A STUDY OF THE CHANGES IN VIRULENCE OF THE PNEUMOCOCCUS AT DIFFERENT PERIODS OF GROWTH AND UNDER DIFFERENT CONDITIONS OF CULTIVATION IN MEDIA.*

By AUGUSTUS B. WADSWORTH, M.D., and MARY BUTLER KIRKBRIDE.

(From the Division of Laboratories and Research of the New York State Department of Health, Albany.)

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Considered broadly, there are two fundamental factors which determine the pathogenic activities of bacteria, growth in the tissues of the host and injury of the tissues locally or systemically. It is not clear in the different infectious processes to how great an extent one phase,—growth or simple multiplication, or pure parasitism—is dependent upon the other phase,—injury resulting from the production of toxic substances. Students of infection and immunity, however, are now drawing each year more precise distinctions between the parasitic and the toxicogenic activities of the agents of infection. Thus in leprosy and tuberculosis on the one hand, the parasitism of the agents is generally recognized to be dominant, while in diphtheria and tetanus, on the other hand, the parasitism has long been known to be extremely limited and largely, if not wholly overshadowed by the action of the powerful toxins which the organisms produce locally. In other infections, notably the bacteriemic infections and especially pneumococcus infection, the full significance of each of these factors is at present indeterminate chiefly on account of our lack of knowledge of the conditions affecting not only the toxicogenic activities of the agents during infection but their parasitism as well.

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Our conception of parasitism connotes adaptation—lacking the necessary adaptation, an organism could not become parasitic. Attention has thus been directed to this fundamental and essential quality which the pathogenic bacteria possess and which distinguishes them from all others. As a result of numerous observations on the effect of animal passage upon bacteria, the virulence, or degree of pathogenicity, has become so intimately associated with one factor in the adaptation of the organism, the medium, that another equally important factor, the growth energy, has hitherto received scant attention. It is the purpose of the following paper to present the results of a preliminary study of this essential relation between the different phases of growth and the degree of virulence of the agent.

Comparatively few observations on increase of virulence produced outside the body tissues are recorded and these are found chiefly in the studies of the early investigators. Thus according to Roger,[1] while the vegetative characteristics and the virulence of the streptococcus diminish progressively during repeated transfers in broth, both the vegetative power and the pathogenic action are again enhanced when serial transplants of the attenuated strain are made in normal rabbit serum. He considered that the action of serum outside the animal organism was therefore almost identical with that of the tissues of the organism. Von Lingelsheim,[2] also working with the streptococcus, was able to confirm these results only within certain limits. He was never able by successive serum transfers to increase the virulence of *Streptococcus pyogenes*, for example, so that a single injection was fatal for mice. Von Lingelsheim found, however, that the virulence was in most instances increased more rapidly when diminishing minimal doses were used and when the transfers were made from animal to animal. In our experience there has occurred sudden and marked decrease of virulence of pneumococcus strains recovered from dead rabbits kept for an interval of several days or weeks at a low temperature. The maintenance of virulence by preservation of pneumococci in dried spleens of mice, as first suggested by Heim,[3] has, however, been used extensively.

While Eyre and Washbourn[4] also reported increase of virulence by animal passage and decrease by prolonged artificial cultivation, they as well as others

[1] Roger, G. H., *Compt. rend. Soc. biol.*, 1890, xlii, 573.

[2] von Lingelsheim, W., *Z. Hyg. u. Infectionskrankh.*, 1891, x, 331; 1892, xii, 308; *Beitr. exp. Therap.*, Heft I, 1899, 1, 49.

[3] Heim, L., *Z. Hyg. u. Infectionskrankh.*, 1905, l, 123.

[4] Eyre, J. W., and Washbourn, J. W., *J. Path. and Bacteriol.*, 1897, iv, 394; *Lancet*, 1899, i, 19.

made further observations on the marked variations that occur in the increase of virulence of different strains of the pneumococcus isolated from cases of lobar pneumonia and from normal persons. In these investigations, however, repeated animal passage was used for increasing the virulence.

Cotoni[5] studied the virulence of the pneumococcus in culture and in the animal body. He stated that by using gelatin and special peptone media containing no body fluids he was able to raise the virulence for mice of certain originally avirulent strains. Fluctuations in virulence of strains carried on *in vitro* were also noted.

