THE VIRULENCE OF STRAINS OF *BACTERIUM COLI* FOR MICE.

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ONE of the aspects of bacteriology which remains obscure in spite of a great volume of research is the precise nature and cause of virulence in micro-organisms. For a given host there are often very large variations in virulence between different strains of the same species. It is with these inter-strain differences in virulence that the present paper is concerned.

An essential property of virulent strains is their ability to grow in the host, whereas non-virulent strains are usually eliminated sooner or later by the host's defences. It seems that virulent organisms must be able to resist one or more of the defence reactions to which avirulent strains succumb. In this light the study of virulence becomes a study of host defence mechanisms and the variation of microbial response to them. Very little information at the molecular level is available on this subject, except for work relating virulence of various species to the possession of a capsule (Avery, 1932). In pneumococci the capsule is composed of polysaccharide material and prevents removal of the organisms by phagocytosis. Injection of bacteria together with mucin or gum tragacanth is well known to increase their apparent virulence and because of the diversity of substances which cause this effect (Olitzki, 1948), one might think that mere mechanical coating of bacteria was the important factor. Against this belief and pointing to a more specific effect is the fact that specific capsular antiserum to pneumococci renders them susceptible to phagocytosis in spite of the fact that the capsule appears even larger than before.

In the case of *Salmonella typhi* Felix and Pitt (1935) have produced evidence that virulence for mice and probably for man is associated with an unstable antigen, the virulence or Vi antigen. This antigen prevents normal O agglutination *in vitro* and it is believed by Felix to act *in vivo* by masking the reaction between normal serum antibodies of the O type and bacteria. Since this combination between normal serum antibodies and bacterial antigens is a necessary preliminary to complement-mediated lysis and also greatly enhances the efficiency of phagocytosis, prevention of combination would increase the virulence of the organism.

It is theoretically possible that some hosts are resistant to infection by certain bacteria because they do not possess all the growth factors and nutrients required by the organisms for growth (Bacon, Burrows and Yates, 1951). It is unlikely that variations in growth requirements can explain many of the observed differences in virulence among the enteric organisms since most strains of *Salmonella* and *Bacterium coli* have little or no organic requirements other than for glucose.

The observations that only 6 per cent of " normal " coliform organisms were inhibited by DL-serine, whereas 65 per cent of the various serological types of

Bact. coli isolated from cases of gastro-enteritis were susceptible to this inhibition (Rowley, 1953), suggested that this might be an *in vitro* reaction paralleling differences in virulence. A similar relationship between serine inhibition and human virulence has been reported by Dubos (1949) for *Mycobacterium tuberculosis*.

Although the relationship between mouse virulence and human pathogenicity may be in doubt (Miles, 1951), these studies, for obvious reasons, were carried out on mice. Twenty strains of *Bact. coli* were selected so as to include representatives of the serine-sensitive and -resistant groups, strains sensitive to other amino acid inhibitions and strains isolated from cases of gastro-enteritis.

MATERIALS AND METHODS.

Of the 20 *Bact. coli* strains, 6 were from a collection of normal faecal coliform organisms ; of the others, 10, prefixed E, were kindly supplied by Dr. Joan Taylor and came from cases of gastro-enteritis ; we are indebted to Dr. Studzinski of Glasgow for 3 others, prefixed A, belonging to serological type O 111 and also derived from cases of gastro-enteritis. All the strains were freeze-dried from a serum broth culture and 6 slopes of Dorset egg medium were inoculated from one of the freeze-dried tubes of each strain. The slopes were kept at 2° and after a slope had been opened 10 times it was rejected and a fresh slope used. In this way the culture used was always a second subculture from the master freeze-dried strain. All the strains would grow readily on a glucose inorganic salt medium containing ammonium salts as the sole nitrogen source (Davis and Mingioli, 1950). The biochemical reactions of the 20 strains, together with their amino acid sensitivities and antigenic types when known, are listed in Table I. All cultures were incubated at 37°.

