THE VALUE AND DURATION OF DEFENCE REACTIONS OF THE SKIN TO THE PRIMARY LODGEMENT OF BACTERIA

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IN all animal infections there is a stage when the infecting microbes, taking advantage of a breach in the surface defences of the host, make their primary lodgement in or on the tissues. The fate of this primary lodgement—its suppression or its persistence and growth into an established infection—is determined in part by the antimicrobial properties of the tissues immediately round it. Little, however, is known of the efficiency of these tissue responses in the early stages of infection, of the part they play in non-specific immunity, or of the physiological and pathological defects in the responses that determine the breakdown of natural resistances to the primary lodgement. Experimentally, it is obvious that suppression of this kind occurs when sub-infective doses of living pathogens are injected; and in nature they may justifiably be assumed to occur as part of the means whereby animals remain healthy in spite of the substantial microbial hazards of their environment.

We have investigated the local defence reactions in the skin of the guinea-pig, and have attempted to measure their efficacy in nine different infections. Our methods are based on the results of a study of the enhancement of infection by local adrenaline (Evans, Miles and Niven, 1948) and shock (Miles and Niven, 1950). The initiation in the guinea-pig of 2 hours' local adrenaline ischaemia or of 2–3 hours' general dehydration shock, at the time of intracutaneous injection of certain bacteria, substantially increased the maximum size attained by the local infective lesion. The increase was equivalent to multiplying the infecting dose by a factor of 10 to 10^6, according to the pathogen used. When, however, the bacterial lesions were 3–4 hours old, the induction of general shock modified the local infection either little or not at all. Such results suggest firstly that the defences inhibited by shock have a high protective value in an untreated lesion, destroying all but 10^{-1} to 10^{-6} of the infecting dose ; secondly, that they are effective mainly within the first few hours of making the primary lodgement ; and thirdly, that during this period they are decisive in determining the full extent of the local infection (Miles, 1953–54, 1956). We now present evidence, based on a study of a greater variety of bacteria and of agents which modify infection, that this early decisive period of antimicrobial reactions is common to a number of experimental infections of the skin.

MATERIALS AND METHODS

"Liquoid" (Hoffmann—La Roche) is sodium polyanethol sulphonate ; Heparin B.P. was used and assumed to contain 90 units per mg. Streptomycin was used as the sulphate, the preparation containing 780 units/mg., and the penicillin was Benzylpenicillin B.P. Shock lasting 3–4 hr. was induced by the intraperitoneal injection of 16–17 ml. 60 per cent glucose/kg. body weight (Miles and Niven, 1950).

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Bacteria.—The strains were *Streptococcus pyogenes* Group C, CN771 of the Wellcome Collection; *Staphylococcus aureus*, Au 21 from Professor Wilson Smith and CN491 of the Wellcome Collection; *Corynebacterium diphtheriae*, var. *mitis*; *Corynebacterium ovis*, Bl/50 from Professor H. R. Carne; *Listeria monocytogenes*, L71 from Professor E. G. D. Murray; *Clostridium welchii*, S.R.9; *Pseudomonas pyocyanea*, "Scudder"; *Bacterium coli*, 88; *Proteus vulgaris*, PR3 (Miles, 1951). They were preserved in the dry state by the method of Stamp (1947).

Guinea-pigs.—Albino guinea-pigs weighing 300–600 g. were used; in any one experiment, the range of weights was usually within 50 g. The skin was depilated as described by Miles and Miles (1952).

Titration of infectivity.—The bacterial growth from 18–20 hr. culture in nutrient or 5 per cent blood broth at 37° was spun down and re-suspended in a volume of liquid equal to that of the discarded supernatant medium. When required, viable counts were made by the method of Miles and Misra (1938). The bacteria in suspension usually remained fully viable in saline (0·85 per cent) for several hours, but when the bacteria died rapidly in saline, saline containing 0·1 per cent peptone or 0·02 per cent purified guinea-pig serum albumin (fraction AP3, Wilhelm, Miles and Mackay, 1955) was used. With both these stabilizers the viable count was constant for several hours in suspensions held at 4°. The albumin is preferable, because unlike peptone, it is non-toxic to the capillary endothelium of the guinea-pig.

Serial 5- or 10-fold dilutions of the suspension were injected intracutaneously in 0·1 ml. volumes; up to 24 infective lesions are readily accommodated on a 350 g. animal. The injection sites were partly randomized over the skin of the trunk, in the rectangular area posterior to the scapula and anterior to the knee joint in the sitting animal, and superior to a line 3–4 cm. on each side of the ventral mid-line. Each dose of bacteria was tested in 3–6 animals, sometimes in duplicate in each animal; the diameters recorded are the means of those from 3–12 lesions.

All nine test bacteria made lesions which reached a maximum size after 20–36 hr., except *C. ovis*, whose lesions slowly increased in size for 2 more days; and, with the exception of the two corynebacteria, none of the strains in the doses used induced a progressive generalized disease. Intracutaneous *C. ovis* and *C. diphtheriae* killed by toxaemia in 3–4 days. Animals given *C. ovis* showed no sign of intoxication after 1 day, when they were usually killed; animals given *C. diphtheriae* were protected against intoxication by 10–50 units antitoxin/kg. intraperitoneally 4–8 hr. after infection.

The diameter and thickness of the region of indurated and erythematous tissues and the diameter of any area of necrosis, were measured after 20–24 hr. Areas of erythema only, which often surround the indurated area, are usually readily distinguished, because the guinea-pig reacts to handling during measurement by a blanching of the skin, during which the peripheral erythema becomes inconspicuous. Most lesions were circular and their diameters were measured to the nearest 0·5 mm. The diameter of elliptical lesions was recorded as $\sqrt{Dd}$, where D and d are the lengths of the major and minor axes. With very skew, heavily enhanced lesions, the extent was outlined on cellophan, the area (A) measured directly on squared paper, and $\sqrt{A}$ recorded as lesion diameter. These maximum or near-maximum lesions did not change much in size between 17 and 25 hr., so that it was possible to make the final definitive measurement of lesion size at one time on the day after experiments in which lesions were initiated as much as 7 hr. apart.

Lesions in the 7–15 mm. range represent a moderate infection, usually ending in central necrosis, the diameter of the necrotic area being up to 30 per cent that of the indurated area. There was usually a feebler, though a well-defined, induration in lesions of 4–7 mm., and useful comparisons were sometimes made in this range. Lesions ranging from 15–30 mm. or more in diameter were less easy to measure precisely because they were usually severely necrotic, and sometimes grossly elongated in shape, with the long axis pointing downwards in the sitting animal, as though their eccentric spread had been determined by gravity.

