1. With a view to ensuring uniformity of action *routine* meat inspection should be made a compulsory duty of all urban sanitary authorities.

2. With a view to safeguarding more adequately the supply in urban districts there should be compulsory inspection of those slaughter-houses in rural districts at which carcasses are dressed for sale in outside areas.

3. Inspectors engaged on meat-inspection work should be required to possess proof of knowledge of the subject.

4. Regulations should be framed dealing not only with the details of inspection of carcasses but with the sanitary requirements of slaughter-houses, facilities for inspection, and perhaps the requirements of ante-mortem examinations.

5. A system of meat marking under the direct control of the Local Government Board should be instituted, and as a commencement its adoption should be voluntary, but permission to adopt should be within the power of the Board to grant or to refuse.

6. In districts where a system of meat-marking is in operation the local authority should have power to require that all meat used in prepared food products should be inspected and approved before being used.

7. In view of the impossibility of satisfactorily examining in this country imported boned meat and meat in the canned form, some of the responsibility which now devolves upon home inspectors should be accepted by the Government, who should appoint inspectors to visit the chief places abroad where the work of boning, canning, and exporting meat generally is carried out. In this way attention could be given to the observance of hygienic requirements and to the quality and character of the meat used.

8. Regulations should be framed to control cold stores in which food products of any kind are stored.

9. Regulations should be framed to ensure that meat is handled in a cleanly manner.

THE PHYSIOLOGICAL AND ANTISEPTIC ACTION OF FLAVINE

(WITH SOME OBSERVATIONS ON THE TESTING OF ANTISEPTICS).

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FLAVINE, or acriflavine, has been much in evidence recently, and it has been claimed for it that it approaches the ideal antiseptic in that it has no deleterious effect on the tissues and that the antiseptic power is increased by admixture with serum. Browning and his co-workers[1] have published a table giving the antiseptic values and the influence on phagocytosis of a number of antiseptic solutions, and they arrange them in order of merit by means of a "therapeutic coefficient," which they define as the ratio of the antiseptic power in serum to the power of inhibiting phagocytosis. The latter is used as an index of the "general toxicity to living matter." They claim for flavine that it has a very high antiseptic value for the common organisms found in wounds, and that in the strength they apply it (1 in 1000) it is practically without harmful effect on the tissues as measured by its action on leucocytes.

This antiseptic has been introduced into practice purely as the result of laboratory experiments, and thus it would be well to make sure that these are without flaw before drawing deductions as to its clinical value.

I propose to set forth in this article the result of some experiments I have made with flavine, which show that the claims which have been made that this substance approaches the ideal antiseptic are based on fallacious experiments, and that by altering the experimental conditions slightly the "therapeutic coefficient" can be altered to an enormous extent. I will show also that the action of flavine on living tissues (as exemplified by leucocytes) is far in excess of its lethal action on bacteria.

The flavine used in these experiments was supplied by the Medical Research Committee and has been tested at the Bland-Sutton Institute to "ensure its being free from toxicity to human tissues and as efficacious in the control of bacterial infections as the sample with which the preliminary experiments and trials were conducted."

[1] Browning, Kennaway, Gulbransen, and Thornton: Brit. Med. Jour., Jan. 20th, 1917.

PART I.—*Action of Flavine on Human Blood.*

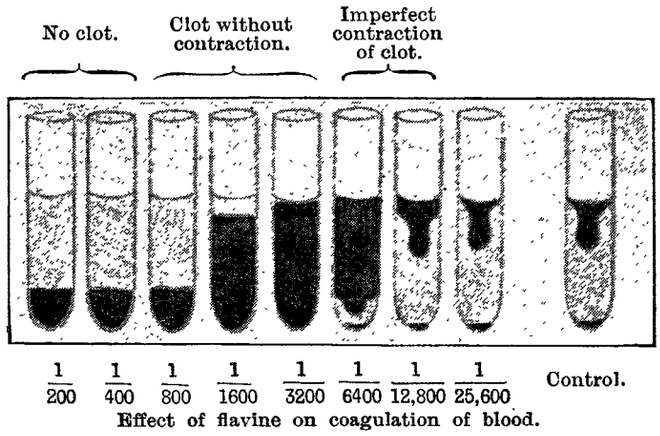
(*a*) *On the red corpuscles.*—There is very marked agglutination of human red corpuscles up to a dilution of flavine of 1 in 3200. A dilution of 1 in 6400 showed only slight agglutination and the higher dilutions showed none at all. This would only be of importance if flavine were used as an intravenous injection, but I hope to produce evidence later that flavine is useless as a therapeutic agent by the intravenous route, so this point need not be further discussed.

(*b*) *On the clotting power of the blood.*

Experiment 1.—My own blood mixed with an equal volume of normal salt solution clotted in 2½ minutes at 37° C. Under the same conditions my blood mixed with an equal volume of flavine (1 in 1000 in normal salt solution) failed to clot in 60 minutes.

