ference of apparent cure-rate between bi-weekly and daily injections was found to be statistically significant (44.4 ± 12.5).[5] It appears likely that the decreased effectiveness of neostibosan and solustibosan following daily injections is related to the increased rate of excretion of antimony. Brahmachari[6] has shown that with repeated injections the amount of antimony excreted by the kidneys was fairly proportional to the amount of antimony present in the tissues, and that when the amount of antimony present in the tissues reached a maximum concentration, its excretion suddenly became increased out of proportion to the amount present in the tissues. With daily injections it seems likely that the amount of antimony present in the tissues reaches a state of maximum concentration rather early; otherwise, the cumulative effect of antimony would have manifested itself.

The higher mortality rate in the group of hamsters treated with neostibosan agrees with our previous experience, although the difference may not be significant.

Conclusions. In the treatment of kala-azar in Chinese hamsters it was found that with daily injections of the drugs under investigation, less antimony in the form of solustibosan than that in the form of neostibosan did not produce the same curative effect, as has been claimed by Kikuth and Schmidt, *i. e.*, the curative effect of solustibosan did not increase with the increase of the frequency of injections. 2. Daily injections of either solustibosan or neostibosan appear to be less effective than bi-weekly or tri-weekly injections.

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Sensitizing Capacity of Polysaccharide of *Monilia tropicalis.*

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Active sensitization of guinea pigs has been generally successful with gram-positive bacteria but not with gram-negative ones. Yet under suitable methods of preparation polysaccharides derived from some of the latter microörganisms are capable of inducing a state of

[5] Fortuyn, A. B. D., *China Med. J.*, 1929, **43**, 31.

[6] Brahmachari, U. N., and Sen, P. B., *Ind. J. Med. Res.*, 1924, **12**, 113.

supersensitivity in guinea pigs.[1] From this it would appear that polysaccharides prepared from gram-positive organisms should possess sensitizing property comparable to or better than that of the intact cell if a proper method of extraction is employed. On the other hand it is also conceivable that the sensitizing capacity of gram-positive organisms may represent a unique function of the bacterial protein. A gram-positive yeast-like fungus, *Monilia tropicalis*, was thought especially suitable for this study because previous studies[2] have shown that a small dose of killed culture of this microorganism sensitized guinea pigs regularly.

Monilia tropicalis was grown on Sabouraud's medium at 37°C for 48 hours. The organisms were collected and the polysaccharide was prepared by 1% acetic-acid hydrolysis of the whole organism.[3] The polysaccharide in 1% solution gave none of the usual reactions of protein and contained 1.12% total nitrogen. A 1:10,000 dilution of the soluble antigen gave a strong Molisch reaction.

Preliminary studies were made to determine the antigenic activity of the polysaccharide. Two rabbits received intravenously a total of 9 mg of the substance, the method of immunization being the same as that employed elsewhere.[4] Seven days after the last injection the sera of these animals were examined for precipitin and complement-fixing bodies. It was found that both sera reacted equally with a 1:10,000 dilution of the antigen in the former test and with a 1:100,000 in the latter.

Two series of guinea pigs weighing 250-300 g each were employed for the study of active sensitization. The first series consisting of 9 animals received 2 subcutaneous and 1 intraäbdominal injection of polysaccharide at 5-day intervals. The total dose was 21 mg per animal. The second series consisting of 7 animals received 3 intra-abdominal injections of 1 cc each using the same time interval. The total dose was equal to 1:5 the growth of a 24-hour agar-slant. Three weeks after the last injection all animals were tested intravenously with 5 mg of the polysaccharide. The results, presented in the first 2 columns of Table I, show that 2 of 9 animals sensitized with polysaccharide exhibited non-fatal shock. On the other hand, all animals sensitized with the whole organism reacted to the intravenous injection of 2-5 mg of the polysaccharide, 5 anaphylactic deaths having

[1] Kurotchkin, T. J., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 113.

[2] Lim, C. E., and Kurotchkin, T. J., *Chinese Med. J.*, Suppl. I, 1936, 256.

[3] Wong, Sam C., PROC. SOC. EXP. BIOL., AND MED., 1938, **38**, 107.

[4] Wong, Sam C., and Kurotchkin, T. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 161.

TABLE I.
Presence of Supersensitivity at Various Time Intervals.

First test	No. animals	Second test	No. animals	Third test	No. animals
Severe shock, recovered after		Mod. shock	1	Death	1
		" "	1	Severe shock	1
1½ hr	1	" "	3	" "	3
Moderate shock, recovered after		No response	4	No response	2*
20 minutes	1				
No response	7				

* 2 died of intercurrent infection after the second test.

been recorded. These results were not included in the table, since no further test was made on the 2 surviving animals.

All the guinea pigs of the first series were retested for the return of sensitivity 2 weeks later. It was found that 5 of 9 animals reacted with moderate but non-fatal shock to the intravenous injection of 10 mg of the polysaccharide. Since the number of sensitive animals has increased, a third testing seems to be indicated. All these animals were tested with the same shocking dose 3 weeks after the second test. It was found that 5 of 7 animals reacted, one typical anaphylactic death having been observed and 4 exhibiting severe but non-fatal shock. The results of the last 2 testings presenting the records of each animal are given in the last 4 columns of the table.

From the above results it is clear that the sensitizing and antigenic properties of the polysaccharide of *Monilia tropicalis* prepared by acetic-acid hydrolysis for guinea pigs and rabbits respectively are decidedly inferior to that of the intact cell. It may seem obvious that the sensitizing capacity of the polysaccharide is low since the weight of intact cells employed compared with that of the polysaccharide is small. Furthermore, large doses of the soluble antigen administered over a comparatively long period of time apparently were necessary to induce a state of supersensitivity in guinea pigs. On the other hand, it is of interest to note that a cellular component of the organism apparently free from proteinous materials *per se* is a complete antigen in the usual immunological sense of the word.

Conclusion. Polysaccharide derived from *Monilia tropicalis* by acetic-acid hydrolysis is capable of inducing active sensitization of guinea pigs and of eliciting the production of antibodies in rabbits. Its capacity in these respects, however, is inferior to that of the intact cell.