THE ENHANCEMENT OF BACTERIAL INFECTIONS BY ADRENALINE

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Received for publication December 12, 1947.

FOLLOWING the observation that an intramuscular injection of adrenaline in a man had apparently induced a *Cl. welchii* gas gangrene at the injection site, Cooper (1946) demonstrated a marked decrease in the minimal lethal dose of *Cl. welchii*, when washed bacilli were injected into the muscle of guinea-pigs together with adrenaline. A 10,000-fold decrease occurred with 62·5 μg. adrenaline in a volume of 1 ml. Adrenaline thus enhances the infectivity of *Cl. welchii* as effectively as 2·5 per cent $CaCl_2$, but does so without the gross damage to the tissues produced by $CaCl_2$ in the concentrations commonly used. We have investigated the nature of this enhancement in experimental infections both by clostridia, and by a number of pathogenic aerobic bacteria. For many of the bacterial species tested, adrenaline decreased the number of living organisms required either to kill the animal, to establish a generalized infection, or to produce infective lesions of a given degree of severity. We have surveyed the infections following the injection of washed living bacteria into the skin of rabbits and guinea-pigs, measuring the size and severity of the local lesion, and into the thigh muscle of guinea-pigs, in this case determining the minimal lethal dose of the bacteria.

The enhancement of infections by adrenaline.

Bacteria in the skin of guinea-pigs and rabbits.—For intradermal tests, ten-fold dilutions of a suspension of washed bacteria, usually from an overnight growth in an optimal medium, were injected in 0·1 or 0·2 ml. quantities within 30 minutes of mixing with equal volumes of 0·85 per cent saline (the S suspension) or a solution of adrenaline in saline (the A suspension). At the same time a viable count was made of the suspension by the method of Miles and Misra (1938), except with suspensions of *Cl. oedematiens*, *Cl. septicum* and *Cl. tetani*, which were counted in nutrient agar shake cultures incubated anaërobically. The skin was depilated the previous night by a brief application of a barium sulphide powder to the clipped skin. When possible, both S and A injections were made into each of the animals, to permit comparisons unaffected by variations between animals. With ten-fold dilutions of culture, relatively large differences between the S and A lesions could be measured by the ratio of the minimal effective doses of the S and A suspensions.

Severity of the inflammatory skin lesion.—Precise comparison of lesions was often difficult, because the dose-response differed in the S and A series. Two titrations in Tables I and II illustrate the difficulties. The lesions are recorded as the areas of moderate, marked and gross inflammation, calculated from

$D^2\pi/4$, where D is the diameter of circular lesions, and $dD\pi/4$ where D and d are the major and minor axes of elliptical lesions. The area of marked inflammation includes that of the gross, and the area of moderate, that of marked and gross inflammation. Moderate inflammation is defined as moderate induration or oedema, and pink hyperaemic skin; marked inflammation as pronounced induration or oedema, and deeply reddened skin; and gross inflammation as very pronounced oedema or induration, and skin either purple with petechial haemorrhages, or yellowish-white as the result of suppuration or necrosis.

These responses are clearly not proportional to the dose. If we select "gross" inflammation as the threshold effect, the S:A ratio for *Ps. pyocyanea* (Table I)

TABLE I.—*The Severity of Inflammation Produced by Graded Doses of* Ps. pyocyanea, *in the Skin of a Guinea-pig, injected with* 2·0 μg. *Adrenaline* (*A*), *and without* (*S*).

Dose.	Day.	Area of inflammation (mm².). Moderate.* S	Moderate.* A	Marked. S	Marked. A	Gross. S	Gross. A
16×10^7	1	800	490	706	415	227	227
16×10^6	1	382	188	314	154	64	64
16×10^5	1	78	223	50	176	12	78
16×10^4	1	3	195	0	78	0	6
16×10^3	1	3	16	0	4	0	0
16×10^7	2	572	314	490	113	314	113
16×10^6	2	154	165	113	132	0	50
16×10^5	2	28	143	20	113	0	13
16×10^4	2	3	71	0	50	0	0
16×10^3	2	3	4	0	0	0	0
16×10^2	2	0	3	0	0	0	0

* For the definition of the degrees of inflammation, see p. 21.

is about $16 \times 10^5 : 16 \times 10^4 = 10:1$ after one day, but 100:1 after two days. "Marked" inflammation gives 10:1 after both one and two days. "Moderate" inflammation is more equivocal: the response to increasing doses of bacteria increases more gradually than with severer lesions. To minimize the effect of the varying dose-response in the S and A series, the S:A ratios were calculated from doses giving marked inflammation at the time of maximal development of the lesions (e.g. at one day for *Ps. pyocyanea*). When there was no marked inflammation in the S series, moderate inflammation was used as the indicating effect. Thus with *Bact. coli* (Table II) the 78 mm.² from 46×10^7 bacilli in the S series is equated with 124 mm.² from 46×10^4 in the A series.

In some cases there was no difference in the end-point in the two series, but for a given dose of bacteria the A lesion was larger. Some strains of *Staph. aureus*, which behaved in this way in rabbits, proved by finer titrations with two-fold dilutions to have S:A ratios ranging from 2:1 to 5:1.

The bacterial strains used were all smooth, and the exotoxin-producers among them were fully toxigenic, except when otherwise stated.

TABLE II.—*The Severity of Inflammation Produced by Graded Doses of* Bact. coli. *in the Skin of a Guinea-pig, Injected with* 2·0 μg. *Adrenaline* (*A*), *and without* (*S*).

Dose.	Area of inflammation (mm.²) after 1 day.					
	Moderate.		Marked.		Gross.	
	S	A	S	A	S	A
46×10^7	78	415	0	346	0	227
46×10^6	50	297	0	240	0	104
46×10^5	20	143	0	104	0	0
46×10^4	2	124	0	78	0	0
46×10^3	2	39	0	0	0	0
46×10^2	2	3	0	0	0	0

TABLE III.—*The Ratio of Minimal Infecting Doses of Washed Viable Bacteria Injected into the Skin of Guinea-pigs with* 2·0 μg. *Adrenaline* (*A*), *and without* (*S*).

	Indicating lesion,* and age in days.	Minimal effective dose of living bacteria.		Ratio.
		S	A	S:A
Cl. septicum (VS 54)	M, 1	$>7 \times 10^7$	7×10^2	$>10^5$:1
Cl. welchii (S.R. 9)	M, 1	6×10^6	6×10	10^5:1
Bact. coli	Mod, 1	46×10^7	46×10^3	10^4:1
Pr. vulgaris	M, 1	63×10^7	63×10^4	10^3:1
Str. pyogenes (Richards)	M, 1	8×10^6	8×10^3	10^3:1
Ps. pyocyanea	M, 1	16×10^5	16×10^3	10^2:1
Staph. aureus (Humphrey)†	M, 1	2×10^8	3×10^6	66 :1
Staph. aureus 21	G, 2	9×10^7	9×10^6	10 :1
Cl. oedematiens (Jolly)	M, 1	9×10^6	9×10^5	10 :1
Br. abortus	Mod, 2	16×10^7	16×10^6	10 :1
B. subtilis	Mod, 1	6×10^6	6×10^5	10 :1
Str. pneumoniae, I	M, 1	29×10^6	29×10^5	10 :1
Staph. aureus 15‡	G, 2	34×10^6	34×10^6	1 :1
V. cholerae‡	Mod, 1	47×10^6	47×10^6	1 :1
Sh. sonnei‡	Mod, 1	11×10^7	11×10^7	1 :1
Sh. flexneri‡	M, 1	18×10^7	18×10^7	1 :1
Cl. histolyticum (CN 920)	M, 1	15×10^5	15×10^5	1 :1
H. pertussis†	M, 1	3×10^4	3×10^4	1 :1

* Mod., M and G = moderate, marked and gross inflammation (see p. 21).
† Tested in rabbit's skin.
‡ Measured as relative sizes of lesions from same dose, ratio A:S was about 5:1.