Experiment 2.—One volume of each of a series of progressive dilutions of flavine were mixed with an equal volume of my blood in small test-tubes and incubated at 37° C. for three hours. The results are shown in Fig. 1, from which it can be seen that 1 in 400 flavine has

FIG. 1.



Effect of flavine on coagulation of blood.

completely inhibited clotting; 1 in 800 had formed a very light clot after the agglutinated corpuscles had sedimented; 1 in 1600 and 1 in 3200 showed loose clots, and here the sedimentation had not proceeded so far before clotting had occurred; 1 in 6400 was the first dilution in which any contraction of the clot was observed; and even in 1 in 12,800 there was shown some deficiency in the contraction of the clot.

It is very difficult to estimate the importance of this inhibitory power on the coagulation of the blood. Sir Almroth Wright has for many years combined with his hypertonic salt solution some citrate of soda to prevent clotting of the lymph in a wound and flavine may be beneficial in this way. This action of flavine, however, must be borne in mind when it is applied to freshly incised wounds which may be oozing and where the inhibitory action on clotting would allow this oozing to continue. Probably the flavine would soon be diluted below the point at which it has any action, but even then the result would be an excessive amount of blood clot which might furnish a nidus for bacterial growth.

(*c*) *Action of flavine on the leucocytes.*—Browning and his co-workers measured the toxic effect of flavine and other antiseptics on the leucocytes by introducing these substances as a fourth volume in an ordinary opsonic mixture and estimating the amount of phagocytosis which had taken place in 20 minutes. A more direct method is to observe the effect of the antiseptic directly on the movements of the leucocytes. Sir Almroth Wright[2] has given us a simple method of observing the emigration of leucocytes, and this method I have used except that instead of blowing out the clots and staining them I have examined them directly at intervals *in situ*.

Experiment 3.—Serial dilutions of flavine are made in normal salt solution, and to each of these dilutions is added an equal volume of blood from the finger. They are rapidly mixed, drawn into Wright's emigration tubes, sealed at one end, and centrifuged before the blood has clotted. After this the tubes are closed and arranged in order on a microscope slide, being kept in place by plasticine. They are then incubated at 37° C., with the portion of the tubes containing the red blood corpuscles downwards, so that any leucocytic emigration into the white clot has to be against gravity. After intervals of one, three, and six hours the tubes are examined with a 2/3-inch or a 1/6-inch objective and the amount of emigration noted.

Using this method I find constantly that a 1 in 2000 dilution of flavine completely inhibits the emigration of the leucocytes, while a 1 in 4000 dilution shows a partial

[2] Wright: THE LANCET, April 17th, 1915.

inhibition. In dilutions weaker than this the leucocytes have emigrated through a distance corresponding to three or four fields of a 1/6-inch objective as they have done in the control without flavine,[3] but in the tubes containing flavine up to a dilution of 1 in 64,000 (the highest dilution observed) the leucocytes appear as rounded bodies in marked contrast to those in the control tube which present a very irregular appearance and show active amœboid movements. In all the flavine dilutions also the leucocytes take up the stain and show a much deeper yellow colour than does the surrounding fluid.

This attraction of the leucocytes for the dye and the absence of amœboid movement naturally lead to the question, "Are these leucocytes in the flavine dilutions dead?" To test this I estimated the phagocytic power of leucocytes after exposure to various strengths of flavine.

Experiment 4.—Serial dilutions of flavine were made in normal salt solution from 1 in 500 to 1 in 32,000. To 0·5 c.c. of each of these dilutions was added 0·5 c.c. of defibrinated human blood. These were mixed and incubated at 37° C. A control tube was also made with equal volumes of defibrinated blood and normal salt solution. After five hours' incubation the tubes were shaken and with capillary pipettes two volumes of the contents of each tube were mixed with one volume of staphylococcus emulsion, incubated for 15 minutes at 37° C. and then proceeded with as in the estimation of the opsonic index. The cocci contained in 100 cells were enumerated (where 100 recognisable cells could be found). The results are shown on Table I.:—

TABLE I.—*Phagocytic Power of Leucocytes after Exposure to Flavine for Five Hours.*

Flavine concentration.	No. of cocci in 100 cells.	Condition of leucocytes.
1 in 1,000	0	Broken up and hardly recognisable.
1 ,, 2,000	0	,, ,, ,,
1 ,, 4,000	0	Mostly destroyed. A few stain well.
1 ,, 8,000	0	,, ,, ,, ,,
1 ,, 16,000	25	Much disintegrated.
1 ,, 32,000	59	,, ,,
1 ,, 64,000	128	,, ,,
Control.	920	Normal appearance.

