

HYPERSENSITIVENESS TO SOLUBLE SPECIFIC SUBSTANCES FROM YEAST-LIKE FUNGI

II. EYE HYPERSENSITIVITY

By E. MOTT, M.D., AND H. D. KESTEN, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York)

(Received for publication, March 17, 1931)

The precipitability by antisera of carbohydrate fractions prepared from several of the yeast-like fungi as described in a previous report from this laboratory (1), and the ability of these fractions to induce anaphylactic death in sensitized animals as reported in the preceding paper (2), are to be looked upon as examples of immune reactions with non-antigenic haptens. As already briefly reported (3), another reaction of the same type consists in eliciting an inflammatory response in a previously locally sensitized eye by subsequent intravenous administration of the carbohydrate fraction derived from the sensitizing organism.

In various studies of sympathetic ophthalmia, attempts have been made to produce an inflammatory reaction in a previously locally sensitized eye, as well as the opposite eye, by systemic administration of the homologous antigen. Kimmel (4) injected serum or uveal emulsion into the vitreous humor of one eye and subsequently reactivated the eye by subcutaneous or intravenous injection of the same antigen. Fuchs and Meller (5) reported the production of an iritis by intravenous injection of human serum into an animal sensitized intraocularly 35 days previously. The reaction took place, however, in only one animal of the series used. Schoenberg (6) injected human serum into the anterior chamber of rabbits, followed in 2 weeks by intravenous injection of the same antigen. This, as well as a similar experiment using tuberculin, was negative. Von Szily (7), working with Arisawa, injected foreign serum between the layers of the rabbit's cornea. Later, with all inflammation absent, intravenous injection of the same antigen caused an "anaphylactic keratitis" in the sensitized eye. Subsequently however, von Szily (8) injected relatively pure pigment from the uveal tract of cattle into the vitreous humor of rabbits. Intravenous injection of large amounts of the same pigment 3 weeks later was without effect. A similar experiment by

Woods (9) on dogs was successful. An inflammatory reaction was observed not only in the previously sensitized eye but in the opposite eye as well. A similar bilateral eye reaction following intravenous injection of horse serum was noted by Riehm (10) in rabbits previously given horse serum into the conjunctival sac or anterior chamber. In their studies of local organ hypersensitiveness Seegal and Seegal (11) injected into the anterior chamber of the rabbit eye such antigens as guinea pig erythrocytes and egg albumen. Intravenous injection of the homologous antigen 13 days later provoked conjunctival and ciliary hyperemia with slight chemosis and moderate lacrimation in the sensitized eye. They were able to elicit the reaction, though less intensely, as long as 8 months after sensitization.

EXPERIMENTAL

The substances heretofore used to sensitize and reactivate the eye have been protein in nature. Similar experiments are reported here, using one of the soluble specific substances or polysaccharides obtainable from microorganisms. Since the uncombined polysaccharides have been found to be non-antigenic, the organism itself was used to sensitize the eye, and the homologous soluble substance was subsequently given intravenously as the reactivator. The organism used was the yeast-like fungus, *Monilia psilosis* Ashford, from which had been prepared a fraction (1) appearing to be essentially a polysaccharide.

A monilia suspension was prepared by washing the organisms obtained from a honey agar (Sabouraud) culture, and heating them 1 hour at 56°C. For some of the injections the suspension was made with heat-killed organisms which had been frozen with carbon dioxide snow and ground until many had become fragmented. The usual strength of the suspension was 10 per cent by volume of moist packed organisms. Under cocain anesthesia a small amount of aqueous humor was removed with a needle and syringe from the anterior chamber of one eye of each of twelve rabbits.¹ Without removing the needle (which had been inserted obliquely through the cornea just anterior to the limbus), a somewhat smaller volume of monilia suspension was injected into the anterior chamber. Control animals were given sterile saline or a 5 per cent saline solution of the monilia soluble substance. For a few days after the injection of the monilia the eye was usually moderately inflamed, and in some cases injected material could be seen in the anterior chamber for as long as 10 days. The reaction was less marked and usually of only 1 day's duration in the rabbits receiving saline or soluble substance.

¹ Dr. A. L. Morgan of the Department of Ophthalmology, Presbyterian Hospital, New York City, very kindly instructed us in the eye technique and assisted with some of the clinical observations.

Except for a small corneal opacity at the site of injection, with often a conspicuous vessel in the adjacent conjunctiva, and a rare posterior synechia, the eye ultimately returned to normal. At various intervals (2 weeks to 8½ months) after

TABLE I

Reactivation of Rabbit Eye by Intravenous Administration of Monilia Soluble Substance at Various Intervals after Local Sensitization with Suspension of Monilia Organisms

Rabbit No.	Anterior chamber of one eye injected with	Eye reaction 5 to 7 hrs. after 50 mg. soluble substance injected intravenously				
		2 to 4 wks.	2 mos.	4 mos.	4½ mos.	8½ mos.
1	0.05 cc., 20% monilia*	0		+		0(b)
3	0.05 " 5% "	0#	0	0		0
5	0.05 " 10% "	0	0	+		0(b)
24	0.1 " 1% "*"	0		+		0
25	0.1 " 0.05% "*"	0		0		0
38	0.03 " 10% "	0	0	0	0	
39	0.05 " 10% "			0	0	
40	0.05 " 10% "	0	0	0(b)	0	
41	0.05 " 10% "			0#	+	
42	0.05 " 10% "		+(c)	+	+	
43	0.05 " 10% "*"			0(b)	0	
45	0.05 " 10% "*"			0#	0	
2	5 mg. soluble substance	0	0(a)			
35	5 " " "	0	0	0	0	
37	4 " " "	0	0	0#	0	
4	0.1 cc. saline	0	0(a)			
34	0.1 " "			0		
36	0.1 " "			0		

* Ground monilia used instead of intact organisms.

