Such a reversal of the effect of a substance with a change in the buffered solvent, and especially with phosphate buffers, has been observed also in blood studies as well as in these muscle investigations. In some way the phosphate ion seems to prevent the caffeine from entering the tissue or of acting upon the tissue with its characteristic effect. It is highly improbable that there is any significant reaction between the caffeine and the phosphate since the caffeine is a very weak base. A reaction with the formation of such a compound as caffeine phosphate would not occur, at least until the pH was far more alkaline than 7.3, which is the figure with reference to frog blood and lymph, and therefore, this value was maintained throughout these experiments.

Summary. 1. The characteristic positive effect of caffeine in delaying the rapidity of fatigue in the gastrocnemius muscle is apparent when administered in an aqueous medium, or in a bicarbonate buffered-Ringer medium. 2. The effect of the medium containing the caffeine is greater than the effect of the medium alone. 3. A reversal of the usual caffeine effect upon the fatigue record of striated muscle occurs when the caffeine is introduced in a phosphate buffered medium, or in a phosphate buffered-Ringer medium. 4. Both phosphate-buffered caffeine solutions cause a negative effect to that of the medium alone. 5. The necessity of discretion in the choice of a buffer in the maintenance of the desired pH of a solvent medium is evident; and, the elimination of an aqueous or Ringer phosphate buffered solution as a solvent for caffeine, in studies of the effect of caffeine upon the fatigue record of the striated (gastrocnemius) muscle, is indicated specifically.

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Occurrence of Rough Pneumococci in Lungs of Patients with Lobar Pneumonia.

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"S" and "R" forms of pneumococci are considered, in a general way, to represent respectively virulent and avirulent phases of the organism. It seemed reasonable to suspect that degradation from

"S" to "R" forms might take place in the mechanism of recovery from pneumococcus infection in living tissues. Wadsworth and Sickles[1] recovered attenuated pneumococci from the blood stream and heart valves of horses previously immunized to pneumococci. Reimann[2, 3] demonstrated the very occasional occurrence of "R" forms in the sputum of patients with lobar pneumonia. Paul[4] produced "R" forms *in vivo* under certain experimental conditions in dogs.

In the present communication is reported the recovery, by direct lung puncture, of "R" variants of the pneumococcus from the lungs of a majority of lobar pneumonia patients studied. Clear cut cases only of lobar pneumonia were studied. Punctures were made at points of maximum consolidation. Lung puncture, in pneumonia, has been shown to be a safe procedure.[5] Material obtained by lung puncture was cultured at once in glucose broth and upon blood agar plates. For the latter, following the work of Sia and Chung,[6] 5% dog blood was used to facilitate recognition of "R" and "S" forms. Colony studies were made after 18-22 hours incubation, longer incubation being used only when growth was retarded. Smears of the lung puncture material were studied with Gram's and Hiss capsule stains. In several cases portions of the puncture specimen were inoculated intraperitoneally into white mice.

Identification of organisms was based upon cultural and microscopic characteristics, and in most cases upon bile solubility and type specific agglutination. In the majority of cases of typical lobar pneumonia, pure cultures were obtained upon lung puncture. Occasionally the pneumococcus was accompanied by *H. influenzae*. This interesting finding is being studied further.

Colonies were examined under the Zeiss colonyscope with oblique illumination. Colonies classed as "R" showed variations in their borders and surfaces from gross irregularity to fine grayish stippling; they were indented and firm in character and moved as a solid mass. "S" colonies presented clear, glistening, rounded, highly refractile surfaces and were quite soft.

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[1] Wadsworth, A. V., and Sickles, G. M., *J. Exp. Med.*, 1927, **45**, 787.

[2] Reimann, H. A., *J. Exp. Med.*, 1925, **41**, 587.

[3] Reimann, H. A., *J. Exp. Med.*, 1927, **45**, 807.

[4] Paul, J. R., *J. Exp. Med.*, 1927, **46**, 807.

[5] Rosenow, E. C., *J. Inf. Dis.*, 1911, **8**, 500. Dochez, A. R., personal communication. Thomas, H. M., and Parker, F., *Arch. Int. Med.*, 1920, **26**, 125. Glynn, E., and Digby, L., Medical Research Council Special Report No. 79, 1923.

[6] Sia, R. H. P., and Chung, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 245.

In Table I, colonies are classified as "R+", "R", and "S" to differentiate between complete rough and less rough or intermediate forms. This variation is of considerable interest. For the discussion below, however, the use of "R" and "S" suffices. Of 24 lung punctures, 11 were done during life and 13 immediately after

TABLE I.

