THE ACTION ON NITRATES AND NITRITES OF DYSENTERY ORGANISMS KILLED BY VARIOUS PROCESSES AND OF FILTRATES FROM FLUID CULTURES.

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It has been shown previously (Logie 1909 and 1910) that the power of reducing nitrates to nitrites is possessed by the majority of organisms belonging to the dysentery group. Thus of 15 different dysentery strains, examined by the author, only one failed to reduce nitrate. This strain did not ferment glucose, mannite, maltose, or saccharose. In the case of these dysentery bacilli which ferment mannite (Flexner, Strong and Y types) the reducing action proceeds further and the nitrite finally disappears. On the other hand, *B. dysenteriae* Shiga which rapidly reduces nitrate to nitrite does not carry the reduction further, even when the cultures are kept for many weeks in the incubator. A number of factors have been found to modify the reducing action of bacteria. Free access of oxygen diminishes the reducing action (Burri and Stutzer 1895, Weissenberg 1897, Logie 1910). Thus if equal amounts of inoculated fluid medium be distributed (*a*) in conical flasks so as to form a thin layer and expose a large surface to the air, and (*b*) in ordinary test tubes, reduction proceeds much more rapidly in the latter. Indeed by filling the flask with oxygen reduction may be quite prevented. On the other hand, anaerobic conditions (*e.g.* filling the flasks or test tubes with hydrogen) do not cause reduction of nitrite by *B. dysenteriae* Shiga, or the other organisms which do not usually reduce nitrite. The presence of glucose does cause *B. dysenteriae* Shiga to

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destroy nitrite, but this is due to the formation of acid from glucose which leads to the liberation of free nitrous acid, which in turn liberates the amide nitrogen of the peptone according to the equation

$$R.NH_2 + HNO_2 = R.OH + H_2O + N_2.$$

The following experiments were undertaken with a view to the further elucidation of the nature of the reducing power of dysentery bacilli. It was necessary to determine whether (1) the bacilli formed some soluble substance which possessed the reducing action (a reductase) or (2) the reducing power was inseparable from the organism and (3) if in the latter case dead bacilli exerted this action.

The action of filtrates.

For this purpose *B. dysenteriae* Flexner and *B. dysenteriae* Celli were selected, as these strains were found to be very active reducers of nitrite. The action of *B. coli* was also tested.

Small Jena glass flasks each containing 100 c.c. of peptone water (1 %) were inoculated with these organisms, and incubated for 24 to 48 hours at 37° C. The cultures were then sucked through small Maassen filters of unglazed porcelain (measuring externally 3·5 in. by 0·6 in.) into filter flasks by means of a water pump. With a sterile pipette the filtrate was distributed in amounts of 5 c.c. in test tubes. To each test tube was added ·025 c.c. of a $\frac{n}{600}$ solution of sodium nitrite. A similar amount of sodium nitrite was added to 5 c.c. of sterile water, the latter being used as a colour control. Some of the tubes of filtrate were reinoculated from agar slope cultures of the homologous organism. Two other controls were used, consisting each of 5 c.c. of the original unfiltered culture. To the one was added ·025 c.c. of an $\frac{n}{600}$ solution of nitrite. To the other a similar amount of $\frac{n}{600}$ nitrate solution. The tubes were then sealed and incubated at 37° C. for from five to 14 days. At the end of that period the tubes were opened, subcultures made, and the presence or absence of nitrite ascertained by means of the test with α naphthylamine acetate and sulphanilic acid. The results were as follows:—

	With nitrate	With nitrite
Filtrate	Slight colour	Nitrite reaction
Filtrate reinoculated	,, ,,	,, ,,
Original fluid culture	No colour	No reaction