Hilbert[6] found that association of *B. diphtheriæ* and streptococcus in culture tended to produce a more rapid and increased production of toxin, a result which he considered due chiefly to changes in the medium occurring as a result of the growth of the streptococcus. Thus streptococcus culture filtrate, he stated, was an excellent medium for toxin production. Attempts to raise the virulence of *B. diphtheriæ* by cultivation with streptococci or other organisms have, however, generally proved unsuccessful. Williams[7] cultivated two avirulent strains with virulent streptococci for 90 generations without any change in virulence. Roger[1] reported that he had been able to make avirulent pneumococci and streptococci virulent by injecting one or more drops of a sterile culture or extract of *B. prodigiosus* into rabbits at the same time. Although non-pathogenic, *prodigiosus* cultures are nevertheless toxic and thus might act as a predisposing agent favoring the development of streptococcus infection. Roger apparently did not test the effect of combining in culture prior to injection.

The latent period of growth of bacteria after transfer to a fresh medium and the diminution in growth due to inhibition after the maximum has been reached, have long been recognized. Müller[8] called attention to variations in the length of the latent period or lag depending upon the age of the parent culture. Barber[9] first found that the latent period might be avoided if an actively dividing organism accustomed to the medium was used for inoculation. Penfold[10] studied the nature of bacterial lag and Ledingham and Penfold[11] have contributed a mathematical analysis of the lag phase in the growth of bacteria, in which they showed that the generation time decreased steadily and uniformly until the minimal length or commencement of the second or logarithmic phase was reached. Chesney[12] has recently made investigations of these phases of bacterial growth under

[5] Cotoni, L., Thèse de Paris, 1912, No. 78.
[6] Hilbert, P., *Z. Hyg. u. Infectionskrankh.*, 1898, xxix, 157.
[7] Williams, A. W., *J. Med. Research*, 1902, viii, 83.
[8] Müller, M., *Z. Hyg. u. Infectionskrankh.*, 1895, xx, 245.
[9] Barber, M. A., *J. Infect. Dis.*, 1908, v, 379.
[10] Penfold, W. J., *J. Hyg.*, 1914, xiv, 215.
[11] Ledingham, J. C. G., and Penfold, W. J., *J. Hyg.*, 1914, xiv, 242.
[12] Chesney, A. M., *J. Exp. Med.*, 1916, xxiv, 387.

different conditions and at different stages of the culture. But the possible relation between these phases of growth and the virulence of the organism has apparently never been studied.

The present study of changes in virulence of the pneumococcus in culture, under different conditions of growth, was undertaken not only for the purpose of obtaining a clearer conception of the immediate factors involved but also in the hope that, as a result of a more precise knowledge of the conditions of culture at different stages of the growth of the pneumococcus, it might be possible to develop better methods for producing immune serum of greater potency. The strain selected for the tests was a standard Type I pneumococcus, which had been maintained at a uniformly high degree of virulence for a number of years by means of frequent animal passage. When the interval between passages exceeded 2 days, the strain was stored in a special semisolid serum medium, in which it had been found that a constant level of virulence could be held for several weeks.[13] Previous tests had, however, shown that a rapid decline of virulence was likely to occur unless the above methods were rigidly observed.

For purposes of comparison the experiments included repeated transfers at 8 and 24 hour intervals of subcultures from the standard strain both in its original virulent and in its later attenuated relatively non-virulent state. The 8 hour transfers were made in order that the latent period and also the period of inhibition of growth might be avoided. The organisms were grown in 1 per cent peptone meat infusion broth prepared by neutralizing to 0.2 per cent acid (phenolphthalein) before heating and coagulating the meat proteins, and also in the same medium to which 5 per cent citrated rabbit blood had been added. One set of the blood cultures was grown in a thin layer of the medium exposed to the air while in the other a surface layer of albolene excluded the oxygen of the air and gave fairly anaerobic conditions of growth after the oxygen absorbed by the corpuscles had been taken up by the pneumococcus.

While it was realized that parallel bacterial counts and acidity and hydrogen ion estimations at different periods of growth in con-

[13] Wadsworth, A., *Proc. New York Path. Soc.*, 1903, iii, 113.

nection with the virulence tests would have been of considerable value, the few tests that it was possible to carry out proved insufficient to allow of definite conclusions.

Reduction of Virulence during Cultivation by Repeated Transfers at Intervals of 24 Hours.

Two series of transfers at 24 hour periods were made in meat infusion broth (Table I). In the first series, undertaken originally for another purpose, only one virulence test was made up to the 17th week when 1 cc. of the 24 hour growth, injected intraperitoneally, failed to kill a mouse. Tested at intervals up to the 32nd week, mice receiving 1 cc. of the culture, with one or two exceptions, not only survived but failed to show any abnormal symptoms.

