army because of valvular disease of the heart. I examined him, found his response to effort good, the heart normal in size, and a well-marked systolic murmur at the apex. I gave him a certificate saying that the heart was perfectly healthy, and that the murmur was physiological, indicative neither of disease nor impairment. I told him to take this certificate to some other recruiting centre than that at which he had been rejected. This he did, was accepted, joined the army, went through his training, carrying his pack and enjoying his route marches. In course of time he was in Gallipoli in the trenches leading a strenuous life and feeling fit. One day in May a shell exploded near him and he was blown over and became unconscious; he was picked up and taken into hospital. After he recovered consciousness he felt quite fit, but, unluckily, a medical officer auscultated his heart, detected a murmur, and at once diagnosed valvular disease of the heart. He was kept on his back for a considerable time, shifted from hospital to hospital, repeatedly examined by medical men, and given treatment, till finally he was examined by a Board in August, 1915, and invalided out of the service because of valvular disease of the heart. He called to see me a few weeks later; the heart was exactly in the same condition as it was a year before, normal in size, with a physiological murmur, as sound a heart as there is in the British army.

A short time ago a highly placed medical official brought his son to see me, as the lad suffered from irregular heart which was resistant to treatment. I found the irregularity to be of the youthful type, and I explained very minutely to the medical official that the irregularity was purely physiological, and I demonstrated to him by what means I separated this form of irregularity from all others, and told him how it was frequently present in the most healthy hearts. A week later he wrote asking me if I could not give him some drug, such as digitalis or strophanthus, to remove the irregularity, as it still persisted. I again wrote, clearly explaining that the condition was a purely physiological one. Nevertheless, he replied that he himself had given the lad a tonic which he hoped might "cure the irregularity."

NOTE ON

A SUPPOSED SOLUBLE TOXIN, PRODUCED IN ARTIFICIAL CULTURE BY THE BACILLUS OF MALIGNANT OEDEMA.

BY

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SOME months ago we made some experiments on the nature of a toxic substance occurring in the filtrates from cultures of the bacillus of malignant oedema, an organism which has acquired special importance during the war, as being one of the spore-bearing anaërobic bacteria concerned in the production of gas gangrene from soil-infected wounds.

Our observations were limited to the cultural filtrate from one particular strain, for the culture of which, as well as for a quantity of the reputed toxin, with which many of our experiments were made, we are indebted to Mr. J. B. Buxton, F.R.C.V.S. The strain was originally obtained from a case of gas gangrene, and its correspondence in all diagnostic points with the true bacillus of malignant oedema has been confirmed by several bacteriologists. From the muscles and areolar tissue of guinea-pigs dying of infection with an organism of this type Roux and Chamberland expressed a juice which, after sterilization by filtration, killed other guinea-pigs with acute convulsant symptoms. Besson prepared a similarly toxic filtrate by growing the organism on sterilized meat, which the digestive activity of the organism rapidly liquefies. The filtered juice of a five or six days culture kills medium-sized guinea-pigs with acute symptoms in a dose of 3 to 5 c.cm. given hypodermically. It is a juice of this kind which has been regarded by Besson and others as containing a characteristic toxin produced by the organism, and attempts have been made to prepare an antitoxic

serum by immunizing animals against it. A broth culture of the organism, however abundant the growth, will not yield a filtrate of such potency.

The material used by us was obtained by growing the organism for five to six days at 37° C. on minced ox-heart, previously sterilized in the autoclave. The culture was started in hydrogen and provision made for the escape of gases, which the culture soon produced in abundance, by a tube leading from the cultural jar into a trap filled with sulphuric acid. The semifluid, evil-smelling product was filtered first through paper-pulp, and then through a Berkefeld candle. A nearly clear brown filtrate was obtained.

This filtrate produced the acute intoxication described by Besson when injected into guinea-pigs, the fatal dose for an animal weighing about 300 grams varying from 3 to 5 c.cm. with different preparations. It was at once evident, however, that its toxic constituent had none of the characteristics of a true bacterial toxin. The relatively large volume of the filtrate required for the production of general symptoms and the almost immediate onset of the symptoms, when the dose was sufficient to produce them at all, suggested rather the non-specific action of some product of putrefactive digestion, and the necessity of using solid meat as a culture medium was suggestive in the same direction. The suggestion was confirmed by the observation that the toxicity of the product was but little affected by brief boiling. Dr. Walpole filtered the fluid under pressure through a collodion film, which was impervious to diphtheria toxin, and the filtrate showed the full toxicity of the preparation.

