THE BEHAVIOUR OF HYPOCHLORITES ON INTRAVENOUS INJECTION AND THEIR ACTION ON BLOOD SERUM.*

BY

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HYPOCHLORITE solutions containing variable amounts of free hypochlorous acid have been employed fairly extensively for the local treatment of infected wounds. Recently one of these solutions, "eusol," introduced by Professor Lorrain Smith and his co-workers, has been administered by intravenous injection with apparently successful results in cases of septicaemia and toxaemia.[1] It was of interest, therefore, to endeavour to determine some of the factors conditioning the action of this solution and related substances.

The object of the following communication is to describe certain experiments which appear to prove that hypochlorite solutions when injected intravenously in quantities similar to those employed for therapeutic purposes cannot exert any appreciable direct germicidal action. It should be noted that, with the exception of certain experiments by Cordova,[2] in which eusol given intravenously was stated to be capable of protecting rabbits from lethal doses of staphylococci and *B. capsulatus*, no direct curative effects have been claimed in cases of septicaemia in which organisms were isolated from the blood. Lorrain Smith, Ritchie, and Rettie state that "the method of intravenous injection may give favourable results in cases of general septic infection in the field, gangrene, tetanus, and other kindred conditions characterized by toxaemia."

1. *Quantitative Relations.*

Eusol has been used for intravenous injection in man in doses ranging from 60 c.cm. to 100 c.cm. An average full dose may be taken to be 100 c.cm., corresponding to 0.27 gram of hypochlorous acid. This amount is at once diluted with four or five litres of blood, so that the possible concentration of hypochlorous acid, assuming that no decomposition of the drug by the blood occurs, is not more than 50 to 65 mg. of hypochlorous acid per litre. Even if this concentration persisted temporarily, which will shortly be shown not to be the case, it is much below the concentration of hypochlorite at which active direct action is observed, when tested against organisms such as *B. coli* or staphylococci suspended in blood, or even in serum.

Direct experiments in which *B. coli* or s aphylococci were mixed with sterile rabbit serum or plasma, and then 70 mg. per litre of hypochlorous acid in the form of eusol added, showed no marked diminution in the number of organisms in the course of two hours when compared with a control experiment in which no hypochlorous acid was added. Precisely similar results were obtained when the hypochlorous acid solution was injected intravenously into rabbits, and the serum taken before and after the injection was tested against *B. coli in vitro*. The following are typical results. The concentration of hypochlorous acid added was 70 mg. per litre in all cases.

Mixture.	Organisms per drop, $\frac{1}{40}$ c.cm., at end of two hours.
1. Normal serum + *B. coli*	780
,, ,, + *B. coli* + HClO	640
2. Normal serum + *B. coli*	1120
,, ,, + *B. coli* + HClO	1280
3. Normal serum + *B. coli*	1640
Serum after injection HClO + *B. coli* ...	1760

The changes are in no case significant. Miss Chick[3] has already shown that normal rabbit serum has practically no action on *B. coli* during the first few hours of action. The above experiments followed the technique described in Miss Chick's paper.

2. *Fate of Hypochlorites on Intravenous Injection.*

Hypochlorous acid or its salts exert their known antiseptic action on account of the active chlorine contained in them. This active chlorine may occur in two forms: that present as unchanged hypochlorite, and chlorine loosely bound to amino compounds, including proteins. Chlorine in both of these forms is able to liberate iodine from an acidified solution of potassium iodide, and hence may be easily detected. The antiseptic value of the chlorine loosely bound to amino compounds, in "chloramine" form, probably varies as the ease with which it can be detached so as to permit of union with other substances, including bacterial protoplasm. But hypochlorites can combine with a variety of substances by reactions of a different kind from that just mentioned. Thus, for example, the chlorine may become attached to carbon atoms present in proteins and other substances, and then loses its power of liberating iodine from potassium iodide, and confers no germicidal properties on the product. Furthermore, it is probable that direct reduction of hypochlorites to chloride may occur in the body, for this is a common reaction *in vitro*. Thus it is seen that hypochlorites may be decomposed and lose their germicidal properties by a variety of reactions. Changes of this nature apparently occur with great rapidity when hypochlorous acid or its salts are injected intravenously, for a much greater quantity of "eusol" per kilo of body weight than is used therapeutically may be injected intravenously into animals without the appearance of a detectable trace of active chlorine in the plasma or serum.

