which from the application in parallel of the ordinary methods of examination without preliminary incubation the existence of pure cultures of the meningococcus was suggested, it was at first difficult to resist the conclusion that contamination must have occurred. And the presence of the involution forms, and of organisms of a diphtheroidal form, made this conclusion doubly difficult to resist. Increasing experience, however, of the value of preliminary incubation of the body fluids in cerebro-spinal fever before inoculation of laboratory media—in attempting to determine their true bacterial content—soon enabled us to refute the frequently made suggestion of contamination as being merely the outcome of inexperience of the method. And this conviction was greatly strengthened by finding that the same types of organisms simultaneously occurred again and again in increasing numbers of cases, and this largely irrespective of whether the fluid examined was cerebro-spinal fluid, blood, or urine. Moreover, the apparent existence of pleomorphism, although, as we have said, not absolutely proved by culture from single individuals, showed itself so constantly in subculturing from single colonies as practically to exclude the possibility of perpetually recurring contamination with the same organisms.

And, finally, the suggestion of contamination we have again found to be quite inadequate to explain the results obtained by repeating the observations with a fresh series of 18 specimens of cerebro-spinal fluid in the summer of 1915. Of these 18 specimens 6 were submitted to filtration, a new Chamberland bougie being in each case employed, and in the majority of the specimens, filtered and unfiltered, submitted to complete examination the same essential results were obtained, and may be seen by reference to the full paper now in the press. The total number of specimens examined amounts, therefore, to 64, of which 11 were filtrates, a Chamberland F filter being employed in 8.

The stage, therefore, we have now reached is this—that the true bacterial content of the body fluids in this disease is by no means adequately represented by the meningococcus. On the contrary—provided that these fluids are thoroughly examined by (1) study of the morphology of their deposits in fresh and in incubated specimens, both filtered and unfiltered; (2) supplemented, after plating, by cultural and biochemical study in, or on, laboratory media inoculated with incubated material, filtered and unfiltered; (3) injection of monkeys with the fresh filtrates—ample justification will be found for directly attacking the problem of the true ancestry of the meningococcus. And I therefore propose to make this the next stage in the investigation of the etiology of the disease side by side with further experimental observations in monkeys.

CONCLUSIONS.

1. There is at present available no adequate evidence to show that the meningococcus of Weichselbaum is itself the causal agent of the disease cerebro-spinal fever.
2. On the contrary, the experimental evidence here recorded distinctly suggests that it is not.
3. The fresh filtrate of cerebro-spinal fluid in the two cases examined contained a virus which was highly infective to monkeys.
4. The true bacterial content of the body fluids in this disease can only, as a routine procedure, be satisfactorily determined by, in the first instance, using the fluids themselves as the optimum medium of growth, combined with injection into suitable animals in the fresh condition.
5. Incubation of the body fluids, infective to monkeys in the unincubated state, appears to destroy their infectivity and, in so doing, to throw some light on the ancestry of the meningococcus.

INFLUENCE OF CONCLUSIONS ON THERAPEUTIC AND EPIDEMIOLOGICAL CONTROL OF THE DISEASE.

1. Although the meningococcus appears itself to be incapable of reproducing the disease, the search for and isolation of carriers of this organism should, for the present, be vigorously pursued as heretofore, since its presence is a valuable danger signal that the true infective agent is, or has been, present.

2. Attempts to prepare antisera to the meningococcus, whether multiple strains are used or not, do not

appear likely to lead to effective therapeutic control of the disease.

3. Confirmation of the infectivity of fresh cerebro-spinal fluid would justify the preparation and trial of effective antisera thereto in the treatment of the disease.

4. Attempts to destroy the meningococcus in the naso-pharynx of contact carriers, as a means of epidemiological control, would appear to be less valuable than an attempt to determine what the true infective agent of the disease really is.

5. In order to discover the true infective agent, whether biologically related to the meningococcus or not, further research is imperative, attention being particularly directed to filtrable organisms in the naso-pharynx and the cerebro-spinal fluid of acute cases.