The symptoms produced are both local and general. Locally a large, oedematous, hæmorrhagic infiltration is produced in the subcutaneous tissue and underlying muscle, in a considerable area surrounding the site of injection. With a non-fatal dose this local effect may be the only one, except for a vague malaise. To the general intoxication small guinea-pigs are much more susceptible than larger ones. Thus 3 c.cm. may cause the death of a guinea-pig weighing 200 grams in 10 to 15 minutes, while 6 c.cm. of the same preparation may cause little more than a local infiltration in a guinea-pig weighing 400 grams. The general symptoms comprise tremors, weakness, and excitability, rapidly passing into extensor convulsions, which usually terminate in death within fifteen minutes of the injection. *Post mortem* the lungs are found to be oedematous, with areas of hæmorrhagic consolidation, and the trachea contains a blood-stained froth.

Though the toxic principle resisted brief boiling, it was rapidly lost if boiling was prolonged, and no concentration of the activity could be effected by evaporation, even at a low temperature and under diminished pressure. The toxic substance was evidently very unstable, or highly volatile. Omitting experimental details, we may say at once that we found that the toxic substance could be distilled readily from the fluid if the latter was made alkaline, and could be collected in acid; but that it was held back if the fluid was made acid to Congo red with sulphuric acid. We were dealing, therefore, with a volatile base. We accordingly made a quantity of the "toxin" alkaline with excess of potash and distilled into normal sulphuric acid. The quantity of volatile bases obtained was surprisingly large. From 150 c.cm. of collodion-filtered "toxin" we obtained volatile bases neutralizing 113.2 c.cm. of normal sulphuric acid. The mixed sulphates, obtained by evaporation of the distillate, were exhausted with absolute alcohol, in which ammonium sulphate is even less soluble than ammonium chloride. From the part insoluble in alcohol, consisting of ammonium sulphate, we obtained ammonia equivalent to 96 c.cm. of normal H₂SO₄; the organic bases, with sulphates soluble in alcohol, were therefore equivalent to 17.2 c.cm. of normal acid. The "toxin" was therefore 0.64 N. in respect of ammonia, and 0.115 N. in respect of other volatile bases. The alkaline residue left after distillation contained large amounts of soaps, and from these, by acidifying and distilling with steam, we obtained volatile fatty acids in quantity corresponding to a normality of 0.75 in the original fluid. This accords with the observation that the "toxin" has but a feebly alkaline reaction.

The question of practical interest was the identification, among the volatile basic constituents, of the toxic principle.

A few experiments showed that the ammonia fraction was entirely responsible for the symptoms produced, and that pure ammonium salts, in dose and concentration equivalent to those of the ammonium salts in the "toxin," produced all the effects described, both local and general, in perfectly characteristic manner. Young guinea-pigs showed, in proportion to their weight, the same excessive sensitiveness to the general, convulsant action of ammonium salts as to those of the toxic culture-filtrate. Neither the organic bases, in the proportion present, nor the fatty acids contributed materially to the effect.

There seems, then, to be no ground for supposing that the bacillus of malignant oedema, in an artificial culture of the type described, produces anything in the nature of a specific toxin. The production by it of a toxic solution from meat depends on its intense proteoclastic and desaminating action. The proteins are rapidly broken down to amino-acids, and from these the amino-groupings are then split off, yielding ammonium salts of fatty acids, which accumulate in surprising concentration. To these must be attributed at least the greater part of the acute symptoms produced by the culture-filtrate in the guinea-pig; though doubtless intermediate products of proteolysis may play some part, especially in the delayed death which sometimes follows recovery from the immediate acute symptoms.

The chief interest of the observation seems to us to be the hint it furnishes as to one of the factors favouring spread of infection with the bacillus, in the tissues of an animal in which it has once obtained a hold. It is well known that with washed spores, or even with a broth culture of this organism, it is practically impossible to produce an experimental infection. Some local tissue injury is needed to give the infection a chance of success. This may be mechanical injury, as by contusion of tissue, or irritation by soil particles; or chemical injury, as by lactic or acetic acid. Among such chemical injuries we may now, with some reason, include the effect of the toxic juice formed by the bacillus from meat, which is essentially that of a strong solution of ammonium salts. Given injured tissues in which the growth may start, it is evident that the localized production of ammonium salts will soon be sufficient to create in the neighbouring tissues conditions favouring the spread of the growth, which may then go on indefinitely.

As to how far the production and absorption of ammonium salts, from a large area of infected tissue, may play a part in the general symptoms attending such an infection, we are not in a position to express any opinion. Nor have we studied the production of ammonia in culture by other members of the group.

We hope that these points may receive attention from those who are studying the effects of this group of organisms in the laboratory or at the bedside, and that this publication of observations, so unexpectedly simple in their outcome, may at least save others the trouble of a similar investigation. Our conclusion should, further, make clear the futility of attempting to obtain an antitoxin to the products of the organism with which our observations deal.