The noteworthy points about intradermal infections (Table III) are :

(*a*) the large minimal effective doses (m.e.d.) in terms of viable bacteria ;

(*b*) the wide range of S:A ratios ; and

(*c*) the haphazard distribution of bacterial species, when arranged in order of susceptibility to adrenaline enhancement.

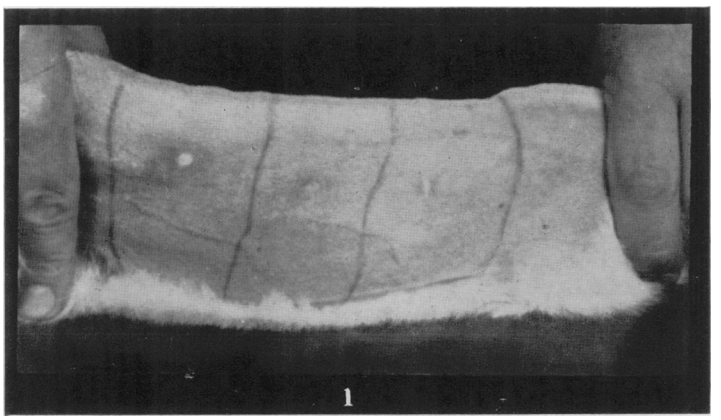

FIG. 1.—Left to right: washed organisms alone, 80, 40, 20 and 10 millions.

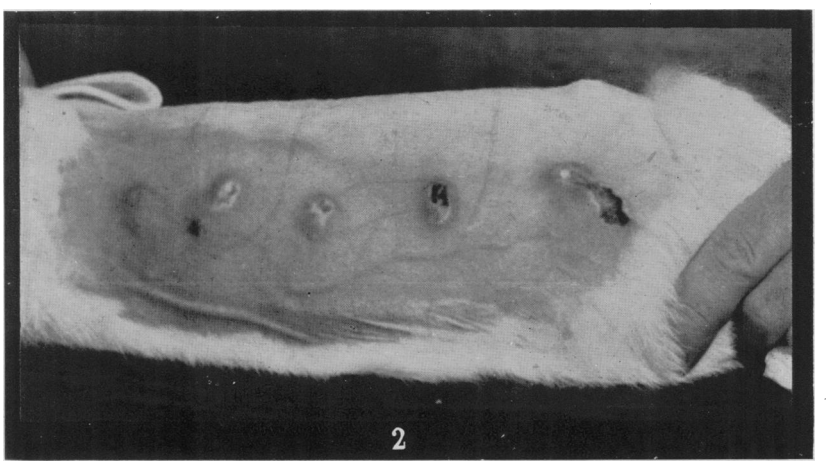

FIG. 2.—Left to right: washed organisms with 2·0 μg. adrenaline, 5, 10, 20, 40 and 80 millions.

FIG. 1 and 2.—Four day *Staph. aureus* infective lesions in the skin of a rabbit.

No safe general conclusion can be drawn about a given species from the results of testing one strain. In all, eight strains of *Staph. aureus* coagulase positive with human plasma were tested, and for these the S:A ratios varied from 5:1 to 100:1 (Figs. 1 and 2). Two strains of *Staph. aureus* from Professor Wilson Smith, both coagulase-positive with guinea-pig plasma, one (No. 15) α-toxigenic, the other (No 21) non-toxigenic, were tested several times in guinea-pigs; infections by No. 21 were consistently more enhanced than those by No. 15. Among the clostridia, the enhancement of *Cl. welchii* and *Cl. septicum* and the relative or total absence of it with *Cl. oedematiens* and *Cl. histolyticum* was striking. *Ps. pyocyanea* had an unexpectedly low m.e.d., and higher doses produced severe but non-ulcerating lesions that persisted, though slowly declining, for 14 days or more. For the bacteria marked ‡ in Table III the 1:1 ratios measured on a ten-fold scale are misleading; although the S and A end-points were the same, the A lesions were more severe than the corresponding S lesions, indicating a two- to five-fold enhancement of infections. Adrenaline had no effect on *H. pertussis* in the rabbit.

Bacteria in the muscle of guinea-pigs.—For the intramuscular tests the procedure was modified in that 0·5 ml. adrenaline solution was injected first, followed by 0·2 ml. of the graded doses of bacteria. Adrenaline enhanced intramuscular infections (Table IV) in the same degree as those in the skin, where the parallel test was made; in addition, *C. diphtheriae* infections were enhanced 100-fold, and *Cl. tetani* not at all.

TABLE IV.—*The Ratio of Minimal Lethal Doses of Washed Viable Toxigenic Bacteria by the Intramuscular Route in Guinea-pigs with 2 μg. of Adrenaline (A), and Without (S).*

Bacterium.	Minimal lethal dose of viable bacteria.		Ratio.
	S	A	S:A
Cl. septicum (VS 54)	$>7 \times 10^7$	7×10	$>10^6$:1
Cl. welchii (S.R. 9)	5×10^7	5×10^2	10^5:1
C. diphtheriae, gravis	15×10^4	15×10^2	10^2:1
Cl. oedematiens (Jolly)	9×10^5	9×10^4	10 :1
Cl. tetani (T 67)	1×10^3	1×10^3	1 :1
Cl. histolyticum (CN 920)	15×10^6	15×10^6	1 :1

Tests of other sites and organisms.—A few other tests were made. In the rabbit skin, 2 μg. adrenaline enhanced the lesions made by vaccinia virus ten-fold; the readings were made after 7 and 10 days. In the mouse there was no enhancement of the LD50 of a highly mouse-virulent *Str. pneumoniae* Type I, given either intraperitoneally or subcutaneously in the ear; 10–15 mice were used for each dose. And in the same animal, Dr. C. H. Andrewes reports that the infectivity of Type A influenza virus by nasal instillation 0·05 ml. virus supension was not altered by adding 100 μg. adrenaline to each ml. of suspending fluid.

The enhancement of intoxication by adrenaline.

The enhancement of infection by bacteria like *B. subtilis*, *Sh. sonnei*, and *Sh. flexneri*, none of which is particularly pathogenic for the guinea-pig, suggested

that the adrenaline might be enhancing intoxication, not infection. It is clear from Table V, however, that though clostridial exotoxins were enhanced two-

TABLE V.—*The Ratio of Minimal Effective Doses of Preparations of Bacterial Endo- and Exo-toxins in the Skin of Guinea-pigs and Rabbits Injected with 2·0 μg. Adrenaline (A), and Without (S).*

Toxin.	Animal.	Indicating reaction.	Ratio of m.e.d., S:A
Cl. septicum	Guinea-pig	Necrosis	4:1
Staph. aureus	Rabbit	,,	4:1
Cl. welchii	Guinea-pig	,,	2:1
Cl. oedematiens	,,	,,	2:1
Cl. histolyticum	,,	,,	2:1
C. diphtheriae	,,	Inflammation	1:1
Br. melitensis	,,	,,	1:1
Pr. vulgaris	,,	,,	1:1
H. pertussis	Rabbit	,,	1:1

to four-fold, *C. diphtheriae* toxin, the endotoxins of *Br. melitensis* (Miles and Pirie, 1939), *Pr. vulgaris* (a fraction corresponding to the F68 fraction of *Salm. typhi-murium* (Raistrick and Topley, 1934)), and *H. pertussis* toxin (Evans and Maitland, 1937) were unaffected. The enhancement of intoxication bears no constant relation to the enhancement of infections, for the S:A ratios for the toxins of *Cl. oedematiens* and *Cl. welchii* are of the same order, whereas the ratios for the infections are respectively 10:1 and 100,000:1. That intoxication, as distinct from invasion by multiplying bacteria, may contribute to the enhancement in infective A lesions was evident in *Cl. welchii* lesions (see p. 26). In the S lesions one hour old there was marked inflammation and leucocytosis, but little necrosis; in the A lesions no inflammation or leucocytosis, but incipient characteristic muscle necrosis.

