HYPERSENSITIVENESS TO SOLUBLE SPECIFIC SUBSTANCES FROM YEAST-LIKE FUNGI

I. Anaphylaxis

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A previous report (1) from this laboratory was concerned with the preparation and properties of a water-soluble fraction, essentially a polysaccharide, from each of five yeast-like fungi. Tested by direct precipitation against the corresponding antisera these polysaccharides exhibited only partial specificity, cross-precipitin reactions being frequent. By absorption of precipitin on the intact mycotic bodies, however, a relatively high degree of specific precipitability was demonstrated for the soluble substances.

The present paper deals with (1) an attempt to induce active sensitization to one of these soluble substances by injection (*a*) of the substance itself, (*b*) of the killed homologous organism; (2) the production with the several fractions of anaphylactic shock in guinea pigs passively sensitized with homologous fungus antiserum; and (3) a comparison of the specificity of the anaphylactic phenomenon with that of the precipitin reaction.

Tomcsik (2), using a soluble specific carbohydrate substance obtained from *B. lactis aerogenes*, induced fatal anaphylactic shock with intravenous doses as small as 0.033 mg. in guinea pigs passively sensitized with homologous rabbit antiserum. Tomcsik and Kurotchkin (3) subsequently reported similar results with carbohydrate haptens from *B. lactis aerogenes*, the pneumobacillus, and a yeast. Attempts to produce active sensitization against the *B. lactis aerogenes* specific substance by repeated inoculation of living and dead bacteria were unsuccessful. Lancefield (4) produced fatal shock in guinea pigs passively sensitized with antistreptococcus serum using a carbohydrate fraction isolated from strepto-

cocci. Inasmuch as all of these fractions contained small amounts of nitrogen it could not be stated definitely that anaphylactic shock had been produced by carbohydrate hapten alone. Similar results, however, were obtained by Avery and Tillett (5) who injected into passively sensitized guinea pigs protein-free specific carbohydrates of Pneumococcus I, II, and III. (The fractions from Types II and III were nitrogen-free as well.) The anaphylactic reaction was type-specific. Attempts to induce active sensitization by injection of the polysaccharides were uniformly negative. Using a specific carbohydrate obtained from the tubercle bacillus and containing 0.3 per cent nitrogen, Enders (6) obtained lethal anaphylactic shock in passively sensitized guinea pigs and also in guinea pigs actively sensitized with dead tubercle bacilli. The minimum lethal dose of carbohydrate was 0.5 mg. for the passively sensitized animals and 2 mg. for the actively sensitized. While the present work was in progress Kurotchkin and Lim (7) reported the production of anaphylactic shock in guinea pigs actively sensitized to *Monilia pinoyi* and *M. psilosis*. Fatal shock followed the injection of 1 mg. of *M. pinoyi* soluble specific substance into guinea pigs actively sensitized with the homologous organism. Sublethal shock was obtained in the case of *M. psilosis* with a dose of 2 mg. of the corresponding substance.

EXPERIMENTAL

Material Employed.—The organisms are those used previously (1), namely: (1) A *Monilia albicans* isolated from an interdigital erosion and identical culturally and morphologically with *Monilia psilosis*. (2) *Monilia psilosis*, Ashford. (3) A monilia similar to *M. parapsilosis*. (4) A strain of *Willia anomala*. (5) A stock strain of *Saccharomyces cerevisiae*.[1]

Antisera were prepared in rabbits by intravenous injections of heat-killed organisms grown in Sabouraud's honey broth. The sera in dilutions of 1:500 agglutinated the homologous organisms, and in addition a number of cross-agglutinations were evident (1).

A water-soluble fraction was prepared from each of the organisms. Protein tests on these fractions are doubtful or negative (1), although small amounts of nitrogen are present[2] (Table I), but they react strongly to the Molisch test, yield reducing sugar on hydrolysis, and are precipitated in high dilution by the homologous antisera. Quantitative solutions of the fractions were made up in sterile salt solution.

