the Cladocera mothers made in the culture medium, other than mere reduction of the available quantity of bacteria, appears to have been the determining factor in influencing the sex of the young produced.

5552

Oral Immunization of Humans Against Pneumococcus, Determined by the Increased Protective Antibody Content of Serum.

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The writer reported[1] that protective antibodies against pneumococcus, type 1, could be found in the sera of rats fed either (1) infected pneumococcus tissue (2) the living organism, or (3) the acid killed organism. Not all sera of rats thus actively immunized were found to contain these transferable antibodies and in those that did, the amounts present neutralized from 10 to 100,000 fatal doses (in 0.20 cc.). Experiments performed on dogs demonstrated similar results. Neither agglutinins nor precipitins could be found in the blood of such orally immunized animals, a statement confirmed recently by Maeji[2] for a rabbit fed type 3 organisms. The absence of agglutinins and precipitins made it appear that a similar condition would possibly be found to exist in humans fed acid killed pneumococci, even if the subjects should be made actively immune by this procedure. Reliance would consequently have to be placed upon the detection of an increased concentration of protective antibodies as a means of determining immunity.

The experiments reported here were done between October, 1928, and October, 1930, and were briefly mentioned elsewhere.[3] The work was interrupted but is now being continued. The results obtained on 14 subjects are reported below.

The sedimented HCl killed organisms (type 1 throughout) were used directly after centrifugation or after desiccation and were administered generally on a fasting stomach. The quantities fed

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[1] Ross, Victor, PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 273.

[2] Maeji, Y., *Acta Scholae Univ. Imp.*, Kioto, 1929-30, **12**, 295.

[3] Ross, Victor, *J. Exp. Med.*, 1930, **51**, 585.

are listed for each subject. Blood was examined before and after the feedings. 0.20 cc. of serum drawn into a syringe containing 0.20 cc. of the diluted culture (type 1) were injected intraperitoneally into mice. The table shows the dose of pneumococci (with and without serum) injected before and after ingestion of the organisms, and the result. In most cases 2 animals were injected with each dose. Only those subjects who showed either a definite increase or a probable one are listed in the table; those which were negative are described briefly in the text.

TABLE I.

Protective power of serum of humans before and after ingestion of pneumococci. S = survived. The number = the day on which the mouse died.

Dose cc.	V. R. before		After 2 days		After 11 mos.		J. P. before		After 2 days		After 8 mos.	
	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum
10^{-9}					S.S		S.3	S.S	S.S	S.S	S.S	S.S
10^{-8}	2	S	2	S	2.2	S.S	2.2	2.2	S.4†	S.S	2.2	2.2
10^{-7}	3	3	2	S	2.2	S.4*	2.2	2.3	2.2†	S.2	2.2	2.2
10^{-6}	2	3	2	S	2.2	S.S	2.2	2.2		S.S	2.2	2.2
10^{-5}		S		3		S.6		2.2		4.5		2.1
10^{-4}		2		3		2.4		2.2		S.2		2.2
10^{-3}	*Ill at time of test; lost 2 gm. in 4 days preceding test.						†Pn. in heart			2.2		1.1

	H. W. before		After 2 days		After 8 mos.		A. J. K. before		After 2 days		After 6 mos.	
10^{-9}	S.3	S.S	S.S	S.S	S.S	S.S	7°.S	1.S	S.S	S.S	S.3†	S.S
10^{-8}	2.2	2.2	S.4†	S.S	2.2	2.2	3.3	7°.S	2.2	S.S	3.2	S.5†
10^{-7}	2.2	2.2	2.2†	S.S	2.2	2.2	3.2	8°.S	2.2	S.S	2.6†	S.S
10^{-6}	2.2	2.3	2.2	2.2	2.2	2.2	2.2	6.3	3.2	S.S	3.3	7†.6†
10^{-5}		2.2		2.2		2.1		4.4		S.3°		3.3
10^{-4}								3.3		2.3		4.3
10^{-3}	†Pn. in heart		°Sterile					2.3		2.2		2.4

	D. P. before		1 day after		C. M. before		1 day after		M. L. before		1 day after	
	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum	Pn. alone	Pn.+ serum
10^{-9}	2†.S	S.S	S.S	S.S	S.2	S.S	S.S		S.S	S.S	S.7‡	S.S
10^{-8}	2.2†	S.2†	2.2	S.2†	S.2	S.S	S.2		2.2	2.2	S.3	S.S
10^{-7}	3.2	S.2	2.2	S.S	2.2	S.S	2.2	S.S	2.2	2.2	3.4	S.2
10^{-6}		2.2	2	2.S	2	S.S		S.S	2.2	2.3	3.2	3.3
10^{-5}		1.2		3.2		7†		S.S		2.2		2.2
10^{-4}		2.2		2.2				3.2				
10^{-3}								1.1				
	†Pn. in heart								‡Contaminant in heart.			