On the other hand, such exotoxins as may be injected with the infecting dose of washed *Staph. aureus* were not demonstrably affected by adrenaline, as the following experiments show. Rabbits received 10,000 I.U. penicillin intravenously 3 hours before, and 2, 4 and 6 hours after the intradermal injection of graded amounts of washed, α-toxigenic *Staph. aureus* (Humphrey) mixed with 2·0 μg. adrenaline. Only the largest dose, 80×10^6 cocci, produced a moderate lesion 50 mm.^{2} in area, whereas in animals without penicillin, the same dose produced a necrotic lesion 445 mm.^{2} in area. A similar result followed the addition of 10 I.U. penicillin to the adrenaline-bacterium mixtures; thus in one rabbit 20×10^6 cocci with penicillin produced no lesion, and without penicillin a 980 mm.^{2} lesion. Clearly, the local intoxication was consequent on the multiplication of the staphylococci, and not due to preformed exotoxin whose action was enhanced by the adrenaline.

We may conclude that the greater part of the adrenaline effect is upon infection as distinct from intoxication by substances contained in the inoculum.

The Mode of Action of Adrenaline.

Speculation about the action of adrenaline in enhancing the infective process must clearly start with the acute and severe constriction of the arterioles that is produced by the relatively large doses used, resulting in a diminution of the blood flow through the capillary vessels distal to them. As a consequence, we may expect not only a diminution of the normal fluid and gaseous exchanges of the tissues, but also of many of the characteristic features of the inflammatory response to infective bacteria; and in particular exudation, and diapedesis of leucocytes, since the continuous supply of blood fluid and cells necessary for these phenomena is cut off.

The visible effects of 2·0 μg. adrenaline intradermally wear off in 1–2 hours, and, as far as the local resistance of the tissues is concerned, there is no after-effect. When graded doses of washed *Staph. aureus* or *Str. pneumoniae* were injected into normal guinea-pig skin and into the site of injection of 2·0 μg. adrenaline made 1 minute, 2 hours and 4 hours previously, the "1 minute" lesion was fully enhanced, the "2 hour" slightly, and "4 hour" lesion not at all. Here there was no gross after-effect of the adrenaline, its action being confined to the period of its manifest physiological activity. Moreover, with *Staph. aureus* in both rabbit and guinea-pig skin, the injection of 2·0 μg. adrenaline 2 and 4 hours after the injection of the bacteria did not enhance the infection, showing that with this organism at least, the infective process cannot be modified by adrenaline after a certain critical period.

As a working hypothesis, therefore, it may be assumed that during the temporary conditions in adrenaline-treated tissue certain bacteria are able to grow or adapt themselves to growth more readily than they do in normal tissue; and that the favourable consequence of adrenaline action is the relative absence of bactericidal substances and cells normally derived from the blood.

It is easy to establish that adrenaline inhibits both diapedesis of leucocytes and exudation.

Inhibition of exudation.

Depilated albino guinea-pigs weighing 250–350 g. were given 0·3–0·4 ml. of 5 per cent pontamine sky blue 6X (Gurr, London) intravenously, and injected intradermally with solutions of various substances known to increase capillary permeability, which was estimated by the marked staining of the injection site by exuded dye-stained blood fluids. Adrenaline, 3·3 μg. in 0·1 ml. injection fluid, inhibited the deep staining of tissues induced in 10–30 min. by 0·1 mg. histamine, by 1 mg. bacteriological peptone, and by maximal sub-necrotizing doses of the toxins of *Cl. welchii*, *Cl. septicum* and *H. pertussis*; and inhibited for 1–2 hours the lesser and more slowly developing stain induced by heavy suspensions of washed *Cl. welchii* and *Staph. aureus* containing about 10^{10} organisms per ml.

Inhibition of diapedesis.

Washed 18-hour living *Staph. aureus* and *Cl. welchii* and *B. subtilis* spores were suspended either in 0·85 per cent saline or 0·5 per cent peptone water, together with a trace of indian ink to mark the site of injection. These suspensions, 0·1 ml., were injected intradermally into depilated albino guinea-pigs.

with 2·0 μg. adrenaline (A) and without (S). The skin bearing the lesions was excised with a 2 mm. margin of normal tissue all round, tied to small pieces of perspex to prevent distortion during treatment, and fixed. After fixation the pieces were trimmed, dehydrated, cleared and embedded in paraffin, and serial sections (6μ) cut perpendicular to the skin surface through the entire lesion. All sections were mounted and every fourth slide stained with haematoxylin and eosin, each succeeding slide with a bacterial stain and intermediate slides according to the particular examination required. Both *Staph. aureus* and *Cl. welchii* were readily identifiable in sections stained with haematoxylin and eosin sections, or by Kirkpatrick's modification of Gram's stain. For the spores of *B. subtilis*, a modification of Armstrong's stain proved effective. Each variation in experimental conditions was tested in four lesions from two guinea-pigs.

With each organism, the S and A lesions 3 minutes old were almost indistinguishable. Spreading through the dermis from the site of the needle puncture (which in no case penetrated beyond the upper layer of the dermis) were irregularly shaped masses of carbon, which diminished in size towards the periphery of the lesion. In the centre they obscured the microbes by reason of their size and density: at the periphery the masses were looser, and microbes could be distinguished. In the S lesions, microphages (polymorphonuclear leucocytes) were slightly more numerous in the capillaries lying between the panniculus carnosus and the subcutaneous fat, and predominated at the periphery of the vessels.

With *Staph. aureus* and *B. subtilis* at 30 minutes, there was general dilatation of capillaries and arterioles between the muscle and the subcutaneous fat in the S lesions, and the normally inconspicuous capillaries in the interstices of the fat and in the upper layers of the dermis were open. Microphages lined their endothelium and many had emigrated into the tissue spaces, and some were accumulating round the carbon and bacteria. At 1 hour these changes were more pronounced. Phagocytosis of the cocci and particularly of the spores was active, and of the carbon particles less so, and by 2 hours the *Staph. aureus* lesions were largely overrun by active microphages.

The A lesions were in sharp contrast. The 30 and 60 minute lesions differed little from the 3 minute: a few microphages lay between the muscle and the fat at 2 hours, and large numbers at 3 hours: but in no case did the migration equal that at 1 hour in the S lesion, nor did it extend, as in the S lesion, to the upper layer of the dermis.

With *Cl. welchii*, as with *Staph. aureus*, there was progressive migration of the microphages and active phagocytosis in the S lesions, and none in the A lesions until after 2–3 hours. The 1 and 2 hour S lesions showed also a thin zone of necrosis of connective tissue round the injection site, characterized by rounding and shrinkage of the cells and disappearance of thier cytoplasmic processes, and nuclear karyolysis. In the A lesion this necrosis was much more extensive, suggesting a much more intense intoxication: fibrocytes were absent round the injection site; and the nearby muscle fibres were invaded by bacteria, had lost striation and, in some cases, sarcous substance.