[1] We are indebted to Miss R. W. Benham of the Medical Mycology Laboratory of the Department of Dermatology, College of Physicians and Surgeons, Columbia University, for the original cultures and for numerous helpful suggestions.

[2] Dumas micro nitrogen determinations were kindly done by the Department of Biochemistry, College of Physicians and Surgeons, Columbia University.

Active Sensitization.—A. An attempt was made to actively sensitize animals by injection of one of the soluble substances.

Using Holobut's method (8), guinea pigs of about 250 gm. weight were given on 10 consecutive days intraperitoneal injections of 10 mg. of the soluble substance from *Monilia psilosis*. 21 days after the last injection the animals were tested by intravenous, intracardiac, or intraperitoneal injection of from 0.25 to 4 mg. of the same soluble substance.

TABLE I

Properties of Polysaccharide Fractions

Soluble substance from organism	N	Highest dilution giving Molisch reaction	Highest dilution giving precipitate with serum of rabbit immunized against homologous organism
	*per cent**		
1. *Monilia albicans*	0.85	1:1,000,000	1:1,000,000
2. *Monilia psilosis*	0.84	1:1,000,000	1:1,000,000
3. *Monilia parapsilosis*	0.65	1:1,000,000	1:1,000,000
4. *Willia anomala*	1.48	1:1,000,000	1:1,000,000
5. *Saccharomyces cerevisiae*	1.78	1:1,000,000	1:1,000,000

* Substances dried over sulfuric acid, analyzed by Dumas micro method.

TABLE II

Absence of Active Anaphylaxis to Monilia psilosis Soluble Substance in Guinea Pigs Sensitized with the Soluble Substance

Guinea pig No.	Sensitizing dose *M. psilosis* polysaccharide, i.p.	Interval	Shocking dose *M. psilosis* polysaccharide	Result
		days	*mg.*	
83	10 daily 10 mg. each	21	2.0 i.c.	No effect
84	10 " 10 " "	21	2.0 i.v.	" "
85	10 " 10 " "	21	4.0 i.p.	" "
86	10 " 10 " "	21	0.25 i.v.	" "

The data are presented in Table II. Although the number of animals was limited because of the difficulty in obtaining the polysaccharide in quantity, the failure of any animal to react following the injection of the soluble substance indicates a lack of sensitivity, and one may tentatively conclude that the soluble substance is non-

antigenic when used in this way. It is possible that a single sensitizing dose would have been more effective, but Avery and Tillett (5) were unable to induce active sensitization in this fashion with pneumococcus polysaccharides.

B. A similar attempt to induce active sensitization to the soluble substance was carried out, using killed monilia bodies as the sensitizer.

250 gm. guinea pigs were given 10 consecutive daily intraperitoneal injections of 0.2 cc. of a 5 per cent suspension of heat-killed washed *Monilia psilosis* organisms grown 72 hours in Sabouraud's broth. (The suspension consisted almost entirely of budding forms with relatively few mycelia.) 21 days after the last injection the animals were given an intravenous or intracardiac shocking dose of from 0.5 to 2 mg. of the homologous soluble substance. Three animals of the series were given a suspension of ground or whole monilia bodies in a dose representing approximately 2 mg. of polysaccharide.

The results are presented in Table III. Of the eight animals given polysaccharide in the series, all but one showed evidences of anaphylactic shock immediately following the shocking injection. Two of them died an anaphylactic death and at autopsy showed the characteristic marked pulmonary emphysema associated with bronchial spasm. The three animals given a shocking dose of monilia bodies also showed symptoms of anaphylactic shock, and one died.

These findings confirm and extend those of Kurotchkin and Lim (7), who recently produced sublethal shock by intravenous administration of a *Monilia psilosis* soluble substance in guinea pigs previously sensitized with the dead fungi. Their inability to produce fatal shock may have been based on a lower degree of sensitization due to less intensive preliminary dosage, or on a less potent soluble fraction.