TABLE I.—*Characters of Strains Used.*

Strain.	Lactose.	Maltose.	Xylose.	Antigenic type.	Amino acid sensitivity.
145	+	+	+	U	Serine, norleucine.
75	−	−	+	U	,, ,,
E2380 (V)	+	+	+	0111 B4 H12	,,
E2846	+	+	+	0111 B4 H12	,,
E.C.	+	+	+	U	,, ,,
21	+	+	+	—	Norleucine, lysine.
E2070	+	+	+	U	,,
A170	+	+	+	0111 B4	,, serine, cystine.
A104	+	+	+	0111 B4	Norleucine, serine.
E1790	+	+	−	U	,, ,,
E1662	+	+	+	0111 B4 H2	,, norvaline.
E3751	+	+	+	0111 B4 H12	,,
E044	+	+	−	044	,,
A172	+	+	+	0111 B4	,, ,, serine, cystine.
147	+	+	+	—	Norleucine, norvaline, cystine.
146	+	+	+	—	Norleucine.
E2206 (AV)	+	+	+	0111 B4 H12	,,
K12	+	+	+	—	,, valine, norvaline.
E2186	+	+	+	0111 B4 H2	Serine, cystine.
197	+	+	+	—	Norleucine, norvaline, lysine.

U = unknown antigenic structure.
All strains are indole +, methyl red +, Vosges-Proskauer −, citrate −, mannitol +.

Test animals.—Most of the work was done using a pure strain of Swiss white mice No. 1, weight limits 18–22 g. For one experiment albino guinea-pigs, weight 300 g. were used.

Virulence tests.

The method used was similar to that described by Smith (1950) and consisted of intraperitoneal injection into mice of 0·2 ml. of a suspension of the organism (14 hr. growth in 10 per cent mouse serum broth) in 2 per cent gum tragacanth or in 1·7 per cent hog gastric mucin (1701 W. Wilson Labs., Chicago) + 0·4 per cent finely powdered charcoal. The gum tragacanth had the effect of increasing the virulence of all the strains about 50-fold, thereby extending the scale over which differences in virulence could be observed. The gastric mucin + charcoal was even more effective in this direction and results were more reproducible. Without virulence-enhancing factors, some of the less virulent organisms would not kill even when 0·2 ml. of an undiluted 14-hr. culture was injected, whereas with the most virulent strains a 1/100 dilution without mucin would kill. For many of the strains 3 10-fold dilutions of the suspensions were injected into 3 groups of 3 mice, *i.e.*, 9 mice in all. This rough method was supplemented with some of the strains by additional determinations using 6 mice in each group.

The results with the gum tragacanth suspensions were all obtained on the same day with one batch of mice; all the organisms had been inoculated in equal numbers into tubes prepared from the same batch of 10 per cent mouse serum broth.

Viable counts on the original bacterial suspension were done in triplicate by the drop method of Miles and Misra (1938). Deaths usually occurred within 24 hr. of injection but final results were read after 48 hr. In the initial stages of the work, heart blood cultures were performed on all dead mice, but since these cultures were always positive for the specific *Bact. coli* injected, the laborious process of isolation and identification was later discontinued.

Toxicity tests.

The organisms were grown for 14 hr. in 10 per cent serum broth, centrifuged and concentrated in saline. The thick suspensions were heated for 30 min. at 80° and samples withdrawn for sterility tests and for dry weight determination after allowing for the Na Cl content. In order to be comparable with the virulence tests, bacterial suspensions were mixed with hog mucin and charcoal in such proportion that the mixture contained 5–30 mg. bacterial dry wt./ml. and 1·7 per cent w/v hog gastric mucin + 0·4 per cent w/v powdered charcoal.
Each suspension was then injected intraperitoneally in 0·2 ml. amounts into 4 mice.

TABLE II.—*Virulence and Toxicity of* Bact. coli *Strains for Mice.*

Strain.	LD_{50} 14 hr. culture + 1·7% hog mucin. organisms.	LD_{50} 14 hr. culture + 2% gum trag. organisms.	Lethal dose killed organisms in mg./dry wt.†	Serine sensitivity. µg./ml.
*145	10^3	10^5	4·4	2
* 75	10^3	10^5	2·3–5	4
*E2380 (V)	4×10^3	—	5·0	—
E2846	2×10^4	—	3·0	500
E.C.	2×10^4	—	2	—
21	4×10^4	10^6	2·5–4·5	30
E2070	10^5	10^5	—	15
A170	5×10^6	10^7	4	15
A104	at least 10^7	10^6	—	4
*E1790	„ 10^7	10^7	4	8
E1662	„ 10^7	2×10^7	—	30
E3751	„ 10^7	—	—	500
E044	„ 10^7	10^8	2·1	250
A172	„ 10^7	—	3·5	15
147	„ 10^7	3×10^7	3·5	8
146	„ 10^7	10^8	3·0	15
*E2206 (AV)	„ 10^7	—	6·0	500
*K12	„ 10^7	—	—	250
E2186	„ 10^7	10^6	2·8	15
197	„ 10^7	4×10^8	2·5	2

* The average of many tests performed on different occasions.
† Figures given killed 1–3 out of the 4 mice used.