RESULTS

The Dosage-response to Pathogenic Bacteria

Plotted against log dose of bacteria, neither diameter, area, nor volume of 24-hr. lesions, nor any of the valid biometrical transformations of these values, fell on a straight line with any consistency over the whole range of easily measur-

able responses, namely of lesions 5–25 mm. in diameter. With most of the test bacteria, however, lesion-diameters between 7 and 15 mm. increased in a roughly linear manner when plotted against log dose, and the response lines were roughly parallel to one another (*cf.* Miles, 1954). We therefore worked as much as possible in this range, and adopted 10 mm. as the standard response at which to compare infectivity. Thus the "skin virulence" of the test pathogens themselves is recorded in col. 1, Table I, as the ED_{10}, the effective dose producing this standard 10 mm. lesion. The comparison at the 10 mm. level of infectivity, of a given pathogen under different conditions, is exemplified in Fig. 1. The slope of the

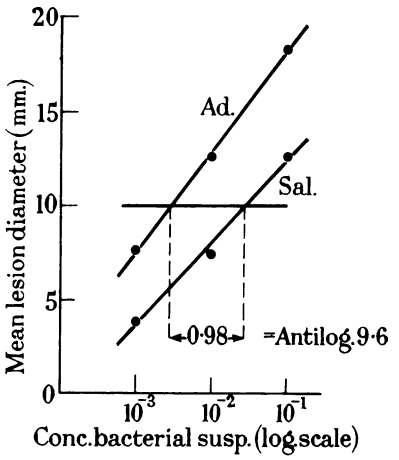

FIG. 1.—Enhancement of *C. diphtheriae* infections by 2 μg. adrenaline (Ad). Sal. = control lesions by bacteria suspended in saline.

C. diphtheriae lesions enhanced by adrenaline, as fitted by eye, is 0·98 distant, on a logarithmic scale, from that of the untreated lesions, and the enhancement is therefore equivalent to an increase in dose of antilog 0·98 = 9·6-fold, an enhancement factor (F) of about 10.

Strictly speaking, the degrees of enhancement or depression determined at the 10 mm. level are valid only for responses in that range ; the conditions under which they are valid are further restricted by the variability of slope from test to test. Nevertheless, the results of repeated comparisons at this and other levels were sufficiently consistent to justify generalizing from them, provided not too much weight is put upon the actual numerical values obtained. In any single experiment, a 5-fold difference in dose was certainly significant, and where smaller differences are cited as significant, they were established by repeated tests.

Although the values for the slope of the dosage-response in the 7–15 mm. range varied from test to test, they clustered round a characteristic figure for each bacterium. Most of the characteristic slopes were similar, lying between 2·3 and 3·0 (Table I, col. 2) ; but that for *L. monocytogenes* was shallower (1·5), and that for *Cl. welchii* steeper (3·4), than the rest. These characteristic values were used in estimating the enhancement of infectivity in experiments where only a single concentration of bacteria was used. Thus staphylococcal lesions in

shocked animals were on the average 4·1 larger than those in the control animals. The *Staph. aureus* slope being 2·3 (col. 1), the enhancement is equivalent to an increase in dose of antilog 4·1/2·3 = approx. 60-fold.

Modification of Experimental Infections

Three properties of each modifier were determined : direct action, duration of effect on the tissues, and efficacy in infected lesions of different ages.

Local modification.—For direct action, the modifier is mixed with the washed bacteria a few minutes before injection ; control injections of bacteria + saline are also made (*cf.* Fig. 1). For the two other tests we used the technique of super-injection (Miles, 1949). In tests of duration, each animal receives a number of 0·1 ml. injections of saline alone and of modifier in saline, at 0, 1, 2 . . . 6 hr. The puncture hole made by the needle is marked by a trace of India ink previously painted on the needle tip. Immediately after the last injection, when the skin bears saline- and modifier-treated areas 6, 5, 4 . . . 0 hr. old, 0·1 ml. bacterial suspension is super-injected into each original puncture hole (*cf.* Fig. 4). In tests of lesions of different ages, several marked injections of bacterial suspension are made at one time, and as the lesions reach the required age, two of them receive super-injections, one of the modifier and one of saline (*cf.* Fig. 5). In the last two tests, with lesions of varied ages, the controls, consisting of sites injected with saline, are required throughout the course of the experiment, because the mechanical disturbance made by the super-injection of 0·1 ml. of liquid may modify bacterial lesions to a slight but definite extent varying with the bacterium and the age of lesion on super-injection (*cf.* the control lesions in Fig. 5B, F).

Systemic modification.—When agents affecting the whole animal are used, their direct action, duration of effect and relative efficacy on infections of different ages are determined in a single test. Bacterial suspension is injected at intervals during a 6 or 7 hr. period into each animal of a batch. The systemic modifier is given to half the animals when the earliest lesion is about 3 hr. old, so that by the time the latest lesions are initiated, the modifier has been acting for 3–4 hr. The curves in Fig. 2 exemplify this test. The lesions initiated 1 hr. after the intraperitoneal glucose are maximally enhanced by the shock (*b*). The mean difference between control and test lesions at this point is 4·1 mm., and as noted above, the enhancement corresponds to about a 60-fold (antilog 4·1/2·3) increase in dose. The shock is effective a little over 1 hr. after the maximum (*b* to *c*), and lesions 2 hr. old when the shock was initiated (*a*) are insusceptible to its enhancing action (see also Fig. 8).

In these examples, the full range of lesion ages was tested in each animal. The procedure is justified when all the corresponding lesions in the control animals are similar in size. In some infections, however, it was evident in the control animals that the earlier lesions had induced a substantial general resistance of the skin to the same suspension injected at the 4th to the 7th hour, so that the diameters of these later lesions, when measured at 24 hr., were smaller than those of the earlier. This has occurred to a mild degree in the experiment in Fig. 2. The change can be allowed for by a direct comparison of the control and test at each point in the time-course. However, in most cases where it occurred, definitive tests were made on a number of separate batches of animals, in which each animal bore lesions differing in age by 2 hr. at the most. This method is more laborious because of the

large number of animals needed to compensate for the inter-batch variation in animal susceptibility which is thereby introduced into the error of the assay of infectivity.