The alteration of tissue oxidation-reduction potentials.

The marked enhancement of infections by *Cl. welchii* and *Cl. septicum* (Tables III and IV) suggests that adrenaline, by cutting off the supply of oxygen, might

induce in the tissues a high reducing intensity favourable to the growth of anaërobic organisms. Accordingly two Eh indicator dyes, methylene blue and indigo disulphonate, were tested in the depilated skin of albino guinea-pigs, in the manner adopted by Fildes (1929) in his investigation of the *in vivo* germination of tetanus spores. Concentrations of 0·01 per cent methylene blue and 0·04 per cent indigo disulphonate proved to be most suitable for intradermal injection in 0·1 ml. volumes. Methylene blue in greater concentrations produced an intense and persistent staining of the superficial layers of the skin ; staining

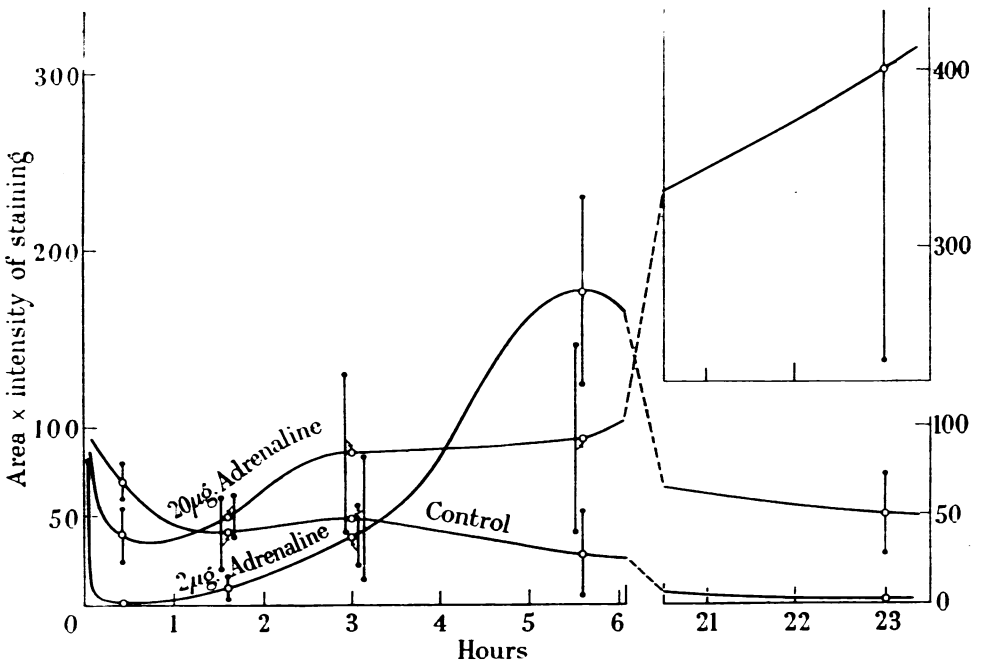

FIG. 3.—The course of staining of guinea-pig skin injected intradermally with methylene blue, with and without adrenaline. Each point represents the mean of readings from six guinea-pigs. The vertical lines indicate the standard deviation of the six observations.

by the 0·1 per cent solution faded from normal skin in 12–20 hours. The intensity of staining was estimated on an arbitrary, approximately logarithmic scale, which was checked by three independent observers against normal guinea-pig skin and filter papers, both stained by graded concentration of the dye. The total amount of staining in skin lesions was measured by intensity × area stained. In each test 2–4 animals were used, repeating the injection in different parts of their flanks and back. Fig. 3 summarizes the essential features of the results with methylene blue, which were similar in all tests made on a total of 35 guinea-pigs. Each point on the curves is the mean of six injection sites in two guinea-pigs. The scatter of values round the means is substantial, especially in the later readings, but there is no doubt that the three curves, for dye alone, and for dye

with 20 and 2 μg. adrenaline, represent three significantly different processes. Within the hour the colour of the control lesion declines, remains approximately stationary till the fourth hour, and, slowly fading, is invisible at 23 hours. With 2 μg. adrenaline there is almost complete or complete decolorization within 3 minutes, which persists for 30–60 minutes, after which the colour grows in intensity until at the fifth hour it is more intense than that of the initial lesion: after this it fades, but is still marked after 23 hours. The same sequence occurs with 20 μg. adrenaline, except that decolorization is complete only at the periphery of the lesion, a small central spot of blue-green staining persisting throughout the whole period; and the subsequent recolouring is intenser and more persistent.

By section of similar lesions at 30 minutes, staining of the whole thickness of the skin in the saline lesions and its complete decolorization by 2 μg. adrenaline was visible to the naked eye: and with 20 μg. adrenaline the residual staining was confined to superficial layers of the skin. In fact, the staining in the 20 μg. adrenaline lesions at 5–23 hours was characteristic of a much stronger concentration of dye injected without adrenaline.

A similar sequence of events was observed in the skin of the rabbit's flank, and the mouse's ear.

It is clear, therefore, that the injected adrenaline lowers the Eh of the tissues to the level where methylene blue is decolorized. No such reversible decolorization could be demonstrated with 0·04 per cent indigo disulphonate (Fig. 4*a*). The dye disappeared within six hours, and the only effect of adrenaline was to prolong the period of staining. The stronger adrenaline lesions recoloured after what appeared to be a temporary decolorization. This was not a true decolorization, for the edges of skin cut through the centre of the lesion were well stained. The recolouring was apparently due to the removal of the injection fluid, which was to some extent masking the staining of the tissues; for the reintroduction of 0·1 ml. saline into established 3-hour dye-lesions led to a similar temporary decolourization.

Because of possible errors in estimating the intensity of staining, the *in vivo* results were confirmed by tests in Thunberg's tubes. Skin from a newly killed guinea-pig was frozen in M 15 phosphate buffer, pH 7·0, cut in 20μ slices on a freezing microtome and made into a 15 per cent (wet wt./vol.) suspension with the buffer. At 37° C., both the suspension itself, and the supernatant fluid from centrifuged suspension, reduced 0·003 per cent methylene blue, but not 0·005 per cent indigo disulphonate. Adrenaline is known to affect tissue reducing systems; low concentrations increase and higher concentrations diminish the reducing power of suspensions of frog muscle (Ahlgren, 1925; Euler, 1927). The same held for the suspension of sliced guinea-pig skin, as the following typical experiment shows:

Concentration of adrenaline μg. ml.	Reduction time (min). of methylene blue at 37°C.
333	83
33	54
3	36
0	42

It is possible, therefore, that adrenaline stimulates the reduction of methylene blue *in vivo*, apart from its vaso-constrictor action (Ball and Chen, 1933); and that the lesser *in vivo* effect of the higher concentrations tested (Fig. 3) were in part due to inhibitory effects of the kind exemplified above, in the test with 333 μg. adrenaline/ml.