Calculation of LD_{50}.

Approximate LD_{50} values were calculated by the method of Reed and Muench (1938). While the small numbers of mice make most of the data very approximate, the differences in virulence between the most and least virulent strains were far greater than the experimental errors. In particular, with some of the strains such as 75, E1790, E2380 and E2206 (see Table II) on which further work has been based, the virulence tests have been repeated many times and the LD_{50} values are those derived from at least 100 mice in each case. For the sake of simplicity strain E2380, which is a typical virulent strain, is referred to as V and E2206, which has the same antigenic structure but is nevertheless avirulent, as strain AV.

RESULTS.

Virulence determinations, using such small numbers of mice for each test, are obviously inaccurate and results from repeat tests sometimes differed by a factor of 5. Nevertheless, the results leave no doubt that there are very large differences in mouse virulence among these strains. The most virulent need only 10^3 organisms to kill, whereas some others do not kill when more than 10^7 organisms are injected.

It is clear from Table II that there is no connection between serine sensitivity and virulence for mice. The results of the toxicity tests are likewise crude, but show that the lethal doses of killed organisms lie within similar limits for the many different strains. This would seem to rule out the production of a heat-stable toxin by the virulent strains only, and since all the strains have simple growth requirements we must look elsewhere for explanations of these great differences in killing power.

Three virulent and 3 avirulent strains were chosen for further work. From Table III it can be seen that strains AV (E2206) and V (E2380) differ greatly in virulence yet possess the same antigenic structure, the same sugar fermentations and are inhibited by the same amino acids. It seems unlikely that mouse virulence is directly connected with any of these properties. This was confirmed for sugar fermentations and amino acid inhibitions by developing mutants changed in various characters and testing the mutants for virulence. Table III shows that in no case was the virulence altered.

TABLE III.—*Characteristics of* Bact. coli *Strains and Mutants.*

Strain.	Lactose.	Maltose.	Xylose.	Antigenic type.	Amino acid sensitivity.	LD_{50} organisms.
75	−	−	+	U	Serine, norleucine	10^3
75 serine-resist.	−	−	+	U	Norleucine	10^3
75 lac. +	+	−	+	U	Serine, norleucine	5×10^3
V15.	+	+	−	U	,, ,,	5×10^6
K12	+	+	+	U	Valine, norleucine	2×10^7
K12 mucoid	+	+	+	U	,, ,,	10^7
K12 valine resist.	+	+	+	U	Norleucine	2×10^7
E2206 (AV)	+	+	+	0111 B4 H12	Serine, norleucine	2×10^7
E2380 (V)	+	+	+	0111 B4 H12	,, ,,	4×10^3

U = Unknown antigenic structure.

Tests for heat-labile extra-cellular factors.

To see if the virulence of some strains might be due to the elaboration of an extra-cellular aggressin or heat-labile toxin, after the fashion of the *Clostridia* (Evans, 1945), 14-hr. cultures of strains 75 and E1790 were centrifuged and the supernatants sterilised by filtration through a gradocol membrane. The sterile

supernatant from E1790 was added to the cells of 75 and vice versa and the organisms re-suspended. Dilutions were made from these and simultaneously of cells from 75 and E1790 which had been washed 3 times in saline. Virulence tests made with these suspensions showed no variations from 75 whether washed, suspended in its own supernatant or in that from E1790. Similarly with E1790 cells. We are left with the conclusion that in these cases virulence is not mediated by some powerful extra-cellular property.

Effect of injecting killed cells together with living ones.

In order to see if virulent cells, when killed, produced a virulence-enhancing effect on injection of avirulent cells, dilutions of *Bact. coli* AV were made so as to straddle the LD_{50} dose and injected together with 10^7 killed, washed *Bact. coli* V. No enhancement of killing was found over the results to be expected from the injection of strain AV alone. This is contrary to the results of a similar experiment by Maaløe (1948) using avirulent strains of *Salmonella typhi murium*.