Bactericidal power of the blood.—Guinea-pig blood obtained by heart puncture was defibrinated, filtered through a little gauze to remove any particles of fibrin, and immediately distributed in 0·95 ml. volumes for the test. Ten-fold dilutions of washed bacterial suspension in saline, 0·05 ml., were added to two sets of tubes. The bactericidal action of the first,

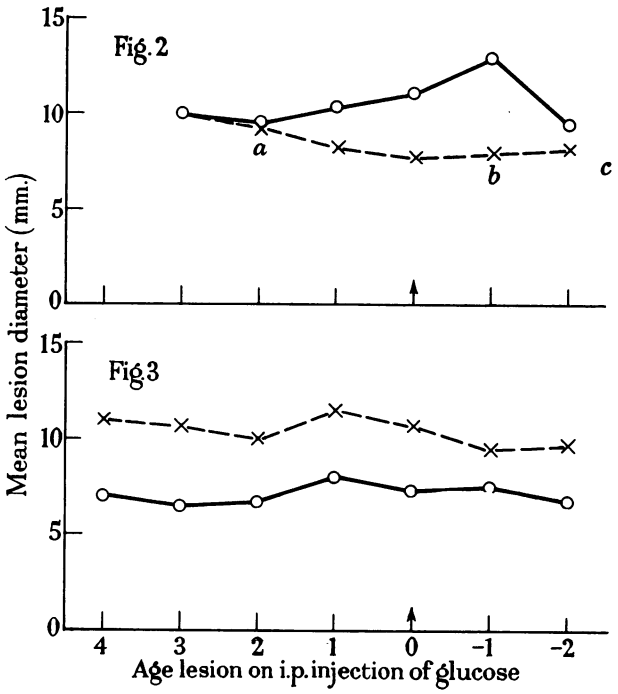

FIG. 2.—Enhancement of *Ps. pyocyanea* infections by dehydration shock, and insusceptibility to enhancement of infections initiated 2 hr. before the shock. Arrow indicates the intraperitoneal injection of glucose.
O———O Test lesions. × — — — × Control lesions.

FIG. 3.—Depression of *P. vulgaris* infections by dehydration shock. The dotted line indicates control lesion diameters. Arrow indicates the intraperitoneal injection of glucose.
O———O Test lesions. × — — — × Control lesions.

control, set was immediately destroyed, and the corpuscles laked, by adding 1 ml. 0·1 per cent Liquoid in distilled water. The second set of blood-bacterium mixtures were agitated for several hours in a water bath at 37°, in open tubes lightly plugged with cotton wool. The incubation period was determined for each bacterium, as the time at which maximum killing occurred, and proved in most cases to be 2 hr. and in others 3 or 4 hr. After incubation, the test mixtures, like the control, were treated with Liquoid solution before plating. The bactericidal power of the blood was estimated by the method of Miles and Misra (1938) from the results of 6-plaque surface plate counts made of the control and the incubated blood-bacterium mixtures; it is expressed (Table I, col. 11) as p, the proportion of the inoculum killed by the blood, total killing being unity. Each bacterium was tested on two or more occasions, and in all cases, blood and serum from the same sample were tested in parallel, to distinguish the contribution of cellular and soluble elements of the blood to its bactericidal effect on a given pathogen.

Other techniques, of experiments incidental to the main investigation, are described in the appropriate sections.

Direct Enhancement of Infection

It is impracticable to display the detailed results of the 19 definitive tests made with each of the nine pathogens. Most of the tests were repeated once, and some of them several times, each time using an average of 6 guinea-pigs. The main results are summarized in Tables I and IV ; and the details of each kind of test are illustrated by examples.

Enhancement by shock

Seven of the nine infections were enhanced by dehydration shock. The duration (D) of the enhancement (*cf.* Fig. 2) is 2–4 hr. from the injection of the glucose. After this period, the skin recovers its normal resistance to the infection (Table I, col. 3). As already reported (Miles and Niven, 1950), infections by *Cl. welchii* are strongly enhanced ; the value 10,000 for the enhancement factor F (Table I, col. 4) is very approximate, because, as in most instances of strong enhancement, the dose-response lines are skew, and the dose-ratios from enhanced and unenhanced lesions are difficult to estimate with any precision. *L. monocytogenes* and *Ps. pyocyanea* and the two cocci are moderately enhanced and *C. ovis* is enhanced only 10-fold. *Bact. coli* infections are recorded as enhanced, but in some tests of these and in all tests of *P. vulgaris* (Fig. 3) and *C. diphtheriae* infections, shock *diminished* the lesion-diameter. The reason for this effect is obscure.

Enhancement by local modifiers

Three local modifiers were used : adrenaline, 20 μg./ml.; Liquoid, 500–1000 μg./ml.; and heparin, 300–500 μg./ml. The corresponding amounts in the standard 0·1 ml. injection volumes, 2, 50–100 and 30–50 μg., are about twice the minimal doses producing a readily detectable enhancement of susceptible infections. Since in these concentrations none of the agents stimulated or inhibited the growth of the nine test pathogens in culture, it may be assumed that they enhance by direct action, not on the bacteria, but on the tissue defences. As the values of the factor (F) of enhancement show (Table I), many of the pathogens take striking advantage of this inhibition of defences.

Adrenaline.—Adrenaline is active only for a short period. The duration of its effect (D, Table I, col. 4) is 1–2·5 hr., after which the skin largely, and in most cases completely, recovers its normal resistance. For example, the streptococcus can take advantage of the change induced by 4 μg. adrenaline for 2·5 hr. after its injection ; and 0·8 μg. is effective only during the first hour (Fig. 4b, c). With *Cl. welchii* infections, D was notably short—about 40 min. (Fig. 4a), although the visible ischaemia of the skin persisted for 2 hr. after injection of the adrenaline. Infections by *Cl. welchii*, *Bact. coli* and *P. vulgaris* are strongly enhanced, and those by *C. ovis* and *L. monocytogenes* relatively feebly so ; the rest are intermediate (Table I, col. 6).

