same amount of a pooled infectious suspension of tunica vaginalis washings, Wilmington strain of murine typhus, developed three types of reaction following a variable period of incubation: (a) fever of variable duration and scrotal swelling, followed by the appearance of specific murine complement fixing antibodies; (b) fever of variable duration and no scrotal swelling followed by the appearance of specific murine complement fixing antibodies; and (c) no febrile reaction or scrotal swelling but followed by the appearance of specific murine complement fixing antibodies (inapparent infection). All guinea pigs showing one of these three types of response were proven immune when challenged subsequently.

2. Guinea pigs inoculated intraperitoneally at the same time with the same amount of pooled infectious brain suspension, Breinl strain of epidemic typhus, developed 3 types of reaction following a variable period of incubation: (a) febrile period of variable duration followed by the appearance of specific epidemic complement fixing antibodies; (b) no febrile reaction but followed by the appearance of specific epidemic complement fixing antibodies (inapparent infection); and (c) no febrile reaction followed by no complement fixing antibodies. This latter group are regarded as "misses" for these guinea pigs were not immune when challenged while those of group a and b were immune.

3. Specific murine or epidemic complement fixing antibodies were demonstrated after the inapparent infections with these 2 types of typhus.

4. Guinea pigs inoculated at the same time with the same amount of pooled infectious blood of Rocky Mountain spotted fever (mild strain) exhibit a febrile reaction of variable duration following a variable period of incubation. In all instances specific complement fixing antibodies to Rocky Mountain spotted fever were demonstrated in convalescent specimens of serum and no complement fixing antibodies with a murine or epidemic antigen.

Conclusion. The specific complement fixation test, using purified rickettsial suspensions as antigen, is recommended as a routine test to differentiate infections in guinea pigs caused by rickettsiae of murine and epidemic typhus and Rocky Mountain spotted fever.

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Studies on Chancroid. IV. The Ducrey Bacillus: Growth Requirements and Inhibition by Antibiotic Agents.*

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The classification of the Ducrey bacillus in the genus *Hemophilus* appears to be based largely on its morphology and on the fact that it grows best in a medium containing blood. Actually the growth requirements and biology of this organism have received very little study. The present article is a report of some observations made in the course of a clinical and laboratory investigation of chancroid. The data presented support the view that there are important differences between the Ducrey bacillus and other members of the genus *Hemophilus*.

Materials and Methods. The base medium used was proteose peptone broth (Difco). Blood was obtained from rabbits by cardiac puncture. Defibrination was accomplished by shaking with glass beads. Erythrocyte suspensions were prepared by washing repeatedly with large volumes of normal saline, until a negative Pandy test was obtained on the supernatant fluid.

X factor was prepared as follows: The clot

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TABLE I.
Growth of Ducrey Bacillus, *H. influenzae*, and *H. parainfluenzae*, in Media Containing Whole Blood and X and V Factors.

Organism	Base medium, plus			
	X factor	V factor	X and V factors	Whole blood
Ducrey No. 1	0	0	0	++++
No. 2	0	0	0	++++
No. 3	0	0	0	++++
No. 4	0	0	0	++++
No. 5	0	0	0	++++
No. 6	0	0	0	++++
H. influenzae 6208	0	0	++++	++++
" " I	0	0	++++	++++
" " II	0	0	++++	++++
H. parainfluenzae 7901	0	++++	++++	++++

Base medium: Proteose peptone broth (Difco).
Whole blood: Defibrinated rabbit blood (concentration 10% in base medium).
H. influenzae 6208, *H. parainfluenzae* 7901: American Type Culture Collection.
H. influenzae I, *H. influenzae* II: Type a strains recently isolated from patients with influenzal meningitis.
This table represents findings after more than three successive subcultures.
Readings based on microscopic examination of stained smears of cultures, after 48 hours' incubation at 34°C.

from 40 ml of rabbit blood was infused in 8 ml of normal saline at room temperature for one hour. This was then boiled for 5 minutes, and filtered through paper. The filtrate was autoclaved for 15 minutes at 15 pounds pressure. This material was added to the base medium in a concentration of 4% to provide an adequate supply of X factor.