Growth curves in vivo.

Experiments *in vitro* had failed to show any differences in growth rate between the strains when growing in 10 per cent mouse serum broth. Attempts were made to study the growth of these organisms *in vivo* by the simple method of washing out the peritoneum at intervals following injection. Model experiments showed that it is possible to recover 50–80 per cent of an injected dose of organisms by sacrificing the mouse, skinning it from tail forwards, opening the peritoneum and washing out with 10 ml. of sterile saline.

Groups of 20 mice were inoculated with the same dose of *Bact. coli* + mucin and charcoal. Two of the mice were sacrificed immediately and bacterial counts done on peritoneal washings. At the same time counts of the dose injected were made. At intervals during the following 16 hr., pairs of mice were killed and counts were made of the numbers of intraperitoneal organisms. Fig. 1 shows the results of such an *in vivo* growth curve using 75 and E1790 as the test organisms. From this it can be seen that there is little difference in growth rate between the two strains in the logarithmic part of the curve. The difference is in the capacity to continue multiplying from a small inoculum; when 8000 organisms of E1790 are introduced they multiply for a few hours and are then slowly eliminated.

Presumably these *in vivo* growth curves are the resultant of two opposing tendencies; these are on the one hand the multiplication of the organisms and on the other their removal or inactivation by host defence mechanisms. The difference between the virulent and avirulent organisms in this case resolves itself into a difference in behaviour towards one or other of these defence mechanisms.

Peritoneal leucocytosis.

Examination of stained peritoneal smears taken from mice at varying intervals following infection with 10^7 organisms of *Bact. coli* V or AV without mucin, showed that with both organisms polymorphonuclear leucocytes began to appear in considerable numbers after about 8 hr. Large numbers of organisms were phagocytosed, but there was no consistent difference between the strains in the

average numbers per leucocyte. The presence of mucin in the peritoneum did not affect the numbers of leucocytes migrating into the cavity to any marked degree, but the extent of phagocytosis was reduced with both virulent and avirulent organisms.

Injection of *Bact. coli* cytoplasm prepared by mechanical disruption of a bacterial suspension with ballotini beads (Cooper, Rowley and Dawson, 1949)

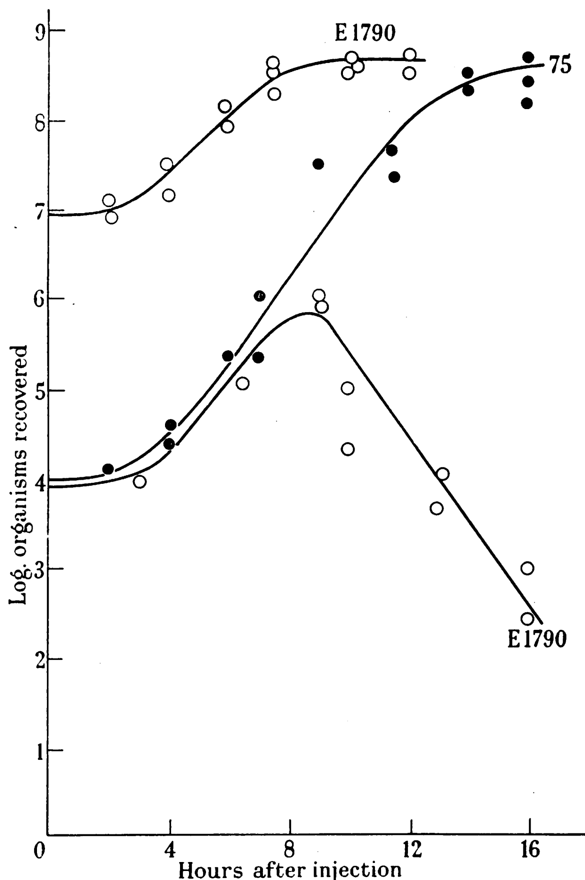

FIG. 1.—Growth of *Bact. coli* strains E1790 and 75 in the mouse peritoneum. 0 = numbers of organisms of E1790 recovered in the total peritoneal washings at varying times following I.P. injection of 8×10^3 and 10^7 organisms. ● = same after I.P. injection of 10^4 organisms of strain 75.

showed that under these conditions both organisms attract similar numbers of leucocytes. In one such experiment injection of 0·4 mg. dry weight of cytoplasm from *Bact. coli* V or AV into each of 2 groups of 10 mice produced after 18 hr. an average of 10^6 leucocytes (mostly polymorphs) per peritoneum irrespective of whether V or AV cytoplasm had been used.