Liquoid.—The duration (D) of the Liquoid effect varied between 1 and 3 hr., except with the *Str. pyogenes*, where it was $>$ 6 hr. Although the main enhancing effect has gone after about 3 hr., a small residual increased susceptibility as a rule remains at the site for some hours after the Liquoid treatment. The highest

TABLE I.—*The Infective Dosage-response in Guinea-pig Skin to 9 Bacteria; the Factor (F) by which Infections are Enhanced by 4 Agents and the Duration of Action (D) in Hours of the Agents; and the Death Rate* (p) *of the 9 Pathogens in Guinea-pig Blood and Serum.*

Test pathogen	Dose response in skin		Enhancement by								Death in	
			Shock		Adrenaline (2 μg.)		Liquoid (50–100 μg.)		Heparin (30–50 μg.)		Blood	Serum
	Approx. slope	ED_{10}	D	F	D	F	D	F	F		p	p
Str. pyogenes Group C	3·0	2×10^6	4	50	2	20	6	160	4		0·70	0·0
Staph. aureus	2·3	2×10^7	4	50	2·5	50	nt	200	1		0·83	0·0
C. diphtheriae, var. *mitis*	2·8	5×10^4	—	(–ve)	1	10	3	3–10	1		0·66	0·0
C. ovis	2·3	5×10^4	2	10	1	2	nt	10	1		0·0	0·0
L. monocytogenes	1·5	1×10^4	2	135	1	2·4	1	20	150		0·50	0·0
Cl. welchii	3·4	5×10^7	3	10^4	0·5	10^5	3	40	1		0·968	0·954
Bact. coli	2·3	1×10^9	2	10	1	10^4	nt	80	1		0·9996	0·9996
P. vulgaris	2·6	5×10^8	—	(–ve)	2	1000	2	1600	1		0·916	0·9995
Ps. pyocyanea	2·8	5×10^6	2	60	2	100	3	10^4	75		0·982	0·846
Column	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		(10)	(11)

nt = not tested.

value of F is recorded for *Ps. pyocyanea*. The rest of the infections are moderately susceptible (Table I, col. 7 and 8).

Heparin.—Heparin was tested less as an effective modifier than as an anticoagulant for comparison with Liquoid. In doses of 50 μg. it slightly enhances *Str. pyogenes*, and strongly enhances *L. monocytogenes* and *Ps. pyocyanea*. The rest were unaffected by doses up to 100 μg. (Table I, col. 9).

That the heparin effect is complex is evident from Table II, which records the effect of graded concentrations of heparin on the two pathogens which it enhances,

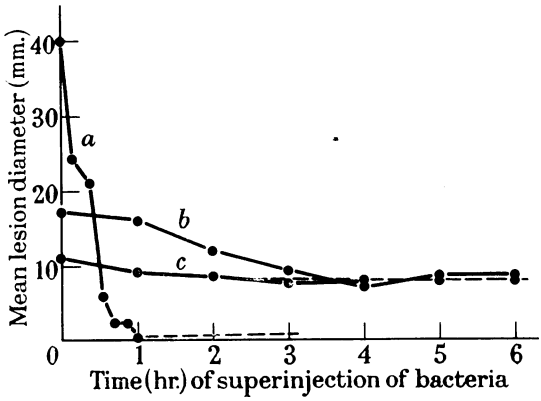

FIG. 4.—Duration of enhancing effect in skin treated with adrenaline at 0 hr.
(*a*) *Cl. welchii* with 2 μg. adrenaline ;
(*b*) and (*c*) *Str. pyogenes* with 4 and 0·8 μg. adrenaline respectively.
The dotted line indicates the mean diameter of control lesions.

Str. pyogenes and *L. monocytogenes*. In both there was a striking *depression* of infection by a concentration of 6 μg./ml. Its significance is obscure, but its occurrence is a warning against too ready deductions from the effect of large doses of a modifier.

TABLE II.—*The Biphasic Action of Simultaneously Injected Heparin on* Str. pyogenes *and* L. monocytogenes *Infections of the Skin.*

Heparin (μg./ml.)	Mean lesion diameter (mm.)	
	Str. pyogenes	*L. monocytogenes*
500	**8·2**	**26·0**
167	6·1	16·0
55	6·5	14·0
18	6·8	13·7
6	*3·5*	*7·5*
2	5·5	10·3
0	5·6	11·3

Diameters of strongly enhanced infective lesions in bold type, and of significantly depressed lesions in italics.

Mode of Action of the Modifiers

The four enhancing agents are not consistently effective on all nine test pathogens. *Cl. welchii* infections, for example, are highly susceptible to shock

and adrenaline, but only moderately so to Liquoid; and *L. monocytogenes*, though well enhanced by shock and heparin, is feebly affected by adrenaline. The two pathogenic cocci are moderately enhanced by all agents, and the two corynebacteria rather feebly so. Like those of *Cl. welchii*, infections by the three Gram-negative bacilli are capable of great enhancement, and vary widely in susceptibility to the various modifiers.

The absence of any strict parallelism between the results with each infection is to be expected, since the various defences inhibited by these agents are unlikely to have the same relative importance for each pathogen. It is nevertheless suggestive that the readiness with which these pathogens are enhanced is broadly paralleled by their susceptibility to the bactericidal action of blood and serum (Table I, col. 10 and 11). *Cl. welchii* and the three Gram-negative bacilli are highly susceptible to both blood and serum, and all are capable of enhancement to 1000-fold or more. The moderately enhanced cocci and *C. diphtheriae* are next most susceptible, but are killed only by whole blood, not by serum. The feebly enhanced *C. ovis* is insusceptible to blood under the conditions of the test. If the modifiers are not inhibiting reactions directly dependent on elements of the blood, they are inhibiting bactericidal actions at least of the same kind as those of blood.

Shock appears to enhance infections because there is in the skin a temporary diminution of blood supply, and of the intravascular pressure necessary for the exudation of plasma bactericidins and of leucocytes (Miles and Niven, 1950). In these respects, of the local modifiers adrenaline most nearly reproduces the ischaemic effect of shock. It presumably shuts off the supply of blood-borne antibacterial substances, both cellular and humoral (Evans *et al.*, 1948). With its transient yet powerful effect in low doses, it is an admirable tool for exploring tissue defences.

Liquoid is a more brutal modifying agent, because even in minimally effective doses it leaves a residual diminution of local defences after the main effect has passed off. It is anticoagulant and anti-complementary, and, by virtue of its anti-complementary powers, readily inhibits the bactericidal action of both plasma and whole blood (von Haebler and Miles, 1938). Unlike shock and adrenaline, it does not inhibit the rapid exudation induced by histamine or leukotaxine, tested locally in the skin of guinea-pigs with pontamine sky blue in their circulation (see Miles and Miles, 1952); moreover, as judged in stained sections, it interfered only mildly with the leucocyte response of the tissues to the infecting bacterium. Its anticoagulant powers must be considered in relation to Menkin's (1940) view that the formation of intravascular thrombi and of an intercellular fibrin mesh-work materially contributes to the localization of infection by inflamed tissues. It is unlikely that the great increase in area of some infective lesions under the influence of Liquoid is due to the inhibition of any defensive coagulation, because heparin, in more effectively anticoagulant doses, does not enhance and has none of the high potency of Liquoid as an inhibitor of the bactericidal effects mediated by complement.

