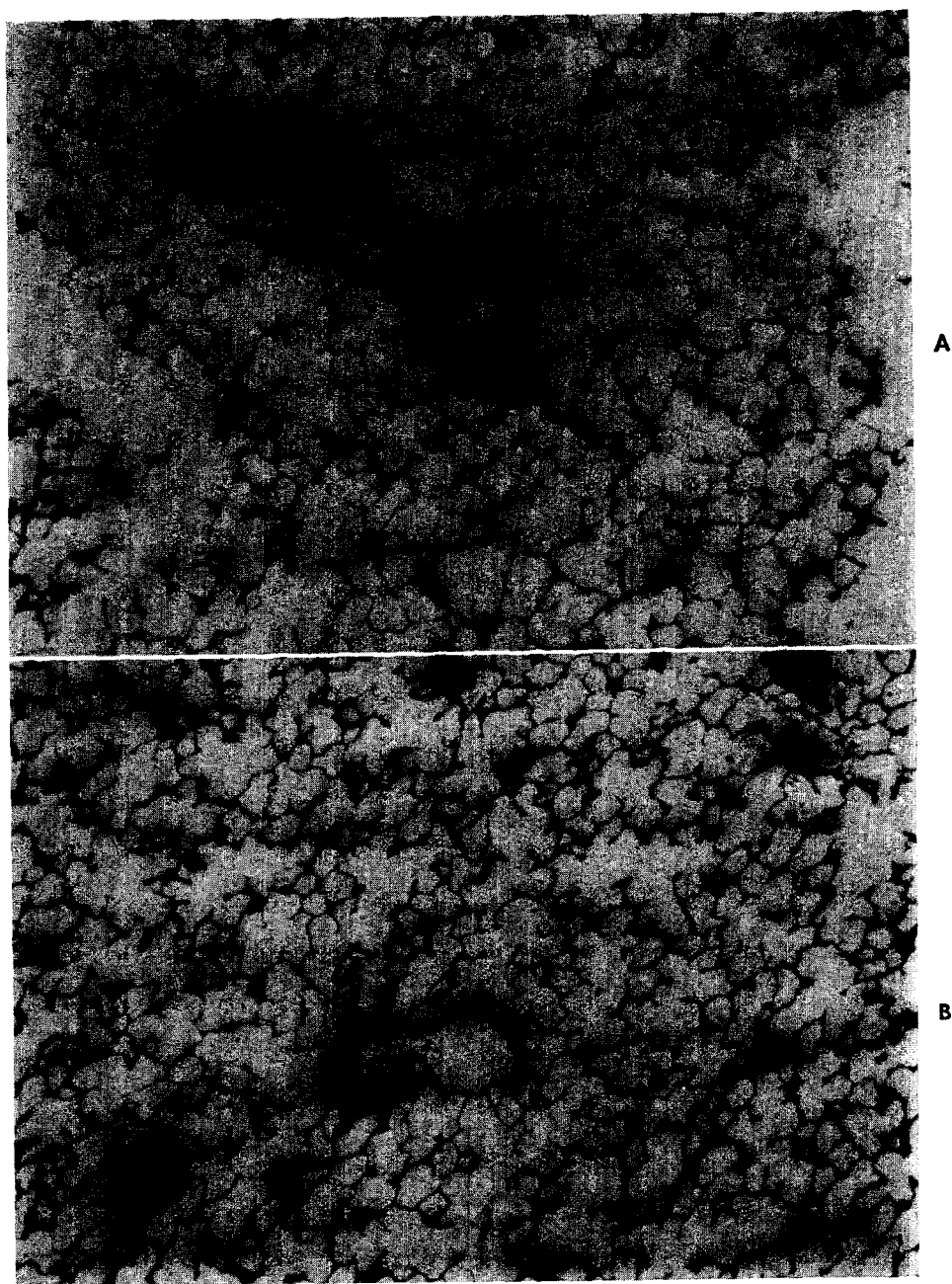


FIG. 7. Guinea pig with immediate reaction to PS from the extracts of *Candida albicans*. Histologic pictures of the lungs approximately 10 minutes after intravenous injection of PS represent acute hyperinflation, bronchoconstriction, and stasis in venules (A). At 48 hours after the inhalation less conspicuous hyperinflation still remained (B). (X40.)