Experiment 5.—The previous experiment was repeated except that the flavine dilutions were made in defibrinated blood from a 1 in 1000 saline solution of flavine. To 0·5 c.c. of each of these dilutions was added 0·5 c.c. of defibrinated blood. Samples were tested, as in the previous experiment, after 5 hours and again after 24 hours. The results are shown on Table II.:—

TABLE II.—*Phagocytic Power of Leucocytes after Exposure to Flavine Dissolved in Blood.*

1. *After 5 hours.*

Flavine concentration.	Cocci in 100 cells.	Condition of leucocytes.
1 in 2,000	0	Hardly recognisable as cells.
1 ,, 4,000	0	,, ,, ,,
1 ,, 8,000	0	One or two with nuclei still visible.
1 ,, 16,000	0	,, ,, ,,
1 ,, 32,000	5*	Either broken up or pycnotic.
1 ,, 64,000	7	,, ,, ,,
1 ,, 128,000	49	,, ,, ,,
1 ,, 256,000	53	,, ,, ,,
1 ,, 500,000	200	Many broken up but some stain normally
1 ,, 1,000,000	192	,, ,, ,,
1 ,, 2,000,000	320	In good condition.
Control.	372	,, ,,

* In 1 cell.

2. *After 24 hours.*—The cells in all the dilutions up to 1 in 2,000,000 were much broken up, and none of them showed any phagocytosis, although in the control tube the leucocytes stained well, and nearly all of them contained ingested cocci.

An experiment similar to Experiment 5 was done substituting carbolic acid for flavine and it was found that a 1 in 2000 dilution only partially destroyed the functions of the leucocytes in 24 hours.

We see from these experiments that flavine, if allowed to act for some hours, has a very powerful leucocidal action and that even a 1 in 2,000,000 dilution will completely destroy all the leucocytes in 24 hours. The leucocidal action, also, is more marked in Experiment 5, in which flavine was acting in a greater concentration of serum, than in Experiment 4, in which equal parts of a saline solution and blood were employed. This indicates that possibly the leucocidal action of flavine is enhanced by combination with serum in the same way as is bactericidal action. The lethal effect of flavine on leucocytes is, as we shall see later, much greater than its bactericidal action.

The importance of the time factor in regard to the estimation of the toxicity of flavine on the leucocytes will be further discussed in connexion with the "therapeutic coefficient."

[3] In this method the leucocytes do not after three hours appear to wander farther away from the red clot when they have no special stimulus attracting them.

PART II.—*Antiseptic Action of Flavine.*

Browning and his co-workers have given the following figures for the antibacterial power of flavine:—

Medium.	Organism.	Lethal concentration of flavine.
Peptone water	Staphylococcus.	1 in 20,000
Serum	,,	1 ,, 200,000
Peptone water	*B. coli.*	1 ,, 1,300
Serum	,,	1 ,, 100,000
Blood	Staphylococcus.	1 ,, 40,000
Pus	Staphylococcus and streptococcus.	1 ,, 2,000

The following experiments will bring out clearly, among other things, that the high antiseptic values for flavine in serum can only be obtained in special conditions, and do not give us a clear indication as to the value of this antiseptic as an application to wounds.

Does the Number of Microbes Introduced into the Antiseptic Solutions make an Appreciable Difference to the "Lethal Concentration" of the Antiseptic?

Browning and his co-workers in their experiments on flavine added 0·1 c.c. of a 1 in 20,000 dilution of a peptone water culture of the organism to 1 c.c. of the antiseptic solution. A loopful of such a dilution of the culture "yielded 20 or more colonies." We see, then, that in all cases they gave the antiseptic only a very few microbes to kill. I made some experiments with a view to ascertaining what difference was made to the apparent lethal concentration of an antiseptic by the addition of varying numbers of microbes.

Experiment 6.—Progressive dilutions of carbolic acid, crystal-violet, and flavine were made in broth, and to 0·5 c.c. of each of these dilutions was added 0·5 c.c. of a staphylococcus emulsion in broth. A control tube was put up in which the antiseptic was replaced by broth, and 10 c.mm. of this control tube yielded 32 colonies when plated on agar. Thus the staphylococcal emulsion used in this case was probably somewhat weaker than that used by Browning. Another set of tubes were put up identical with the last, except that the staphylococcal emulsion used was 5,000,000 times as strong. The tubes were incubated at 37° C. At intervals 10 c.mm. samples were taken out and transferred to broth tubes. The results are shown in Table III.:—

TABLE III.—*Effect of Variation of the Strength of the Bacterial Emulsion on the "Lethal Concentration" of an Antiseptic (in Broth).*

CARBOLIC ACID.							CRYSTAL VIOLET.							FLAVINE.						
Concentration of antiseptic.	Strong emul. of staph.*			Weak emul. of staph.			Concentration of antiseptic.	Strong emul. of staph.			Weak emul. of staph.			Concentration of antiseptic.	Strong emul. of staph.			Weak emul. of staph.		
	Time of action of antiseptic (in hours).							Time of action of antiseptic (in hours).							Time of action of antiseptic (in hours).					
	2	5	23	2	5	23		2	5	23	2	5	23		2	5	23	2	5	23
1 in—							1 in—							1 in—						
100	+	O	O	O	O	O	10,000	+	+	O	O	O	O	1,000	+	+	O	O	O	O
200	+	+	+	+	O	O	20,000	+	+	+	O	O	O	2,000	+	+	O	O	O	O
400	+	+	+	+	+	+	40,000	+	+	+	+	+	O	4,000	+	+	O	O	O	O
800	+	+	+	+	+	+	80,000	+	+	+	+	+	O	8,000	+	+	+	O	O	O
							160,000	+	+	+	+	+	O	16,000	+	+	+	+	+	O
							320,000	+	+	+	+	+	O	32,000	+	+	+	+	+	O
							640,000	+	+	+	+	+	O	64,000	+	+	+	+	+	O
							1,280,000	+	+	+	+	+	+	128,000	+	+	+	+	+	O
							2,560,000	+	+	+	+	+	+	256,000	+	+	+	+	+	O