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The control series to which no addition of nitrite or nitrate had been made gave no reaction with the test. The slight nitrite reaction in the tubes containing nitrate seemed to be due to the difficulty of obtaining a nitrite-free solution. It will be noted that the tubes of filtrate re-inoculated from agar cultures of the homologous organism behaved very much like the filtrate alone, while the tubes of unfiltered fluid culture show complete reduction both of nitrate and of nitrite. No reduction by the filtrate could be detected even when it still contained some organisms. In the reinoculated tubes of filtrate, growth tends to be very scanty, and this no doubt accounts for the difference between these tubes and those containing unfiltered culture. Thus it appears that these bacilli do not produce a reducing substance which diffuses out into the surrounding medium to any marked degree. It is known that in the process of filtration substances present in solution may be retained to some extent by the substance of the filter. Serum complement and rennin may be thus retained, but as Muir and Browning (1909) have shown for serum complement, such action tends to occur only at first, the filter latterly becoming permeable. In the above experiments the amount of fluid passed through the filter was considerable in proportion to the size of the latter, and it is most unlikely that in this instance the whole of the reducing substance has been retained by the filtrate. The conclusion therefore seems to be justified that no soluble reducing substance is produced.

In studying the effect of dead organisms experiments were made with organisms killed by heat, by certain antiseptics and by acetone.

The action of organisms killed by heat.

In the case of organisms killed by heat the following method was employed. Cultures were made on agar of *B. dysenteriae* Flexner, *B. coli*, and *Staphylococcus aureus*, and after 24 hours incubation at 37° C. these were made into emulsions with sterile water. The emulsions were considerably denser (at least three or four times) than bouillon cultures of the same organism after 24 hours at 37° C. and contained 750 to 1500 million organisms per 1 c.c. As it was desirable that nitrate or nitrite from the agar or the condensation water should not be carried over into the emulsion, the cultures were removed by means of a platinum loop, and carefully rubbed down on the sides of the tubes containing the sterile water. The emulsions were made as homogeneous as possible. The emulsion having been prepared the

sides of the test tubes above the emulsion were heated in the bunsen flame till all the material on the side of the tube was carbonised. The tube was then plugged with sterile cotton wool, and immersed in a water-bath at 56° C. Cultures were made about every ten minutes, and it was found possible to sterilise four emulsions at a time, the subcultures being made in rotation from each. As a rule, cultures taken after heating for one hour at 56° C. were quite sterile, but in one case when *B. coli* and *B. dysenteriae* Flexner were kept at 54° C. all the cultures gave growths.

The subcultures were made by taking loopfuls of the emulsion and smearing them on sloped agar, and it was possible by this means to trace the gradual diminution in the number of living organisms. Thus in one experiment with *B. coli* the subcultures gave the following result after 24 hours at 37° C.

Cultures taken after	Result after incubation for 24 hours at 37° C.
16 mins.	Copious growth.
26 ,,	Fairly abundant growth. Numerous confluent colonies.
36 ,,	31 colonies, of 0·5 mm. to 3 mm. diam.
46 ,,	Only four colonies. One of 1 mm. and three of 2·5 mm. diam.
56 ,,	No growth.
66 ,,	,, ,,
80 ,,	,, ,,

In this case the temperature of 53° C. to 55° C. had been maintained for one hour and 20 minutes. It is to be noted that a marked degree of inhibition is possible with ultimate growth. Whether this is an indication that different individuals in the cultures possess very variable degrees of resistance to heat, must be left undecided (v. H. Chick 1910). Thus in one experiment a subculture which showed only three colonies of *B. dysenteriae* Flexner after 24 hours at 37° C. showed six after 48 hours, and nine after some days, while one which seemed sterile the first day had developed one or two colonies a few days later.

It was thus important that more than one subculture should be sterile, before deciding that the culture had been killed, and it was always necessary to test the sterility at the end of the experiment before applying the test for nitrite. On one occasion a *Staphylococcus aureus* emulsion which had given no growth either on the last three controls or on a control agar slope inoculated from the dense emulsion after it had been incubated at 37° C. for 24 hours, gave after a fortnight's incubation quite a copious growth of *Staphylococcus aureus*, when a small loopful was smeared on agar and incubated at 37° C. for 24 hours.