V factor was prepared as follows: 20 g of baker's yeast were emulsified in 80 ml of distilled water, by rubbing in a glass mortar. The reaction was adjusted to pH 4.6 by addition of N/1 HCl. The mixture was boiled for 10 minutes and then centrifuged at 2000 r.p.m. for 20 minutes. The clear supernatant material was used as a source of V factor, being added to the base medium in a concentration of 10%.

All cultures were incubated at 34°C. Liquid media were distributed in 1-ml quantities, and cultures were inoculated with 1 loopful of material.

Requirement of X and V Factors. Hemophilus influenzæ has been shown to require for growth 2 agents which are present in erythrocytes. One of these, originally called the X factor, is heat-stable and is known to be hemin. The other, called V factor, is heat-labile, and is replaceable by coenzyme I or II.[1] *H. parainfluenzæ* requires only the V factor.

Six strains of Ducrey bacillus were tested for growth in the presence of X and V factors. Three strains of *H. influenzæ* and one of *H. parainfluenzæ* were included in the experiment. The results of the test are shown in Table I. It will be seen that all 10 organisms were able to grow in the medium in the presence of defibrinated blood. On the other hand, addition of X factor alone was not sufficient to support growth of any of the 10 organisms. *H. parainfluenzæ* was able to grow in a medium containing V factor alone. The 3 strains of *H. influenzæ* and *H. parainfluenzæ* all grew in a medium containing both X and V factors, but none of the Ducrey strains would grow in it. Thus, although the Ducrey bacillus resembles true members of the genus *Hemophilus* in that growth is supported by the presence of blood in the medium, it differs by inability to grow in a medium containing only X and V factors. One must conclude from this either that the X and V factors are not the substances essential for growth of the Ducrey bacillus, or that some other factor which is present in whole blood in addition to X and V factors is needed for growth.

Growth in Media Containing Cells Alone, or Serum Alone. The constituents of whole blood necessary for the growth of *H. influenzæ* and *H. parainfluenzæ* are present in the ery-

[1] Lwoff, A., and Lwoff, M., *Proc. Roy. Soc., London, Series B*, 1937, **122**, 352, 360.

TABLE II.
Growth of Ducrey Bacillus, *H. influenzae* and *H. parainfluenzae* in Media Containing Blood Serum or Washed Erythrocytes.

Organism	Base medium, plus	
	Blood serum	Washed erythrocytes
Ducrey No. 1	++++	++++
No. 2	++++	++++
No. 3	++++	++++
No. 4	++++	++++
No. 5	++++	++++
No. 6	++++	++++
H. influenzae 6208	0	++++
" " I	0	++++
" " II	0	++++
H. parainfluenzae 7901	0	++++

This table represents findings after more than 3 successive subcultures.

Readings based on microscopic examination of stained smears of cultures, after 48 hours' incubation at 34°C.

throcytes, not in the blood serum. It is known, however, that the Ducrey bacillus will grow in a medium enriched only with blood serum. A demonstration of this is the experiment shown in Table II. The concentration of serum required for growth of Ducrey bacilli appears to be relatively high. Optimum growth was obtained in media containing from 25 to 50% serum although some of the strains tested survived when the concentration was reduced to as low as 10%. The fact that Ducrey bacilli will grow in serum broth, a medium which will not support growth of either *H. influenzæ* or *H. parainfluenzæ*, together with the evidence in the preceding section, suggests that neither X nor V factor is essential for growth of the Ducrey organism.

The Ducrey bacillus can also be cultivated in a base medium enriched only with erythrocytes. The volume of erythrocytes necessary is relatively large: 10% or greater.

It would appear that enrichment with whole blood provides a better medium for growth of the Ducrey bacillus than is obtained with either cells or serum alone since growth can be maintained indefinitely in a base medium containing only 3% of defibrinated blood. It is probable, therefore, that whole blood contains more than one growth substance utilized by Ducrey organisms, and that these substances are present in both erythrocytes and serum. Further evidence bearing on this will be given in the next section.

Heat Stability of Growth Factors in Erythrocytes and Serum. The substances in rabbit blood serum essential for growth of Ducrey bacilli are not destroyed by heating at 100°C for 30 minutes. Twenty per cent serum broth heated in this manner will support the growth of Ducrey bacilli through repeated transfers without apparent change. Growth does not occur, however, in serum broth which has been autoclaved at 15 pounds pressure for 20 minutes.

