proof that the mode of action of these drugs in pneumococcal infections is fundamentally the same. Moreover, it suggests the futility of attempting therapy with a second of these compounds when the pneumococcal infection becomes resistant to treatment with any one of these drugs.

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A New Material (Lygranum) for Performance of the Frei Test for Lymphogranuloma Venereum.

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No completely satisfactory product for the performance of the Frei intradermal test for the diagnosis of lymphogranuloma venereum has yet been obtained. The materials commonly employed for the purpose are a suspension, in sterile physiological saline, of human pus or mouse brain containing the heat-inactivated virus of the disease. Many critical reports upon the value of these materials, which are termed Frei antigens, have already appeared, *e. g.*[1] It was felt that a suspension of elementary bodies of the virus of lymphogranuloma venereum might be the ideal agent for the performance of the Frei test. Accordingly, this virus was grown in the yolk sac of the developing chicken embryo[2] according to the method of Cox[3] and a considerable yield of elementary bodies obtained. One cc amounts of suspensions of virus-infected yolk sac showing many elementary bodies were inoculated into the yolk sac of eggs of 6 days' previous incubation. As soon as the embryos were moribund or dead the yolk sacs were collected, washed lightly to remove excess yolk and then weighed. They were ground thoroughly in a mortar with sterile quartz fragments and sufficient sterile physiological saline added to make a 10% suspension. This suspension was centrifuged for 1 hour at 2500 RPM in the cold and the sediment discarded. The super-

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1 Grace, A. W., *Arch. Dermat. and Syph.*, 1939, **39**, 347.

2 Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

3 Cox, H. R., *Pub. Health Rep.*, 1938, **53**, 2241.

natant was further centrifuged for 2 hours at 12,500 RPM in the cold. The resulting supernatant was discarded and the sediment resuspended in 0.1% formalin-saline to a volume 20 times that of the original 10% yolk sac suspension. Later, phenol to a final dilution of 0.25% was also added. After 2 days the suspension was tested for bacteriologic sterility and for freedom from active virus by inoculation into the brains of mice and into the yolk sacs of developing hen's eggs. This inactivated suspension has been designated Lygranum antigen.[4]

The normal control material was produced in exactly the same manner with the exception that normal yolk sac from 10-day embryos was used as the starting material. This material has been termed Lygranum control.

During the Spring of 1940, tests with Lygranum control and with Lygranum antigen were performed upon 36 subjects. Twenty-six of these presented signs which were compatible with a previous or existing infection with lymphogranuloma venereum in the individual or in the marital partner. This group is referred to in the paper as "clinically lymphogranulomatous". The remaining 10 persons had neither history nor signs of the disease in themselves or in the marital partner.

Each individual was also tested at the same time with suspensions of normal and lymphogranulomatous mouse brain and all tests were performed by the intradermal inoculation of 0.1 cc of material. Exact measurement of both principal diameters of the central papule or indurated area produced by the inoculation was made with a millimeter scale at the end of 48 hours in 18, and at 72 hours in 18 persons. The significant element in the reaction is the size of the central papule or indurated area which is shown in the Table. The size of the accompanying erythematous area is without diagnostic value.

In the Table are shown the results of the tests of Lygranum and mouse brain controls and antigens upon non-lymphogranulomatous and lymphogranulomatous subjects as well as the clinical condition of each individual.

The results of the tests of Lygranum control upon 35 non-lymphogranulomatous and lymphogranulomatous persons and of Lygranum upon 9 non-lymphogranulomatous subjects indicated that the upper limit of the diameter of non-specific papules produced by these materials was 5 mm and that papules of 5 mm or less, as measured on both principal diameters, represented negative reactions. Papules of

[4] McKee, C. M., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 410.

TABLE I.

