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### Effect of Formaldehyde upon the Sensitizing Property of Monilia.

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In a previous work<sup>1</sup> we were able to show that regularly constant sensitization of guinea pigs could be produced through the inoculations of heat-killed agar cultures of different moniliae. A comparative study of the sensitizing capacity of the *Monilia pinoyi* and *Monilia psilosis* demonstrated that while the former fungus could sensitize guinea pigs even after a single injection of a moderate dose of the culture, it was necessary to use repeated injections of the latter in order to obtain a successful sensitization of the animals. In view of the close serological and morphological relationship between these two species such a difference in the sensitizing capacity was difficult to explain.

On the basis of the above study the suggestion occurred to us that the sensitizing capacity of different organisms such as moniliae may represent an independent function of the fungus protein. If such be the case, it would seem reasonable to expect that this function of the fungus may be diminished or completely destroyed through the use of a suitable procedure. It is known that the addition of formaldehyde to bacterial products, such as toxins, or to bacterial vaccines brings about a considerable diminution or destruction of the

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<sup>1</sup> Kurotchkin, T. J., and Lim, C. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **28**, 223.

toxic principle. Of especial significance is the fact that within certain limits, the removal of the toxic principle by formaldehyde is not associated with the decrease or disappearance of the antigenic property of bacterial toxins or vaccines.

Considering this peculiar action of formaldehyde it seemed of interest to test its effect upon the sensitizing power of the monilia cultures. For our experiment, *Monilia pinoyi*, the agar culture of which possessed a high sensitizing power, was selected. The fungus was grown for 48 hours at 37°C. and then its growth was washed off with normal saline. A sufficient quantity of fungous suspension was thus prepared. The bulk of the suspension was then divided into 6 equal parts. One part was exposed to 75°C. of heat in water bath for 30 minutes and then incubated for 20 days at 37°C. The remaining 5 parts of the monilia suspension received the addition of formaldehyde in concentrations of 1:5, 1:10, 1:50, 1:100, and 1:500 respectively. These were left in the incubator for 10 days and then each suspension was centrifuged, washed with normal saline and resuspended in the same volume of saline as used for the original preparation. With each suspension a series of young guinea pigs of approximately equal weight was sensitized by giving 3 intraperitoneal injections, each consisting of 1 cc. of the respective suspension. One control series of guinea pigs was sensitized by injections of heat-killed monilia suspension prepared from a 48-hour agar growth. Another control series was sensitized by injections of heat-killed monilia suspension kept in incubator for 20 days before use.

TABLE I.  
Showing sensitization of guinea pigs with formalin or heat-killed cultures of *Monilia pinoyi*.

Series	No. of animals	Kind of vaccine used for sensitization Monilia culture treated with formaldehyde	Result
1	3	1:5	No symptoms
	2		Slight shock
	2		Fatal shock
2	9	1:10	No symptoms
	1		Slight shock
3	7	1:50	No symptoms
	3		Moderate shock
4	5	1:100	No symptoms
	1		Slight shock
5	4	1:500	No symptoms
	3		Fatal shock
	6		No symptoms
6	1	Heat-killed and incubated 20 days at 37°C.	Moderate shock
	2		" "
7	10	Heat-killed freshly prepared	Fatal shock

Two weeks after the last injection all animals were tested for hypersensitivity by injecting one or 2 mg. of the specific carbohydrate obtained from *Monilia pinoyi*. The result of the experiment is given in Table I. It will be seen that freshly prepared heat-killed culture of the *Monilia pinoyi* produced sensitization of guinea pigs in all instances. In contrast to this, the heat-killed culture which was kept in the incubator for 20 days before use showed a definitely lower sensitizing capacity, since out of 7 animals only one developed moderate shock. Formalin-treated suspensions gave somewhat variable results. The addition of formaldehyde in a concentration of 1:5 produced no appreciable effect upon the sensitizing power of the monilia culture. With the next concentration of 1:10 a marked diminution of this power occurred. Concentrations of 1:50 and 1:100 have also brought about a definite decrease of the sensitizing power. The result with a concentration of 1:500 was inconclusive in view of the fact that almost 50% of animals were thrown into anaphylactic shock.

In the interpretation of these results it should be borne in mind that the individual response of guinea pigs to the anaphylactic shock may vary. We found, however, that when *Monilia pinoyi* is used as a sensitizing agent, the failure to produce anaphylactic shock through subsequent injection of the specific carbohydrate was quite exceptional, and, therefore, the absence of the reaction in animals cannot be attributed to their individual resistance. From the data in this study, it seems evident that when monilia culture is kept in contact with certain concentrations of formaldehyde ranging from 1:5 to 1:100 its sensitizing power is distinctly diminished. Similar effect has been observed with heat-killed cultures of the same monilia suspended in normal saline and then incubated for some time. From this latter fact, it follows that the sensitizing power of the monilia may be partially destroyed by ageing. It seems, therefore, justifiable to conclude that the effect of formaldehyde upon monilia is comparable in certain respects to its effect upon anatoxins.