No.	Day of disease	Type	Stained Smears: Pneumococci	Stained Smears: Capsules	Dog Blood Agar Plates: Hr. growth	Dog Blood Agar Plates: Amt. growth	Dog Blood Agar Plates: R+	Dog Blood Agar Plates: R	Dog Blood Agar Plates: S	+ Broth growth	R = recovered; D = died	Remarks
1	24	II	+	—	48	++		+	+	+	D	*H. Influenzae(?)*
2	6	II	+	+		0				++	D	
3	6	II	+	+	18	++		+	+	++	D	Staphylococcus, *H. Influenzae*
4	4	II	+	+	24	++	+	+	+	++	D	*H. Influenzae*
5	8	gIV	0	0		0				0	D	Acute pericarditis. Pneumonia resolving
*6a	8	II	+	+	20	+			+	+++	—	
6b	9	II	+	+	22	++		+++	++	++	D	*H. Influenzae(?)*
*7	6	I	0	0		0				+	R	Cultured after crisis
*8a	4	II	+	+	18	++			+	+++	—	
8b	8	II	+	+	48	+		++	+	+++	D	S colonies virulent; R, avirulent
*9	11	gIV	0	0		0				0	R	Cultured during lysis
*10	7	II	+	—	48	+			±	+	R	Massive empyema
*11a	3	VII	+	—	24	+	++			++	—	Peculiarly "tufted" R colonies
11b	4	VII	+	—	18	+	++	+		++	D	
12	8±	II	+	+	40	+	+	+	+	++	D	
13	11	VIII	+	+	20	+		+	+	+	D	Confluent Bronchopneumonia
*14	7	gIV	±	—		±				0	R	Cultured 24 hrs. after crisis
15	6	II	+	+	20	++			+	+	D	Cultured at autopsy. Mixed growth
16	3	II	+	+	48	+	+	+	+	++	D	
*17	3	gIV	+	0		0				0	R	Cultured during crisis
*18	2	II	+	+	24	++	+		++	+++	D	Mixed growth. *H. Influenzae*
19	5	II	+	+		0				++	D	
*20a	3	II	+	0	22	+		+		++	R	Pnc. of low virulence. Restored by mouse passage
*20b	4	II	+	—	20	++	+	+		++	R	

* Studies from living patients.

death. Positive cultures of pneumococci were obtained in all but 4 cases, each of which was in crisis or lysis at the time of puncture. Slight broth growth was obtained once after crisis (Case No. 7) but could not be subcultured. There were 16 successful colony

studies. In 10 done post mortem "R" colonies occurred alone once, "S" colonies alone once, and "R" and "S" 8 times. Six successful colony studies from living patients showed "R" colonies alone in 3 instances, with one subsequent death; "S" alone in 2 with 2 deaths; and "R" and "S" in one which resulted in death. In the total series "R" colonies were recovered 13 times and "S" colonies 12.

Case No. 20 is of particular interest in that pure cultures growing variant "R" colonies were isolated twice during life. The first puncture was obtained at the height of the disease, the second at the onset of crisis. Colonies obtained from the latter were more nearly completely rough than those obtained from the former. Both strains showed very low virulence for mice and inagglutinability in type specific serum, but were restored to virulence and agglutinability by mouse passage.

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Effect of Anterior Pituitary Upon Production of Red Pigment in the Salamander Pseudotriton ruber ruber (Soninni).

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A marked change in coloration occurs at the time of metamorphosis in most salamanders. We have obtained evidence that this change is not produced by the thyroid hormone in the case of red pigment formation. Thyroidectomized larvae of *Hemidactylium scutatum* will assume the red coloration of the adult although they do not metamorphose.[1] Larvae of the red salamander *Pseudotriton ruber* when precociously metamorphosed with thyroid extracts are yellow and not red. Since the anterior pituitary is known to play a part in normal metamorphosis we have tested the effect of anterior pituitary implants upon red salamanders which have been precociously metamorphosed with thyroid solutions.

Fifty larvae of *Pseudotriton ruber,* 30 to 50 mm. in length, were metamorphosed in a solution of 1:15,000 desiccated thyroid. Before transformation was completed 7 of the largest specimens of the same size (Group A) were selected and each of 4 given subcutaneous

[1] Noble, G. K., and Richards, L. B., *Anat. Rec.*, 1931, **48**, 58.