In the second series virulence tests were made at the end of the 2nd, 3rd, 6th, and 8th weeks, when the transfers were discontinued. In 3 weeks 0.000001 cc. did not kill in less than 46 hours; on the contrary a mouse receiving 0.1 cc. survived. By the 6th week still more complete loss of virulence had occurred, 1 cc. of the 24 hour growth failing to kill.[14] Thus, it was shown that, except in rare instances in which slightly greater susceptibility in the individual mouse or irregularities in cultural growth might be important factors, marked reduction in virulence had been established.[15]

[14] Marked differences in the power to retain virulence in artificial medium are shown by different strains of the pneumococcus. In our experience a Type I strain cultivated in plain meat infusion broth medium and transferred at 24 hour intervals for 14 months has killed in 42 hours, when 0.000001 cc. of the 24 hour growth is inoculated into mice. Transferred at 48 hour intervals for nearly 1 year 0.000001 cc. has even proved fatal in approximately 2 days. A standard Type II and a Type III strain also required repeated transfers for many weeks before a marked decrease in virulence was obtained.

[15] Later tests of the attenuated Type I culture during continued transfers in broth for more than a year have shown at times evidence of slight fluctuations, 1 cc. for one or two successive tests proving fatal to mice. These fluctuations appear, however, to be infrequent and transitory.

TABLE I.

Decrease in Virulence by Repeated Transfers in Broth at 24 Hour Intervals. Results of Virulence Tests on Mice Inoculated with 24 Hour Growth.

Medium and series.	Parent culture 18 hr. growth.	Transferred every 24 hrs. for.													
		2 wks.	3 wks.	4 wks.	6 wks.	8 wks.	17 wks.	18 wks.	20 wks.	21 wks.	23 wks.	24 wks.	30 wks.	30½ wks.	32 wks.
Plain broth medium; 1st series.	0.000001 cc. D.*—46 hrs.			0.01 cc. D. 41 hrs.			1 cc. L.	1 cc. L.	1 cc. L. 1 " "	1 cc.D. 54 hrs.	1 cc. L. 1 " "	1 cc. L. 0.6 " "	1 cc.D. 48 hrs.	1 cc. L. 1 " "	1 cc. L. 1 " D. 30 hrs.
Plain broth medium; 2nd series.	0.000001 cc. D. — 46 hrs.	0.001 cc. L.	0.1 cc. L.		1 cc. L.	1 cc. L. 1 " "	Discontinued.								

* In the tables D. indicates died, L., lived.

Maintenance of Virulence during Rapid Cultivation by Repeated Transfers at Intervals of 8 Hours.

Cultures of the standard virulent strain after recent passage through mice were used in all the series of these experiments. Three series of tests were made in plain broth and in the two blood media previously described (Table II). In the first series after 2 weeks, or 42 transfers, virulence was maintained; after 12 weeks, or 252 transfers, mice receiving 0.000001 cc. succumbed after a slightly increased interval. In the second series virulence held in both blood cultures up to the 5th week, when owing to contaminations they were discontinued. The third series was continued until the 13th week, or for 273 transfers. The organisms grown in the plain broth medium maintained their virulence unimpaired. Some fluctuations occurred in the blood cultures, although, with one exception, all mice inoculated with 0.000001 cc. succumbed. These slight irregularities, occurring in this and other tests in which the medium contained 5 per cent blood, were probably due in part to the medium, as at times separation of the blood elements and the broth occurred, affecting the growth of the culture.

The results of this experiment stand in striking contrast to those of the previous experiment. In one the virulence of the pneumococcus was maintained for 13 weeks with little or no loss; in the other virulence decreased rapidly and in 6 weeks was practically lost. In one the pneumococcus was under rapid cultivation; in the other the pneumococcus was cultivated more slowly. In the first experiment and in one series of the second experiment the same plain broth medium was used, to which had been added no special enriching material. It was thus shown that the presence of blood, serum, or other tissue fluid is not essential and that the virulence of the pneumococcus may be maintained by rapid cultivation alone. This is of special significance and suggested further experiments to determine the effect of rapid cultivation on cultures which had been attenuated and were practically avirulent.

TABLE II.

Maintenance of Virulence by Repeated Transfers in Broth at 8 Hour Intervals. Results of Virulence Tests on Mice Inoculated with 8 Hour Growth.