* Staph. = Staphylococci; emul. = emulsion.
+ = Growth on subculture. O = Complete sterilisation.

Experiment 7.—Two series of experiments were made, the only difference being that in the one case the staphylococcal emulsion was 100,000 times stronger than in the other. Progressive dilutions of flavine were made in serum, and one volume of each of these dilutions was thoroughly mixed with an equal volume of staphylococcus emulsion in serum, incubated in capillary tubes at 37° C. for 24 hours, after which the contents were blown out into broth tubes. The results were as follows.

Staphylococcus emulsion.	Strength in which flavine acted on the bacteria.						
	1/2000	1/4000	1/8000	1/16000	1/32000	1/64000	Control
Strong ...	Growth	Growth	Growth	Growth	Growth	Growth	Growth
Weak ...	0	0	0	0	Growth	Growth	Growth

These two experiments show clearly that the number of microbes on which an antiseptic has to act either in broth or serum plays an important part in the determination of the lethal concentration of an antiseptic. We see also from Experiment 6 that in respect of changes in the number of microbes flavine is much more sensitive than is carbolic acid. It follows from this that, as the antiseptic has to act in wounds both heavily and lightly infected, this factor must be taken into account in any comparison of antiseptics.

What is the Concentration of Flavine which will Inhibit the Growth of Microbes in Serum?

Experiment 8.—Progressive dilutions of flavine were made in serum. To one volume of each of these dilutions was added one volume of serum which had been implanted with the microbe used in the test.

As some microbes grow badly in serum and do not macroscopically show evidence of growth, I added to the serum in some cases 1/20th of its volume of trypsin (which was just in excess of the quantity necessary for the neutralisation of the antitryptic power of the serum) and sometimes 1/50th of its volume of 50 per cent. glucose solution. Trypsin made the serum a good cultivation medium, and the glucose was acted on by the microbes with the production of acid in the case of cocci and acid and gas in the case of *B. coli* and *B. proteus*.

These experiments were conducted in miniature test-tubes, and after the mixtures had been made in the tubes melted vaseline was poured on the surface of the cultivation fluid to make a layer about 1 cm. deep.

TABLE IV.—*Inhibitory Action of Flavine on the Growth of Microbes in Serum.*

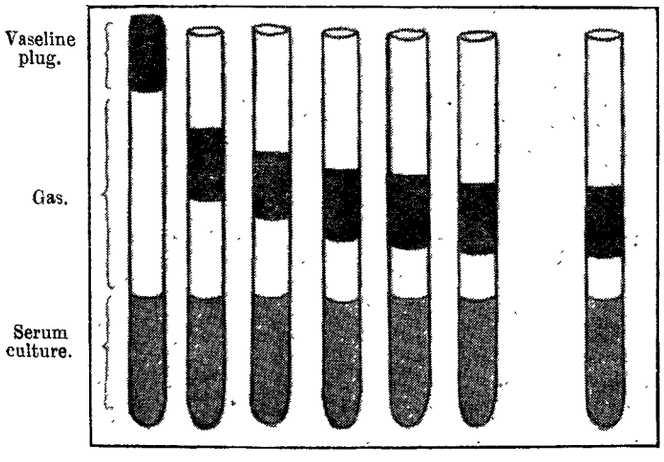
No.	Organism.	Medium.	Time of action of antiseptic.	Lowest concentration of flavine in which no growth occurred.	Greatest concentration of flavine which showed growth.
1	Staphylococcus.	Serum+Trypsin+Glucose.	3 days	1/80,000	1/160,000
2	,,	,, ,, ,,	7 ,,	1/16,000	1/32,000
3	*B. coli.*	,, ,, ,,	18 hrs	—	1/1000
4	*B. proteus.*	,, ,, ,,	36 ,,	1/1000	1/2000
5	,,	Serum + glucose	7 days	1/4000	1/8000
6	*B. coli.*	,, ,,	7 ,,	1/2000	1/4000

This vaseline protected the fluid from outside contamination, and at the same time it was forced up the tube by any gas that was formed in the cultivation fluid it served as an easy method of measuring the amount of gas and giving some index of the amount of growth.

In these experiments the number of microbes implanted was not estimated, but in all cases it was considerable (usually a loopful of bacteria from an agar culture was emulsified in about 3 c.c. of serum).

The results are set forth in Table IV.

FIG. 2.