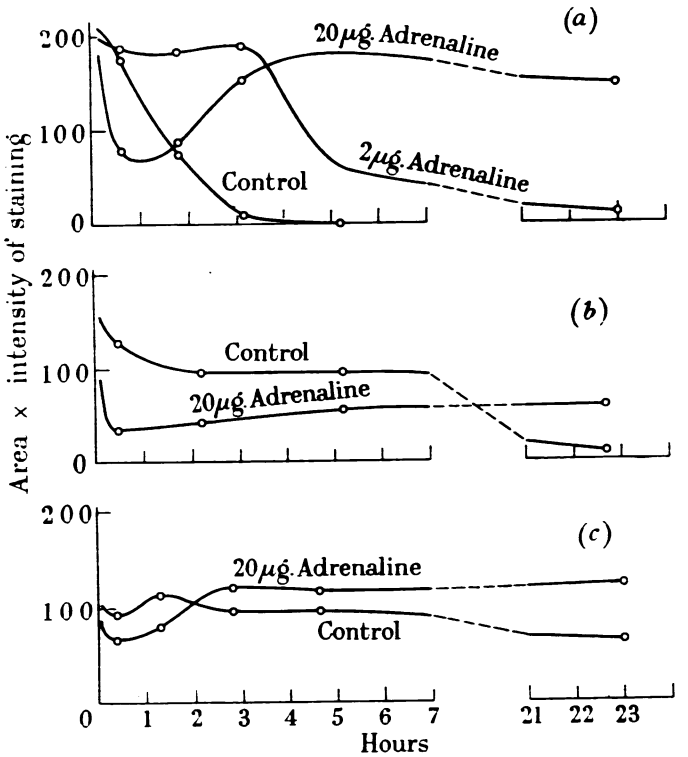

FIG. 4.—The course of staining of guinea-pig skin injected intradermally with various dyes, with and without adrenaline. Each point represents the mean of readings from at least three guinea-pigs. (*a*) Indigo disulphonate. (*b*) Trypan blue. (*c*) Pontamine blue.

The significance of increased reducing intensity in the tissues.

Whatever the mode of action of the adrenaline on the oxidation-reduction systems of the skin, the decolorization of methylene blue indicates a certain tissue Eh, whose magnitude will depend on the pH of the tissues. No exact measurements of pH were made, but tests in skin lesions 30 minutes old with brom-thymol-blue indicated pH levels of 7·0–7·2 in lesions made with 2 μg. adrenaline and with saline; and with phenol red, pH levels 7·0–7·2 in the adrenaline and 7·2–7·4 in the saline lesions. Even allowing for the errors in these colour estimations due to absorption of the dyes to tissue proteins, we may assume that the tissues pH lay well within the range 6·5–7·5. In this range, the 98 per cent reduction of methylene blue indicates an Eh of —0·023 to —0·058 volts, and of indigo disulphonate, an Eh of —0·148 to —0·197 (Fildes, 1929).

Since the skin tissue slices *in vitro* at pH 7·0 failed to attain the Eh (−0·1 volts) necessary for even the partial reduction of indigo disulphonate, it is probable that the Eh of the adrenaline-treated tissues *in vivo* was in the region of −0·04 to −0·1 volts.

It is problematic how far an Eh of this magnitude would aid the growth of aerobic organisms. Growing cultures of many pathogens attain Eh levels of this order and lower; but as Hewitt (1936) points out, such reducing intensities may be the accompaniments of growth, and not necessarily conditions for its initiation and maintenance. Moreover, among the aerobes tested (Table III) there is no association between enhancement of infection by a bacterial species and its capacity to produce high reducing intensities in artificial culture. Thus, though *Bact. coli*, cultures of which can attain Eh values as low as −0·4 volts, is enhanced, so are *Proteus vulgaris* and *Ps. pyocyanea*, which do not attain low values in culture (Hewitt, 1936).

With regard to the clostridia, the Eh induced by adrenaline approaches the level of −0·2 volts, at which growth of these anaerobes starts, and may be expected to facilitate the attainment of this critical level as the infection develops. Nevertheless, we have little evidence that the diminution of Eh was a major factor in enhancing the clostridial infections.

Moreover, adrenaline has a marked effect on only two of the six clostridial infections examined (Tables III and IV); and we have observed some enhancement of *Cl. welchii* infections with 0·15 μg. adrenaline, an amount which induced no detectable decolorization of methylene blue, though, like larger amounts, it retarded the removal of the dye from the skin.

The fixation of dyes by adrenaline.

The fixation of methylene blue.—The retardation by adrenaline of the removal of methylene blue and to some extent of indigo disulphonate from the skin suggests that the enhancement of local infections might be due to a similarly retarded removal of injected bacteria. Immediately after injection, the removal of dye must in great part be due to fluid drainage; after which removal by phagocytes presumably plays an increasingly important part. Adrenaline must affect the early stages of the removal, since even 0·15 μg., whose effect passes off with the hour, does so. It is pertinent to inquire what physiochemical effect adrenaline might have on the dispersion and retention of dyes and bacteria in the tissues.

At 37° C., strong adrenaline (1 mg./ml.) is a rapid precipitant of indian ink and slowly agglutinates washed suspensions, 10^9/ml., of *Cl. welchii*, *Staph. aureus* and *B. subtilis* spores; but concentrations of 0·2 mg./ml. have little effect even after 12 hours either on ink or bacterial suspensions. Nevertheless, when a dilute solution of methylene blue was injected with adrenaline in this concentration into the skin of a transilluminated mouse's ear, the spread of the area of obvious staining round the site of injection observed under a low-power microscope was much less than that with methylene blue alone, as though the affinity of dye and tissues was enhanced. However, adrenaline in concentration 5–500 μg./ml. did not materially increase the readiness with which a suspension of fresh skin slices absorbed methylene blue from solution. Neither did adrenaline appear to act as a mordant; for both dried sections of fresh skin, and filter

paper, were less readily stained by mixture of dye and adrenaline than by dye alone.

Adrenaline as a precipitant of tissue substances.—A possible mode of action is suggested by the observation that adrenaline precipitated extracts of finely sliced fresh skin. With a given extract, precipitation occurred only over a narrow range of adrenaline concentrations, and with dilution of the extract, the optimally precipitating concentration was shifted linearly to a corresponding lower concentration of adrenaline. Thus, in one test, 1 mg./ml. adrenaline optimally precipitated neat extract, 0·2 mg./ml. a 1/5 extract, and 0·04 mg./ml. a 1/25 extract. The precipitable material is evidently released when the skin is torn or sliced, for neither guinea-pig serum nor guinea-pig plasma was precipitated by adrenaline in concentrations ranging from 0·1 to 1000 μg./ml.

During injection into the skin of the adrenaline-bacterium mixtures, the tearing apart of the connective tissues by the injection fluid might liberate low concentrations of precipitable substance; and if adrenaline were absorbed preferentially by the tissues round the site of needle entry, the concentration of adrenaline in the advancing edge of the injection fluid might be low enough to precipitate this substance, with a consequent entrainment of bacteria in, or adsorption to, the precipitate.

The entrainment of bacteria in optimal adrenaline-skin extract precipitates was microscopically demonstrable *in vitro*. *In vivo* entrainment of this kind is obviously difficult to determine. But the following experiments demonstrate the possibility. A strip of filter paper 1 cm. wide is immersed for 0·5 cm. at its lower end in a reservoir of fluid under test, and the height climbed by the fluid is compared with that reached by substances in solution or in colloidal suspension. Changes in the absorption of the substance to the filter paper are inferred from the change in proportional height climbed. In this technique, which we modified from Bedson and Bland (1929), the filter paper represents the injected tissue, and the capillary climb the movement of injection fluid from the needle point outwards. Saline suspensions of washed living *Staph. aureus*, *Cl. welchii* and *B. subtilis* spores containing about 10^{10} viable cells per ml. were tested alone and mixed with adrenaline, and allowed to climb up strips of Whatman No. 1 filter paper. The paper was either untreated, or soaked in skin extract and dried rapidly *in vacuo* over P_2O_5. The extract was the liquid left after centrifuging a suspension in phosphate buffer of skin slices prepared as for the Thunberg tube experiments (p. 28). The height climbed by the bacteria was determined by cutting the strips into 0·5 cm. lengths, and pressing each firmly on to the surface of appropriate agar media. After 2 days' incubation *Staph. aureus* appeared as minute colonies in the filter paper, and the height climbed could be estimated to within 0·2 cm. The greater spreading power of *Cl. welchii* and *B. subtilis* colonies resulted in confluent growth over each section of strip, so that estimates were possible only to within 0·5 cm.