Medium and series.	Transferred every 8 hrs. for.								
	2 wks.	4 wks.	5 wks.	6 wks.	7 wks.	9 wks.	11 wks.	12 wks.	13 wks.
Plain broth medium; 1st and 2nd series.	1st series: 0.000001 cc. D. 38 hrs.		2nd series: not tested, growth too slight.					1st series: 0.000001 cc. D. 50 hrs.	
Plain broth medium; 3rd series.		0.0001 cc. D.—29 hrs. 0.000001 cc. D. 100 hrs.	0.000001 cc. D.—53 hrs.	0.000001 cc. D. 32 hrs.	0.000001 cc. D. 42 hrs.	0.000001 cc. D.—38 hrs.	0.000001 cc. D. 42 hrs.		0.000001 cc. D. 32 hrs.
Plain broth + citrated 5 per cent normal rabbit blood; aerobic; 1st and 2nd series.	1st series: 0.000001 cc. D.—31 hrs.		2nd series: 0.000001 cc. D. 30 hrs.					1st series: 0.000001 cc. D.—63 hrs.	
The same medium; 3rd series.		0.000001 cc. D. 30 hrs.		0.000001 cc. D. 40 hrs.	0.000001 cc. D. 95 hrs.	0.000001 cc. D.—38 hrs.	0.000001 cc. D. 42 hrs.		0.000001 cc. D. 54 hrs.

Plain broth + citrated 5 per cent normal rabbit blood; anaerobic; 1st and 2nd series.	1st series: 0.000001 cc. D. 23 hrs.		2nd series: 0.000001 cc. D. 33 hrs.					1st series: 0.000001 cc. D. 50 hrs.	
The same medium; 3rd series.		0.000001 cc. D. 44 hrs.		0.000001 cc. D. 40 hrs.	0.000001 cc. D. 40 hrs.	0.000001 cc. D.—38 hrs.	0.000001 cc. D.—42 hrs. 0.000001 cc. D.—47 hrs.	0.000001 cc.* D. 5 days, 6 hrs.	0.00001 cc.* D. 40 hrs. 0.00000 1cc.* L.

* Growth slight and unsatisfactory, owing to separation of blood elements and partial clotting.

TABLE III.

Increase in Virulence by Repeated Transfers in Broth at 8 Hour Intervals. Results of Virulence Tests on Mice Inoculated with 8 Hour Growth.

Medium and series.	Parent culture 24 hr. growth.	3 transfers.	9 transfers.	21 transfers.				
Plain broth medium; 1st series.	1 cc. plain broth. L.	1 cc. L.	1 cc. D.—36 hrs.	1 cc. L. 0.5 " " 0.01 " "				
	Parent culture 24 hr. growth.	3 transfers.	9 transfers.	18 transfers.	30 transfers.	42 transfers.	63 transfers.	84 transfers.
Plain broth medium; 2nd series.	1 cc. plain broth. L.	1 cc. D. 45 hrs. 1 " L.	1 cc. D.—31 hrs. 1 " "—40 " 0.5 " " 48 "	1 cc. D.—42 hrs. 0.5 " " 50 " 0.1 " "—42 " 0.1 " "—54 " 0.01 " "—56 "	0.1 cc. D. 5 days, 9 hrs. 0.01 cc. D. 41 hrs. 0.001 " L. 0.001 " "	1 cc. D. 44 hrs. 0.5 " " 33 " 0.5 " "—42 " 0.1 " "—66 "	1 cc. D. 30 hrs. 0.5 " L. 0.1 " " 0.01 " D. 71 "	1 cc. D.—88 hrs. 1 " " 5 days, 17 hrs. 0.5 cc. D. 7 days, 10 hrs. 0.5 cc. L. 0.1 " " 0.1 " "
	Parent culture 24 hr. growth.	3 transfers.	9 transfers.	21 transfers.				
Plain broth + citrated 5 per cent normal rabbit blood; aerobic; 1st series.	1 cc. plain broth. L.	1 cc. D.—32 hrs.	1 cc. D. 26 hrs.	Not tested.				