The nitrite solution which had been added was sterile. This capacity of organisms so delicate even as those of the dysentery group to revive after a somewhat lengthy period of inhibition, is a point which must be borne in mind in all experiments where it is necessary that sterilisation should be effected with a minimal amount of disturbance of function, as for instance in obtaining endo-enzymes or vaccines. It seems that this is an important source of possible fallacy, especially as organisms may revive to some extent during the experiment, and yet perhaps die off before the end.

The emulsions having been made and sterilised as above described, an amount of nitrite enough to make an $\frac{n}{60,000}$ solution was added, and the tube sealed to exclude air, as it has been shown that in the course of incubation a considerable amount of nitrate may be absorbed from the air. A similar tube of sterile water containing the same amount of nitrite was sealed as a control, and the tubes were incubated for several days or even weeks. At the end of the period of incubation the tubes were opened, subcultures made and the presence or absence of nitrite ascertained by the usual test. In this way it was found that reduction might apparently take place with dead cultures of *B. dysenteriae* Flexner and *B. coli*. In three experiments out of 15, reduction was obtained, but the amount of reduction was very slight when compared with that obtained with living fluid cultures, containing considerably fewer organisms, even when the latter had been growing for several days before nitrite was added. In the other 12 experiments no reduction was detected even after incubating for a week at 37° C. In none of the experiments was a growth of organism obtained from the heated emulsion, either at the beginning or at the end of the experiments. But the possibility cannot be excluded that in the positive experiment some of the bacilli at least had retained for a time a slight degree of vitality. Furthermore the degree of nitrite reaction obtained was so slight as to suggest that it might be due to some experimental error. Thus the dead bacilli have little if any action in reducing nitrates and nitrites.

The action of organisms killed by antiseptics.

The action on nitrites and nitrates of organisms killed by antiseptics was also studied, five antiseptics being used, viz. sodium fluoride, tetrachlor-o-biphenol, tetrabrom-o-kresol, p-chlor-o-kresol and hexabromdioxy-diphenyl-carbinol. The four latter, for which we are indebted to the kindness of Professor Ehrlich, are antiseptics of high value, whose

action on *B. diphtheriae* and some other organisms has been studied by Bechhold and Ehrlich (1906). Briefly it may be stated that a 1 % solution of tetrachlor-o-biphenol ($OH . Cl_2C_6H_2 - C_6H_2Cl_2 . OH$) poured over a 24 hour agar culture kills *B. diphtheriae* in less than two minutes and *B. coli* in less than five, while it inhibits the growth of *B. diphtheriae* in bouillon culture in a strength of $\frac{1}{400000}$. A 1 % solution of phenol does not, even when allowed to act for 60 minutes, completely kill an agar culture of *B. coli*. Similarly a 1 % solution of tetrabrom-o-kresol ($C_6Br_4 . CH_3 . OH$) kills an agar culture of *B. diphtheriae* in less than two minutes and of *B. coli* in less than five, while in the strength of $\frac{1}{200000}$ to $\frac{1}{160000}$ it inhibits the growth of *B. diphtheriae* in bouillon. A 1 % solution of hexabrom-dioxy-diphenyl-carbinol

$$(OH . C_6HBr_3 - CHOH - C_6HBr_3 . OH)$$

kills *B. diphtheriae* (agar culture) in less than two minutes, but a 3 % solution takes over an hour to kill *B. coli*. It must be remembered that the molecule of hexabrom-dioxy-diphenyl-carbinol weighs twice as much as the molecule of tetrachlor-o-biphenol and more than seven times that of phenol, so that strictly speaking a 7·3 % solution of hexabrom-dioxy-diphenyl-carbinol corresponds molecularly to a 1 % solution of phenol.

Taking phenol as a standard and measuring the power of inhibiting the growth of *B. diphtheriae*, Bechhold and Ehrlich found that 0·7 of a molecule of tetrachlor-o-biphenol, 0·9 of a molecule of tetrabrom-o-kresol, and 0·6 of a molecule of hexabrom-dioxy-diphenyl-carbinol were each equivalent to 1000 molecules of phenol. The theoretical interest of this lies in the relationship established between the chemical composition and the antiseptic power. The introduction of the halogen atoms (Cl, Br, etc.) or the uniting of two phenol groups either directly or by means of a CHOH group increases the antiseptic power of the molecule.