Lister Institute of Preventive Medicine, November, 1915.

THE ANTISEPTIC ACTION OF HYPOCHLORITES:

THE ANCIENT HISTORY OF THE "NEW ANTISEPTIC."

BY

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THE "new antiseptic" announced in the *Times* of August 8th, and subsequently by many other newspapers, was discovered in 1788 by the French chemist, Berthollet.¹ He obtained a liquid with bleaching and disinfecting properties by the action of chlorine upon aqueous alkalis. The composition of the mixture was obscure, but Berthollet presumed that additive compounds were formed between the halogen and alkali—for example, Na_2OCl_2 .

Berzelius in 1808 expressed the view that the product formed was a mixture of a salt of an unknown acid of chlorine with sodium chloride. The discovery of this acid

—hypochlorous acid—in 1834 by Balard² definitely settled the composition of Berthollet's fluid as a mixture of sodium chloride and sodium hypochlorite.

Four years after Berthollet's discovery—that is to say, in 1792—the corresponding potassium salts were made commercially by Percy at the Javel works near Paris by passing chlorine into crude potashes. The product was sold as eau de Javel,³ and its use as a disinfectant was advocated by Berthollet and by Guyton de Morveau.

In 1820 Labarraque³ prepared a fluid similar to Berthollet's by the action of chlorine (1 mol.) on an aqueous solution of sodium carbonate (2 mols.). This preparation containing much free alkali became known as "Liquor de Labarraque," or "Labarraque's disinfecting fluid." It is related that Labarraque, who was pharmacist first to the Emperor and subsequently to Louis XVIII, achieved great renown on the death of the latter monarch, for, thanks to the disinfecting and deodorizing value of his "liqueur," he was able to proceed with the embalming of the royal body, which was so profoundly decomposed that no one was able to approach it until after the application of the hypochlorite solution.

At a later date solutions of the hypochlorites of sodium and potassium were largely replaced by the more stable solid "chloride of lime," a combination of calcium chloride and calcium hypochlorite. It is interesting to note that it was with this substance that Semmelweis in 1846 succeeded in eradicating endemic puerperal fever from his clinic.

Subsequently the liquor de Labarraque and eau de Javel, as well as the liq. sodae chlorinatae of the *Pharmacopoeias*, were generally prepared by the decomposition of chloride of lime with the carbonates or sulphates of the alkali metals, and this simple process has been the subject of innumerable patents applied for long after the process was well established in common practice.⁴

In 1859 Charles Watt discovered that sodium hypochlorite could be formed by the electrolysis of sodium chloride solution, and a similar electrolytic process was patented by Andreoli in 1890. Since then scores of different modifications for the electrolytic preparation of hypochlorites have been advocated.

Other hypochlorite preparations, such as "extrait eau de Javel," "esprit de Javel," "essence de Boulogne," "chlorozone," "hermitine," "chloros," etc., need not be referred to in detail, since they represent hypochlorite preparations of varying purity and stability.

Meanwhile the hypochlorites, and by implication hypochlorous acid—for the latter is liberated from its salts by the carbon dioxide of the atmosphere—were finding an ever-extending application as general disinfectants. They were also employed for the sterilization of polluted supplies of potable water, and to a lesser extent for surgical dressings and for oral antiseptics. I am informed that "liquor sodae chlorinatae" has been used at St. Thomas's Hospital as a dressing for amputation stumps as long as sixty years ago. In more recent times weak hypochlorite solutions have been used with more or less success for removing sloughs and for controlling the smell of offensive wounds. As a skin disinfectant for use in pathological laboratories hypochlorite solutions have been used by Professor Delépine for a generation past.

The great drawback to the more general use of hypochlorites as antiseptics has been the strongly irritating character of the commercial solutions. In the early part of the present war some French surgeons made use of the ordinary "eau de Javel," but there were many accidents owing to its caustic action. It was for this reason, therefore, that it seemed worth while to try and obtain a solution of hypochlorite with fewer objectionable qualities. This was done in January of the present year, and after the solution had been in constant use for many months a short communication written by me was presented at the French Academy of Sciences. The essential parts of the communication are the following:

"Parmi les antiseptiques qui ont déjà été étudiés dans le traitement des plaies infectées, les hypochlorites surtout remplissent les conditions qui viennent d'être énumérées. Mais malheureusement les hypochlorites du commerce ont une composition très inconstante et contiennent généralement de l'alcali libre ou du chlore libre.

* At the present time in France the term "eau de Javel" refers usually to sodium hypochlorite solution and not the potassium salt.