Table III records the potency ratio of Liquoid and Heparin B.P. in four *in vitro* tests made at 37°. The anticoagulant potency was measured in freshly-drawn heart blood of the guinea-pig, and the anti-complementary potency in a system containing 3 M.H.D. of guinea-pig complement and sheep R.B.C. sensitized with 2 M.H.D. of antibody. The antibactericidal action on defibrinated guinea-pig blood was compared using as test bacteria the two cocci and *Bact. coli*

TABLE III.—*The Relative Inhibiting Potencies of Liquoid and Heparin.*

Inhibition of	Ratio of minimal effective concentrations	
	Liquoid	Heparin
Complement	1	15
Chemotaxis	1	>30
Bactericidal power of blood (4 bacteria tested)	1	>10 to >27
Coagulation of blood	1	0·2

and *Ps. pyocyanea*. Chemotaxis of guinea-pig leucocytes, from 3-hr.-old peritoneal exudates, was observed in sealed slide-cells containing starch granules (Comandon, 1920); 330 μg. but not 110 μg./ml. of Liquoid totally inhibited the chemotaxis, which was unaffected by 1 per cent heparin. Weight for weight, heparin was about 5 times as potent as anticoagulant, and had from 1/10th to < 1/30th the potency of Liquoid in the three other respects. That is, in equal anticoagulant doses, Liquoid has more than 25 to 150 times the inhibiting action of heparin on complement, chemotaxis and the bactericidal power of blood.

Susceptibility to Enhancement and Age of Infection

In nearly all the infections, shock and the three local modifiers enhanced most actively during the first 1½ hr. after the primary lodgement. After this, the susceptibility of the lesions declined, and none of the enhancing agents had much effect after 5 hr. Fig. 5A is characteristic of the insusceptibility of streptococcal infections more than 2 hr. old to super-injected adrenaline. The other 8 infections behaved similarly, except those with *P. vulgaris* (Fig. 5B). Here the initial susceptibility to enhancement, as with the others, declined rapidly within the hour, but older lesions were strikingly susceptible to enhancement. A similar, though slight, secondary rise occurred in one of three tests with *Bact. coli*.

As with adrenaline, initial susceptibility to Liquoid enhancement as a rule declined within the first few hours of infection, but a residual susceptibility to Liquoid was maintained (Fig. 5C, D), or returned (Fig. 5E) after a short period of insusceptibility. In none of these cases, however, was the residual enhancement more than a fraction of the direct enhancement produced by simultaneous injection of bacteria and modifier. *C. diphtheriae* lesions (Fig. 5F) were most susceptible when 2–3 hr. old, but like the rest were insusceptible at the 5th hour.

The picture of enhancement by shock was clear cut; lesions initiated 2–5 hr. before the shock were unaffected by it. The example in Fig. 2 is characteristic of the seven infections susceptible to enhancement.

The Concept of the Decisive Period

The 24-hr. lesions, in terms of whose diameters the infectivity of a bacterium is measured, represent the maximum development of local infection, the extent of which is decided by defences active at the site of the primary lodgement. The increasing insusceptibility of the infection to enhancement may be interpreted in one of two ways. Either the agent is active in older lesions, but the defences it inhibits are no longer operative or, if operative, no longer decisive; or the defences in question are still decisive in older lesions, but have become topographically or pharmacologically inaccessible to the agent. If the first explanation is correct,

the period of useful activity of a given set of defences, defined as those inhibited by the particular modifier, may be determined from results like those set out in Table IV. It is almost certainly correct in shock, which deprives the primary lodgement of its supply of bactericidal elements of the blood. Other consequences of shock are theoretically possible. Thus, a systemic tissue poison might be generated, whose movement into the older lesion might be prevented by, *e.g.*, thrombosis of blood vessels, changes in permeability of the vessel walls, or extravascular events like coagulation in the infected tissues. There is, however, no evidence of such an enhancing toxin in shock. Moreover, if there were, there is

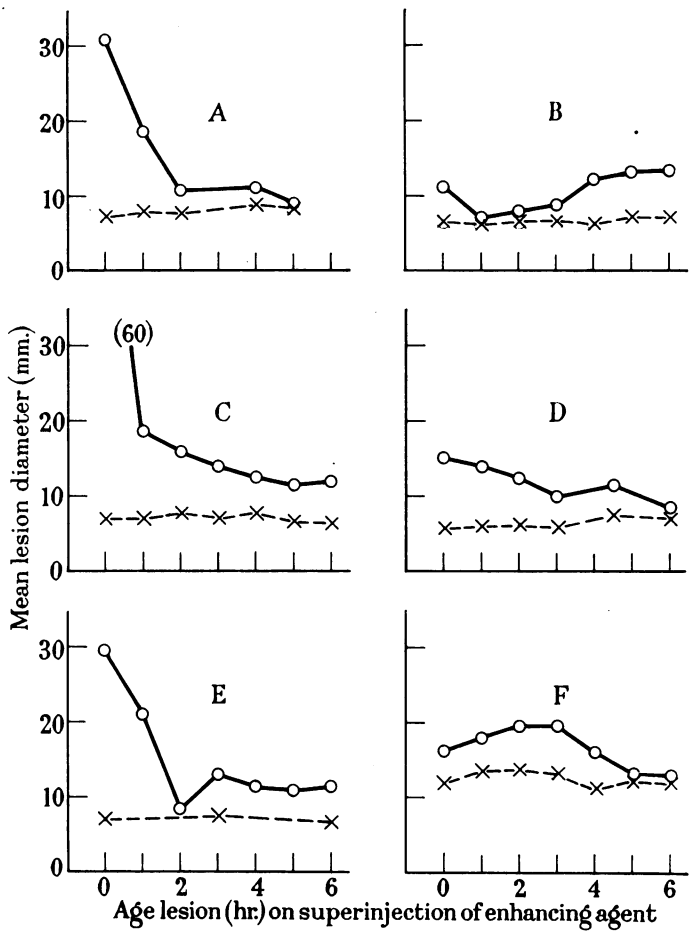

FIG. 5.—The change in susceptibility of various infections to local enhancement with increasing age of lesion.