That leucocytes played an indecisive rôle in defending against this artificial infection, in the presence of mucin, is shown by an experiment in which peritoneal leucocytosis was produced in mice by injection of *Bact. coli* AV cytoplasm as above. The mice were challenged 18 hr. later by inoculation with varying doses

of organism V + mucin and charcoal. As a result the survival time of the mice was increased but the final mortality figures were the same as in a normal control group (Table IV).

TABLE IV.—*Survival of Mice when Challenged Intraperitoneally after Induction of Leucocytosis.*

Numbers of *Bact. coli* V injected.	Leucocytosed mice dead at 24 hr.	Leucocytosed mice dead at 48 hr.	Normal control mice dead at 24 hr.
10^5	3/4	4/4*	4/4
2×10^4	2/4	4/4*	4/4
2×10^3	0	3/4*	4/4

* The numbers include those which had died at 24 hours.

Effect of Mouse Blood on Bact. coli *Strains* in vitro.

Mice were anaesthetised with ether, bled aseptically from the heart and the blood defibrinated with a bent glass rod. Quantities of 0·5 –1 ml. of this blood were pipetted into bijou bottles and various dilutions of organisms AV and V were added, contained in 0·1 ml. saline. The dilutions were designed to cover a range of 50–50,000 organisms/ml. blood and the bottles rotated in a machine kept at 37° (Robertson and Sia, 1924). Samples of 0·1 ml. were withdrawn at intervals during 4 hr. and spread on nutrient agar plates for counting. In all such experiments no bactericidal action was observed and after a few hours the organisms began to multiply irrespective of their virulence.

A similar test using defibrinated guinea-pig or human blood from several normal individuals showed in every case that at least 500 organisms/ml. blood were killed within 30 min. This observation together with the knowledge that one of the differences between human and mouse blood is the lack of detectable haemolytic complement in the latter, tempted speculation that this might account for the failure of normal mouse blood to kill *Bact. coli in vitro*. Table V shows the results of an experiment in which *Bact. coli* AV and V were rotated in bijou bottles containing mouse blood and fresh or heat-inactivated guinea-pig serum, and human blood as a control. It can be seen from Table V that addition of 0·4 ml. fresh guinea-pig serum to 0·7 ml. defibrinated mouse blood produces a system which is rapidly bactericidal to the avirulent strain, but has little or no effect on the virulent strain V. When the guinea-pig serum is heated to destroy complement, all bactericidal power is lost. In Fig. 2 the data from several survival curves of this type are collected and show the striking difference in behaviour of the two strains.

Similar differences in survival rates, reflecting their differences in virulence, were found between strains 145 and 75, and E1790 and E3751.

When tested in guinea-pigs for virulence in the same manner as for mice, strain 75 had an LD_{50} of 5×10^4 and strain E1790 of 10^8 organisms. The inter-strain difference in virulence is of the same order in guinea-pigs as in mice, suggesting that the same defence mechanisms are involved. In the guinea-pig, normal serum which contains complement is actively bactericidal *in vitro*, and when diluted suitably will differentiate between virulent and avirulent strains. Although the relationship between mouse virulence and resistance to the bactericidal effects of mouse blood and complement-containing sera have been shown,

