

phosphate to the plasma of the mammary blood, and this S might be oxidized and similarly returned as sulfate to the blood.

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**Immunity to pneumococcus afforded rats by feeding them
the germ.**

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In December it was reported¹ that rats became resistant to pneumococcus injections if fed on the tissues of other rats which had been killed by the same organism. It was shown that such animals tolerated 1000 or more times the dose of pneumococci that proved fatal for control rats. It was suggested that the living germs present in the tissue being fed might be the real cause of this increased resistance. Attention was also called to the fact that experiments had been done in which pneumococci were fed with the object of seeing whether a similar immunity could be built up in this manner. Our experiments at that time indicated that feeding the cocci from 50 cm. of culture per day to a rat produced a decided degree of immunity. It is our intention in the present report to give a representative experiment (our latest), in tabular form and to show that an immunity almost as good as, if not quite equal to that produced in the tissue feeding experiment, can be produced by feeding live pneumococci. Tests have also been made in which the dead germs have been fed, and are included in the table. The immunity built up by this latter method, is not so good as that obtained with living organisms, when fed in equal quantities.

Each of the rats listed in the accompanying table received the germs from approximately 50 cm. of a 24 hour culture of pneumococcus Type I, daily, for about 20 days. The organisms were grown in meat infusion media. The culture was centrifuged, the

¹ Ross, Victor, *PROC. SOC. EXP. BIOL. AND MED.*, 1925, xxiii, 183-185.

germs suspended in 0.1 per cent gelatin solution, stirred, and cracker meal added and mixed. This was spread out on a shallow dish and given to the rats several hours after the last meal. In the experiments with dead germs, the suspended pneumococci in gelatin solution were heated to 80° C. for two hours, then cultures were made, and, if no growth occurred after 48 hours incubation, the material was fed after mixing with cracker meal. The feeding of live and dead pneumococci was carried on simultaneously. The two groups of rats were kept in separate cages. The weights of the rats increased at the usual rate and the animals were as active as controls.

In order to test the resistance of the animals, they were injected intraperitoneally with pneumococcus, Type I. The volume injected was in all cases 0.20 cm., although the actual number of organisms varied. A 24 hour broth culture was used. In the table are given the number of the rat, whether it is control or experimental animal, the kind of germs fed, *i. e.*, living or dead, the weight, the quantity of culture injected, and the result.

The control rats die of 0.00001 cm. and 0.000001 cm. Of the rats which were fed live germs, only one died of 0.0001 cm. Such rats generally survive 0.001 cm. and 0.01 cm. Of the rats fed on the germs killed by heat, none survived 0.001 cm. Some survived 0.0001 cm. although quite as frequently they died of this amount and even of 0.00001 cm. There is thus clear indication of the immunizing effect of the live germs and evidence for some effect of the dead germs. Experiments are in progress in which larger amounts of dead germs are being employed, with the object of seeing whether an immunity as good as that produced by the living organisms can be created. It is not unlikely that the method of killing the germs is important in determining their immunizing properties. Different methods of doing this are therefore being tried. Avirulent germs are also being fed. No suggestion as to the manner in which immunity by feeding is created is offered. Our earlier germ feeding experiments showing the effects of increasing quantities will be published in detail shortly.

The work of Killian² and of Eguchi³ has just come to our notice. The former found, in a small group of mice, that heat

² Killian, H., *Z. f. Hyg., u. Infektionskrankh.*, 1924, cii, 279-286.

³ Eguchi, Ch., *ibid.*, 1925, cv, 74-90.

Table Showing Increased Resistance to Pneumococcus of Rats which were fed Pneumococci

*Indicates a new day.