The *B. dysenteriae* Flexner emulsions used by the author contained so many organisms that they can hardly be used for comparison with Ehrlich and Bechhold's results. It was found, however, that tetrachlor-o-biphenol, tetrabrom-o-kresol and p-chlor-m-kresol completely killed the emulsions in 24 hours at 37° C. in a strength of $\frac{1}{5000}$ to $\frac{1}{10000}$, while hexabrom-dioxy-diphenyl-carbinol required a strength of $\frac{1}{4000}$ to sterilise the emulsion and sodium fluoride a 2 % solution. On one occasion a dense emulsion of *B. dysenteriae* Flexner in a saturated NaF solution (4–5 %) gave copious growths from platinum loopfuls smeared on agar even after 12 hours and 24 hours incubation of the emulsion. A dense

emulsion of *B. dysenteriae* Flexner in normal saline solution was killed in 30 hours by a $\frac{1}{2000}$ dilution of hexabrom-dioxy-diphenyl-carbinol, and by a $\frac{1}{15000}$ dilution of p-chlor-m-kresol. It was also killed in the same time by a $\frac{1}{500}$ solution of phenol, but not by $\frac{1}{1000}$ solution. For the dysentery group also, phenol is therefore a much less efficient antiseptic.

The organisms were grown on 2½ % agar in large Kolle flasks and transferred by means of a platinum scraper to 0·85 % salt solution in a strong sterile test tube. The latter contained a quantity of glass beads, by means of which the culture could be broken up and thoroughly emulsified by shaking. In practice it was found that even better results were obtained by using a fairly wide test tube and raking the beads about by means of the scraper. The emulsion always contained from five to ten millions of bacteria; that is to say, many times the number which occurs in any peptone water or bouillon culture. It was, indeed, quite thick and creamy, so that objects were invisible through a test tube containing it, whereas type can be made out through a bouillon culture.

Of each of the phenol derivatives, 0·1 grm. was dissolved with the least possible amount of NaOH solution and the volume was made up to 100 c.c. with sterile water, and diminishing amounts of this 0·1 % solution were added to a series of sterile 4 in. by ½ in. test tubes, the bulk being made up in each case to 1 c.c. with sterile salt solution. One cubic centimetre of bacterial emulsion was then added to each tube and the tubes were sealed with paraffin. After 24 hours incubation the tubes were opened, subcultures taken and 0·2 c.c. of sodium nitrate solution $\left(\frac{n}{500}\right)$ added. The subcultures were made by withdrawing 0·2 c.c. of the contents of the test tube and adding it to an amount of peptone water sufficiently large to make the antiseptic ineffective. With the amounts of antiseptic used, 5 c.c. of peptone water is sufficient, but in some experiments 200 c.c. were employed. The nitrate having been added, the tubes were sealed and incubated for from three to ten days. They were then reopened, subcultures again made, and the test with α-naphthylamine acetate and sulphanilic acid applied for nitrites. The following is an account of such an experiment:

The growth from six 5 in. by 5½ in. Kolle flasks was made into an emulsion with 40 c.c. salt solution. Six series of tubes were prepared, series (*a*) containing tetrachlor-o-biphenol, series (*b*) tetrabrom-o-kresol, and series (*c*) p-chlor-m-kresol in dilutions of $\frac{1}{5000}$, $\frac{1}{10000}$, $\frac{1}{20000}$, $\frac{1}{40000}$. Series (*d*) contained hexabrom-dioxy-diphenyl-carbinol in dilutions of $\frac{1}{2000}$, $\frac{1}{4000}$, $\frac{1}{10000}$, $\frac{1}{20000}$, and series (*e*) sodium fluoride in 2 %, 1 %, 0·5 %,

and 0·04 % solution. To each tube was added 1 c.c. of *B. dysenteriae* Flexner emulsion. Four controls (5 *a*, 5 *b*, 5 *c* and 5 *d*) containing bacilli but no antiseptic were also put up.