The growth-supporting properties of erythrocytes are more easily destroyed by heat than are those of serum. Ten per cent erythrocyte-broth would not support growth of any of 6 strains of Ducrey bacillus after heating at 65°C for 30 minutes. Four of the 6 strains tested would not grow in this medium after it had been heated at 60° for 30 minutes.

The difference in heat stability of the growth factors in erythrocytes and serum provides further evidence that the agents present in the two constituents of blood are not the same.

Importance of Moisture for Growth of Ducrey Organisms on Solid Media. A striking peculiarity of members of the Ducrey group is their dependence on moisture for growth on the surface of a solid medium. We have discussed this elsewhere, in an article dealing with the cultural diagnosis of chancroid.[2] Usually no visible growth occurs on the surface of an inoculated blood agar plate placed in an ordinary incubator. On the other hand, a plate which has received a similar inoculum and which is incubated at the same temperature inside a closed jar containing a moist sponge will show a good growth of Ducrey colonies at the end of 24 hours. *H. influenzæ* and *H. parainfluenzæ* show no such rigid dependence on moisture in the air.

Satellite Phenomenon. Most members of the genus *Hemophilus* exhibit more luxuriant growth on the surface of a solid medium in the vicinity of colonies of certain other bacteria, particularly staphylococci. This enhanced growth is called the "satellite phe-

[2] Beeson, P. B., and Heyman, A., *Am. J. Syph., Gon. and Ven. Dis.*, in press.

TABLE III.
Growth of Ducrey Bacillus, *H. influenzae*, and *H. parainfluenzae* in Media Containing Varying Concentrations of Tyrothricin.

Organism	Dilution of tyrothricin						
	4000	8000	16000	32000	64000	128000	0
Ducrey No. 1	0	0	0	+	+++	+++	++++
No. 2	0	0	0	0	++	++++	++++
No. 3	0	0	0	0	++	+++	++++
No. 4	0	0	0	0	0	++	++++
No. 5	0	0	0	++	+++	+++	++++
No. 6	0	0	0	++	++	++++	++++
H. influenzae 6208	0	++	++++	++++	++++	++++	++++
" " I	0	++	++++	++++	++++	++++	++++
" " II	0	+++	++++	++++	++++	++++	++++
H. parainfluenzae 7901	+	++	++++	++++	++++	++++	++++

Medium: 10% defibrinated blood in base medium.
This table represents findings after more than three successive subcultures.
Readings based on microscopic examination of stained smears of cultures, after 48 hours' incubation at 34°C.

nomenon." It is thought to be due to liberation of growth substances by the staphylococci. No such change is noted in Ducrey colonies, whose gross appearance is not altered by proximity to staphylococcus colonies on the surface of a solid medium.

Dialysis of Growth Factors. Repeated attempts were made to demonstrate the growth factors for Ducrey organisms in dialysates of either defibrinated rabbit blood or fresh rabbit serum. Blood and serum were dialyzed against distilled water, physiologic salt solution, and proteose peptone broth. Sterilization of dialysates was accomplished by Seitz filtration, a procedure which does not affect the growth-stimulating properties of serum broth. None of the dialysates obtained would support growth, either alone or when added to the base medium.

Bacteriostasis by Antibiotic Substances. The 3 strains of *H. influenzæ* and 1 strain of *H. parainfluenzæ* were compared with 6 strains of Ducrey bacillus in respect to inhibition of growth by penicillin, tyrothricin, streptomycin,† and streptothricin. Other workers have already reported that penicillin exerts a pronounced bacteriostatic effect on Ducrey organisms.[3] We have repeated that work and our findings agree entirely. In addition we find a similar difference in respect to inhibition by tyrothricin. The results of a test are shown in Table III, where it will be observed that the typical *Hemophilus* organisms were able to grow in much greater concentrations of tyrothricin than was the case with strains of Ducrey bacillus. This is further evidence of a basic difference between Ducrey bacilli and true *Hemophilus* organisms. In respect to streptothricin and streptomycin, no significant difference in susceptibility was found.