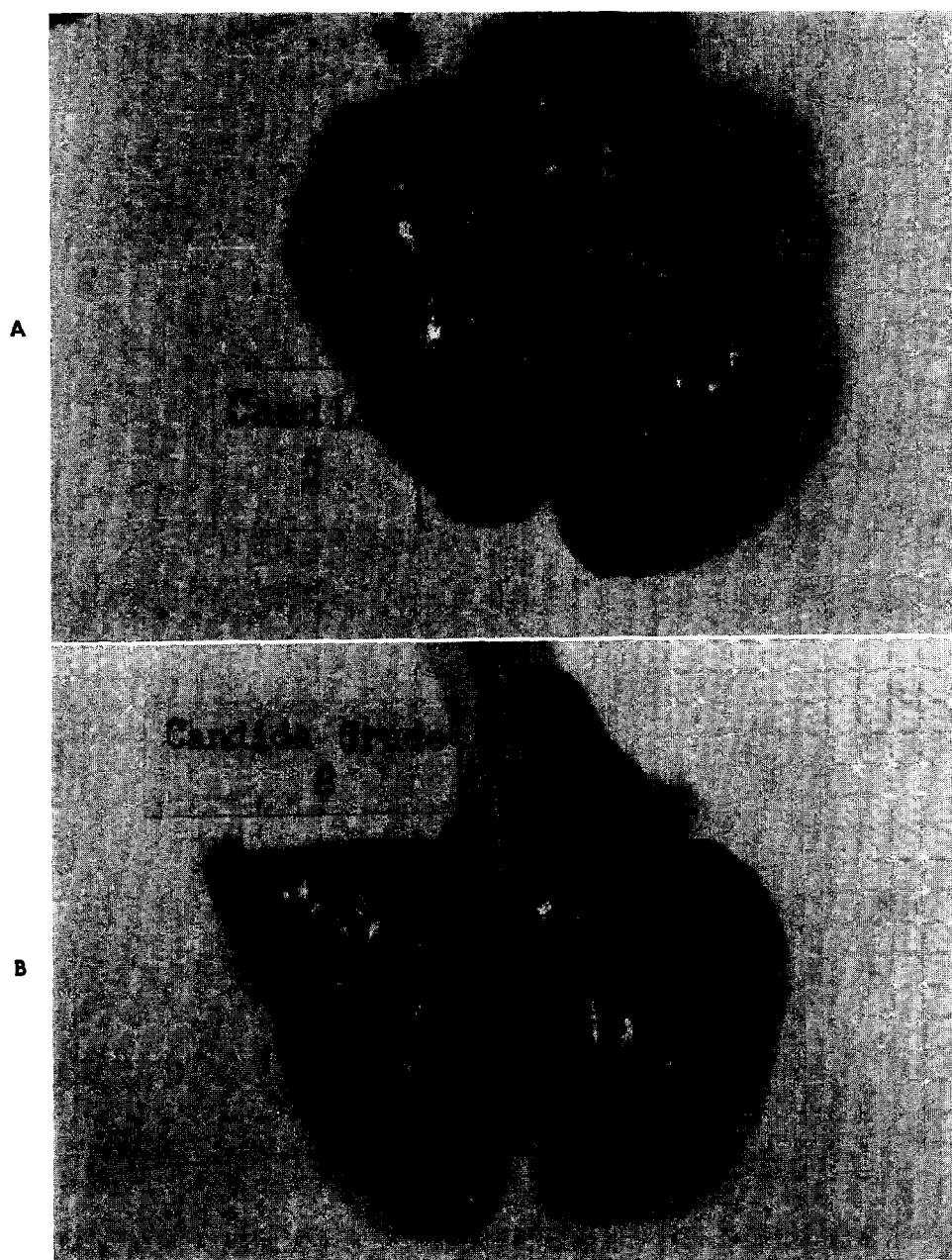


FIG. 8. Pictures of the lungs 48 hours after the exposure to Prot (A) or crude extracts (B) of *C. albicans* showing multiple red-brownish areas with irregular limits.