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STUDIES IN ANTISEPTICS (II):

ON CHLORAMINE: ITS PREPARATION, PROPERTIES, AND USE.*

BY

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REFERENCE was made in a paper on the use of certain antiseptic substances for the treatment of infected wounds, recently published in this *JOURNAL*,¹ to a number of compounds containing chlorine linked to nitrogen and possessing strong germicidal properties. Substances containing this type of linkage were studied because analogous compounds were believed to be formed by the action of the actively germicidal hypochlorites upon proteins and some other nitrogenous cell constituents. After studying a very large number of substances of the type referred to, a selection has been made of the one which on the whole seemed to possess the greatest number of desirable properties and which experience showed to be practically useful. The substance chosen is para-toluenesodium-sulphochloramide, and possesses the following structure:

The systematic name of this substance is somewhat unsuitable for general use, so that it has been decided to assign the more convenient name "chloramine" to the compound, thus indicating its membership in the group of substances containing the NCl linking. If, in future, other members of the group should come into use, it is suggested that the present compound should be known as chloramine-T to indicate its relationship with toluene.

Properties.

Chloramine, the preparation of which is described in a later section of this paper, is a white solid, crystallizing in prisms containing three molecules of water of crystallization. The solid has a very faint chlorous odour, while solutions of it are without smell. It is freely soluble in water, a saturated solution at room temperature containing

* This investigation was carried out on behalf and with the support of the Medical Research Committee.

about 15 per cent. of the salt. It may be dissolved in two parts of boiling water. The aqueous solution reacts faintly alkaline and has a bitter taste. It is an extremely stable substance, and in solid form may be preserved indefinitely, while aqueous solutions show no significant decomposition after keeping for several months.

Chloramine has no corrosive action even in concentrated solutions. It neither precipitates nor coagulates proteins such as blood serum, a property which is of great practical importance in the treatment of infected wounds. Chloramine is practically non-toxic, guinea-pigs and rabbits tolerating as much as 1 gram per kilo given subcutaneously, with no symptoms other than a moderate local reaction due to the injection of a strong solution.

The germicidal action of chloramine is intense, and when tested against the ordinary organisms found in infected wounds it is found to be about as powerful as an equal weight of sodium hypochlorite. The molecular weight of chloramine is about four times that of sodium hypochlorite, so that the germicidal action of one molecule of chloramine is about four times as great as that of a molecule of sodium hypochlorite. In addition, it is much less irritating than the latter substance, and may be used safely at a concentration five to ten times as great.

Table showing the Germicidal Action of Chloramine on Several Common Organisms.

Two drops of a fresh culture of the organisms were suspended in 5 c.cm. of fluid, either water or 50 per cent. horse serum, and the antiseptic was allowed to act two hours at room temperature. In comparison, a few figures for sodium hypochlorite and phenol are added.

	Chloramine.	Sodium Hypochlorite.	Phenol.
Staphylococci in water ...	1: 500,000— 1: 1,000,000+	1: 500,000— 1: 1,000,000+	1: 250— 1: 500+
Staphylococci in serum ...	1: 1,500— 1: 2,500+	1: 1,500— 1: 2,000+	1: 50— 1: 100+
<i>B. pyocyaneus</i> in water ...	1: 200,000— 1: 400,000+	1: 100,000— 1: 1,000,000+	1: 200— 1: 400+
<i>B. pyocyaneus</i> in serum ...	1: 1,250— 1: 2,000+	1: 2,500— 1: 5,000+	1: 25— 1: 50+
Streptococci in water ...	1: 1,000,000— 1: 2,500—		
Streptococci in serum ...	1: 5,000+		
<i>B. capsulatus</i> in water ...	1: 10,000,000— 1: 2,500—		
<i>B. capsulatus</i> in serum ...	1: 5,000+		

Complete sterilization is indicated by —, while + indicates that organisms survived.

Practical Uses.

Chloramine has already been used as an antiseptic for a variety of purposes with decidedly satisfactory results. It is not proposed at the moment to do more than outline some of its applications, leaving the detailed records of clinical results to others.