The sixth series (*f*) consisted of five tubes each containing one of the antiseptics in the same amount as the first tube of its series, and to this series autoclaved emulsion (120° C. for 15 minutes) was added. A sixth tube of each set contained the same amount of antiseptic as the first tube, but no bacilli, the volume being made up to 2 c.c. with salt solution. Each tube therefore contained 2 c.c. of fluid. After incubation for 24 hours the tubes were opened, subcultures made and sodium nitrate added to make an $\frac{n}{5000}$ solution. The tubes were sealed and incubated for ten days. They were then opened and tested for nitrite. The results of the various tests are comprised in the table on p. 369.

It will be noted that of the salt solution controls (tubes 6 *ag*, 6 *bg*, 6 *cg*, 6 *dg*, and 6 *gg*), two (6 *ag* and 6 *dg*) show traces of nitrite. This may be due to some slight reducing action of the antiseptic or to solution of nitrite from the glass of the test tube during the prolonged incubation; it might also result from some imperfection in the sealing of the tube which allowed absorption from the air to occur. This very slight colour, however, is of quite a different order from the marked reaction given by tubes 3 and 4 of series (*a*), 3 and 4 of series (*b*), 2, 3 and 4 of series (*c*), 3 and 4 of series (*d*), or 2 and 3 of series (*e*).

The slight trace of nitrite found in the first tube of each series and in all the tubes of the 6th (*f*) series, may be due to nitrite carried into the emulsion with the bacilli or to one of the causes suggested above in the case of the salt solution control. It is of the same order as the traces found in the salt solution control and must be considered an experimental error. In this connection one must remember the extreme delicacy of the α-naphthylamine reaction. Studying each series by itself, we find that first we have tubes which show only a trace of nitrite and give no growth in the sterility control. Then come one or two tubes which give a very marked nitrite reaction, indicating reduction of the nitrate. These all give growth when tested by culture. Finally in the tube containing no antiseptic, the reaction varies from a distinct colour to nothing at all. In these the reduction of nitrate to nitrite has occurred just as in peptone water or bouillon culture and the nitrite has subsequently been reduced. It thus appears that a series of tubes containing diminishing amounts of antiseptic give very similar results to a series of sample tubes taken at intervals

from a peptone water or bouillon culture. The first tubes give no reaction owing to non-reduction of the nitrate. The later tubes give a strong reaction, owing to the reduction of nitrate to nitrite,

Antiseptic	No. of tube	Amount of antiseptic	Sterility: before nitrate added	Sterility: at end of experiment	Result of test for nitrite
Tetrachlor-o-biphenol	1 *a*	$\frac{1}{5000}$	—	—	Trace
	2 *a*	$\frac{1}{10000}$	—	—	,,
	3 *a*	$\frac{1}{20000}$	G	G	Very marked
	4 *a*	$\frac{1}{40000}$	G	G	,, ,,
	5 *a*	None	G	G	Very faint trace
	6 *ag*	$\frac{1}{5000}$	—	—	Trace
Tetrabrom-o-kresol	1 *b*	$\frac{1}{5000}$	—	—	Faint trace
	2 *b*	$\frac{1}{10000}$	—	—	,, ,,
	3 *b*	$\frac{1}{20000}$	G	G	Very marked
	4 *b*	$\frac{1}{40000}$	G	G	,, ,,
	5 *b*	None	G	G	Distinct
	6 *bg*	$\frac{1}{5000}$	—	—	0
p-chlor-m-kresol	1 *c*	$\frac{1}{5000}$	—	—	Trace
	2 *c*	$\frac{1}{10000}$	G	G	Marked
	3 *c*	$\frac{1}{20000}$	G	G	Very marked
	4 *c*	$\frac{1}{40000}$	G	G	,, ,,
	5 *c*	None	G	G	Trace
	6 *cg*	$\frac{1}{5000}$	—	—	0
Hexabrom-dioxy-diphenyl-carbinol	1 *d*	$\frac{1}{2000}$	—	—	0
	2 *d*	$\frac{1}{4000}$	—	—	0
	3 *d*	$\frac{1}{10000}$	G	G	Marked
	4 *d*	$\frac{1}{20000}$	G	G	Distinct
	6 *dg*	None	—	—	Trace
Sodium fluoride	1 *e*	2 %	G	—	Trace
	2 *e*	1 %	G	G	Marked
	3 *e*	·5 %	G	G	,,
	4 *e*	·04 %	G	G	Faint trace
	5 *e*	None	G	G	0
	6 *eg*	2 %	—	—	0
Organisms autoclaved + tetrachlor-o-biphenol	1 *fa*	$\frac{1}{5000}$	—	—	Faint trace
Tetrabrom-o-kresol	2 *fb*	$\frac{1}{5000}$	—	—	,, ,,
p-chlor-m-kresol	3 *fc*	$\frac{1}{5000}$	—	—	,, ,,
Hexabrom-dioxy-diphenyl-carbinol	4 *fd*	$\frac{1}{2000}$	—	—	,, ,,
Sodium fluoride	5 *fe*	2 %	—	—	,, ,,
NaCl sol. control	6 *gg*	None	—	—	0