Growth of *B. proteus* in serum + flavine, showing the great increase of growth (as evidenced by increased gas-production) in the stronger dilutions of flavine. (⅓ actual size.)

Experiment 4 of Table IV. requires special mention. At the end of 36 hours the tubes containing the three strongest concentrations of flavine—namely, 1 in 1000, 2000, and 4000—were opened and examined. Pure growths of *B. proteus* were obtained from all of them. The remaining tubes were left standing on the bench, and after one month it was noticed that they showed a gradual increase in the amount of growth as evidenced by the gas formation, from the control tube, in which there was no flavine, to the tube containing the greatest concentration of flavine (1 in 8000). The diagram (Fig. 2), drawn to scale, represents the height of the gas column in the various tubes.

The increase in the gas formation in the stronger flavine concentrations indicates that in some way flavine had favoured the growth of the microbes, and the regularity of the increase further proves that it is not accidental but is actually due to the flavine, as the contents of the tubes are identical except as regards this substance. Experiment 5 of Table IV. confirmed this result.

Antiseptic Value of Flavine in Serum.

It has been shown in Table IV. when dealing with the inhibitory action of flavine on the growth of microbes in serum that staphylococci can grow in 1 in 32,000 flavine, *B. coli* in 1 in 1000, and *B. proteus* in 1 in 2000. Experiment 7 also shows that sterilisation of a strong staphylococcus emulsion is not effected by a 1 in 20,000 dilution of flavine, although when using a weak emulsion 1 in 160,000 is sufficient to kill all the microbes. (This last figure is practically the same as the figure given by Browning as the lethal concentration of flavine in serum on staphylococci.) In another experiment in which I used a large number of staphylococci I found that growth resulted after a 24-hour exposure to a 1 in 2000 dilution of flavine in serum. Usually, however, when a fairly strong staphylococcal emulsion is used complete sterilisation is effected in 24 hours by a 1 in 4000 to a 1 in 16,000 dilution.

Flavine 1 in 8000 in serum sterilised a *B. coli* emulsion containing 1500 million living organisms per c.c., while 1 in 64,000 sterilised an emulsion of 4000 *B. coli* per c.c.

Antiseptic Value in a Non-serous Medium.

Table III. gives details of the antiseptic value of flavine in nutrient broth, from which it appears that in 23 hours a 1 in 8000 dilution failed to sterilise a strong emulsion of staphylococcus, while a 1 in 256,000 was capable of sterilising a very weak emulsion.

It was found that when flavine was added to a 48-hour-old broth culture of staphylococcus in sufficient quantity to make a 1 in 1000 solution it was unable to kill all the cocci in 24 hours at 37° C. Carbolic acid 1 in 100 rendered a similar culture sterile in less than six hours.

Antiseptic Power of Flavine in Blood in Vitro.

Experiment 9.—Progressive dilutions of a 1 per cent. flavine solution were made in defibrinated human blood and one volume of each of these was mixed with one volume of the same blood containing staphylococci. Two sets of experiments were made, the only difference being that the staphylococcal emulsion was 100,000 times as strong in the one as in the other. The mixtures were incubated at 37° C. for 24 hours and then planted into broth. The results were as follows:—

Staphylococcus emulsion.	Concentration of flavine.					—
	1/2000	1/4000	1/8000	1/16000	1/32000	Control.
Strong	0	Growth	Growth	Growth	Growth	Growth
Weak	0	0	0	,,	,,	,,

This experiment shows that the antiseptic action of flavine is much weaker in blood than it is in serum.

Action of Flavine on Pus.

When strong solutions of flavine are mixed with equal parts of pus they cause a very marked agglutination of the pus cells, and after incubation it can be seen that the pus in such a mixture has become mucoid in consistency. This mucoid alteration of the pus, which is probably due to the destructive nature of flavine on the cells, is noticeable up to a 1 in 6000 dilution. In wounds treated with flavine this mucoid appearance of the pus is sometimes manifest, and it may be that this will interfere with the distribution of the antiseptic. As in wounds, however, there appear to be so many factors which interfere with the action of flavine that it is unnecessary to lay much stress on this.

Antiseptic action in pus.—In these experiments flavine in saline solution was thoroughly mixed with pus from wounds either in equal parts or in the proportion of 1 part of flavine to 9 parts of pus. The specimens of pus were obtained from wounds in different stages of healing, and they differed materially in the nature and the number of microbes contained in them. No account was aken of he spore-bearing anaerobes, as we were dealing here only with the action of

flavine on the vegetative forms of bacteria. In all cases the antiseptic was allowed to act on the pus for 24 hours at 37° C., after which a sample of the mixture was planted on agar or into broth. Seven specimens of pus were subjected to the action of flavine in a concentration of 1 in 1000 and in only one of these was sterilisation effected. In the other six specimens the resultant cultures did not differ as regards the variety of microbes from the control cultures. Staphylococci, Streptococci, coliform bacilli, *B. pyocyaneus* and *B. proteus* all survived the action of flavine. We see, then, that flavine is a relatively weak antiseptic in pus, and that in only one case was a concentration of 1 in 500 (twice the strength recommended for use in wounds) capable of sterilising an equal volume of pus.