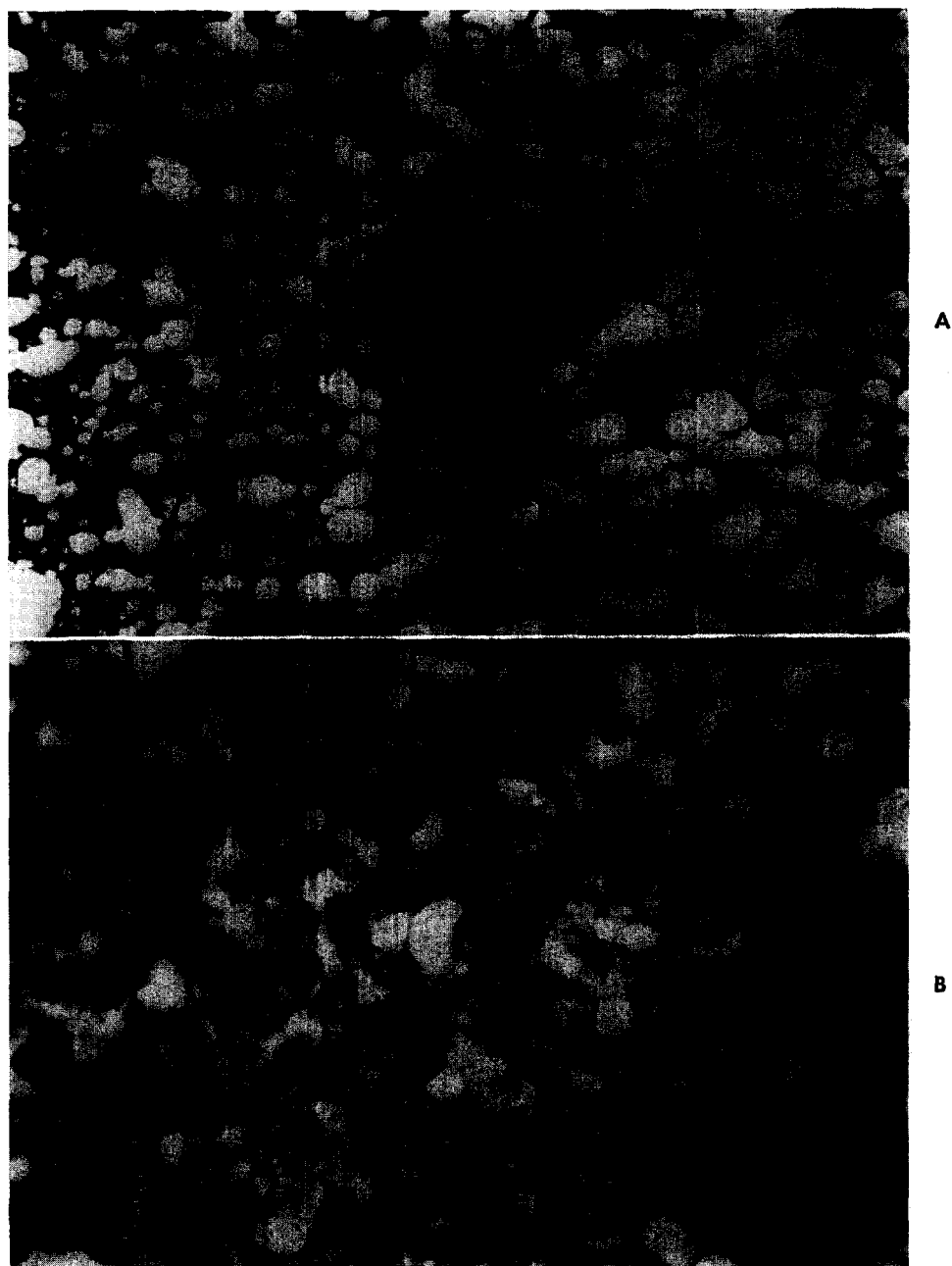


FIG. 9. Photomicrographs of the lungs 48 hours after the inhalation of Prot. Note the intensive perivascular and peribronchial reaction composed of mononuclear and polymorphonuclear cells, mostly lymphocytes with minor numbers of macrophages and moderate numbers of eosinophilic polymorphs, infiltrating the adjacent interstitial tissue. (A, X40.) (B, X400.)