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while the last tubes give no reaction owing to complete reduction of the nitrite. From this it appears that no reduction occurs when the antiseptic is present in sufficient amount to kill the organism, while when the amount is less than is sufficient to kill the organism the reduction may yet be impeded, so that either no reduction occurs or the action stops at the formation of nitrite in place of proceeding to complete destruction of the latter. When the amount of antiseptic is very small it may have practically no effect, as *e.g.* in tube 4*e* of the above experiment where only a faint trace of nitrite is left when 0·04 % sodium fluoride was used. It may be remarked that while tube 1 of series *e* gave growth in the subculture made immediately before the addition of nitrite, this tube was sterile at the end of the experiment. This probably accounts for the non-reduction of nitrate, the organism having died off too soon after the nitrate was added. This series (*e*) shows all gradations from non-reduction of nitrate in the first tube (2 % NaF) to almost complete reduction of nitrite in the fourth (0·04 % NaF). That the absence of colour in the first tube is not due to complete reduction of nitrate to nitrite followed by destruction of nitrite was shown by adding nitrite $\left(\frac{n}{20,000}\right)$ to tubes of emulsion containing these amounts of the various antiseptics, and incubating at 37° C., when no reduction was found to occur.

The action of organisms killed by the acetone process.

The "Buchner-Albert-Rapp" acetone process was originally devised as a method of fixing yeast cells without destroying their power of fermenting sugar. In other words, its purpose was without destroying the fermenting enzyme itself to check the action of such proteolytic or other enzymes as might destroy the fermenting enzyme, and at the same time to destroy all power of proliferation. It was a modification of Albert's alcohol ether process which had been used for the same purpose but was subject to the disadvantage that alcohol interfered with the fermenting enzyme. In the Buchner process the yeast is washed, freed from water as far as possible by submitting it to a pressure of 50 to 100 atmospheres, and treated for about 15 minutes with acetone. It is then filtered free from acetone with the aid of pressure and suction, treated with a second quantity of acetone, freed from this and finally treated for a short time with ether. Having been freed as far as possible from ether, it is spread on filter paper and left exposed to the air for an

hour. It is then, to prevent it absorbing moisture from the air, placed in the incubator for 24 hours at 37° C. in order that the last traces of ether may evaporate. The final product is an almost white dry powder, which is found to contain only 5·5 % to 8·5 % of water. In adapting this method, the author was unable to make use of high pressure, but otherwise the method is the same. The bacilli were grown on 2½ % agar in Kolle flasks and made into an emulsion with sterile water. About four times the bulk of acetone was then added and the bacilli immediately began to clump. They were allowed to settle and the supernatant fluid was then pipetted off, more added, and the mixture agitated for several minutes. The bacilli were again allowed to settle and the fluid changed. Finally ether was substituted for the acetone and after two washings the bacilli were dried. It is possible to shorten the process and obtain a larger yield by centrifugalising the mixture, to aid the precipitation of the organisms. Usually the emulsion was made in a large Jena glass test tube and transferred beads and all to a sterile 100 c.c. Jena flask for further treatment. The glass beads proved useful, not only in mixing the bacilli with the acetone but also in making them into an emulsion when they came to be used for experiments.