It is obvious that there is something in pus which prevents the action of flavine on the microbes. The concentration required to kill microbes in pus is far greater than that required in serum, although the liquor puris is of a serous nature. The simplest explanation of this is that the pus cells have a greater attraction for flavine than have the microbes. In this connexion some experiments were made on the "quenching" power of pus on flavine.

Experiment 10.—Equal quantities of flavine (1 in 500) and pus were mixed and immediately centrifuged. The antiseptic power of the supernatant fluid was then tested on staphylococcus emulsified in serum and compared with the lethal power of fresh dilutions in serum of flavine (from a 1 in 1000 solution in normal saline) on the same emulsion of staphylococci. The "quenched" flavine undiluted was just capable of sterilising this serum in 24 hours, but a twofold dilution was insufficient, whereas with fresh flavine complete sterilisation was effected by a 1 in 8000 dilution.

We see then that by contact with an equal volume of pus a 1 in 500 dilution of flavine loses 7/8ths of its antiseptic power almost immediately.

Experiment 11.—Flavine dilutions 1 in 100, 200, 400, and 800 were mixed with equal volumes of pus and centrifuged immediately. The intensity of the colour in the supernatant fluids were tested by putting equal sized drops of these on a white filter paper and matching these with a series of similar drops of progressive dilutions of fresh flavine. By this method it was found that the two strongest flavine dilutions exhausted with pus had lost half, the 1 in 400 dilution had lost 2/3rds, and the 1 in 800 had lost about 5/6ths of the colour intensity.

These two experiments show clearly that flavine by intimate contact with pus loses a large part of its power practically immediately.

The results of the absorption of flavine by pus cells naturally leads to the question of the affinity of other cells of the animal body for this substance, and the following experiments were designed to give some indication of what happens when flavine is injected into the body.

Browning and his co-workers state that flavine (1 in 1000) can be administered intravenously without toxic effect, and because of this non-toxic action flavine has been used intravenously as a therapeutic measure in conditions like streptococcal septicæmia in the hope that the dye will have a directly lethal effect on the cocci in the blood.

Experiment 12.—A rabbit, weighing 3 lb., was injected intravenously with 10 c c. of flavine (1 in 1000). Blood was taken before, immediately after, and again 15 minutes after the injection. The sera which separated from these blood specimens did not show the slightest trace of the canary-yellow colour of flavine or any fluorescence, and none of them had the slightest bactericidal effect on staphylococcus or inhibitory power on the growth of this organism. The urine, which was bright yellow, had no bactericidal power on staphylococcus.

We see therefore that flavine is immediately removed from the blood, which does not acquire any bactericidal action, and that therefore there is no justification for the therapeutic use of flavine intravenously as an antiseptic.

Browning and his co-workers have recommended the injection of flavine into the tissues as a means of preventing the spread of infection. As it is impossible to recover the flavine injected this way, I tried to get some idea of the fate of this substance when injected into closed spaces by introducing it into the pleural and peritoneal cavities of animals.

Experiment 13.—Two c.c. of flavine (1 in 100) were injected intraperitoneally into a three quarters grown rat. Two hours afterwards the rat was killed, and it was found that all the tissues of the body with the exception of the brain were coloured bright yellow. Serum from the heart blood showed not a trace of the yellow colour of flavine. The urine was bright yellow. The peritoneal fluid, urine, and blood were found to be without antiseptic power on staphylococcus.

Experiment 14.—Two c.c. of flavine (1 in 100) were injected into the pleural sac of a full-grown rat. Death resulted within two hours. All the tissues were bright yellow except the brain. The blood serum was distinctly yellow. The pleural fluid and the blood serum failed to kill in 24 hours a few staphylococci.

It follows from these experiments that when flavine 1 per cent. is introduced into a closed cavity in the animal body it loses its antiseptic power within two hours. Much more rapidly, then, will the (1 in 1000) solution recommended for use lose its potency.

It might be argued that when flavine is introduced into the body it is immediately taken up by the muscles, connective tissues, &c., and that these are the tissues in which flavine would be useful in combating a severe local infection. On theoretical grounds it is unlikely that when these tissues have such an affinity for the dye they will give it up to bacteria, but I tested this in the following experiment.

Experiment 15.—22 c.c. flavine (1 in 1000) were injected intravenously into a small rabbit. Death resulted immediately. Within half a minute blood was drawn from the heart and serum from this was distinctly yellow. Five minutes later another sample of blood was taken from the subclavian artery and showed no trace of yellow colour, thus proving that all the dye injected had been taken up by the tissues. All the organs and muscles were a bright yellow colour. A leg was amputated and into one of the muscles a small quantity of a broth culture of *B. perfringens* was implanted. In four hours at 37° C. there was obvious growth of the organism with gas formation. Portions of all the organs were planted with small amounts of *B. perfringens* and growth resulted in every case.