Summary. In a comparative study of several strains of Ducrey bacillus with *H. influenzæ* and *H. parainfluenzæ* a number of significant biologic differences have been noted. The Ducrey bacillus will not grow in a medium which supplies both the X and V factors. Unlike these other members of the genus *Hemophilus,* the Ducrey bacillus will grow in a medium enriched only with blood serum. It will also grow in a medium enriched only with erythrocytes. The agents in serum and in erythrocytes which support growth of the Ducrey bacillus differ in heat stability; that in serum withstands 100°C but not autoclaving, while that in erythrocytes is inactivated by heating to 65°C. The addition of both erythrocytes and serum to the base medium provides a more favorable medium for the growth of the Ducrey bacillus than either one alone. Ducrey colonies will not develop on the surface of a solid medium unless the humidity of the atmosphere is very

† Courtesy of Dr. S. A. Waksman.

[3] Mortara, M., Feiner, R. R., and Levenkron, E., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 163.

high. The "satellite phenomenon" is not observed in colonies of Ducrey bacillus. Attempts to separate Ducrey growth factors from whole blood or serum by dialysis have not been successful. The Ducrey bacillus differs significantly from *H. influenzæ* and *H. parainfluenzæ* in susceptibility to inhibition by penicillin and by tyrothricin.

Miss Elizabeth Roberts gave technical assistance in this work.

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Muscles Consisting of a Single Motor Unit After Poliomyelitis.

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During an investigation concerning the possible increase of muscle force in paretic muscles by means of increased motor nerve branching[1] the action potentials of more than 1000 different paretic muscles were obtained. Among these the records of 36 muscles in 26 different patients were remarkable for their simplicity and they resembled closely records produced by single motor units in fully[2,3,4] or partially innervated muscles,[5] though no measures were taken to insure the recording of units. The behavior of these muscles was analyzed by repeated tests.

Methods. In routine electrograms, the patient contracted the muscle as strongly as possible for 2 or 3 seconds and then released the tension quickly. Electrodes, consisting of solder pellets of 5 mm diameter were attached to the skin with tape, electrode paste being used to insure a good contact. The action potentials were amplified and recorded by a Matthews oscillograph. Nearly all of the muscles to be described were found in patients who had undergone the treatments intended to enhance the nerve branching.[1] In all cases the paresis was at least of one year standing.

Results. The short lasting, strong contractions of all muscles under consideration were accompanied by a single row of action potential spikes, (Fig. 1) which were in general, rather evenly spaced and often grew considerably in height, especially the first 10 spikes. In by far the majority of the cases the voluntary control of the muscle was good, in only a few were there signs of poor control as evidenced by an irregular and low frequency of the spikes and difficulty in maintaining the contraction even for a short time.

The maximum frequency of the spikes reached in this way has been measured for all these muscles and was found to vary between 20 and 60 per second, the mean being 40. In accordance with the data of Gibson and Mills[3] it was observed that 2 or even 3 spikes could follow each other closely, (0.01 second), after which there was usually a somewhat longer delay before the appearance of the next spike. Therefore the maximum frequency was computed by using the time interval of at least 10 consecutive spikes. Since most of the 15 different muscles used in the investigation are represented only a few times and these are only larger muscles in which the influence of the contraction of neighboring muscles on the electrogram is negligible, the material is not very suitable to show whether or not different muscles have different inherent maximum frequencies.[5] It may be significant, however, that the deltoid which is represented 5 times, showed a maximum of 60 in 3 and of 40 in 2 of these cases, and has thus a mean frequency greater than that of the mean maximum given above.

[1] Billig, H. E., van Harreveld, A., and Wiersma, C. A. G., *J. Neuropath. and Exp. Neurol.*, 1946, in press.

[2] Smith, O., *Am. J. Physiol.*, 1934, **108**, 629.

[3] Gilson, A. S., and Mills, W. B., *Am. J. Physiol.*, 1941, **133**, 658.

[4] Denslow, J. S., and Hassett, C. C., *Am. J. Physiol.*, 1943, **139**, 652.

[5] Lindsley, D. B., *Am. J. Physiol.*, 1935, **114**, 90.