A.—*Str. pyogenes* . Adrenaline, 2 μg.
B.—*P. vulgaris* . ,, ,,
C.—*Str. pyogenes* . Liquoid, 100 μg.
D.—*P. vulgaris* . ,, ,,
E.—*Cl. welchii* . ,, ,,
F.—*C. diphtheriae* . ,, ,,

O———O Test lesions. × — — — × Control lesions.

TABLE IV.—*The Effective Proportion (P_e) of the Inoculum in 9 Intracutaneous Infections Surviving Defences Inhibited by 4 Agents, with the Period in Hours (DP) during which these Defences are Decisive, and the Proportion Surviving Treatment with Antibiotics, with the Period (Hr.) during which the Infections are Susceptible to the Antibiotics.*

	Defences inhibited by								Bacteria inhibited by			
	Adrenaline (2 μg.)		Liquoid (50–100 μg.)		Heparin (30–50 μg.)		Shock		Penicillin 10^4 u.		Streptomycin 12×10^3 u.	
Test pathogen	P_e	DP	P_e	DP	P_e	DP	P_e	DP	P_e	DP	P_e	DP
Str. pyogenes Group C	0·05	4	0·006	2	0·25	4	0·02	2	0·1	3	0·02	>8
Staph. aureus	0·02	2	0·005	4	1·0	—	0·02	4	0·07	3	0·004	5
C. diphtheriae, var. *mitis*	0·1	4	0·1	4	1·0	—	—	—	0·05	3	0·01	4
C. ovis	0·5	1	0·1	3	1·0	—	0·1	3	0·007	3	0·0005	5
L. monocytogenes	0·42	2	0·05	1	0·006	nt	0·007	5	1·0	—	1·0	—
Cl. welchii	0·00001	3	0·025	3	1·0	—	0·00008	3	0·1	3	1·0	—
Bact. coli	0·0001	1	0·013	1	1·0	—	0·1	4	1·0	—	0·1	3
P. vulgaris	0·001	1	0·0006	5	1·0	—	—	—	1·0	—	0·01	3
Ps. pyocyanea	0·01	2	0·0001	3	0·013	2	0·017	3	1·0	—	0·02	3
Column	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)

nt = not tested.

no major hindrance to its entry into the lesion ; because no thrombosis, either of blood or lymphatic vessels, occurred in any of the nine local infections during the first 4–5 hr. and except in the centre of those lesions destined to become necrotic, there was no histological evidence of intercellular formation of fibrin (Burke and Miles, to be published).

The local modifiers, being super-injected, reach all parts of the lesion. Four-hour-old lesions appear to be as susceptible as the 0-hour-old lesions to adrenaline vaso-constriction, as judged by the occurrence and duration of superficial blanching of the skin. Moreover, continuing vaso-constriction for 1–2 hr. of the arterioles underlying 4-hr. streptococcal and staphylococcal lesions is directly observable under low power microscopy in the ear of the guinea-pig. If the first explanation of insusceptibility is to be accepted, it remains to show that the constriction impedes blood supply to older lesions. For this purpose we used a modification

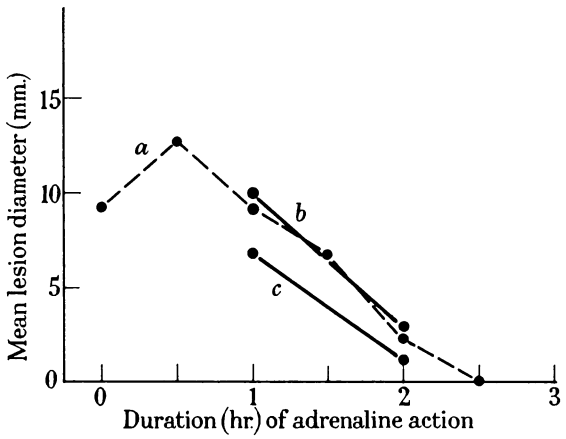

FIG. 6.—Duration of ischaemia induced by 0·1 ml. of 20 μg./ml. adrenaline, in (*a*) normal skin, (*b*) 0-hour, and (*c*) 4-hour streptococcal lesions. The diameters are of areas not filled by intra-arterial injection of a suspension of carbon.

of the India ink method of Miles and Miles (1952). Intracutaneous injections of 0·1 ml. adrenaline solution are made at intervals of 15 or 30 min. into the area of skin supplied by the left axillary artery. When the skin bears lesions 0–3½ hr. old, the artery is cannulated under anaesthesia, the returning vein opened, and a non-irritant suspension of carbon particles injected at a pressure (80–100 mg. Hg) of the same order as that in the main arteries of the animal. A dark area of skin is produced, in which white patches indicate regions supplied by occluded vessels.

The extent and duration of the occlusion induced by 2 μg. adrenaline was measured in uninfected skin and in skin bearing 0- and 4-hr. streptococcal lesions. The occluded region shrinks (Fig. 6), and in untreated skin the vessels are fully patent after 2–2½ hr. The vessels of infected skin also recover within 2 hr., when the drug and the streptococci are injected together ; and in 1½ hr., when the drug is super-injected into 4-hr. lesions. The circumstances in which the drug lasts on the average for 2 hr. are of course those of direct enhancement. In the 4-hr. lesion the ischaemia passes off a little more rapidly. However, the ischaemia

in 4-hr. lesions may be made to last for 2 hr. by using concentrations of 40–60 μg./ml. and this higher dose of adrenaline enhances such lesions either little or not at all. That is, the failure of the 20 μg./ml. to enhance 4-hr. lesions cannot in any substantial part be attributed to the relative insusceptibility of the inflamed region. Inflamed vessels are reported (Meltzer and Meltzer, 1903) as being insusceptible to adrenaline. We have no evidence on this point about individual vessels ; the inflamed region as a whole certainly becomes ischaemic when the drug is injected. Some of the ischaemia is due to constriction of the larger blood vessels on and in the panniculus carnosus, which at 4–5 hr. is not conspicuously inflamed ; but we cannot say whether the drug's action is confined to such vessels.

We conclude that in both shock and adrenaline enhancement, infections become progressively insusceptible because the defence reactions inhibited by these two agents become less and less decisive as the lesion ages, and are largely immaterial to the outcome of local infection after 4–5 hr.

The choice between the two explanations is more difficult to make for Liquoid enhancement. The inhibitor reaches the inflamed tissues, and there is no reason to suppose that the complement or leucocytes of the inflammatory exudate will be insusceptible to its action. But it is possible that the complement and leucocytes of a 4-hr. exudate are in excess of the amounts inhibited by the standard enhancing dose, 100 μg. of Liquoid ; because on injection, 0·1 ml. of a liquid fills about 0·25 ml. volume of skin, so that the average concentration of super-injected Liquoid is of the order of 0·04 per cent ; this is only about twice the minimal concentration required to destroy the relatively limited bactericidal power of defibrinated blood (von Haebler and Miles, 1938).