This experiment shows that flavine introduced intravenously, even in a dose far greater than would be given to man, is incapable of conferring on the tissues any inhibitory power against the growth of a microbe such as *B. perfringens*.

The great affinity which muscle tissue exerts towards flavine can be shown also by grinding up 1 gm. of muscle with 1 c.c. of flavine 1 in 1000 and testing the colour intensity of the resulting free fluid. This colour will be found to be reduced to about one-eighth of its original strength.

The Action of Flavine in Wounds.

Browning has scouted the suggestion that the potency of an antiseptic in pus gives much indication as to the value of such an antiseptic in a wound.[4] Of course, in dressing a wound all the pus in the cavity should be washed away before a fresh dressing is applied, but the exudation from the walls of the wound will consist of a serous fluid with a larger or smaller number of cells and a variable number of microbes, some inside the cells and some free. This fluid, then, is not far removed from pus, and it is in this fluid that the antiseptic applied is first going to act. Also the antiseptic and the fluid are not going to be intimately mixed as in test-tube experiments, but the antiseptic has to penetrate into the fluid by diffusion. Thus the test tube experiment appears to be the less severe test for the antiseptic.

The action in this purulent exudate is only going to throw light on what is happening in the cavity of the wound, and any antiseptic, before it can begin to approach the ideal, must act beyond the cavity in the infected walls of the wound. Here it would have to act in a tissue composed largely of cellular elements and bathed in a serous fluid. No claims were made as to the penetrative powers of flavine, but in the light of the experiments quoted above, which show that the muscular tissues and the leucocytes have a very great power of reducing the potency of flavine, it is hardly conceivable that any penetrative power can exist. Any antiseptic action of flavine, therefore, would be confined to the cavity of a wound, and, as it must act in the cavity in a fluid akin to pus, we have seen from the above experiments on its antiseptic action in pus that its potency when applied as is recommended in a 1 in 1000 dilution can only be very weak, much weaker, indeed, in regard to the concentration used than would be carbolic acid.

We have also seen that flavine, although it appears to have little action on the leucocytes in a few minutes, has a most destructive effect when its action is prolonged over some hours, an effect greatly in excess of its lethal action on bacteria. Let us consider what this may mean in a wound cavity. The leucocytes when they first appear would not be hindered from ingesting the microbes. Soon, however, they would themselves fall victims to the toxic effect of the flavine, and it is likely that this would occur before they had time to destroy the microbes ingested. The flavine itself would be unlikely to have killed the microbes before they were ingested, as its lethal effect on bacteria is a very slow one. It has been shown that the ingestion of microbes by leucocytes serves as a protection of such microbes against antiseptic solutions. Thus the slow toxic action of the flavine on the leucocytes, instead of being a virtue, may actually allow the bacteria to be protected against any antiseptic powers which flavine may possess.

"Therapeutic Coefficient."

Lastly, we might shortly consider the value of the so-called "therapeutic coefficient" as an index of the value of an antiseptic. This has been defined by Browning and his

[4] Browning: Brit. Med. Jour., Feb. 3rd, 1917.

co-workers as the concentration which reduces phagocytosis 50 per cent. divided by the concentration just sufficient to kill bacteria in serum. A consideration of the text of their article, however, shows that it really amounts to a comparison of the toxicity to leucocytes with the toxicity to bacteria (acting in serum). The experiments which I have detailed above show clearly that in regard to flavine this coefficient, as arrived at by the authors of the expression, gives information so misleading that as a practical test it is quite valueless. In calculating the toxic power of substances towards leucocytes they apparently paid no attention to the time factor, but in all cases used 20 minutes only as the time during which the action on leucocytes was observed. It would seem obvious that just as antiseptics act quickly or slowly on bacteria, so they will act quickly or slowly on leucocytes. Thus in the 20 minutes allowed only the antiseptics which acted rapidly on the leucocytes (and probably also on the bacteria) showed anything like their true effect. If we take flavine as an instance of how neglect of the time factor permitted quite wrong conclusions to be drawn, we see that the numerator of the "coefficient" in 20 minutes is 1 in 500, whereas it is over 1 in 2,000,000 if 24 hours is the time allowed. Thus by a small alteration in the experimental method we get an alteration of the coefficient by at least 4000 times to the detriment of flavine.

Again, the "lethal concentration" on bacteria in serum is a very variable quantity, and it has been shown above that by altering the strength of the bacterial emulsion the lethal concentration to staphylococcus can be varied from 1 in 2000 to 1 in 160,000, or a variation of 80 times.

We see, therefore, that in respect of flavine the numerator of the "therapeutic coefficient" is subject to a variation of at least 4000 times and the denominator to a variation of 80 times. This is quite sufficient to show that the coefficient is worthless as a standard of antiseptic value. Browning and his co-workers in their calculations of the "therapeutic coefficient" of different antiseptics happened to choose all the conditions which favoured flavine—namely, a long period of action on the microbes, a serous medium in which to test the bactericidal action, a few microbes to kill, and a short period of action on the leucocytes. Thus they obtained a coefficient for flavine 800 times better than that given by carbolic acid. If, however, they had used many microbes and had allowed the antiseptics to act on the microbes and leucocytes alike for 24 hours they would have found that carbolic acid gives a coefficient at least ten times better than flavine where serum is the medium employed, or 250 times better if the lethal concentration were estimated in pus.