First of all a series of fresh but badly infected shell wounds containing dirt, clothing, and shell fragments were studied. The wounds were exposed, cleaned mechanically in the usual fashion, and lightly packed with gauze, leaving a narrow rubber tube or tubes passing to the bottom or recesses of the wounds. By means of these tubes 10 to 15 c.cm. of a 3 to 4 per cent. solution of chloramine was squirted at frequent intervals into the wounds by means of a glass syringe so as to moisten the whole surface of the cavity. The results were clinically similar to those observed in the early treatment of infected wounds with sodium hypochlorite,² with the exception that sloughs are dissolved somewhat more rapidly by the hypochlorite than by the chloramine. The majority of these wounds, though undoubtedly infected at the start, could be rendered aseptic after three to five days when treatment was commenced early. The wounds so treated were severe cases, including a number of fractures of the femur and humerus.

But the properties of chloramine seemed to indicate that it might find a more valuable application in cases where the more generally used antiseptics were either too irritating or too feeble. Accordingly it was used in a large number of cases of jaw and mouth injuries which are so apt to become extremely foul. Chloramine was used in 1 to 2 per cent. solution as a mouth wash, and a 2 per cent. solution was also squirted into the external wound cavities through short rubber tubes lightly sur-

rounded by gauze packing. The results were very encouraging. Some of these cases have been described by Surgeon Fisher, R.N., of H.M.H.S. *Iewa*, in a recent issue of this JOURNAL. As an antiseptic mouth wash 1 to 2 per cent. chloramine has been found to be of value in a variety of septic mouth cases.

In addition chloramine at 0.5 per cent. has been used for the irrigation of bladder and uterus in septic cases and the results are stated to be encouraging. A few cases of chronic urethral infections which had been unsuccessfully treated with silver preparations did well with injections of 1 to 2 oz. of chloramine four times daily, beginning at 0.5 per cent. strength and then increasing later to 1.5 to 2 per cent.

A practical point which may prove to be of value is the fact that gauze may be readily impregnated with large quantities of chloramine. It is possible, for example, to get as much as 10 grams of chloramine in a four-fold roll of gauze 1 yard by 4 in. The use of this impregnated gauze for packing infected wounds is being investigated at the present time by Sir Berkeley Moynihan. There are very few substances of high antiseptic value which can be successfully used for impregnating gauze. Chloramine gauze obviously should not be moistened before use or the antiseptic will dissolve out. It can be used dry for lightly packing and subsequently moistened if necessary when in position.

Preparation and Cost.

Toluene-sodium-sulphochloramide (chloramine) was first prepared by Chattaway³ by the action of sodium hydroxide upon toluene sulpho-dichloramide, a substance obtained by the action of acetic acid on toluene sulphonamide dissolved in bleaching powder solution. A more economical method for its preparation consists in dissolving p-toluene sulphonamide (1 mol.) in a 5 per cent. cold alkaline solution of sodium hypochlorite (1.2 mol.), warming gently if necessary, filtering, and adding $1\frac{1}{2}$ volumes of saturated brine. The chloramine crystallizes out of solution as a white shining meal of crystals, and is filtered off, washed with brine, and dried in the air.

The toluene sulphonamide necessary for the preparation is prepared by acting upon para-toluene sulphonic chloride with ammonium carbonate. The sulphonic chloride is a by-product in the manufacture of saccharin, and in ordinary times can be obtained very cheaply. But even when made directly from toluene, the finished chloramine should be a relatively cheap antiseptic, especially when its high germicidal action is considered. The cost of a 2 to 3 per cent. solution such as is commonly used should be considerably less than that of 10 volume hydrogen peroxide. It is anticipated that the manufacture of the substance on a commercial scale will be undertaken shortly by some British firm.

Mode of Action.