Case No.	Sex	Color	Size of papule (mm) produced by: Mouse brain Control	Mouse brain Antigen	Lygranum Control	Lygranum Antigen	Interpretation of reaction: Mouse brain Antigen	Lygranum Antigen	Clinical condition
					Clinically Lymphogranulomatous Individuals.				
1	M	W	5x5	38x22	Nil	30x30	Positive	Positive	Purulent inguinal adenitis, 1915, abscess of thigh.
2	F	Col	3x3	21x19	Nil	25x22	"	"	Active proctitis, rectal stricture, colostomy
3	M	W	6x6	16x15	Nil	15x14	"	"	No evidence of lymphogranuloma venereum. Wife has active lymphogranuloma venereum.
4	M	W	5x4	13x11	Nil	14x10	"	"	Active proctitis, rectal stricture.
5	F	Col	4x4	12x12	Nil	11x9	"	"	Active proctitis, rectal stricture.
6	M	Col	Nil	11x11	Nil	14x12	"	"	Active proctitis.
7	M	Col	5x5	11x11	Nil	13x13	"	"	Purulent inguinal adenitis.
8	M	W	Nil	11x11	Nil	10x9	"	"	Active proctitis, rectal stricture.
9	M	Col	Nil	11x10	Nil	19x15	"	"	Purulent inguinal adenitis.
10	M	W	6x6	10x10	Nil	13x12	"	"	Active proctitis.
11	M	W	Nil	10x9	Nil	9x8	"	"	Perianal fissure.
12	M	Col	3x3	9x9	Nil	7x7	"	"	Primary syphilis with inguinal adenitis.
13	M	W	3x2	9x9	Nil	6x6	"	"	Generalized adenopathy.
14	M	W	Nil	9x8	Nil	18x13	"	"	Inguinal adenitis in 1925.
15	M	Mex	5x3	9x8	4x3	11x9	"	"	Healed proctitis, rectal stricture.
16	M	W	6x5	9x7	Nil	7x6	"	"	Active proctitis, rectal stricture.
17	M	W	5x4	8x7	Nil	8x7	"	"	Active proctitis.
18	M	W	6x6	7x6	Nil	6x6	Doubtful	"	Active proctitis, rectal stricture.
19	M	W	Nil	7x6	Nil	6x6	"	"	Active proctitis, rectal stricture, elephantiasis of penis and scrotum.
20	M	Col	5x4	6x6	Nil	8x7	Negative	"	Primary syphilis with inguinal adenitis.
21	M	W	4x4	6x6	Nil	7x6	"	"	Purulent inguinal adenitis 1920.
22	M	W	4x4	6x6	3x2	7x6	"	"	Active proctitis, carcinoma of rectum.
23	M	W	5x4	6x5	Nil	7x5	"	Doubtful	Perianal abscesses and sinuses.
24	M	W	5x4	6x5	Nil	5x3	"	Negative	Perianal fissure.
25	M	W	Nil	4x4	Nil	6x4	"	"	Active proctitis.
26	F	W	Nil	3x3	Nil	2x2	"	"	Active proctitis, rectal stricture.
					Non-Lymphogranulomatous Individuals.				
1	M	W	3x3	6x5	Nil	5x4	Negative	Negative	Pruritus ani.
2	M	W	4x3	5x5	Nil	Nil	"	"	Ruptured appendix.
3	M	W	4x4	5x4	Nil	5x4	"	"	Periurethral sinus.
4	F	W	3x3	4x4	Nil	Nil	"	"	Congestive cardiac failure.
5	F	W	3x3	4x4	Nil	Nil	"	"	Constipation.
6	M	Red	3x3	4x4	Nil	4x4	"	"	Secondary syphilis.
7	M	Col	3x3	4x3	Nil	5x4	"	"	Chancroid.
8	F	W	3x2	3x3	Nil	3x2	"	"	Primary syphilis.
9	F	Col	3x3	3x3	Nil	Nil	"	"	Ventral hernia.
10	M	W	4x3	4x3	10x10	10x10	"	"	Primary syphilis.

diameter 6 mm or larger, produced equally with Lygranum control and antigen in the same person probably represent a reaction of hypersensitivity to chick material. In such instances the lymphogranulomatous status of the individual must be determined by tests with antigens prepared from mouse brains or human pus.