The experiments detailed in this paper show that the theoretical basis for the use of flavine is thoroughly unsound, and that there seems nothing to specially recommend this substance as an antiseptic for use in septic wounds.

Summary.

1. Flavine strongly agglutinates human red corpuscles up to a dilution of 1 in 3200.
2. Flavine has a strong anticoagulant effect on human blood.
3. Flavine in a concentration of 1 in 2000 completely inhibits leucocytic emigration.
4. Flavine has a very destructive action on leucocytes, and if the action on leucocytes and bacteria be each tested for 24 hours its leucocidal action is far in excess of its bactericidal action.
5. The number of microbes on which an antiseptic has to act has a very marked effect on the "lethal concentration" of the antiseptic.
6. This alteration of the "lethal concentration" with the number of microbes varies in degree with different antiseptics, and appears to be especially marked in the case of certain aniline dyes. This factor must in all cases be taken into account in any comparison of antiseptic values.
7. When many microbes are used it requires a much greater concentration of flavine to effect sterilisation than that given as the "lethal concentration" by Browning and his co-workers.
8. In serum under certain conditions staphylococci will grow in 1 in 32,000 flavine, *B. coli* in 1 in 1000, and *B. proteus* in 1 in 2000.
9. Flavine 1 in 8000 appears to aid the growth of *B. proteus*.
10. Flavine 1 in 500 is usually unable to sterilise in 24 hours an equal volume of pus from a wound.
11. Flavine injected intravenously in large doses immediately disappears from the blood, which acquires no bactericidal power, and is taken up by the tissues, which become yellow but acquire no inhibitory power on the growth of bacteria.
12. Flavine 1 in 100 injected into the pleural or peritoneal cavities loses its antiseptic power within two hours.
13. Flavine is rapidly absorbed by pus or muscle tissue.
14. The so-called "therapeutic coefficient" of flavine can be changed with slight variations of the experimental method by at least 300,000 times.
15. If the antiseptic is allowed to act on staphylococci and leucocytes alike for 24 hours and the ratio is taken of its toxicity to both of these, carbolic acid has a coefficient 10 times better than flavine when the antiseptic acts on the microbes in serum and 250 times better when the bactericidal action is estimated in pus.

NERVOUS UNREST IN THE INFANT AS A CAUSE OF THE FAILURE OF BREAST-NURSING.

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IN THE LANCET of Sept. 27th, 1913, I published a paper upon the causes of the failure of women to nurse their infants at the breast. I then urged that in the first establishment of the milk-flow the part played by the mother was essentially a passive one, and that her contribution towards a successful result was limited to providing a nipple sufficiently large for the child to grasp firmly. If the nipple was well developed the causes of failure were commonly to be found not in the mother but in the child. I maintained that we had to distinguish between circumstances which prevented or delayed or interfered with the establishment of milk during the early weeks of lactation and circumstances which shortened the period of efficient lactation in the later months, and that the former—save in the all-important question of the nipple—were concerned chiefly with defects in the child, while the latter were commonly dependent upon the condition of the mother.

Among the causes which interfere with the suction of the infant, and so with the proper establishment of the milk-supply, I enumerated the following: (*a*) Dyspnœa from nasal catarrh, adenoid vegetation, the snuffles of congenital syphilis, bronchitis, or congenital heart disease. (*b*) Infective or toxæmic states of all sorts, with or without pyrexia. (*c*) Conditions such as cleft palate, facial paralysis, Bednar's aphthæ, or stomatitis which interfered mechanically with suction. (*d*) Prematurity and congenital debility. Further, I referred to the adverse influence of mixed feeding instituted soon after birth in the hope of preventing the supposed ill-effects of the period of physiological inanition which precedes the "coming-in" of the milk, and I laid stress upon the danger of chilling the infant, a disaster which is apt to occur in weakly babies unless carefully graduated artificial heating takes the place of the warmth of the mother's body.

The dyspnœic child lets go the nipple from exhaustion and because he cannot breathe and suck at the same time. The premature child and the child deprived of sufficient heat are sometimes too somnolent to sustain a sufficient effort. The infected child may have no appetite or desire for food. The child who has sucked once or twice from an easily running bottle may obstinately refuse the exertion of pulling at a still empty breast. The child with a cleft palate or facial paralysis cannot maintain the necessary vacuum.

Nervous Unrest in the Infant.

I wish now to call attention to another defect in the child which, in my experience, is not uncommonly a cause of failure to nurse successfully—the inhibition of the sucking reflex by the infant's nervous unrest and excessive emotional display. In the young infant the act of suction is not voluntary but reflex. It proceeds perfectly, as has often been observed, in an acephalic monster which is provided only with medullary