The possibility that the shock is caused by protein contamination of the polysaccharide fraction is remote. Unless all the nitrogen present, 0.84 per cent, represents protein nitrogen (an improbable assumption in the light of the negative protein tests), the amount of protein in a 2 mg. dose of soluble substance would be rather less than the minimum lethal anaphylactic dose of purified protein, 0.05 or 0.1 mg., as given by Wells (9). The results in passively sensitized animals provide further evidence in favor of the conclusion that the shock is induced by polysaccharide independent of possible protein contaminant.

Passive Sensitization.—As already reported (1), the rabbit antisera prepared against each of the five yeast-like organisms contain precipitins for the homologous soluble substance, and also for one or more of the heterologous substances. Separation of the precipitins can be

TABLE III

Active Anaphylaxis to Monilia psilosis Soluble Substance in Guinea Pigs Sensitized with Monilia Organisms

Guinea pig No.	Sensitizing dose killed *M. psilosis* i.p.	Interval	Shocking dose *M. psilosis* polysaccharide i.v. or i.c.	Result	Autopsy
		days			
54	10 daily 0.2 cc. 5% suspension	21	0.5 mg. i.v.	Cough, dyspnea, convulsions	—
55	" "	21	1.0 " "	Fur ruffled, cough, scratches nose	—
57	" "	21	2.0 " "	Fur ruffled, cough, dyspnea	—
75	" "	21	1.0 " "	Cough, dyspnea, restlessness, urination, defecation, ataxia	—
76	" "	21	1.0 " i.c.	No effect	—
77	" "	21	2.0 " i.v.	† 4 min.	Typical
78	" "	21	0.5 " "	Cough, marked dyspnea, ataxia, inability to stand	—
79	" "	21	2.0 " "	† 3½ min.	Typical
80	" "	21	0.2 cc. 20% suspension ground *M. psilosis* i.v.	Cough, dyspnea, defecation, pulmonary edema	—
81	" "	21	" "	† 4 min.	Typical
82	" "	21	0.4 cc. 10% suspension whole *M. psilosis*, i.v.	Dyspnea, urination, defecation	—

effected by absorption with the organisms. It was deemed of interest to sensitize animals passively with each of the five antisera and to investigate the ability of the several polysaccharide fractions to induce anaphylactic shock. To test each soluble fraction against each antiserum involved, accordingly, the use of twenty-five series of guinea pigs. A complete protocol of one series follows.

Guinea pigs of approximately 250 gm. weight were sensitized by intraperitoneal injections of 0.5 or 1.0 cc. of serum from a rabbit immunized against *Monilia psilosis*. 24 hours later each pig received 0.01 to 1.0 mg. of the homologous soluble substance intravenously or intracardially. Control animals were injected with normal rabbit serum and tested with the same soluble substance.

The results appear in Table IV. Of the eight animals sensitized with *Monilia psilosis* antiserum, six died in anaphylactic shock in 3½

TABLE IV

Passive Anaphylaxis to Monilia psilosis Soluble Substance in Guinea Pigs Sensitized with Monilia psilosis Rabbit Antiserum

Guinea pig No.	Sensitizing dose monilia antiserum i.p.	Interval	Shocking dose *M. psilosis* polysaccharide i.v. or i.c.	Result	Autopsy
	cc.	*hrs.*	*mg.*		
146	1.0	24	1.0 i.v.	† 5 min.	Typical
149	1.0	24	0.75 "	† 4 "	"
148	1.0	24	0.25 "	Cough, snuffles, dyspnea, fur ruffled, legs weak	—
60	0.5	24	0.2 "	† 3½ min.	Typical
17	0.5	24	0.1 i.c.	† 4 "	"
204	0.5	24	0.05 i.v.	† 3½ "	"
63	0.5	24	0.01 "	† 3½ "	"
62	0.5	24	0.01 "	Cough, fur ruffled, bucking, dyspnea, urination, defecation	—
	Normal serum				
73	0.75	24	2.5 i.c.	No effect	—
50	0.5	24	3.0 "	" "	—
37	0.5	24	2.0 i.v.	" "	—
36	0.5	24	0.5 i.c.	" "	—
93	0.5	24	0.1 i.v.	" "	—
213	0.5	24	0.1 "	" "	—