The product thus obtained at first formed a porous and friable cake, adherent to the bottom and sides of the flask, but on shaking the flask this cake was readily broken up by the glass beads to a fine yellowish grey powder. Its action on nitrites and nitrates was tested by making it into an emulsion with sterile water and incubating it with nitrate or nitrite solution. Equal amounts of emulsion and of sodium nitrate $\left(\frac{n}{50}\right)$ or sodium nitrite $\left(\frac{n}{10,000}\right)$ solution were placed in a sterile test tube. Similar amounts of emulsion and nitrate or nitrite solution were placed in separate tubes, and all the tubes were sealed. The emulsion and nitrite or nitrate solution which had been incubated separately were mixed immediately before testing and formed a control upon the tubes in which the emulsion had been incubated along with the salt. The following gives the result of such an experiment. The tubes were sealed in the flame, the lower end being immersed in cold water during the process and for some time after:

	Result when tested with naphthylamine acetate and sulphanilic acid
Emulsion and nitrate incubated together	Faint colour
Emulsion and nitrate incubated separately	Faint colour
Emulsion and nitrite incubated together	Marked colour
Emulsion and nitrite incubated separately	Marked colour

There is thus no difference between the solutions which were incubated along with the bacilli and the corresponding ones incubated separately. That is to say there has been no reduction of nitrate or nitrite by organisms killed with acetone.

Summary and Conclusions.

The organisms examined have been killed, (*a*) by temperatures which should not have destroyed the reducing enzyme, (*b*) by sodium fluoride and four other antiseptics, derivatives of phenol, and (*c*) by acetone. In each case there has been total destruction of the reducing power, any apparent production of nitrite from nitrate being too slight to justify the assumption that it is due to anything more than experimental error. Filtrates of fluid cultures have also failed to reduce. The results may be summed up as follows:

(1) The reduction of nitrite by certain dysentery bacilli is not due to a soluble extra-cellular enzyme.

(2) Dysentery bacilli and *B. coli* killed by a minimal degree of heat have little if any effect in reducing nitrites.

(3) Organisms killed by antiseptics or by acetone do not reduce nitrates and nitrites.

(4) Even where all the organisms are not dead, reduction may be prevented by antiseptics.

(5) The high antiseptic value of tetrachlor-o-biphenol, tetrabrom-o-kresol, p-chlor-m-kresol and hexabrom-dioxy-diphenyl-carbinol has been established for the organisms of the dysentery group.

REFERENCES.

Albert, Buchner, und Rapp (1902). Herstellung von Dauerhefe mittels Aceton. *Ber. d. chem. Gesellsch.* xxxv., 2376–2382.

Burri und Stutzer (1895). *Centralbl. f. Bakteriol.*, Abt. 2, i., 257, 350, 392, and 422.

H. Chick (1910). *Journ. of Hygiene*, x., 237.

Ehrlich und Bechhold (1906). Beziehungen zwischen Chemischer Konstitution und Desinfektionswirkung. *Zeitschr. f. physiol. Chem.*, xlvii., Hefte 2 and 3.

Logie (1909). *Journ. Pathol. and Bacteriol.*, xiv., 146.

—— (1910). *Journ. of Hygiene*, x., 143.

Muir (1909). *Studies in Immunity*, Oxford, 1909, 93.

Muir and Browning (1909). *Journ. of Pathol. and Bacteriol.*, xiii., 232.

Weissenberg (1897). *Arch. f. Hyg.*, 1897, xxx., 274.