The following is a typical experiment. Under ether anaesthesia cannulae were placed in the carotid artery and femoral vein of a rabbit weighing 1,820 grams. Eusol of about half-strength (1 c.cm. = 1.3 mg. hypochlorous acid) was injected into the vein, and almost immediately afterwards blood was drawn from the artery into citrate solution, rapidly centrifuged, and the plasma tested as promptly as possible for active chlorine by adding potassium iodide, acetic acid, and excess of dilute starch paste. Control experiments showed that the citrate did not interfere with the reaction; 7 c.cm. of the eusol were injected fairly rapidly, and then the first sample of blood was withdrawn. Further samples of blood were taken after each additional injection of 2 c.cm. of eusol. Although haemolysis was detectable in all samples of blood, and was very marked in the later ones, no trace of active chlorine was found in the plasma obtained after the injection of 19 c.cm. of the hypochlorite solution, and only a doubtful reaction was obtained after further additions which finally killed the animal.

The volume of blood in the rabbit used in this experiment may be taken as approximately 125 c.cm., so that it is calculated that at least 200 mg. of hypochlorous acid may be added to each litre of circulating blood before any detectable trace of active chlorine appears in the plasma. This amount is more than three times the therapeutic dose. It may be noted incidentally that the minimal addition of hypochlorous acid to blood serum that will produce a positive reaction for active chlorine confers no added germicidal properties.

It might be suggested that in the foregoing experiment active chlorine could not be detected in the blood plasma on account of its being taken up by the cells. But special experiments showed that blood serum from rabbits and sheep required the addition of from 35 to 80 mg. of hypochlorous acid per litre before any active chlorine persists. Finally, it may be noted that if circulating or drawn blood is mixed with an amount of hypochlorous acid just sufficient to yield a plasma or serum giving a definite positive reaction for active chlorine, the latter rapidly disappears in the course of a short space of time.

From the foregoing experiments it appears in the highest degree improbable that any direct germicidal effects can be ascribed to hypochlorous acid or its salts when injected intravenously in the prescribed therapeutic doses.

3. *Other Effects of Hypochlorites when given Intravenously.*

When hypochlorite solutions are injected intravenously into rabbits a certain amount of haemolysis is observed even with doses as low as 1.5 c.cm. of eusol = 4 mg. of hypochlorous acid per kilo of body weight. With larger doses the haemolysis becomes more intense and the

* The work here referred to was done with the assistance of a grant from the Medical Research Committee. The experiments were made in the laboratories of the Committee's Department of Biochemistry and Pharmacology. I am indebted to Dr. H. H. Dale, F.R.S., for much helpful advice and for facilitating my work in every way possible.

coagulability of the blood becomes diminished.* When lethal doses of hypochlorite preparations or chloramine-T are given intravenously a marked increase in the pericardial fluid and oedema of the lungs is commonly observed. It appears that in large doses the substances act as definite endothelial poisons.

Since the experiments already referred to appear to prove that hypochlorite preparations given intravenously can exert no appreciable direct germicidal action, it appeared conceivable that the favourable clinical results stated to follow their employment might be due to an indirect action in stimulating the production of antibodies. Thus far I have only had the opportunity of investigating the effects of hypochlorites given intravenously on the antitrypsin of the blood. The results seem of interest since Wright and others have suggested that antitrypsin plays an important part in the normal antibacterial properties of blood. Whether eusol or other hypochlorite preparations act as a stimulus for the production of other non-specific antibacterial and antitoxic substances is a question that requires further investigation.