to 5 minutes. At autopsy they showed the characteristic marked pulmonary emphysema. The other two animals exhibited definite symptoms of shock, including coughing, bucking, scratching of nose, ruffling of fur, dyspnea with deep abdominal breathing, urination, and defecation. When retested the following day, they were antianaphylactic. Control animals were symptom-free. The minimum lethal

dose of soluble substance was 0.01 mg. Contaminating protein as a possible factor in the production of shock can be definitely excluded, as this dose of soluble substance is less than the usual minimum lethal anaphylactic dose of pure protein (0.1 or 0.05 mg.).

A similar series of guinea pigs was sensitized to each of the five antisera and tested 24 hours later with the homologous soluble substance. Further, animals sensitized with each antiserum were tested with each of the four heterologous soluble substances.

The dose of sensitizing serum was 0.5 cc. in all but an occasional instance when 1.0 cc. was given. The shocking dose of soluble substance varied from 0.01 to 2.5 mg. Any animal which did not show evidence of shock following heterologous soluble substance was retested, usually on the same day, with the homologous, in order to be certain that sensitization had been effective. An occasional such animal failed to react to the homologous substance and was rejected from the series. Occasionally, also, an animal given the shocking dose into the heart died with a hemopericardium. Such an animal was also rejected. Most of the injections of soluble substance were given into a superficial vein of a hind leg.

Table V contains in abridged form the data of these experiments. Anaphylactic death was regularly obtained in animals sensitized with each of the five antisera and shocked with the respective homologous soluble substance. The minimum lethal dose of soluble substance was 0.1 mg. or less. The table includes the results of retesting with homologous substance on the same day those animals negative to heterologous substance. Most of the animals that recovered from shock were also retested a day or two later and found to be refractory.

As Table V indicates, in many instances a heterologous soluble substance also induced anaphylactic shock in a sensitized animal. This is more readily appreciated from Table VI, columns A, which are a summary of Table V, each of the twenty-five sets of animals being recorded as a single result. Because the minimum lethal dose of homologous substance was 0.1 mg. or less, the results are indicated as ++, representing series in which 0.1 mg. or less induced lethal shock, or + for series in which more than 0.1 mg. of soluble substance was required to kill.

In view of the more or less generally accepted identity of precipitin and sensitizing antibody (10) it is of interest to compare the above passive anaphylactic reactions with the precipitin potency of the anti-

TABLE V

Anaphylactic Reactions to Fungus Soluble Substances in Guinea

24 hrs. later. Shocking dose of soluble substance prepared from	Guinea pigs sensitized by intraperitonea		
	1. *Monilia albicans*	2. *Monilia psilosis*	3. *Mon*
1. *Monilia albicans*	0.05 mg. ++ 0.05 mg. ++ 0.03 mg. ++ 0.02 mg. ++	1.0 mg. ++ 0.1 mg. ++ 0.05 mg. ++ 0.05 mg. + 0.05 mg. +	2.0 mg. ++ 0.5 mg. ++ 0.1 mg. ++ 0.05 mg. −0
2. *Monilia psilosis*	1.0 mg. ++ 0.1 mg. ++ 0.05 mg. ++ 0.05 mg. + 0.02 mg. +	1.0 mg. ++ 0.75 mg. ++ 0.25 mg. + 0.2 mg. ++ 0.1 mg. ++ 0.05 mg. ++ 0.01 mg. ++ 0.01 mg. +	2.0 mg. ++ 1.0 mg. ++ 0.75 mg. + 0.45 mg. ++ 0.15 mg. + 0.15 mg. −0
3. *Monilia parapsilosis*	0.05 mg. ++ 0.05 mg. ++ 0.02 mg. +	3.0 mg. ? / 0.075 mg. H ++ 2.5 mg. −0 / 0.075 mg. H ++ 1.0 mg. ? / 0.1 mg. H ++ 1.0 mg. −0 / 0.75 mg. H ++	1.0 mg. ++ 0.07 mg. ++ 0.05 mg. ++
4. *Willia anomala*	2.5 mg. −0 / 0.05 mg. H ++ 0.5 mg. −0 / 0.05 mg. H +	2.0 mg. −0 / 0.1 mg. H ++ 1.0 mg. −0 / 0.03 mg. H ++ 0.5 mg. −0 / 0.05 mg. H ++	4.0 mg. −0 2.0 mg. −0? 1.0 mg. −0
5. *Saccharomyces cerevisiae*	2.0 mg. −0 / 0.05 mg. H + 1.0 mg. −0 / 0.1 mg. H ++ 0.5 mg. −0 / 0.05 mg. H ++	2.0 mg. −0 / 0.05 mg. H ++ 1.0 mg. −0 / 0.05 mg. H ++ 0.5 mg. −0 / 0.075 mg. H ++	2.0 mg. + 1.0 mg. ++ 0.5 mg. + 0.1 mg. −0 0.05 mg. −0