1 cc. 10 per cent suspension ground monilia given intravenously instead of soluble substance.

(a) 1 mg. of soluble substance instead of 50 mg.

(b) 10 " " " " " " 50 "

(c) 25 " " " " " " 50 "

the original injection, monilia soluble substance, usually 50 mg. in 5 per cent solution, was injected into an ear vein. A smaller dose was used in a few instances, and in four cases ground monilia organisms were injected. Both eyes of each animal were examined at intervals after the injection. A slight increase in visi-

bility of the conjunctival vessels was common, particularly in the albino rabbits. This was presumably due to handling and was ignored. When a positive reaction occurred, it reached a maximum in 5 to 7 hours after the intravenous injection of soluble substance and was characterized by marked hyperemia of the circumcorneal conjunctival vessels and often of the vessels of the iris and nictitating membrane.

It was soon obvious that the usual incubation period of 2 weeks was not sufficiently long for reactivation of the eye to be successful, the first positive eye reaction being noted at the end of 2 months. Tested 4 or 4½ months after the original eye injection, five of the twelve rabbits exhibited a positive reaction in the sensitized eye (Table I). The opposite eye was invariably negative and served as a control. Of these five animals one was positive as well at the end of 2 months and of 4 months. Control animals were negative, as were also those rabbits sensitized with monilia but given monilia bodies intravenously instead of soluble substance. One of the latter exhibited a definite eye reaction 2 weeks later when given soluble substance. Five animals tested at 8½ months were negative, although three of these had yielded good reactions at 4 months. No difference was noted between rabbits sensitized with whole and with ground monilia.

One-fourth of the experimental group and one-third of the control animals were albino. Of probably no significance except as a matter of coincidence was the observation that all of the reactors were pigmented animals. The primary inflammatory reaction, however, was, on the whole, more marked and of longer duration in the albino animals.

DISCUSSION

As already stated, monilia suspension was visible in the anterior chamber after injection, sometimes for as long as 10 days. Although not visible grossly, it is quite possible that antigen remained present locally for some time longer. This probably accounts for the inability to elicit a reaction as early as 2 to 4 weeks after the original injection.

Inasmuch as the polysaccharide fraction used in the work contained, as previously reported (1), a small amount of nitrogen (averaging 0.6 per cent in the samples used), it cannot be stated unqualifiedly that the monilia-sensitized rabbit eye can be reactivated by subsequent intravenous injection of the homologous protein-free polysaccharide. The negative character of protein tests would indicate, however, that very little, if any, of the nitrogen is present as protein. The above results, furthermore, are in harmony with those of Tomcsik (12), Tomcsik and Kurotchkin (13), Lancefield (14), Avery and Tillett (15), and Enders (16), who found that bacterial carbohydrates, nitrogen-free in the case of Types II and III pneumococci, had the property of

producing anaphylactic shock in guinea pigs passively sensitized with rabbit immune serums prepared against the corresponding organisms. Analogous results with monilia soluble substance are presented in the preceding paper (2). Of interest also is the work of Julianelle (17) who was unable to demonstrate eye reactions to pneumococcus soluble specific substance applied to the scarified rabbit cornea following intracutaneous sensitization with killed pneumococci. His procedure is the converse in a sense, of that reported above. We are inclined, therefore, to interpret the eye reaction to the monilia polysaccharide as another manifestation of the ability of a bacterial hapten to elicit an immune reaction in a properly sensitized medium, in this case the actively sensitized eye.

SUMMARY

The anterior chamber of the rabbit eye was sensitized by the local injection of heat-killed *Monilia psilosis*. Subsequent intravenous injection of a polysaccharide fraction prepared from the same organism elicited a reaction in the sensitized eye in five of twelve rabbits.

BIBLIOGRAPHY

1. Kesten, H. D., Cook, D. H., Mott, E., and Jobling, J. W., *J. Exp. Med.*, 1930, **52**, 813.
2. Kesten, H. D., and Mott, E., *J. Exp. Med.*, 1931, **53**, 803.
3. Mott, E., and Kesten, H. D., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 320.
4. Kümmel, R., *Arch. Ophth.*, Leipsic, 1910, **77**, 393; 1911, **79**, 528.
5. Fuchs, A., and Meller, J., *Arch. Ophth.*, Leipsic, 1914, **87**, 280.
6. Schoenberg, M. J., *New York State J. Med.*, 1914, **14**, 493.
7. von Szily, A., *Klin. Monatsbl. Augenheilk.*, 1915, **54**, 1.
8. von Szily, A., *Klin. Monatsbl. Augenheilk.*, 1916, **56**, 197.
9. Woods, A. C., *Arch. Ophth.*, New York, 1918, **47**, 161; *J. Am. Med. Assn.*, 1921, **77**, 1317.
10. Riehm, W., *Arch. Augenheilk.*, 1928, **99**, 438; *Deutsch. med. Woch.*, 1929, **55**, 907.
11. Seegal, D., and Seegal, B. C., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 390.
12. Tomcsik, J., *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 812.
13. Tomcsik, J., and Kurotchkin, T. J., *J. Exp. Med.*, 1928, **47**, 379.
14. Lancefield, R., *J. Exp. Med.*, 1928, **47**, 843.
15. Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49**, 251.
16. Enders, J. F., *J. Exp. Med.*, 1929, **50**, 777.
17. Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 633.