In its chemical and biological reactions chloramine closely resembles the hypochlorites in many respects. Its antiseptic action, while largely due to its contained chlorine, is not simply due to the liberation of sodium hypochlorite from the chloramine, for its antiseptic action is four times as great as that of the hypochlorite which could be obtained from it. It is believed that the antiseptic action of hypochlorites is due to their capacity for attacking proteins and related bodies, with formation of substances containing chlorine linked to nitrogen. Chloramine itself appears to act in the same way, for when brought into contact with proteins, such as blood serum, or with peptones or amino-acids, it parts with its chlorine, which attaches itself to the nitrogen of the second substance. With the simplest amino-acid, glycine, the reaction can be represented as follows:

In this particular case the chloramino-acetic acid formed is unstable, and breaks up to give formaldehyde and other products.

Practically, the matter may be stated as follows: Chloramine represents an active antiseptic containing a store of chemically combined chlorine in a form which is quite stable and non-irritating under ordinary circumstances. But when brought in contact with proteins and similar cell constituents containing basic (NH_2) groups, it acts as a chlorinating agent losing its chlorine to the basic

substances, and thereby exerting its antiseptic action as needed.

Chloramine, being a highly reactive substance, should not be mixed with other antiseptics. Both alcohol and hydrogen peroxide are decomposed by it.

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GASSING ACCIDENTS FROM THE FUMES OF
EXPLOSIVES.*

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CASES of gas poisoning account every year for a good many deaths in the mines of the Rand, and for a considerably larger number of cases of temporary disablement and loss of working time.

The irritant acid fumes, commonly known as nitrous fumes, consist of a mixture of oxides of nitrogen. They belong to the group of respiratory irritant poisons in which chlorine, the vapours of bromine and ammonia, sulphuretted hydrogen and sulphurous acid, are also included. All of these cause, when inhaled, a somewhat similar train of symptoms. Fatal poisoning by nitrous fumes has occurred when persons have inhaled the acid fumes from a vessel containing nitric acid, which has been accidentally broken. In mines, poisoning from this source only occurs after the use of explosives in cases where the detonation has been imperfect, and particularly where burning of explosives has taken place. Nitric oxide (NO) is first formed, but this gas is rapidly oxidized, and nitrous fumes consist chiefly of nitrogen peroxide¹ (NO₂). This again quickly reacts with water vapour to form nitrous acid. What is actually inhaled is mainly a mixture of nitrous and nitric acids. Nitrous fumes are the red irritant fumes with the smell of which most miners are familiar. The smell is that of fuming nitric acid. The gas is intensely poisonous, and of all mine gases it is the most treacherous. We do not know the downward limit of its toxicity. Exposure to as little as 0.05 per cent. of nitric oxide for half an hour was found by Haldane to cause death in mice after about twenty-four hours, with the typical sequence of symptoms, and the symptoms were the same whether nitric oxide or the fumes of burning dynamite were added to the air. It was found that with small percentages of nitrous fumes the haemoglobin was normal at the time of death. With higher percentages (and more rapid death) the blood was found to be more or less chocolate coloured, and to contain methaemoglobin. Under the latter circumstances nitrous fumes act like carbon monoxide by disabling the haemoglobin, and may in concentration produce rapid death. Where the exposure is to small amounts death is due solely to the lung inflammation and its immediate consequences.² It is with this latter form of poisoning that we are locally familiar. Half of the amount mentioned is the highest figure (0.026 per cent. reckoned as NO₂) found by Mann in mine air after blasting,³ but the presence of the gas in poisonous amounts is probably not so very uncommon in ordinary blasting practice. I shall give some evidence for this statement later. What is certain is that quite a brief exposure to small quantities of nitrous fumes is sufficient to produce serious and even fatal poisoning. Air which contains enough nitrous fumes to cause feelings of irritation in the nose or air passages is very dangerous.

Dr. D. Macaulay was the first to direct attention to the important part played by nitrous fumes poisoning in gassing accidents on the Rand, and to give an adequate account of its symptoms and treatment.⁴

SYMPTOMS.