Results V. R., Before Oct. 23, 1928. Protection against 1 m.l.d. Since 10^{-9} cc. was not used for control mice the possibility that the serum protected against 10 m.l.d. is not excluded. The survival of

the 10^{-5} cc. serum mouse is probably not significant in view of the death of the 2 mice injected with 1/10 and 1/100 of this quantity, and is probably owing to an unusual resistance on the part of this animal. *2 days after last feeding:* Protection against 100 m.l.d. Since 10^{-9} cc. was not used for control mice it is possibly equivalent to 1000 m.l.d. 11 *mos. after.* Definite survival of serum mice receiving 100 m.l.d. and of 1 of 2 injected with 1000 m.l.d. *Comment:* It appears that this subject developed antibodies which persisted for at least 11 months. There were neither agglutinins nor precipitins. Moist growth from 300 cc. on Oct. 31, Nov. 1, 3, 5, 7; 500 cc. on 8, 9, 10, 12, 14; 1L on 15, 16, 17, and 21.

J. O., Before Dec. 4, 1928. Samples of blood taken at 2 different times were examined. The first showed protection against at least 10,000 fatal doses; the second against a similar quantity. 3 *days after.* Survival against 10,000 m.l.d. 10 *mos. after.* Protection against approximately 10,000 m.l.d. *Comment:* No increase. There were neither agglutinins nor precipitins. Moist growth from 330 cc. on Dec. 19, 20, 21, 22, 24; 500 cc. on 26, 27, 28, 29, 30.

R. B. Before Feb. 1, 1929. One of 2 serum mice survived 1 m.l.d. 1 *day after:* Both serum mice survived 10^{-8} cc. which killed 1 of 2 controls. 8 *mos. after:* Both serum mice survived 1 m.l.d. *Comment:* Probably no antibody increase. Moist growth from 300 cc. on Feb. 7, 8, 9; 500 cc. on 11, 13, 16; 1L on 18, 19, 20.

A. M. Before Feb. 1, 1929. There is protection against 100 m.l.d. 1 *day after:* No change. 8 *mos. after:* The same. Dosage was same as for R. B.

J. P. Before Feb. 28, 1929. There is possibly protection against 1 m.l.d. *2 days after:* One of 2 serum mice succumbed to 10^{-7} cc., although both receiving 10 times this dose survived. Since 1 of 2 serum mice survived 10^{-4} cc. we might balance it against the death of the 10^{-7} cc. mouse. This leaves the protection at probably 100 m.l.d. The deaths from 1000 m.l.d. are delayed. 8 *mos. after:* There is no protective power. *Comment:* Protection rose from 0 to 100 m.l.d. with a return to original value in 8 months or sooner. Moist growth from 300 cc. on Mar. 4, 6, 7, 8; 500 cc. on 8, 9, 11, 12, 13, 14; 1L on 15, 16, 17.

H. W. Before Feb. 28, 1929. There is possibly protection against 1 m.l.d. 10^{-9} cc. killed 1 of 2 controls and neither serum mouse. Control mice before and after are same as for J. P. *2 days after:* Protection against 10 m.l.d. if 10^{-8} cc. which killed 1 of 2 controls is the m.l.d. 8 *mos. after:* No protection. *Comment:* There is very probably an increase in antibody content with a return to the

original value in 8 months or sooner. Dosage was same as for J. P.

E. L. A. Before Apr. 5, 1929. One of 2 serum mice survived 1 m.l.d. *2 days after:* Both serum mice survived 1 m.l.d. (10^{-8} cc.) but one died of 10^{-9} cc. *6½ mos. after:* 1 of 2 serum mice survived 1 m.l.d. *Comment:* No increase in protective power. Moist growth from 300 cc. on Apr. 18, 19, 20.