The decisive period of the inhibitable defences

The decisive periods (DP) for shock- and adrenaline-inhibitable defences are listed in Table IV. Each figure represents the longest period found in 2, 3 or 4 determinations. The values range from 1–5 hr. The Liquoid results are also listed under the heading DP ; and although the evidence is equivocal that the period of susceptibility to Liquoid enhancement is necessarily decisive with respect to Liquoid-inhibitable defences, it is striking that these DP values, and indeed those for heparin enhancement, fall within the 5-hr. range. Of the 27 estimates of the DP, 25 are 4 hr. or less ; and the mean value is 2·8 hr.

The significance of the decisive defences in the various infections depends of course on their magnitude. For example, the proportional importance of adrenaline-inhibitable defences in the course of the *Str. pyogenes* infection is much greater than in the *C. ovis* infection ; the enhancement factor of the first is 50, and of the second, 2. The adrenaline treatment gave 49/50ths of the injected streptococci an opportunity to infect which was denied to them in the control lesion ; that is, in untreated skin, all but 0·02 of the inoculum was killed, removed or otherwise rendered non-pathogenic by the adrenaline-inhibitable defences. The corresponding figure for *C. ovis* is 0·5.

These last values, the reciprocals of the enhancement factor F (Table I), are entered in Table IV as P_e, the effective proportion of the inoculum surviving these early defences. They clearly show that the defences are substantially effective in the nine infections. They are most effective against the Gram-negative bacilli and *Cl. welchii*, least against *L. monocytogenes* and the two corynebacteria, and intermediately so against the two cocci.

The decisive period with antibiotics

No transient stimulants—as opposed to inhibitors—of the defences were available to test the concept of the decisive period with respect to suppression rather than the enhancement of infection. But suppression of infection by antibiotics provides a means of testing whether the early period of infection is in any way as decisive for the bacteria as it is for the defences. The intravenous injection of a single dose of 10^4 units of benzylpenicillin or of 12×10^3 units of streptomycin sulphate, made 0–1 hr. before the intracutaneous injection of the bacteria, suppressed the test infections to the degree indicated by the P_e values in col. 9 and 11 of Table IV. (Since the lesions were measured at 24 hr., the late-developing toxicity of penicillin for the guinea-pig could be ignored.) Here P_e, the effective proportion of the inoculum surviving the antibiotic, is the reciprocal of the factor by which infectivity is *depressed* (*cf.* Fig. 7). Several of the infections were insusceptible to the antibiotics in the doses used and P_e therefore is unity.

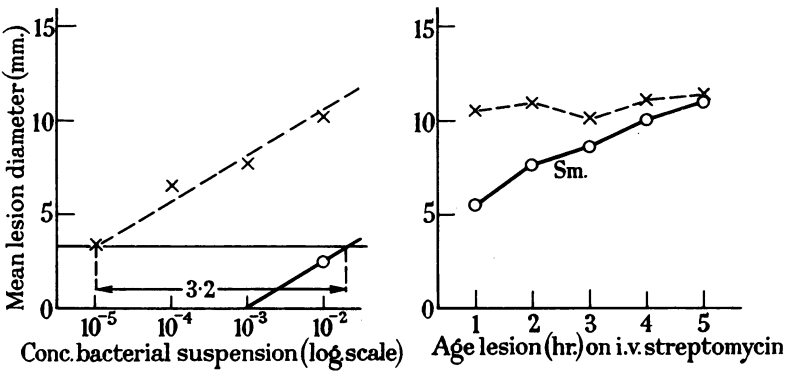

FIG. 7.—Depression of *C. ovis* infections by 12×10^3 units streptomycin, given intravenously at the time of the intracutaneous injection of the bacilli.

FIG. 8.—Decreasing susceptibility of *C. ovis* infections to 12×10^3 units streptomycin with increasing age of the bacterial lesions at the time of the intravenous injection of streptomycin.

O———O Test lesions. × – – – × Control lesions.

The method of determining the decisive period was similar to that for shock. All but one of the infections which were susceptible when initiated at the time of the single intravenous dose of antibiotic were, as Fig. 8 exemplifies, completely insusceptible when the lesions were 3–5 hr. old. *Str. pyogenes* infection is the exception; here, lesions up to 8-hr. old continued to be susceptible to streptomycin. The decreasing efficacy of antibiotics with increasing age of infection is a commonplace of chemotherapy, but it is striking to find that the decisive period of their efficacy in a wide variety of infections coincides with that of the defences. The coincidence is consistent with what we know of the state of the lesions at the end of the decisive period. Leucocytes are plentiful, and phagocytosis in most cases is pronounced. Bacteria destined to survive the decisive period may well be intraleucocytic at this time, and to that extent protected from antibiotics arriving from the blood stream.

DISCUSSION

The nine experimental infections of the skin were surveyed as the first step of a larger investigation of non-specific resistance to microbial infection. Defence against an external microbial hazard entails either the prevention of any lodgement of the pathogen whatsoever, or its destruction in the primary lodgement before it can produce a manifest infection. The intracutaneous injection of the pathogen is equivalent to a successful primary lodgement, so that our analysis can reveal nothing about defences against the *act* of lodgement ; but it reveals rapid, and in some cases highly effective, defences against bacteria newly introduced into the tissues. The exact numerical values upon which we base this conclusion are clearly to be taken as indicating only the magnitude of an effect ; they are not precise measurements of it. None the less, they are reliable enough for measurement of efficacy of the body defences in orthodox terms of the dose-response to infection. On this basis it appears that, according to the pathogen used, from 50 to 99·999 per cent of the inoculum is rendered ineffective. The defences responsible for this effect come into play mainly within the first 2 hours of infection, and appear to be complete within 5 hours.

A few of our results are inconsistent with this generalization, but the striking similarity of most of them, obtained with widely differing infections and modifiers of infection, justifies our regarding it as broadly established for intracutaneous infections of this kind. It is possible that the generalization also applies to primary lodgements in other superficial regions of the body like the respiratory and alimentary tracts ; and perhaps to secondary lodgements in various organs, like those occurring during the course of an established infection, as metastatic events in as yet uninfected tissue : but we have as yet no evidence on these aspects of the problem.