The results of the tests with mouse brain control and antigen were in accordance with the published literature[1] upon this subject. Nonspecific papules of average diameter of 4 mm were produced in 28 of the 36 non-lymphogranulomatous and lymphogranulomatous individuals tested with control material and in all of the 10 non-lymphogranulomatous persons tested with antigen. The criteria for the evaluation of reactions produced with mouse brain emulsions were as follows: positive, a papule 7 mm in diameter or larger; upper limit of negative, a papule 6 mm in diameter.

a. Ten Non-Lymphogranulomatous Subjects Tested with Lygranum Control and with Lygranum Antigen.

Lygranum control produced no papular reaction in 9 persons. Lygranum antigen showed no papular reaction in 4, and papules of maximum and average size of 5 x 4 mm and 4 x 4 mm respectively, in 5 persons.

One non-lymphogranulomatous person showed a reaction that was unusual in that the responses to both Lygranum control and Lygranum antigen were identical and appeared as erythematous papules 10 mm in diameter. There was no pruritus or vesiculation at the sites of the tests nor was there any previous history of hypersensitivity to egg. The reaction persisted for the same length of time as a positive Frei response of the same papule size. The reaction to mouse brain antigen was negative. This unusual result was regarded as a manifestation of abnormal sensitivity to chick material.

b. Twenty-six Clinically Lymphogranulomatous Subjects Tested with Lygranum Control.

Twenty-four persons gave no papular reaction. The remaining 2 individuals developed papules measuring 4 x 3 mm and 3 x 2 mm respectively.

c. Twenty-six Clinically Lymphogranulomatous Subjects Tested with Lygranum Antigen and with Mouse Brain Antigen.

There were 22 positive, 1 doubtful, and 3 negative reactions with Lygranum antigen and 17 positive, 2 doubtful, and 7 negative reactions with mouse brain antigen. Seventeen individuals gave positive reactions with both Lygranum and mouse brain antigen. The 2 doubtful reactions with mouse brain were positive when Lygranum was used. The doubtful reaction with Lygranum was negative with mouse

brain antigen. The 3 negative reactions with Lygranum were also negative when mouse material was employed.

The quality of the positive reaction to Lygranum was similar to that produced by mouse brain antigen. The erythematous area and papule were, in most instances, approximately the same size with each antigen in the same person. The papule which was obtained by the use of Lygranum was, however, sometimes a little lower and slightly less erythematous than that given by mouse brain antigen. The duration of persistence of the papules was approximately the same with both antigens in the same person. The exact time taken for a reaction to disappear depended upon the strength of the response —a stronger reaction remaining visible for a longer period than a weaker one.

Conclusions. Lygranum antigen is superior to mouse brain antigen in sensitivity and specificity in the performance of the Frei test for lymphogranuloma venereum. Lygranum control is superior also to mouse brain control in producing non-specific reactions in only 2 of 35 persons as compared with 28 of 36 in the case of mouse brain control. In the group of 36 tested only one individual showed a reaction which could be regarded as resulting from hypersensitivity to chick material.

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Influence of Local Applications of Potassium Chloride on Action Current of the Mammalian Heart.*

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In experiments previously reported, it was found that application to the surface of the heart (cat, dog, rabbit) of squares of filter paper soaked in M/10 or M/5 KCl solutions, produced changes in the electrocardiogram similar to those which follow various types of injury to the heart, except that the potassium effects quickly subsided following removal of the solution.[1] Generally accepted views of the action of potassium ions in the heart and elsewhere suggest that the

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1 Kish, B., Nahum, L. H., and Hoff, H .E., *Am. Heart J.*, 1940, **20**, 174.