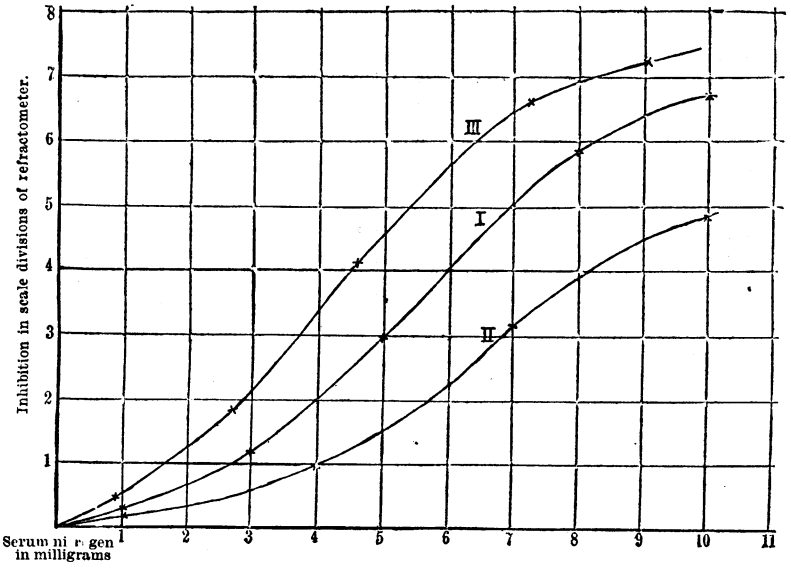

CHART 1.—Showing reduction in antitryptic action of blood after injection of eusol, followed by increase above normal. I, Before injection; II, immediately after; III, twenty-four hours after.

For the experiments on the effect of hypochlorite given intravenously on the antitryptic action of the blood I enjoyed the co-operation of Dr. G. S. Walpole (and his assistant). An exceptionally precise method devised by him was employed, the details of which will be published shortly. The method essentially consists in the estimation by means of the dipping refractometer of the products of tryptic digestion, resulting from the interaction of constant quantities of trypsin and casein in the presence of varying amounts of blood serum. I am much indebted to Dr. Walpole for permission to make use of his method and results.

It was found that when small amounts of hypochlorite, either in the form of eusol or neutral sodium hypochlorite, are injected intravenously into rabbits, a fall is observed in the antitryptic action of blood serum taken immediately after the injection. But if another sample is taken twenty-four hours after the injection, it is commonly found that recovery in antitryptic action has proceeded to a point significantly in excess of the original value before hypochlorite was given. A typical example is shown in Chart I, in which the actual inhibition of tryptic digestion due to antitrypsin, and expressed in scale units of the refractometer, is plotted against the varying amounts of serum, which, for uniformity, are reckoned in milligrams of total nitrogen. The curve marked I represents the initial antitryptic action of the blood serum; that marked II the antitryptic action of the blood serum taken immediately after the injection of 5 c.cm. of eusol (= 14 mg. of hypochlorous acid) into the ear vein of the rabbit, which weighed 1,940 grams; and that marked III, the antitryptic action of the blood serum taken twenty-four hours after the injection.

Similar effects were found to follow the injection of comparable amounts of sodium hypochlorite. The action of hypochlorites in causing a primary fall in the antitryptic action of the blood serum, followed by a secondary increase, is shared by many other substances.

4. *The Action of Hypochlorites and of Chloramine-T on Blood Serum.*

The rate of decomposition of hypochlorites by blood serum is a question of some importance in connexion with the local treatment of infected wounds, since the results afford some indication of the way in which the active mass of the antiseptic may become rapidly diminished. In the case of both eusol and neutral sodium hypochlorite a curious fact was observed—namely, that a small amount of serum may bring about the disappearance of more active chlorine in a given time than will larger amounts of the same serum. This somewhat paradoxical reaction, which was independently observed by Dr. Harden, is best illustrated in the form of a chart showing the disappearance of active chlorine titratable with potassium iodide and sodium thiosulphate, when a constant volume of hypochlorite is mixed with varying amounts of serum, and allowed to stand for thirty minutes at 20°. Similar results were obtained when the reaction was allowed to continue for a longer period. The typical results shown in Chart 2 were obtained by mixing 5 c.cm. of neutral hypochlorite containing 12.6 mg. of hypochlorous acid with varying amounts of sheep serum.

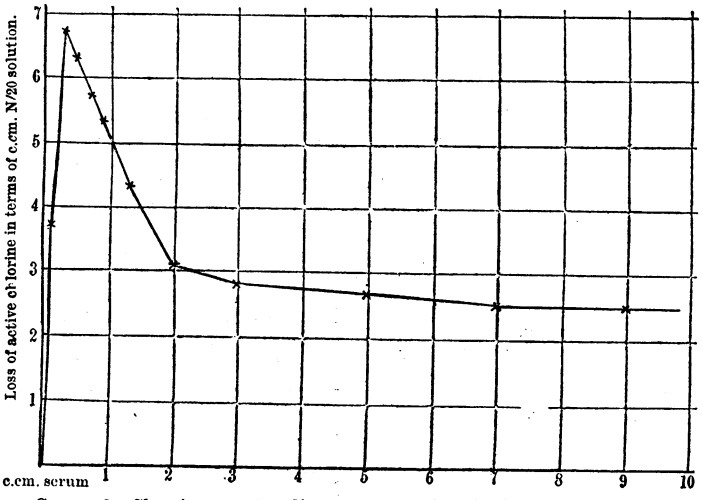

CHART 2.—Showing greater disappearance of active chlorine from hypochlorite solution when treated with small amounts of serum than with large.