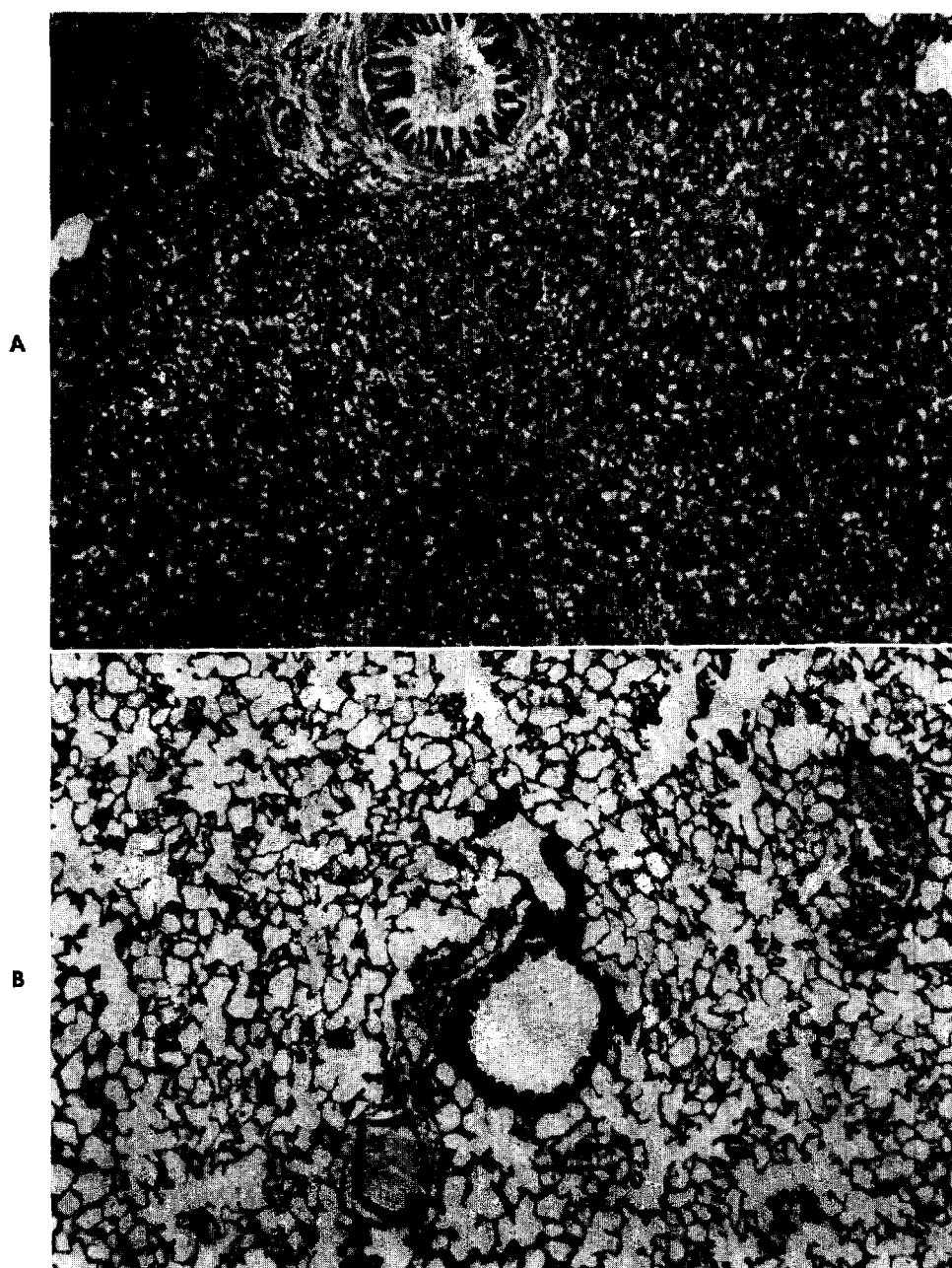


FIG. 10. Photomicrographs of the lungs of a guinea pig with hypersensitivity to crude extract (A) and a guinea pig without immunization (B). Lungs 48 hours after exposure to the extracts of *C. albicans* show lymphocytic infiltration into peribronchial and perivascular tissue in sensitized pig and no remarkable change in unsensitized control pig. (X40.)

rabbits immunized with *C. albicans*. In the experiments in this report, the skin of the pigs which had received two injections of 10 mg. of *C. albicans* reacted in the same manner as those in the above studies to intracutaneous injections of 10 μ g of PS and 3 μ g Prot.