The test pathogens are at first sight a little remote from the microbial hazards of the natural environment. With the exception of the *Str. pyogenes*, which was isolated from a guinea-pig infection, and perhaps the *Staph. aureus*, a species sometimes infecting the skin and eyes of the laboratory guinea-pig, our test bacteria are not natural pathogens of the animal. None of them was virulent enough to infect in doses of a few cells, and only the *Str. pyogenes* and the *C. ovis* could establish a severe general infection. We contend nevertheless that they are sufficiently representative of environmental pathogens, because, except in fulminating epidemics, such pathogens are likely to be in reasonable equilibrium with a stable population of a host species—infecting a small proportion and killing a smaller one—and therefore neither highly infective nor highly virulent.

We have already noted the broad correlation between susceptibility of infection to enhancement and the susceptibility of the infecting bacterium to the bactericidal power of the blood, which suggests that the bactericidal actions inhibited by the enhancing agents are those of blood, or one of the same kind as those of leucocytes and plasma. There is also a suggestion of a broad correlation between this susceptibility and the virulence of the bacteria, as indicated by the dose producing a 10 mm. lesion in the skin (ED_{10}, Table I). It follows that the higher the ED_{10}, the greater the " kill " in the primary lodgement. Thus with *Bact. coli* the ED_{10} is 10^9 and the P_e for adrenaline is 10^{-4}, so that the estimated dose of bacilli surviving the decisive period is 10^5. *Str. pyogenes* has a lower ED_{10} of 2×10^6, and a proportionately lower P_e of 0·02, and the dose surviving the decisive period is 10^4. Most of the ED_{10}'s appear to be diminished to effective doses of the order of 10^3–10^5.

One more numerical association may be noted. Maximum killing of the bacteria in defibrinated blood occurred without exception in 2–4 hours, after which the surviving bacteria began to multiply. The decisive period of 3–4 hours, for the action *in vivo* of defences which, according to our analysis, are mediated by bactericidal elements of the blood, is about the same. The association may be coincidental, but it is also consistent with the view that the effective blood elements mobilized *in vivo* after a short period undergo no substantial renewal from the circulation and elsewhere, and in this respect resemble the sample of blood in a bactericidal test. That is, at 4 hours, the defensive responses characteristic of the first hours of the infection are no longer made.

We do not imply that the short decisive period is applicable to all local defences. It was in each case determined with a dose of modifier equal to that used to establish the enhancement factor, and is therefore valid only for defences of the degree and kind affected by the various modifiers. The modifiers were selected for their transient action and used in a few multiples of minimally effective doses. An analysis of the defences with stronger and with other modifiers might well reveal different decisive periods, but our conclusions would not be invalidated because we define the defences to which the short decisive period applies in terms of the modifiers. We have not tested other modifiers systematically, but the few so tested do not indicate another decisive period. Nevertheless, other important defence systems with different time courses are by no means ruled out. Lesions older than 4 hours are indeed modifiable. Thus adrenaline enhances 5-hour *Ps. pyocyanea* lesions (Fig. 5, B); and in the two infections *depressed* by shock (Fig. 3), lesions 6 hours old or more are as strongly affected as those initiated during shock.

The rapid initial death of a large proportion of the survivors of the inoculum and the defences already mobilized together appear to determine the final size of the local lesion. But the important events of the primary lodgement are by no means over. In the first place, though the participants in the reaction are by now decisively gathered together, the reaction is not over. A moderate oedema and erythema at 4 hours in most cases becomes a heavy induration with necrosis. In the second place, the relation of the various stages of the primary lodgement and its dissemination to other parts of the body, and the part played by systemic reactions in containing the local infection as the lesion matures, is almost entirely unknown. We have evidence that thrombosis at the periphery of the lesion plays no part in localizing it.

Lastly, we would emphasize the simplicity of intracutaneous lesions for studying infection and resistance. The information from a single depilated guinea-pig, on which 3–4 doses each of several infecting bacteria can be titrated, far exceeds that from an animal systemically infected with one kind of bacterium, from which only two simple measures of infection can be obtained—one a continuous variate, time to death, the other a quantal response, death itself. The guinea-pig yields at any one reading as many estimates of infectivity as there are lesions; and there may be 24 or more, all in a piece of tissue substantially uniform in susceptibility to the infecting agents. By contrast, titrations of equal statistical weight, using, for example, a quantal response in systemically infected mice, could be obtained only from several multiples of 24 mice. When means of the skin responses of 4–5 guinea-pigs are used, the precision of the results may be equivalent to that from several hundred mice. The intradermal method,

as will be evident from our few variations in technique, is moreover capable of great and cheaply achieved experimental flexibility.

SUMMARY

The fate of the primary lodgement of infecting microbes was studied in experimental infection of the skin of the guinea-pig by nine different bacteria.

With all nine pathogens, the mean diameters of mature lesions were roughly linear with respect to log dose, within a restricted range of dose-responses; for a given pathogen, the slopes of the dosage-response lines were roughly parallel, thus facilitating numerical comparisons of infectivity under the influence of modifying agents.

Local adrenaline, Liquoid, and general dehydration shock, enhanced nearly all the infections to a degree varying with the pathogen and the enhancing agent. In general, *Bact. coli*, *Ps. pyocyanea*, *P. vulgaris* and *Cl. welchii* were most strongly enhanced, *Str. pyogenes* and *Staph. aureus* less strongly enhanced, and *C. diphtheriae*, *C. ovis* and *L. monocytogenes* moderately enhanced.

The enhancing agents inhibit either the access to the lesion, or action in the lesion, of bactericidal elements of the blood. The degree of enhancement is in general correlated with susceptibility of the pathogen to the bactericidal action of blood and serum *in vitro*.

Enhancement ranged from 2- to 100,000-fold. The body defences inhibited by the enhancing agents are accordingly responsible for the " killing " from 50 to 99·999 per cent of the bacteria originally constituting the primary lodgement.

Infections several hours old are insusceptible to enhancement. It appears that at this stage the defences inhibitable by the enhancing agents no longer have any decisive effect on the subsequent development of the lesion. The period during which these defences are decisive varied between the first 2 and the first 5 hours of infection.

The short decisive period applies also to the bacteria in the lesions. Infections 3–5 hours old were insusceptible to intravenous antibiotics in doses that were effective when given earlier in the infection. At this stage there was no obstruction to the blood supply of the lesions.

This work was begun, and E. M. Miles' part in it completed, at the National Institute for Medical Research, Mill Hill, N.W.7. We are indebted to the Nuffield Trust for financial help in its prosecution.

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