++ = Anaphylactic death in 3 to 5 minutes.
+ = Sublethal anaphylactic reaction.
/ = Retested but with homologous (H) soluble substance.
* = Retested 3rd day after sensitization.

TABLE V

nces in Guinea Pigs Passively Sensitized with Fungus Antiserum

ized by intraperitoneal injection of rabbit antiserum prepared against

3. *Monilia parapsilosis*	4. *Willia anomala*	5. *Saccharomyces cerevisiae*
2.0 mg. ++ 0.5 mg. ++ 0.1 mg. ++ 0.05 mg. −0 / 0.05 mg. H ++	2.0 mg. −0 / 0.25 mg. H* + 0.5 mg. −0 / 0.25 mg. H* ++	1.5 mg. ++ 0.5 mg. −0 / 0.2 mg. H + 0.1 mg. −0 / 0.2 mg. H +
2.0 mg. ++ 1.0 mg. ++ 0.75 mg. + 0.45 mg. ++ 0.15 mg. + 0.15 mg. −0 / 0.05 mg. H +	2.0 mg. −0 / 0.25 mg. H* ++ 1.0 mg. −0 / 0.1 mg. H + 0.5 mg. −0 / 0.25 mg. H* +	2.5 mg. −0 / 0.2 mg. H + 1.0 mg. −0 / 0.25 mg. H ++ 0.1 mg. −0 / 0.25 mg. H ++ 0.1 mg. −0 / 0.1 mg. H ++
1.0 mg. ++ 0.07 mg. ++ 0.05 mg. ++	2.0 mg. −0 / 0.1 mg. H ++ 0.5 mg. −0 / 0.15 mg. H* +	2.0 mg. −0 / 0.2 mg. H + 0.1 mg. −0 / 0.25 mg. H ++
4.0 mg. −0 / 0.05 mg. H ++ 2.0 mg. −0? / 0.05 mg. H + 1.0 mg. −0 / 0.05 mg. H ++	0.1 mg. ++ 0.1 mg. ++ 0.05 mg. + 0.05 mg. + 0.03 mg. +	0.5 mg. ++ 0.2 mg. + 0.1 mg. +
2.0 mg. + 1.0 mg. ++ 0.5 mg. + 0.1 mg. −0 / 0.03 mg. H ++ 0.05 mg. −0 / 0.05 mg. H +	2.0 mg. − 0 / 0.15 mg. H + 1.0 mg. ? / 0.1 mg. H −0 1.0 mg. ? 0.5 mg. −0 / 0.1 mg. H + 0.5 mg. −0 / 0.15 mg. H +	2.0 mg. ++ 1.0 mg. ++ 0.25 mg. ++ 0.1 mg. ++ 0.1 mg. + 0.05 mg. +.