Up untreated paper, the climb of bacteria was not affected by adrenaline: but up treated paper, adrenaline markedly diminished it. Table VI summarizes a representative experiment. In repeated tests the percentage heights climbed varied with the different preparations of bacterial suspensions, and the particular batch of treated filter paper; and although the retardation of climb, indicating greater absorbability of the bacteria to the fibres of the filter paper coated with

extract. was usually greatest with highest concentrations of adrenaline (200 μg. ml.) tested, sometimes lower concentrations (40 μg. ml.) were more effective, again suggesting an optimally, not a maximally, effective concentration of adrenaline. These experiments demonstrate at least that adrenaline can alter the charge on tissue substances, making them readier to absorb, or be adsorbed to, bacteria.

The fixation of other dyes.—Though adrenaline-treated tissue substances may thus entrain bacteria in the experimental adrenaline lesions, it is unlikely that the holding of methylene blue in such lesions is due to such a process. As noted above, adrenaline did not mordant the methylene blue; if anything, it made it a less good tissue stain. Nor did it affect the climb of methylene blue up filter paper treated with skin extract: the percentage climb of the dye up the treated filter paper used in the experiment recorded in Table VI was

TABLE VI.—*Modification by Adrenaline of the Absorption of Bacteria to Filter Paper Treated with Extract of Guinea-pig Skin.*

Suspension.		Height (cm.) climbed by—		Percentage climb by bacterium.
Bacterium.	Concentration of adrenaline.	Liquid.	Bacteria.	
B. subtilis spores	*nil*	12·0	10	83
	20 μg. ml.	11·0	5·5	50
Staph. aureus	*nil*	10·0	4·5	45
	20 μg. ml.	10·0	2·0	20
Cl. welchii	*nil*	9·0	3·0	33
	20 μg. ml.	7·0	1·0	7

20 per cent both in the presence and absence of adrenaline. For comparison with the methylene blue lesions pontamine sky blue 6X and trypan blue, neither of them Eh indicators, were tested in guinea-pigs' skin. Curves, typical of tests on a total of 8 and 10 animals per dye, are included in Fig. 4 (*b* and *c*). The curves with 2 μg. adrenaline are not recorded; they were similar to the control, but at a lower level in the graphs. As with indigo disulphonate, 20 μg. adrenaline produces an initial drop in staining followed by an increase, which is presumably due, not to the reversal of a true decolorization, but, as suggested on p. 28, to an unmasking of colour as the injection fluid drains away from the injection site. Apart from this apparent decolorization, there is in each case a retardation of the disappearance of the dyes, most marked in indigo disulphonate, which disappears most quickly in control lesions, and least in trypan blue, which disappears most slowly in the control lesions.

This difference in behaviour is paralleled by the affinity of the dyes for skin tissue, which was determined with sufficient accuracy by mixing solutions of the dyes with suspensions of freshly sliced skin washed in saline, rapidly filtering in a Buchner funnel after a measured interval, and measuring the residual dye colorimetrically with due correction for the dye absorbed by the filter paper. Thus in 5 minutes a given suspension of slices removed 30 per cent of 0·02 per cent solution of methylene blue; and the corresponding percentages for equimolar solutions of the other dyes were indigo disulphonate, 33 per cent;

pontamine blue, 72 per cent; and trypan blue, 78 per cent. Moreover, when skin slices stained in this way for one minute by methylene blue and pontamine blue were immediately extracted with warm saline or guinea-pig serum at 37° C., only a trace of pontamine blue was recoverable, whereas a substantial amount of methylene blue could be extracted. But when the stained slices were held at 0° C. for 70 minutes, the methylene blue was fixed, for warm saline extracts contained only 20–40 per cent of the amount of dye extractable at one minute. In contrast, no visible dye could be extracted from pontamine blue-stained slices even after 5 minutes at 0° C.

In the light of these facts the persistence of methylene blue in the skin may be accounted for as follows: On injection, the dye is in the traumatic tissue spaces created by the injection fluid, and immediately begins to drain into the lymphatics. The tissues are stained, but at first this stain is partly removed—leached away—by the fluid constantly flowing from the blood capillaries to the lymphatics across the tissues. This natural flow of fluid is increased by the exudate that follows the endothelial damage by the saline solution of the methylene blue injected. The more irreversibly fixed portion of the dye is slowly removed by histiocytes and phagocytes, so that the lesion is colourless after 24 hours. On the other hand, when adrenaline cuts off the flow of fluid from capillaries to lymphatics, little leaching of dye takes place; a greater amount is firmly fixed to the cells, and persists longer in the lesion. With dyes of greater tissue affinity less leaching is in any event possible in the control lesions, and consequently less enhancement of staining under the influence of adrenaline.

The importance of these observations and the hypothesis derived from them lies in their application to bacteria. Quite aside from maintaining a supply of antibacterial substances and cells in the infected tissue, the inflammatory exudate may have a purely mechanical effect in carrying bacteria to the lymphatics before they become fixed to the tissues. If this were so, adrenaline, by inhibiting the flow, would ensure that more of the initially introduced bacteria were held in the local lesion.

The fixation of bacteria by adrenaline.

The hypothesis of local fixation of bacteria to the tissues by adrenaline was tested by comparing the numbers of bacteria, viable and total, in S and A lesions of various ages. Viable counts were made of extracts of whole lesions after cutting them into thin uniform sections. The lesions were made in the skin of guinea-pigs by washed suspensions of *Staph. aureus* 21 and *Cl. welchii* Type A. They were excised under chloroform anaesthesia, frozen in sterile M 15 phosphate buffer pH 7·0, containing 0·05 per cent liquoid (von Haebler and Miles, 1938), to inhibit any antibacterial substances from the blood. With sterile precautions, each lesion was cut into 20μ sections on a freezing microtome turned vertically, so that the sections fell directly into a sterile collecting tube. The sections from each lesion were suspended in a known volume of the liquoid buffer, subjected to a standard shaking, and a viable count was made of the resulting fluid extract. The A:S ratio indicates that at 2–3 hours the *Staph. aureus* counts are slightly and the *Cl. welchii* counts considerably higher in the A lesions (Table VII*a*). But the higher counts could be due, not to a fixation of the bacteria in the presence of adrenaline, but to a greater rate of killing in the S lesions by bactericidal

fluids and phagocytes. The contribution of such tissue bactericides to the result in the S lesion was estimated by measuring the bactericidal power of the animal's serum and blood, representing the source of many of the bactericidal elements in the inflammatory exudate. A sample of blood was taken from each of the guinea-pigs tested half an hour before the intradermal injections, and defibrinated. The samples were pooled, part of the pool was spun to obtain serum, and the

TABLE VIIA.—*The Number of Viable Bacteria Extracted from Infective Lesions in the Skin of Guinea-pigs formed with 2 μg. adrenaline (A), and without (S).*