	Parent culture 24 hr. growth.	3 transfers.	9 transfers.	18 transfers.	24 transfers.	36 transfers.	78 transfers.	
The same medium; 2nd series.	1 cc. plain broth. L.	1 cc. D. 25 hrs. 1 " " —49 "	1 cc. D.—31 hrs. 1 " " 42 " 0.5 " " 4 days, 5 hrs.	1 cc. D. 8 days, 5 hrs. 0.5 cc. D. 6 days, 18 hrs. 0.1 cc. L. 0.1 " " 0.01 " "	1 cc. D. 30 hrs. 0.5 " L. 0.1 " " 0.1 " "	1 cc. D. 33 hrs.* 0.5 " L.	1 cc. D.—24 hrs. 1 " " —40 " 0.5 " " —48 " 0.5 " L.	

	Parent culture 24 hr. growth.	3 transfers.	9 transfers.	21 transfers.				
Plain broth + citrated 5 per cent normal rabbit blood; anaerobic; 1st series.	1 cc. plain broth. L.	1 cc. D.—32 hrs.	1 cc. D. 72 hrs.	1 cc. D. 31 hrs. 0.5 " L. 0.1 " D.—41 hrs.				

	Parent culture 24 hr. growth.	3 transfers.	9 transfers.	18 transfers.	30 transfers.	42 transfers.	84 transfers.	
The same medium; 2nd series.	1 cc. plain broth. L.	1 cc. D.—41 hrs. 1 " " 31 " 0.5 " L.	1 cc. D. 48 hrs. 1 " " —5 days, 6 hrs. 0.5 cc. D.—40 hrs.	1 cc. D. 36 hrs. 0.5 " L. 0.1 " " 0.1 " D. 8 days, 18 hrs.	1 cc. D. 19 hrs. 0.5 " L. 0.1 " " 0.1 " "	0.1 cc. D.—42 hrs. 0.5 " L.	1 cc. D. 18 hrs. 1 " L. 0.5 " D. 47 hrs. 0.5 " L.	

These results indicate fluctuations in virulence of the 8 hour growth possibly due to slight changes in the technique of making the transfers, in the medium, or in the inoculation of the cultures. It is difficult to maintain uniform conditions throughout 84 transfers, especially when blood medium is used. The results of such slight changes would naturally be more evident in the 8 hour than in the 18 or 24 hour growth. In each table the complete experiment is recorded in order to show these variations. Obviously, whenever the method of transferring the culture fails to maintain the vegetative power, the virulence also will fail.

* Staphylococcus contamination. Culture continued from preceding 8 hour transfer.

Increase of Virulence during Rapid Cultivation by Repeated Transfers at Intervals of 8 Hours.

The effect of rapid transfers on the culture which had lost its virulence after repeated seedings at 24 hour intervals in plain broth was studied. 1 cc. of this culture for some time had proved to be innocuous for mice. Seedings of a 24 hour growth were made in its own broth and in the two blood media (Table III). In the preliminary series the virulence was tested at the end of the 1st, 3rd, and 7th days. After three 8 hour transfers mice receiving 1 cc. of the blood cultures died in less than 32 hours. Control mice receiving 1 cc. of uninoculated blood medium were apparently unaffected by the inoculation. After nine transfers each mouse, including that inoculated with the culture in plain broth, succumbed. 1 cc. of the anaerobic blood was fatal after twenty-one seedings, but the same amount of the plain broth culture failed to kill. The aerobic culture in 5 per cent blood broth was not tested. While these results were incomplete and showed considerable irregularity, they were considered of sufficient significance to warrant repetition.

In the second series transfers were made at 8 hour intervals and the virulence was tested from time to time for approximately 4 weeks. The plain broth and anaerobic blood cultures were transferred 84 times; the aerobic culture in blood 78 times. After three 8 hour seedings in media with or without blood five out of six mice inoculated with 1 cc. died in less than 49 hours; after nine transfers five died in less than 48 hours, the sixth after several days. 0.5 cc. was also fatal. In all subsequent tests, with one exception, 1 cc. of the 8 hour growth killed, though in a few instances death was considerably delayed. In these as in the other tests recorded pneumococci were invariably obtained from the heart's blood at autopsy. While the cultures containing blood showed more immediate return of virulence, the increase in virulence was on the whole most marked in the plain broth cultures, 0.5, 0.1, and even 0.01 cc. at times causing death. Thus, without animal passage the virulence of the attenuated culture, 1 cc. of which had previously failed to kill, was increased by rapid transfers in plain broth at 8 hour intervals, so that inoculation of 0.1 and 0.01 cc. was fatal.

TABLE IV.

Fluctuations in Virulence of Attenuated Culture during the 24 Hour Period of Growth.