sera used to sensitize the animals. Columns B, Table VI, comprise a summary of the titration of precipitins in each of the sera against each of the soluble substances. The result in each instance is arbitrarily indicated as ++ when a definite precipitin ring was formed between the serum (diluted 1:1) and a 1:1,000,000 or 1:100,000 solution of the soluble substance, and as + when a precipitate formed with a 1:10,000 or 1:1,000 dilution of soluble substance. Comparison of these results

TABLE VI

Comparison of Passive Anaphylaxis with Precipitin Reactions Using Fungus Antisera and Soluble Substances

Soluble substance from organism	*M. albicans* antiserum		*M. psilosis* antiserum		*M. parapsilosis* antiserum		*Willia anomala* antiserum		*Saccharomyces cerevisiae*	
	A. Passive anaphylaxis	*B.* Precipitin titer	*A.* Passive anaphylaxis	*B.* Precipitin titer	*A.* Passive anaphylaxis	*B.* Precipitin titer	*A.* Passive anaphylaxis	*B.* Precipitin titer	*A.* Passive anaphylaxis	*B.* Precipitin titer
1. *M. albicans*	++	++	++	++	++	+	0	0	+	+
2. *M. psilosis*	++	++	++	++	+	+	0	0	0	0
3. *M. parapsilosis*	++	++	0	0	++	++	0	0	0	0
4. *Willia anomala*	0	0	0	0	0	0	++	++	+	++
5. *Saccharomyces cerevisiae*	0	0	0	0	+	+	0?	+	++	++

++ = *A.* 0.1 mg. or less of soluble substance killed, *or*
B. Precipitin ring with 1:1,000,000 or 1:100,000 dilution of soluble substance.

+ = *A.* Required more than 0.1 mg. to kill, *or*
B. Precipitin ring with 1:10,000 or 1:1,000 dilution of soluble substance.

with the anaphylactic response shows a relatively close correspondence between the two phenomena.

DISCUSSION

Although it has not been possible to sensitize actively a limited series of guinea pigs to the soluble polysaccharide-containing fraction of *Monilia psilosis* by repeated injection of the soluble substance, such a hypersensitivity can be demonstrated after repeated injection of the killed organisms. As little as 2 mg. of the soluble substance adminis-

tered intravenously precipitates fatal anaphylactic shock Furthermore, a high degree of hypersensitivity to the polysaccharide fraction of each of the five yeast-like fungi studied can be readily induced by passive sensitization with rabbit antiserum. From 0.01 to 0.1 mg. of soluble substance induces fatal anaphylactic shock in such passively sensitized guinea pigs. The fractions are apparently non-toxic to non-sensitized guinea pigs. Even if all of the trace of nitrogen present in the material represented protein, which is improbable in the light of the negative protein tests, the amount of protein so administered would be too small to produce anaphylactic death.

Cross-anaphylactic reactions occur between the several antisera and soluble substances, requiring in some cases a larger dose of heterologous soluble substance to kill than of homologous. In other words, the reactions are not highly specific, which is in contrast to the pneumococci (5). The ability of a given serum to passively sensitize a guinea pig to the several polysaccharide fractions parallels the precipitin content of the serum. There are minor quantitative differences but not sufficiently significant to throw doubt upon the identity of the precipitin and the sensitizing antibody.

Accordingly the method of passive anaphylaxis would appear to be of no additional assistance in distinguishing between the yeast-like fungi. It is no more specific than the precipitin or agglutinin reactions. The method of absorption of agglutinin or of precipitin (1) remains the most highly specific of those used.

SUMMARY

1. The polysaccharide fractions from each of five yeast-like fungi produce rapid, fatal anaphylactic shock in guinea pigs passively sensitized with antiserum from rabbits immunized against the killed organisms.

2. Cross-anaphylactic reactions with heterologous polysaccharide fractions are frequent. They parallel closely the cross-precipitin reactions, thus adding evidence in favor of the identity of precipitin and sensitizing antibody.

3. The polysaccharide fraction from *Monilia psilosis* produces anaphylactic death in guinea pigs actively sensitized with killed homologous organisms, but an attempt to sensitize actively with the polysaccharide fraction was unsuccessful.

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