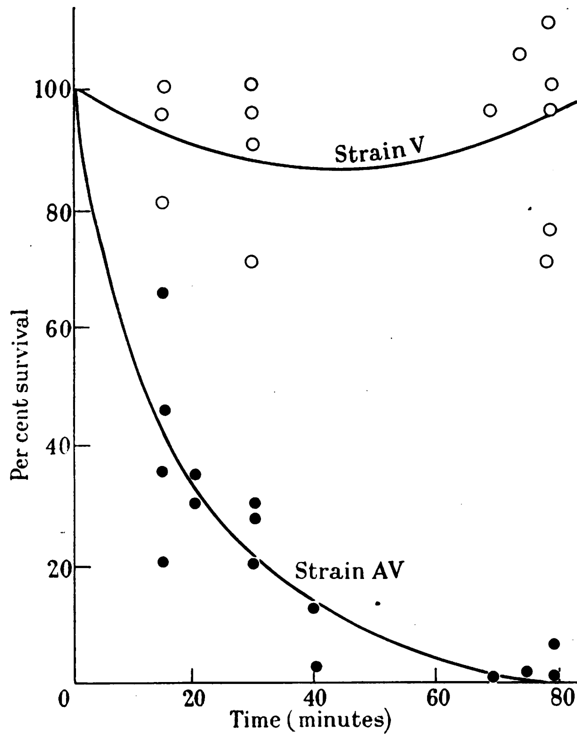

FIG. 2.—Survival of *Bact. coli* strains V and AV in mouse blood and guinea-pig serum. 0 = strain V. ● = strain AV. Experimental details as in Table V.

TABLE V.—*Survival of* Bact. coli *Strains A V and V* in vitro.

Time mins.	Human blood.		Mouse blood + heated serum.		Mouse blood + unheated serum.	
	AV.	V.	AV.	V.	AV.	V.
5	22	50	75	65	84	56
20	0	0	40	60	52	46
35	0	0	66	64	12	33
75	0	0	> 100	> 100	6	65

To 0·7 ml. defibrinated mouse blood, 0·4 ml. sterile guinea-pig serum (active or inactivated by heating) was added, followed by 700 AV or 600 V in 0·1 ml. saline. Figures are numbers of colonies obtained by plating out 0·1 ml. of the mixture.

this does not conclusively prove that this is the mechanism by which the mouse itself differentiates between virulent and avirulent strains, since there is no bactericidal action by normal defibrinated mouse blood, nor does this possess any haemolytic complement.

Bactericidal effect of sera.

Earlier experiments on the survival of strains 75 and E1790 in normal mouse serum + 10 per cent guinea-pig serum had failed to show any killing. This was now found to be due to the anticomplementary action of mouse serum, each ml. of mouse serum being able to neutralise 25–30 haemolytic units of complement, and since guinea-pig serum contains approximately 80 units of complement, about 50 per cent of guinea-pig serum must be added to mouse serum to give any free complement. Mouse serum with sufficient added guinea-pig serum to give free complement differentiates between virulent and avirulent strains like mouse blood + serum.

It is doubtful whether the anticomplementary property develops in mouse serum during the processes of obtaining it, or whether it exists *in vivo*. In any case the test for mouse complement, using a sheep cell rabbit antiserum indicator, is by no means infallible, and it is possible as pointed out by Cushing (1945) that complement activity might be found by using a different indicator system.

Bactericidal power of mouse blood following infection.

If the differences in mouse virulence of these *Bact. coli* strains are to be accounted for in terms of their reaction to complement, then either complement must be present in mouse blood *in vivo* or it must appear in the course of the infection. Complement may only occur locally, as suggested by Maaløe (1948), or it may be generalised throughout the blood and tissue fluids of the animal. It may be significant that the *in vivo* growth curves in mice (Fig. 1) showed that discrimination between virulent and avirulent strains by the host did not appear until some hours after the start of the infection. This suggested that the bactericidal factor, whether it be complement or not, only appears in the mouse as a result of the stimulus of infection.

To test this suggestion, 30 mice were inoculated with 10^7 AV without mucin. Two mice were sacrificed every second day and their serum titrated for haemolytic complement activity and for anticomplementary activity to guinea-pig serum. On the same day 3 more mice were bled and the defibrinated blood tested for bactericidal power towards *Bact. coli* AV. No trace of complement appeared and the anticomplementary power of the mouse serum stayed constant at 30 antihaemolytic units/ml. On the other hand, by the 12th day the defibrinated mouse blood had marked bactericidal powers (Table VI). This Table also shows that the different behaviour of strains AV and V is quantitative rather than qualitative. Thus, although normal mouse blood + guinea-pig serum does not kill strain V, convalescent mouse blood with the same amount of added guinea-pig serum is markedly bactericidal to strain V, although not so rapidly as to strain AV. Any effect on the counts due to clumping of the bacteria should be the same with both strains. This protection by convalescent blood is also found *in vivo* since immunisation of mice with either strain V or AV will protect the mice against a subsequent challenge 12 days later with over 100 lethal doses of *Bact. coli* V. It seems likely that both these effects are due to the common antigenic structures of these two strains and that the increased bactericidal activity of convalescent blood is a function of increased antibody content.