In our study¹³ of the allergic reactions of the respiratory tract of guinea pigs sensitized with egg albumin, it was found that respiratory conductance of the animals decreased immediately after exposure to the aerosolized antigens. Also, groups of guinea pigs sensitized passively with guinea pig anti-egg albumin serum or the γ_1 globulin fraction thereof reacted as did the pigs which were actively immunized. The decreased conductance returned to normal in all animals in one hour after exposure. In the guinea pigs which were challenged shortly after the sensitization with the γ_2 globulin fraction, the decrease in conductance lasted for 2 to 4 hours. There was no definite change in respiratory frequency in any of the above animals. Control exposure of nonsensitized animals gave little change in respiratory conductance and frequency. Kemme and Kuijpers¹⁴ reported tachypnea in guinea pigs sensitized with tuberculin after inhalation of tuberculin and attributed this phenomenon to a delayed-type allergic reaction on the basis of histologic investigation. This tachypnea was not inhibited by prednisolone given intramuscularly in doses of 0.5 mg. per 100 Gm. of body weight 48 hours, 24 hours, and 4 hours before and 20 hours after exposure or by dibenzheptopene (Brontine) given subcutaneously in doses of 0.06 and 0.6 mg. per 100 Gm. of body weight 30 minutes before and 20 hours after challenge. In our previous study^{13, 15} of guinea pigs immunized with killed tubercle bacilli in paraffin-oil, an immediate decrease in respiratory conductance was noted in some of the pigs which were exposed to old tuberculin spray but in none exposed to purified protein derivative (PPD). At 24 hours after the exposure, all these animals exhibited a respiratory frequency 1.5 to 2 times higher than that before treatment, and this tachypnea was not accompanied by any change in respiratory conductance. The tachypnea was not noted after old tuberculin inhalation in the control animals. This reaction of a delayed type was not transferred by the serum of the immunized animals, but it was accomplished with cells from the spleen and lymph nodes of the animals. Histologic examination of the lungs of the sensitive guinea pigs exposed to tuberculin or PPD by inhalation showed a distinctive picture of large collections of mononuclear cells with a moderate number of eosinophils in peribronchial and perivascular areas and extending into the interstitial tissues. These changes are similar to those seen in the present investigation after the exposure to Prot or crude extracts of *C. albicans* and also resemble findings previously presented by Pernis¹⁶ concerning delayed hypersensitivity in guinea pigs sensitized with ovalbumin. From these observations it was concluded that the immediate allergic reaction in the respiratory tract of guinea pigs elicited a decrease in conductance due to bronchospasm, and 24 hours later the delayed reaction evoked tachypnea caused by exudation, edema, and cell infiltrations in the lungs.

In this report, guinea pigs were exposed to the aerosols of PS, Prot, or crude extract of *C. albicans* after sensitization by intraperitoneal injections of the organism. The reactions of the respiratory tract in the animals following ex-

posure to PS were an immediate decrease in respiratory conductance and those after challenge by Prot or crude extract were dual reactions of an immediate decrease in conductance and a delayed increase in respiratory frequency. The results seem to have demonstrated that the allergens in extracts of *C. albicans* can be divided into two groups, that is, PS elicits an allergic reaction of the immediate type and Prot provokes dual reactions of immediate and delayed types in the respiratory tract as well as in the skin of the guinea pigs sensitive to the organism.

In some of the animals exposed to the crude extracts the conductance decreased immediately after the challenge, returned to normal levels in one hour, and again fell 3 to 4 hours later. It is interesting that this phenomenon of an intermediate reaction occurring at 3-4 hours after the challenge was also seen in patients with asthma on skin and inhalation tests with the extracts of *C. albicans*.^{5, 17} The time of the onset suggested an Arthus phenomenon. A detailed report on this subject will be published elsewhere.

The immediate decrease in conductance after exposure to PS, Prot, or crude extract seemed to be due to airway constriction. Constriction of bronchi and bronchioles and hyperinflation were conspicuous after intravenous injection of PS into sensitized animals. This hyperinflation still remained in slight degree in one of 3 animals at 48 hours after exposure by inhalation. The delayed tachypnea without change in conductance following exposure to Prot or crude extract requires explanation. Decrease in lung compliance due to cell infiltrations in interstitial tissues and alveolar spaces, fever due to inflammation, and hypoxemia are all probable causes of this reaction. It is of particular interest that this phenomenon might provide a better understanding of immunological mechanisms in human asthma. Alteration in arterial PO_2 without change in PCO_2 demonstrated in this experiment would help to define the functional nature of the pulmonary insufficiency. However, further study would be necessary before a more definite conclusion could be drawn from these observations.

The authors thank Dr. Y. Oshima for his encouragement and guidance in this work and for reviewing the manuscript. We also wish to acknowledge the technical assistance of Miss Tsuneko Saito, Miss Hifumi Ikuma, and Miss Miyuki Mizunaga.

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