Medium and test.	Parent culture 24 hr. growth.	Tested at.			
		4 hrs.	6 hrs.	8 hrs.	24 hrs.
Plain broth medium; 1st test.	*	A.† 1 cc. L. B. 1 " D. 7 days.	A. 1 cc. D. —36 hrs. B. 1 cc. D. —36 hrs.	A. 1 cc. D. —34 hrs. B. 1 cc. L.	A. 1 cc. L. B. 0.6 " "
The same medium; 2nd test.	*	Not tested.	1 cc. D.—34 hrs. 1 cc. D.—18 hrs. 0.5 cc. L, 0.5 " " 0.1 " "	1 cc. D.—81 hrs. 1 cc. L. 0.5 " " 0.5 " "	*
The same medium; 3rd test.	*	Not tested.	1 cc. D. 52 hrs. 1 " " 25 " 0.5 " " L. 0.5 " " "	Not tested.	*
The same medium; 4th test.	1 cc. L, 1 " "	A, 1 cc, L, B, 1 " "	A. 1 cc. D. 18 hrs. B. 1 cc. D. 48 hrs.	A. 1 cc. D. —29 hrs. B. 1 cc. D. 16 hrs.	A. 1 cc. L. B. 1 " "

*For a period of 6 weeks before and after these experiments control tests of this culture in broth after growth for 24 hours at 37°C. failed to kill mice in doses of 1 cc. with few exceptions; see Table I, tests from the 17th to the 32nd week.

†A, culture seeded from 24 hour growth. B, inoculated from the same culture as A.

As it was thought that the virulence of both the aerobic and anaerobic cultures containing 5 per cent rabbit blood might be further increased if the blood was reduced to $2\frac{1}{2}$ per cent, since the higher percentage might retard growth, a fresh series was commenced. The virulence of the third and ninth 8 hour generations from the avirulent culture was tested, but no marked change in virulence from that recorded in the previous tests resulted. In all 8 hour cultures and especially in those containing blood, growth is

occasionally sufficiently delayed to affect materially the dosage or number of organisms inoculated, with resulting irregularities.

Fluctuations in Virulence during the 24 Hour Period of Culture.

In order to ascertain whether the attenuated 24 hour broth culture might not itself pass through a period of increased virulence, coincident with that of maximum growth, mice were inoculated with the culture on the 4th, 6th, and 8th hours after seeding. The results are given in Table IV. The most marked change was shown at the 6 hour period, all six mice inoculated with 1 cc. dying in less than 52 hours, though four others receiving 0.5 cc. survived. One of two receiving 1 cc. of the 4 hour growth died in about 7 days while two of four inoculated with the 8 hour culture also died. Two controls which received the same culture after the usual 24 hour inoculation were not affected. It therefore appeared that an increase in virulence actually occurred, though this was less marked than in the subcultures which had been transferred repeatedly at frequent intervals for a longer period.[16]

SUMMARY AND CONCLUSIONS.

It has been possible by rapid transfers alone, not only to maintain the virulence for mice of the pneumococcus in artificial media but also to restore a certain degree of virulence to cultures previously rendered non-virulent by less rapid transfers in the same medium. For these results the presence of enriching fluids such as blood or serum is not required. In addition it has been shown that attenuated cultures, which had been repeatedly demonstrated to be avirulent for mice at the 24 hour period of growth, exhibited marked pathogenicity if injected during, or especially at the commencement of the period of maximum growth when the growth energy may be

[16] In a later test of the fluctuations in virulence during the 24 hour period of growth, the mice which had received the culture at the end of the 6 and 8 hour growth periods died promptly of pneumococcus infection, indicating that this culture possessed as much virulence as those transferred repeatedly at short intervals. The medium, however, was not from the same lot as that used in the previous tests.

considered at its height. In these cultures, however, the increase in virulence was usually less than in others transferred repeatedly at frequent intervals.

The significance of these results is not necessarily limited to pneumococcus infections. Other lines of investigation are suggested which may possibly help to clarify certain conceptions of the relation which the different activities of the bacterial cell as an agent of infection bear to one another and to the host in various infectious diseases. Although the pneumococcus may offer an especially striking example for purposes of demonstration by experiment, it is not probable that the close relation between the vegetative power or growth energy of the pneumococcus and its pathogenic power is peculiar to this organism. The vegetative power may depend upon many conditions affecting both the host and the bacterial agent of infection. Different species of bacteria may acquire or develop it in different degrees under different conditions. But it must assuredly form the basis not only of the essentially parasitic but also of the more special toxicogenic activities of the bacteria.