Bacterium.	Guinea-pig number.	Age of lesion (min.).	Number of viable bacteria from lesion. A	Number of viable bacteria from lesion. S	Ratio. A:S
Staph. aureus	1	120	$16{\cdot}0 \times 10^3$	$4{\cdot}8 \times 10^3$	3·3:1
	2	150	$18{\cdot}8 \times 10^3$	$3{\cdot}1 \times 10^3$	6·3:1
	3	180	$13{\cdot}3 \times 10^3$	$4{\cdot}7 \times 10^3$	2·8:1
Cl. welchii	4	130	$10{\cdot}0 \times 10^5$	$2{\cdot}3 \times 10^3$	430·0:1
	5	160	$5{\cdot}0 \times 10^5$	$4{\cdot}0 \times 10^3$	125·0:1
	6	200	$10{\cdot}0 \times 10^5$	$1{\cdot}3 \times 10^3$	769·0:1
B. subtilis (spores)	7	150	81×10^3	56×10^3	1·4:1
			56×10^3	8×10^2	7·0:1
	8	210	19×10^3	7×10^3	27·0:1
			78×10^3	13×10^3	6·0:1
	9	240	66×10^3	34×10^3	1·9:1
			69×10^3	26×10^3	2·7:1
,, ,,	10	2	19×10^4	47×10^4	0·4:1
	11	2	29×10^4	14×10^4	2·2:1
	12	35	14×10^4	6×10^4	2·3:1
	13	40	20×10^4	14×10^4	1·4:1
,, ,,	14	2	$9{\cdot}0 \times 10^4$	$13{\cdot}6 \times 10^4$	0·6:1
	15	30	$12{\cdot}6 \times 10^4$	$9{\cdot}3 \times 10^4$	1·3:1
	16	30	$8{\cdot}0 \times 10^4$	$6{\cdot}5 \times 10^4$	1·2:1

bactericidal power of serum and blood, for the suspension of living organisms employed in the skin injections, tested by the method of Miles and Misra (1938).

Table VIIB summarizes the results. Guinea-pig serum did not kill *Staph. aureus* in 2 hours at 37° C., but defibrinated blood killed about 90 per cent of the smaller inoculum. *Cl. welchii* was highly susceptible both to serum and blood, the two smaller inocula being destroyed, and the largest diminished by 97 per cent. Neither organism is therefore a good indicator of fixation, since both are probably destroyed in the exudate, which at 2–3 hours is abundant in the S lesions. Indeed, the parallelism between susceptibility to the bactericidal power of guinea-pig blood, and the degree to which adrenaline enhances infections by these two organisms (Table III), suggests that the enhancement might be

TABLE VIIB.—*The Bactericidal Power of Pooled Fresh Serum and Defibrinated Blood of the Guinea-pigs against the Bacteria used for Inducing Skin Lesions.*

Organism.	Guinea-pig number.	Number bacteria/ml. seeded into test mixture.	Number bacteria/ml. surviving after 2 hr. at 37°C. in—	
			Serum.	Blood.
Staph. aureus	1–3	2,250	2,460	2,250
		225	360	**38**
Cl. welchii		21,500	**1,050**	**700**
	4–5	2,150	**100**	**0**
		215	**0**	**0**
B. subtilis	7–9	500	550	600
(spores)		50	50	45

Significantly diminished counts are indicated in heavy type.

predominantly due to transient protection of the bacteria from the inflammatory exudate.

We may note here that adrenaline does not appear to affect the bactericidal power of the exudate directly. The bactericidal power of pooled defibrinated guinea-pig blood against *Staph. aureus* and *Cl. welchii* was unaltered in the presence of adrenaline in final concentrations of 8 and 80 μg./ml.

The spores of *B. subtilis* were selected for their resistance to these bactericidal effects, at least for the period of the experiments. A washed suspension of spores was heated for 2 hours at 56° C. to kill the few vegetative forms detectable in stained smears. It proved (Table VIIB) to be unaffected by defibrinated blood. But, though from this viewpoint *B. subtilis* spores are a suitable indicator organism of fixation, the A:S ratios of *subtilis* lesion counts at 2–4 hours (Table VIIA, guinea-pigs 7–9) were too variable to provide satisfactory evidence. All indicated fixation by adrenaline, but about half the ratios were of the same order as those from 2-minute lesions (guinea-pigs 10, 11, 14), which ranged from 0·4:1 to 2·2:1. If this range represents the experimental error in determining a ratio that on *a priori* grounds should be 1:1, the values at 30–40 minutes (guinea-pigs 12, 13, 15, 16) are not significant, except that, like those for guinea-pigs 7–9, all four of them are consistently greater than 1:1, indicating higher counts in the A lesions. But even supposing the counts in the S lesions were significantly lower, they might have resulted, not from relative absence of fixation, but from a number of instances of non-lethal ingestion of two or more spores by one phagocyte (i.e. plural phagocytosis), each intraphagocytic aggregate giving rise only to one colony in the count plate.

In fact, phagocytic aggregation of bacteria was obvious in stained sections of 30 and 60 minute lesions by *Staph. aureus*, *Cl. welchii* and by *B. subtilis* spores. In all three cases the S lesions appeared to contain fewer bacteria than the corresponding A lesions. Nevertheless, in the *aureus* and *welchii* lesions the observed aggregations were insufficient to account for the relative paucity of bacteria in the S lesions; but the *subtilis* S and A lesions at one hour contained approximately the same number of spores, and differed only in the preponderance of intracellular spores in the S lesion. In lesions 2 and 3 hours old the bacteria

were also more numerous in the A series, and here it was clear, especially after 3 hours, that numbers exceeded those originally introduced, and that multiplication *in situ* had occurred.

The experiments recorded in Table VIIA were repeated on other occasions with substantially the same results. We conclude from these crude estimates of bacterial numbers that plural phagocytosis was not wholly responsible for the diminution of S counts in early *Staph. aureus* and *Cl. welchii* lesions: that the bacteria were being removed by other means; and that both phenomena were inhibited by adrenaline. The experiments with *B. subtilis* are inconclusive. We tried also to measure fixation of these spores by counting the number of viable spores that escape in a given time from A and S lesions in mice. The S and A injections were made in the right and left ears respectively, and spore counts made of extracts of frozen sections of the corresponding cervical lymph nodes. Here again, though there were indications that more spores drained into the lymph nodes from the S lesions, the experimental error was too great to warrant a definite conclusion. Nevertheless, the method appears to be worth refining, and we are attempting to do so.

DISCUSSION.

Renaud and Miget (1930) described in rabbit's skin a marked enhancement of *Cl. welchii* infections by 100 μg. adrenaline; a lesser enhancement of staphylococcal and streptococcal infections; and an increased survival of *B. subtilis* in the skin lesions. Brocard (1940) confirmed the result with *Cl. welchii* in guinea-pigs; his strain by itself was feebly virulent, and though small doses killed when injected with adrenaline, the bacteria recovered from the cadaver had not increased in virulence. Our extension of Cooper's quantitative study of the adrenaline effect reveals that among the species studied, striking degrees of enhancement were confined to *Cl. welchii* and *Cl. septicum*, though substantial enhancement occurred with a number of aerobic organisms. The enhancement is due to bacterial proliferation: the degree of enhancement of toxins is relatively small, though, as Mason (1936) first demonstrated with *Cl. chauvoei* toxin, it may be substantial with certain exotoxins.

It must be emphasized that we have tested a small number of species, mainly in the skin and muscle of the guinea-pig, an animal for which few of the organisms were pathogenic in the sense either that natural infections are common, or that artificial infections with small numbers produce a progressive disease. Moreover, many of the results may be characteristic of the strain used, and not of the species, though with *Staph. aureus*, the enhancement was of approximately the same order in the eight coagulase-positive strains tested.