The explanation of this curious action of small amounts of serum on hypochlorite is to be found, I believe, along the following lines. When the mass of the protein in the blood serum is relatively small compared to that of the hypochlorite the amount of chlorine taken up per molecule of protein is large, and the product becomes unstable and is readily hydrolized. This hydrolysis has the effect of liberating from the protein more amino-acid complexes, which can react with additional hypochlorite. Now Langheld has shown that amino-acids react with hypochlorites to form the sodium salts of mono- and di-chloramino-acids, and it is now known that these

* It has been stated that only eusol can be injected with safety, and that sodium hypochlorite must not be used. I have been unable to detect any significant difference between the effects of eusol and sodium hypochlorite prepared according to the formula given in the BRITISH MEDICAL JOURNAL, August 28th, 1915.

substances decompose spontaneously to give aldehydes or nitriles respectively, with liberation of carbon dioxide, while the chlorine is converted into inactive chloride.

$$\text{R. CHNH}_2\text{.COOH} \xrightarrow{+\text{NaClO}} \text{R. CHNHCl.COONa} \xrightarrow{+\text{H}_2\text{O}} \text{R.CHO} + \text{NaCl} + \text{CO}_2 + \text{NH}_3$$

$$\text{R. CHNH}_2\text{.COOH} \xrightarrow{+2\text{NaClO}} \text{R. CHNCl}_2\text{.COONa} \dashrightarrow \text{R. CN} + 2\text{NaCl} + \text{CO}_2$$

I have observed that when small amounts of serum are acted on by relatively large amounts of hypochlorite, both gas production and aldehyde formation are much more pronounced than when the relative mass of serum is large. The fact harmonizes with the suggested explanation of the reaction between hypochlorites and serum. When the mass of serum is large in relation to the hypochlorite, it appears that the smaller amount of chlorine taken up by the protein does not confer on the product such instability as is seen in the case of more highly chlorinated substance. Chloramine-T, when added to serum, does not react like hypochlorite, but is decomposed to a smaller extent, varying directly with the relative mass of serum.

The above experiments are of a preliminary character, and it is hoped that some of the practical questions arising from them may be more closely investigated.

REFERENCES.

[1] J. Lorrain Smith, Ritchie, and Rettie, BRITISH MEDICAL JOURNAL, November 13th, 1915, p. 716. [2] Cordova, BRITISH MEDICAL JOURNAL, May 6th, 1916, p. 651. It should be noted that the lethal doses of *B capsulatus* and staphylococci recorded by Cordova in his control experiments are very much smaller than those generally observed. [3] Miss Chick, *Journal of Hygiene*, *12*, p. 414, 1913.

Reviews.

LOCALIZATION BY X RAYS AND STEREOSCOPY.

Localization by X Rays and Stereoscopy[1] is the title of a book by Sir J. MACKENZIE DAVIDSON which is published at an opportune time, seeing that the exact localization of foreign bodies within the human body is of such vital importance when so many wounded have to be dealt with accurately and with rapidity. Many time-saving methods of localization have been devised during the past eighteen months, mostly based on Davidson's original cross-thread method; few, if any, of these are quite so accurate. The stereoscopic method, also a discovery of the author, has not been, and is not, used so extensively as it should be. The simplicity of technique in the taking of such radiographs, the theory, and the way to apply the results are all well set out in this work, which, after dealing with the *x*-ray tube and secondary radiations and protection, describes in detail the use of stereoscopic radiographs. The last chapter, and one to which the author directs especial attention, is on the precise localization of foreign bodies in the eye and the orbit. If anything was wanting in the previous chapters to convince the reader of the accuracy possible in localization, this last chapter would supply that want, for in dealing with foreign bodies in these situations Davidson's method may be said to stand alone, its simplicity and accuracy being little short of marvellous.