The symptoms of poisoning by nitrous fumes are very characteristic. At the moment of exposure they are slight. A man who inhales nitrous fumes has a sense of

irritation in the nose and throat, and of constriction, and perhaps pain, in the chest. There is headache, smarting of the eyes, and there is coughing. The latter is a characteristic sign. But these immediate irritant effects may not be severe, and commonly pass off altogether in a short time. The man may feel quite well and may continue work. He leaves the mine, has his supper perhaps, and goes to his room. Then, in from perhaps four to eight hours afterwards (very rarely later), acute symptoms suddenly come on, and progress with alarming rapidity. In a typical severe case there is marked and increasing distress in breathing, with coughing, and often severe pain in the chest. The cough is at first dry, and auscultation may at this stage reveal no moist sounds. But this condition is speedily followed by the expectoration of a copious frothy, rather fluid, blood-stained spit. The lungs become waterlogged, and auscultation now reveals copious moist sounds. There is cyanosis and marked dyspnoea and distress, followed, unless the case is promptly treated, and very often in spite of all treatment, by collapse, unconsciousness, and death *within a few hours*. Dr. Macaulay states that "the temperature is at first normal or subnormal, but, if the gas goes on for several hours, a rise takes place." I well remember the first case of this condition which I saw, now more than ten years ago. A miner was taken ill suddenly in his room on one of the mines at 11.30 p.m. I saw him at 1 a.m. He was cyanosed and in much distress. His lungs were full of moist sounds and one base was dull. The wooden floor of his room was covered with a blood-stained fluid expectoration. He died at 5 a.m. I could not reconcile his condition with that of any form of acute pneumonia which I have seen, but it agreed at every point with the published descriptions of nitrous fumes poisoning. It is probable, I think, that a certain number of cases returned as "pneumonia" amongst underground workers are really cases of nitrous fumes poisoning.

The typical sequence of symptoms in nitrous fumes poisoning is therefore this:

1. *Initial symptoms* of irritation occurring at the moment of exposure and usually comparatively slight. Nitrous fumes never, in our experience of ordinary gassing accidents, produce partial or complete unconsciousness at the time of exposure, as does carbon monoxide, although they may do so if present in massive amounts, and cases of rapid death from the concentrated fumes of burning explosives may be partly due to this cause.

2. *A latent period of several hours' duration*, during which the patient may and commonly does feel quite well.

3. *The sudden onset after that interval of acute symptoms*, due to a rapidly progressive inflammatory oedema of the lungs. The appearance of this well marked symptom sequence is of great diagnostic significance.

The *post-mortem* signs are also definite. There is an intense injection of the trachea and bronchi, which is characteristic. The lungs are intensely oedematous, often enlarged and tense with oedema. A copious frothy, blood-stained fluid exudes from them on section, and may be seen to occupy the air passages. There may be patches of incomplete consolidation, subpleural haemorrhages may be observed, and blood-stained fluid in the pleural cavities or the pericardium. The right heart and the great thoracic and abdominal veins are engorged with dark, thick, sometimes almost tarry, blood. The abdominal veins especially are often very greatly distended.⁴

There is one other point. Miners from time to time complain that after slight exposure to blasting fumes—perhaps from blasting at some distance from their working place—they are attacked the same night with difficulty of breathing, and perhaps slight spitting of blood, which pass off in a few hours. The affected man thinks he has had an attack of "asthma" or "bronchitis." He has really had a slight attack of nitrous fumes poisoning. The most sedulous care must be taken that, if any casual blasting has to take place before the end of the shift, the water blast shall be invariably employed immediately after, and that the fumes are thoroughly dispersed. Precautions must be taken, also, in the general blasting at the end of the shift, in order to obviate the risk of inhalation of drifting fumes of this dangerous gas by men waiting on the stations before being "hauled" up the shaft.⁵

Finally, I may state that the intensely irritating character of nitrous fumes has suggested that the repeated

* Extracts from a paper on accidents from poisonous or asphyxiating gases in mines, dealing generally with the subject, and also with the organization of the use of rescue appliances in mines, read before the Witwatersrand Branch of the British Medical Association, and published in full in the *Medical Journal of South Africa*.