A. J. K. Before Apr. 5, 1929. Controls same as for E. L. A. before and after 2 days. Of the serum mice those receiving 1 and 10 m.l.d. can be said to have survived since the 2 mice which died a delayed death of 10^{-8} and 10^{-7} cc. contained no pneumococcus at autopsy. The serum mouse which died of 10^{-9} cc. must also be counted as a survivor since death in 1 day from this dose is unlikely. *2 days after:* Protection against 1000 m.l.d. (10^{-5} cc.). One of 2 serum mice dying of 10^{-5} cc. was sterile. *6 mos. after:* 1 of 2 controls died of 10^{-9} cc. If we disregard this death and the delayed death of 1 of 2 serum mice which died of 10^{-8} cc. we find a return to the initial value of 10 fatal doses. *Comment:* It appears that the protective power of this individual increased decidedly 2 days after the last of 3 successive daily feedings and returned to the original value after 6 months or sooner. Dosage same as for E. L. A.

D. P. Before Sept. 9, 1930. One of 2 controls died of 10^{-9} cc. Among the serum mice 1 of 2 survived 1 m.l.d. and 1 of 2, 10 m.l.d., if 10^{-8} cc. is taken as the m.l.d. or 10 times these amounts if 10^{-9} cc. is taken as the m.l.d. *1 day after:* 1 of 2 serum mice survived 1 m.l.d., 2, 10 m.l.d., and 1, 100 m.l.d. *Comment:* There seems to have been some increase though it is not certain. Desiccated growth from 1 L. on Sept. 9, 400 cc., on 10th, 400 cc. on 11th.

H. L. Before Sept. 16, 1930. 1 of 2 serum mice survived 1 m.l.d. (10^{-9} cc.) and 10 m.l.d. respectively. *1 day after:* Only 1 of 2 controls died of 10^{-8} cc., whereas both serum treated animals succumbed, although 1 after 4 days. *Comment:* It would seem as if a slight drop in protective power had taken place, but since this occurred in the neighborhood of 10^{-8} and 10^{-9} cc., doses where irregularities occasionally are found, the change was probably not real. Desiccated growth from 1 L. on Sept. 16, 400 cc., on 17 and 18.

C. M. Before Sept. 23, 1930. Protection against 100 m.l.d. if 10^{-8} cc. is taken as the m.l.d. for controls (only 1 of 2 died but 1 of 2 succumbed to 10^{-9} cc. also). If 10^{-9} cc. is the m.l.d. then the antibody content is 1000 m.l.d. *1 day after:* Only 1 of 2 controls died of 10^{-8} cc. If this is the m.l.d. then there is protection against 1000 m.l.d. *Comment:* It seems as if an increase may have taken place

but this is not certain. Desiccated growth from 1 L. on Sept. 23, 400 cc. on 24, 25.

M. E. Before Sept. 23, 1930. Same controls as for C. M. before and after. Both serum mice succumbed to 10^{-8} cc. and one died of 10^{-9} cc., so there was no protection. 1 *day after:* No change in survival among serum mice. *Comment:* No change. Dosage same as for C. M.

S. F. Before Oct. 7, 1930. No protection. 1 *day after:* No protection. *Comment:* No change. Desiccated growth from 1 L. on Oct. 7; 400 cc. on 8, 9.

M. L. Before Oct. 7, 1930. No protection. 1 *day after:* 1 of 2 control mice injected with 10^{-8} cc. lived as against both serum mice. Both controls died of 10^{-7} cc. only 1 of 2 serum mice. *Comment:* There is possibly an increase but in view of the experience with H. L. one cannot be certain.

Summary and Discussion. There appears to be clear evidence for an increased antibody content in the sera of subjects V. R., J. P., and A. J. K. In the individuals H. W., D. P., and M. L. there also seems to have been some increase. In C. M. the result is in doubt. No change apparently took place in the remaining persons. Where the initial value for a serum is 100 to 10,000 m.l.d. (C. M., A. M., and J. O.) an increase equal to less than 900 to 90,000 m.l.d. cannot be detected by the method used. This may account for an apparently unaltered concentration of antibodies in some instances. It would seem, therefore, that human beings may develop protective antibodies following ingestion of dead pneumococci (type 1). The appearance of such antibodies in the sera of rats which have been actively immunized against the pneumococcus by similar treatment suggests that those persons who developed antibodies also became actively immune. The absence of a change in antibody content in the others may not necessarily mean the lack of an increased resistance to the organism, for the animal experiments also seem to indicate that in some individuals active immunity may be produced without such a change.