On p. 22 will be found a description of a new *x*-ray couch which for simplicity of design, for adaptability to war work at the front, for ease and cheapness of construction, and for efficiency in doing what it is meant to do, will be hard to beat. Accompanying this is a very ingenious screen instrument which, when used with this couch or others of similar design, will enable the operator to read off on a scale the depth of a foreign body under a marked skin spot. In advocating stereoscopy attention is drawn to "the misleading single picture." With a knowledge of what is going on at the present time, with much of the war work being done by unskilled and even untaught *x*-ray operators, and with surgeons inexperienced in radiography, greater stress might well have been laid on this point. If the author had suggested that for a surgeon to act on the showings of a single radiograph when searching for a bullet was almost criminal, he might not have been going too far. The illustrations are excellent and an important feature. Many diagrams help materially in the understanding of the *x*-ray descriptions, and at the end are a number of very beautiful reduced stereoscopic radiograms which can be viewed with one of the small, cheap stereoscopes so much in vogue. Whilst the expert in radiography can learn much that is of value from this publication, it is nevertheless of even more value to the surgeon, and especially to those surgeons who at the present time are attached to the army.

[1] *Localization by X Rays and Stereoscopy*. By Sir J. Mackenzie Davidson, M.B., C.M.Aberd. London: H. K. Lewis and Co., Ltd. 1916. (Roy. 8vo, pp. 83; 26 plates, 61 figures. 7s. 6d. net.)

MEDICAL ETHNOLOGY.

Medical Ethnology,[2] by Dr. WOODRUFF, will interest students of anthropology and possibly also those of tropical medicine. The work was begun, the author tells us in the preface, as a revision of the first edition of *The Effects of Tropical Light on White Men*, but it was necessary to change the title because so many other factors besides pigmentation have entered into the discussion of the reasons for the differences between the present races and sub-races of men.

The subjects discussed in the twenty chapters include the effects of light on man, the question of pigmentation, actino-therapy, the cause of the extinction of migrants, ethnic psychology, etc. One thing the work brings out very clearly is the slender basis on which rests the hope that the white man will ever be able to inhabit the tropics—that is to say, to live there for good, breeding and propagating his species, even when all the diseases have been eradicated from them. Dr. Woodruff gives a good example of the mania amongst some people to deny that climate can affect any one. "The most notorious case was that of Civil Service Commissioner Washburn, who stated that the climate was not harmful if one was moral and sober, and that if he did break down recovery was possible there. Within a few months he himself collapsed and had to go away to get well." Similar cases can easily be quoted from British colonies, one example that is often mentioned being the sad end of a man who was trying to get other people's leave cut down, but before he had progressed far in his endeavour was himself removed by death. The advocates of the white man for the tropics seem to forget all about neurasthenia, irritability of temper, and loss of memory, or evidently do not consider them as diseases. These are, however, the very things that finish off so many people and render them unfit for further efforts in a hot climate. Make the tropics healthy by all means, so that the constant stream of migrants to them may have a chance of returning home again fit and well, but do not delude white people into the belief that if disease is stamped out they can make a permanent home there. In writing his book Dr. Woodruff has quoted largely from the literature, and incidentally has perpetuated many of the mistakes to be found in it—for example, the part on heat exhaustion, on p. 136, is hopelessly involved, and even distinctly misleading. Again, we cannot agree with him in all he says as regards light as compared with heat in producing symptoms. Statements also, such as that cases of chronic malaria have been cured without quinine by a five-minute dose of *x* rays to the enlarged spleen, would have been better omitted. The question of why certain races are found in some parts of the world and not in others is, however, a fascinating one, and a perusal of Dr. Woodruff's book will give the reader plenty of food for thought.

[2] *Medical Ethnology*. By C. E. Woodruff, A.M., M.D. London: W. Heinemann. 1916. (Med. 8vo, pp. 328. 10s. net.)

NOTES ON BOOKS.

READERS of the BRITISH MEDICAL JOURNAL will have studied with profit and amusement the articles on the *Minor Horrors of War* written by the Master of Christ's College, Cambridge. The articles have been collected into two volumes, of which the first was reviewed in our issue of April 17th, 1915. The second series, entitled *More Minor Horrors*,[3] contains fourteen chapters, of which ten have already appeared in this JOURNAL. The book gives very readable accounts of such pests as cockroaches, the bot-fly, the mosquito, the biscuit weevil (so familiar to readers of Clark Russell's nautical novels), the fig-moth, the stable-fly, rats, and the field-mouse. There are

[3] *More Minor Horrors*. By A. E. Shipley, Sc.D., Hon. Sc.D.Princeton, F.R.S. London: Smith, Elder, and Co. 1916. (Cr. 8vo, pp. 177; 49 figures. (Paper, 1s. 6d. net; cloth, 2s. net.)