Rat No.	Control or Experm'tal	Germs fed. Live or dead	Wt. gm.	Quantity Injected cm.	Result D=dead
*—	C	—	103	2.10 ⁻³	D. 1 day
227	E	L	109	2.10 ⁻³	D. 1 day
239	E	D	103	2.10 ⁻³	D. 1 day
*—	C	—	113	10 ⁻⁵	D. 2 days
—	C	—	123	10 ⁻⁴	D. 1 day
222	E	L	125	10 ⁻⁵	Survived
214	E	L	125	10 ⁻⁴	D. 2 day
250	E	D	125	10 ⁻⁵	Survived
247	E	D	130	10 ⁻⁴	Survived
*—	C	—	120	10 ⁻⁶	D. 1 day
—	C	—	127	10 ⁻⁵	D. 1 day
*—	C	—	113	10 ⁻⁵	D. 1 day
—	C	—	123	10 ⁻⁴	D. 1 day
*—	C	—	137	10 ⁻⁵	D. 4 days
218	E	L	132	10 ⁻⁴	Survived
217	E	L	141	2.10 ⁻³	Survived
249	E	D	127	10 ⁻⁴	D. 2 days
236	E	D	151	2.10 ⁻³	D. 2 days
*—	C	—	142	10 ⁻⁵	D. 2 days
215	E	L	145	10 ⁻³	D. 2 days
225	E	L	170	10 ⁻²	D. 2 days
252	E	D	155	10 ⁻⁴	Survived
246	E	D	133	10 ⁻⁴	D. 4 days
*—	C	—	145	10 ⁻⁵	D. 2 days
—	C	—	143	10 ⁻⁵	D. 2 days
229	E	L	158	10 ⁻⁴	Survived
220	E	L	143	10 ⁻⁴	Survived
232	E	L	153	10 ⁻³	Survived
230	E	L	160	10 ⁻³	Survived
238	E	D	127	10 ⁻⁴	D. 2 days
242	E	D	146	10 ⁻⁴	D. 2 days
237	E	D	166	10 ⁻³	D. 2 days
234	E	D	165	10 ⁻³	D. 1 day
*—	C	—	125	10 ⁻³	D. 2 days
—	C	—	135	10 ⁻⁵	D. 2 days
215	E	L	157	10 ⁻³	D. 2 days
219	E	L	151	10 ⁻³	Survived
224	E	L	174	10 ⁻³	Survived
221	E	L	175	10 ⁻²	Survived
240	E	D	140	10 ⁻⁵	D. 3 days
253	E	D	191	10 ⁻⁴	D. 2 days
235	E	D	175	10 ⁻⁴	D. 2 days
245	E	D	160	10 ⁻⁴	D. 2 days
*—	C	—	125	10 ⁻⁶	D. 3 days
—	C	—	132	10 ⁻⁵	D. 3 days
231	E	L	130	10 ⁻³	Survived
228	E	L	145	10 ⁻³	Survived
223	E	L	140	10 ⁻³	Survived
220	E	L	172	10 ⁻²	D. 2 days
216	E	L	180	10 ⁻²	Survived
244	E	D	130	10 ⁻⁵	D. 2 days
243	E	D	157	10 ⁻⁴	Survived
248	E	D	154	10 ⁻⁴	D. 2 days
241	E	D	142	10 ⁻⁴	D. 2 days
251	E	D	173	10 ⁻³	D. 2 days

killed pneumococci, when orally administered, were valueless as an immunizing agent and that the living germ was only slightly better. Eguchi finds that *young* mice can be immunized using dead pneumococci.

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The histology of local streptococcus immunity.

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The two most significant results of our studies over several years on localized streptococcus infections in the rabbit would seem to be, first: That, under properly controlled conditions, local infection is followed by a local form of immunity;^{1, 2} and second, that local protection in the pleural cavity, whether in a form of increased resistance (broth) or of specific active or passive immunity, is associated with an increase in the number of clasmatocytes or tissue macrophages there present.^{2, 3} A third study would associate clasmatocytes with the formation of antibodies.⁴

Repeated attempts to simulate conditions in the body by the action of clasmatocyte exudates outside the body have been confusing. The transfer of the entire pleural exudate of a clasmatocyte type, from a broth-protected to a normal animal, transfers no protection. Exudates of both polymorphonuclear and clasmatocyte type do not in their entirety destroy even a minimal number of streptococci in the test tube, although it may be shown that the supernatant fluids of both exudates are bactericidal, and furthermore, that the acid cell extracts of both types of cell will destroy streptococcus. (Unpublished observations.) But these extracorporeal phenomena do not account for the occurrences in the animal body, since they are much slower in effect, and, since the polymorphonuclear exudate yields more highly bactericidal

¹ Gay and Rhodes, *J. Infect. Dis.*, 1922, **xxxi**, 101-115.

² Gay and Morrison, *J. Infect. Dis.*, 1923, **xxiii**, 338-367.

³ Gay and Clark, *J. Infect. Dis.*, 1925, **xxxvi**, 233.

⁴ Gay and Clark, *J. Am. Med. Assn.*, 1924, **lxxxiii**, 1296.