In spite of the failure to detect haemolytic complement in mouse blood the evidence strongly suggests that a small amount of complement must be present

TABLE VI.—*Survival of* Bact. coli *V and AV in Normal and Convalescent Defibrinated Mouse Blood, with and without added Guinea-pig Serum.*

Time mins.	Normal mouse blood 0·7 ml. + guinea-pig serum 0·4 ml.		Convalescent mouse blood 1 ml.		Convalescent mouse blood 0·7 ml. + guinea-pig serum 0·4 ml.	
	AV.	V.	AV.	V.	AV.	V.
Start	78	48	61	58	75	68
15 mins.	78	61	42	44	11	29
30 mins.	18	49	22	34	0	5
75 mins.	2	57	1	23	0	4

Figures are numbers of colonies obtained by plating 0·1 ml. samples. Initial counts were 70 colonies AV/0·1 ml. and 60 V/0·1 ml.

Convalescent mouse blood obtained from mice injected 12 days previously with 10^7 AV.

in convalescent mouse blood and that this is responsible for its bactericidal effect. This evidence was obtained by demonstrating that treatment of convalescent mouse serum or guinea-pig serum with zymosan, which is known to inactivate the third component of complement (Bier, Leyton, Mayer and Heidelberger, 1945), simultaneously removes all bactericidal properties from the sera.

DISCUSSION.

It has been found that among *Bact. coli* strains there are a few which possess very much greater virulence for mice and guinea-pigs than the majority. In the first analysis the difference between these two groups is reflected by the capacity of the virulent strains to grow up and multiply from a small inoculum ; in other words, the growth rate of the virulent bacteria *in vivo* must exceed the overall rate of destruction by the body's defences.

Since the use of mucin in this system increases virulence about 500 times and most strains of *Bact. coli* are relatively avirulent without mucin, then mucin must reverse one of the effective body defences, probably phagocytosis. This may be analogous to the case of streptococci, reported by Hadley and Wetzel (1943), where there was a great increase in mouse virulence in passing from the rough to the smooth encapsulated form, this increased virulence being presumably due to resistance to phagocytosis. A further even larger increase in virulence was obtained by continued animal passage, without changing in any apparent way the state of dissociation or antigenic properties of the organism. The similarities to the present findings with *Bact. coli* are that addition of mucin increases virulence of all strains, possibly by providing them with an artificial capsule, and enabling them to resist phagocytosis ; over and above this effect however, there are great differences in virulence which appear to be related to resistance to some unstable serum factor, probably complement.

If there are several distinct host defence mechanisms, it is reasonable to suppose that against any given microbial species some of the mechanisms will play a predominant rôle over the others. If the majority of the strains of such a species are avirulent then the occasional virulent strain will presumably be one which is resistant to the one or more normally dominating defence reactions. In the event of the difference in virulence between two strains being due to a different behaviour towards *one* defence reaction, then the magnitude of the difference in virulence will be a measure of the predominance of that particular defensive

reaction over all the others. Conversely, where there are great changes in magnitude between the virulence of two strains of the same organism it seems likely that there is a key defence reaction among the several available, which is involved in the struggle against that organism. The present model infection of mice with *Bact. coli* strains provides such a case since there are a few strains which are about 10,000-fold more virulent than the majority. This great increase in virulence is associated with resistance to the bactericidal effect of normal mouse blood plus complement-containing sera, as has been described by Maaløe for *Salm. typhi murium* (1948). It is suggested that resistance or otherwise to this effect is the major determinant of mouse virulence in *Bact. coli.*

SUMMARY.

Twenty strains of *Bact. coli* have been tested for mouse virulence by intraperitoneal injection together with 1·75 per cent hog gastric mucin. Six of these strains were virulent in comparison to the remainder. This virulence is not due to the possession of a heat-stable toxin since all the strains have approximately the same toxicity when killed.

In vivo studies show that virulence is a reflection of the capacity to continue multiplying in the peritoneum from a small inoculum. This capacity for sustained growth *in vivo* is correlated with greater resistance towards the *in vitro* bactericidal powers of complement-containing sera. It is suggested that this resistance to complement is an important determinant of mouse virulence in *Bact. coli.*

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