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Specific Soluble Substance of Pneumococcus in Lungs of Dogs Recovered from Experimental Lobar Pneumonia.

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That the soluble specific substance of the pneumococcus may remain in tissues infected with this organism even after the inflammatory exudate has disappeared was first suggested by the studies of Dochez and Avery[1] on the excretion of "S" substance in the urine of cases of lobar pneumonia in human beings. They found that the soluble specific substance was excreted in some cases for days or weeks after recovery from the disease. In some instances this continued excretion of "S" could be correlated with delayed resolution, but in other cases this was not the explanation. It would seem probable that in these latter instances "S" remained stored in the pulmonary tissues for some time after the other constituents of the inflammatory exudate had apparently disappeared.

A direct approach to this problem cannot be made in human beings because of obvious difficulties. However, the availability of an experimental animal in which a pneumococcus pneumonia may be induced makes possible the determination of "S" substance in the lungs at various intervals after recovery. The present report is a record of preliminary studies of the persistence of "S" substance in the lungs of dogs recovered from experimental lobar pneumonia.

Healthy adult male dogs were infected with Type I pneumococci by the method of Terrell, Robertson, and Coggeshall.[2] Some of these dogs had been previously "vaccinated" by spraying formalized Type I pneumococci into the bronchial tree. Two dogs in the group had been similarly treated with a 0.9% salt solution as controls. The "vaccination" procedure had been carried out in the course of a separate experiment but the dogs after recovery were utilized for this study. The other animals in the series had received no preliminary treatment.

After inoculation the dogs were examined regularly with the fluoroscope to determine the progress and extent of the pulmonary lesion and to ascertain as accurately as possible by this means the

[1] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26**, 477.

[2] Terrell, E. E., Robertson, O. H., and Coggeshall, L. T., *J. Clin. Invest.*, 1933, **12**, 393.

TABLE I

Dog No.	Condition of dog before infection	Time of killing animals: Days after inception of infection	Time of killing animals: Days after disappearance of lesion	Highest dilution of tissue extract giving precipitin reaction: Infected Lung	Highest dilution of tissue extract giving precipitin reaction: Uninfected Lung	"S" substance in blood serum and Urine: Serum	"S" substance in blood serum and Urine: Urine	Histological appearance of infected lung
17G	Normal	2		1-320	0	+	+	Uniform exudate of polymorphonuclear cells
32H	"	4		1-384	0	+	+	Uniform exudate of polymorphonuclear cells
34H	"	6		1-384	0	0	+	Late stage resolution. Exudate principally macrophages
25F	"	12	8	1-512	0	0	0	Thickened alveolar walls
6F	Vaccinated	13	7	1-384	0	—*	—	Still slight macrophage reaction
5F	"	13	7	1-384	0	—	—	Most areas normal. A few alveoli contain detached mononuclear cells
23F	Saline "Vaccinated"	18	10	1-480	0	0	0	Alveolar walls in some areas still thickened and some of the septal cells are swollen
18F	Vaccinated	25	15	1-240	0	0	0	Alveolar walls still slightly swollen. No exudate
14F	Saline "Vaccinated"	57	50	1-128	0	0	0	Normal
28F	Normal	70	60	1-256	0	0	0	"
20F	Vaccinated	109	98	0	0	0	0	"
17F	"	109	96	1-128	0	0	0	"

*— = Determinations not made.

time of completion of resolution. At different intervals after inoculation the animals were sacrificed and the previously infected and normal lobes were removed for study. Histological sections were prepared for microscopic examination.

For the demonstration of "S" substance the lung tissues were frozen in liquid air and crushed to a fine powder in an apparatus previously described.[3] Two volumes of 0.9% salt solution were added to each gram of tissue. The brei was then placed in the ice box for extraction. Twenty-four to 48 hours later the material was centrifuged and the supernatant fluid filtered through a small Seitz pressure filter. Type I antipneumococcus serum was then overlaid with the clear filtrate in varying dilutions, and the highest dilution showing a precipitin reaction determined. It is to be noted that the lowest dilution tested thus was 1-3.

A purely qualitative determination for the presence of "S" in the blood serum was made by the method described by Amoss.[4] Urine removed from the animal at the time of death was tested for "S" by simply overlaying a small amount over antiserum.

The data obtained are summarized in Table I. It should be kept in mind that the pneumonic lesion in dogs passes through the stages of development and regression more rapidly than in human beings. Furthermore, there occurs the same variation in rate of these changes in individual dogs as one sees in a series of cases of the human disease. Thus clearing of the lesion as determined by fluoroscopy may be complete in 4 days, as in dog 25 F, or may not take place for 13 days, as in dog 17 F. Fluoroscopic examination, of course, is not adequate to demonstrate actual restitution to normal, histologically, but is sufficiently accurate for the comparative purposes of this study.

The data obtained thus demonstrate that "S" substance may be present in detectable amounts in previously infected lobes 2 to 3 months after the inflammatory exudate has disappeared. The question may, of course, be raised as to the rôle vaccination may have played in causing retention of "S" substance in some of these cases. Experiments on normal animals are now in progress to check this point. The lung of a normal animal, dog 28 F, however, contained "S" substance 2 months after resolution had taken place.

Soluble substance was not found in the uninfected lobe at any stage of the disease where the lowest dilution tested was 1-3. In

[3] Graeser, J. B., Ginsberg, J. E., and Friedemann, T. E., *J. Biol. Chem.*, 1934, **104**, 149.

[4] Amoss, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 23.

several cases, not included in this series, the tissue extract from the uninfected lobes has been concentrated to equal an undiluted tissue extract and "S" substance was not demonstrable.

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Hemoglobin Studies. I. In Rachitic Chickens: Effect of Ultraviolet Irradiations.*

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A study of the effect of ultraviolet irradiation on the amount of hemoglobin of the blood was made on 3 pens of White Leghorn chickens (10 chickens each pen) grown in complete absence of sunshine. The room, 20′ x 30′, received moderate lighting from two 60 watt Mazda lamps. Small brooders supplied with 60 watt carbon filament lamps furnished the brooder heat for individual pens.

Pen 1, the normal controls, received 2 minutes daily irradiation from a quartz mercury arc lamp at 30 inches distance. Pen 2 had no ultraviolet irradiation. Pen 3 received no ultraviolet until after hemoglobin studies on rachitic chickens were made.

The irradiated chickens developed smooth plumage, yellow shanks and bills, and the male birds large red combs. (These organs do not develop until later in the females.) All of these chickens showed every sign of normal growth.

The non-irradiated chickens were less than 2/3 the size of the controls (Table I). Many showed extreme weakness and deformed

TABLE I.
Comparative Size, Hemoglobin and Blood Calcium of Normal Irradiated (Control), Non-irradiated and Rachitic Irradiated (Healing) Chickens.

Pen	Wt. in gm.	Hb 14.5 = 100%	Blood Ca mg. per 100 cc.	Hb later	
				10 days	25 days
I	315	10.3	12.6	10.3	10.9
II	197	8.41	6.73	8.00	dead
III	203	6.52		8.41	11.6

joints and bones due to rickets. The feathers were rough and the shanks and bills were light, almost white, in color.

At 7 weeks of age hemoglobin studies were made. These aver-

* Aided by a grant from the Heckscher Research Foundation.