The mode of action of adrenaline was studied mainly with *Cl. welchii* and *Staph. aureus*, representing markedly and moderately enhanced bacteria; and with *B. subtilis*, which was chosen as a feebly enhanced organism whose spores were insusceptible to tissue bactericides for the first few hours after injection. Upon these infections, adrenaline appears to act as might be expected, namely, through its being a powerful transient vasoconstrictor. The enhancement is determined in the 2–3 hours' vasoconstriction induced by the doses used, and there is no evidence of any after-effect. The adrenaline ischaemia results in a

deficient supply of oxygen, blood fluids and blood cells, so that the reducing intensity of the tissue increases, and both the diapedesis of leucocytes and the fluid exudation characteristic of the inflammatory response are inhibited. The absence of exudate almost certainly entails absence of antibacterial substances, and may also entail absence of a fluid flow which would otherwise dilute the bacterial toxins and assist in the transfer of the injected material to the lymphatic capillaries.

Except for the striking difference in numbers of bacteria visible in early lesions with and without adrenaline, our direct evidence for the last mechanism is not good, owing to experimental difficulties we hope to overcome. If it proves to exist, we must recognize that in the early stages of infection, *dispersion*, not localization, of the invading bacteria is an effective defence. This view gains some support from the observations of Duran-Reynals (1935), that in rabbit's skin the minimal infecting dose of many bacteria may be made innocuous if it is dispersed over a larger area of skin by injection with spreading factor: and by observations like those of Sobernheim and Murata (1924), who found that in guinea-pigs the subcutaneous minimal lethal dose of *B. anthracis* was smaller than the intravenous; and of Lange and Gutdeutsch (1928), who observed the same phenomenon in mice with pneumococci, streptococci and pasteurellae. In these cases it appears that the immediate dispersion of the infective material throughout the potentially bactericidal tissues has for the animal a greater survival value than its localization. The matter requires investigation with organisms of varying virulence and different natural modes of attack: but on *a priori* grounds there is a case for dispersion as an early defence mechanism and for the speculation that adrenaline may act by opposing it.

In addition to the consequences of vasoconstriction, a direct physico-chemical action of adrenaline, either on the bacteria or the tissues, cannot be excluded. Adrenaline is a precipitant of certain unidentified tissue substances: it directly enhances the reducing intensity in preparations of fresh guinea-pig skin, and in high concentration it agglutinates bacterial suspensions.

The artificial adrenaline effect is achieved only by physiologically enormous concentrations, and though it may bear on the natural functions of adrenaline in resistance to infection, there is nothing in our results to indicate any immediate connection. Systemic effects of the intensity that results in local enhancement would require very large doses of adrenaline, and enhancement is likely to be practicable only in relatively dense tissues where the necessary degree of local vasoconstriction can be maintained for several hours. The inefficacy of adrenaline in influenza virus infections of mouse lung, and pneumococcal infections of the mouse peritoneum, is in accord with this view.

We have referred throughout the paper to enhancement of *infection*, to avoid the current ambiguities (Proceedings, 1947) about the term *virulence*. As the word is sometimes loosely used, in the sense of an increased efficacy of the parasite in the host, virulence is enhanced by adrenaline; but in the more exact sense of an attribute of the parasite *per se* virulence does not appear to be affected. In this more precise sense, virulence is usually measured by a particular end-result in the host-parasite relationship; and, given a reasonably uniform group of test animals, it is possible to say that one bacterial strain is more virulent than another. However, the demonstration that the strain isolated

from an animal dead of an adrenaline-enhanced infection was more virulent than the original strain (Brocard, 1940, *supra*) would not necessarily mean that adrenaline had directly affected the bacteria. The increase in virulence might have been due to an adrenaline-induced increase in the susceptibility of the tissues, which permitted the more virulent variants of the strain to survive and initiate infection. Moreover, absence of any increase in virulence after such passage would not exclude a temporary, direct action of adrenaline on the injected bacteria. We have not therefore attempted to compare virulence in this way. Our analysis of the adrenaline effect, though incomplete, nevertheless strongly suggests that adrenaline directly affects tissue susceptibility, and not the virulence of the parasite. In particular, we would stress the demonstrable inhibition of exudation and diapedesis of phagocytes by adrenaline, and the parallelism between the degree of enhancement of *Cl. welchii* and *Staph. aureus* on the one hand, and their susceptibility to blood phagocytes and the bactericidal substances of the serum on the other (Tables III and VII*b*). For these infections at least, it seems to us unnecessary to postulate any substantial alteration of bacterial virulence by adrenaline. In this respect adrenaline resembles mucin, but differs from it in that it does not protect the bacterium against the tissue defences, but inhibits their mobilization.

We investigated the action of adrenaline in the first place as a means of enhancing experimental infections for testing chemotherapeutic substances. In animals and with bacteria for which it is effective, it has the advantage of doing little but temporarily inhibiting a natural tissue response; and in this it is a marked contrast, for example, to $CaCl_2$, which acts only in concentrations that grossly damage the tissues. But its value for this purpose is limited to a few species; and though tests of other pathogenic bacteria may reveal useful degrees of enhancement, the transience of the adrenaline effect means that enhancement will be confined to those infections for establishment of which the first few hours of the inflammatory response are critical.

SUMMARY.

1. The injection into the skin or thigh muscles of guinea-pigs of 2 μg. adrenaline renders these tissues more susceptible to some microbial infections. The resulting enhancement of infection by strains of various pathogens tested was as follows: *Cl. septicum* and *Cl. welchii*, 100,000-fold; *Bact. coli*, *Pr. vulgaris*, *Ps. pyocyanea* and *Str. pyogenes*, 100- to 10,000-fold; *C. diphtheriae* and *Staph. aureus*, 10- to 100-fold; *B. subtilis*, *Br. abortus*, *Cl. oedematiens* and *Str. pneumoniae*, about 10-fold; *Sh. flexneri*, *Sh. sonnei* and *V. cholerae*, 2- to 5-fold. Infections by strains of *Cl. histolyticum* and *Cl. tetani* were not enhanced. In the skin of the rabbit, *Staph. aureus* was enhanced 100-fold, vaccinia virus 10-fold and *H. pertussis* not at all.

2. Adrenaline chiefly enhances infection, not intoxication. Bacteria-free toxins were slightly enhanced. Two- to five-fold enhancement of crude toxins of *Cl. histolyticum*, *Cl. oedematiens*, *Cl. septicum* and *Cl. welchii* was observed.

3. The ischaemia induced by 2 μg. adrenaline leads to a diminution of tissue Eh and an inhibition of inflammatory diapedesis of leucocytes and exudation of blood fluids. These effects last for about 2 hours, and there is no detectable after-effect on the susceptibility of normal tissue to infection.

4. The temporary failure of mobilization of bactericides in adrenaline-treated tissue appears to be the chief factor in the enhancement of infections by adrenaline. There is some evidence of an early removal of bacteria from the injection site which is retarded by adrenaline, with a consequent increase in the severity of the local lesion. The removal of certain vital dyes is also retarded by adrenaline.

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THE PATHOLOGY OF PHENYLDICHLOROARSINE POISONING IN RABBITS.

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Received for publication December 20, 1947.

PHENYLDICHLOROARSINE is a vesicant resembling lewisite in its general action. Application of large enough doses to the skin may result in death. With a view to establishing the major effects contributing to the lethal action of the vesicant and the way in which systemic poisoning is brought about, a microscopic examination of the various organs was carried out.

METHODS.

The rabbits used were of both sexes. Before applying the vesicant, an area on the back was freed from fur by clipping as closely as possible with scissors.