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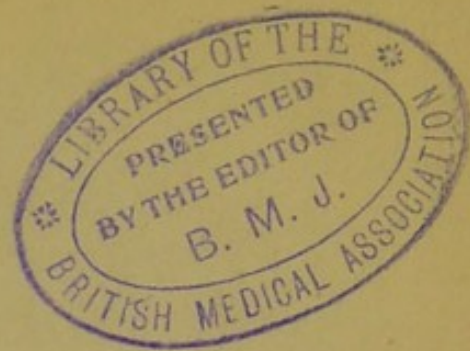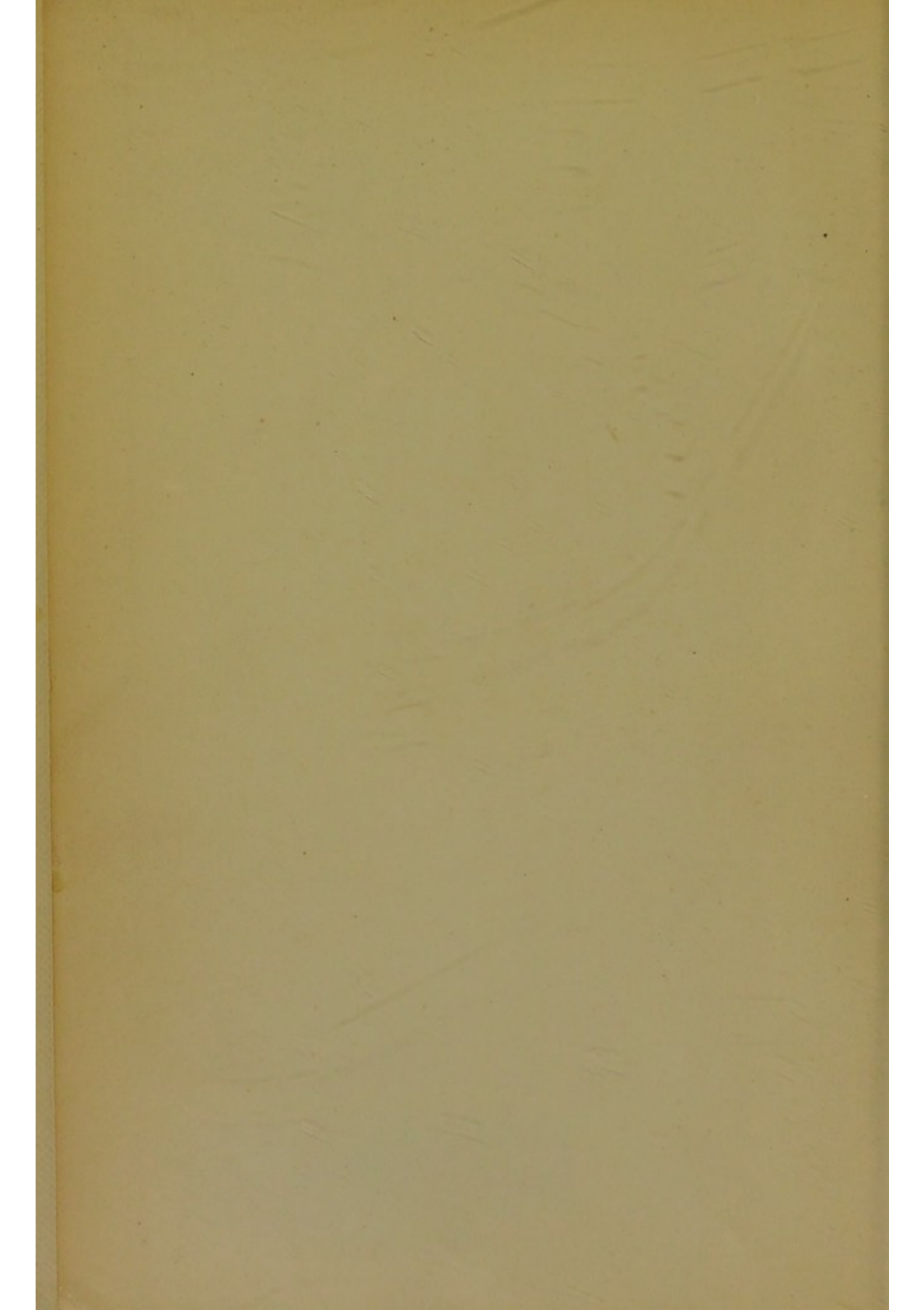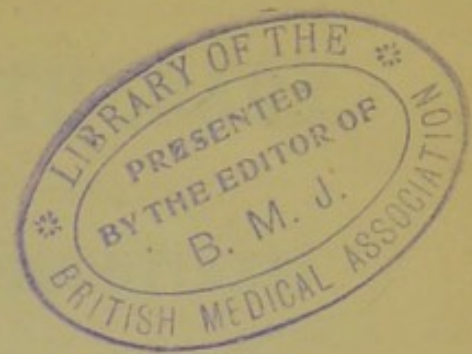
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SERUM AND VACCINE THERAPY

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BACTERIAL THERAPEUTICS AND PROPHYLAXIS

BACTERIAL DIAGNOSTIC AGENTS

By

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SECOND EDITION

PREFACE

THE SECOND EDITION

In this second edition the text has been revised throughout, and while the general character and scope of the first edition has been retained, much new matter has been incorporated. The great advances in the treatment of disease by bacterial vaccines have necessitated the inclusion of a new chapter on this subject. A chapter on sour milk is also added.

For further information on immunity, bacteriological technique, and pathological data the reader is referred to the writer's 'Manual of Bacteriology' and 'Pathology.' The preparation of therapeutic sera is exhaustively treated in the 'Handbuch der Technik und Methodik der Immunitätsforschung,' edited by Professor Dr. Kraus and Dr. Levaditi (Gustav Fischer, Jena, 1909).

I have to thank Mrs. Allan Macfadyen for permission to make use of figures 13 and 26 from 'The Cell as the Unit of Life.'

I am indebted to my friends and colleagues Mr. Laurie McGavin, for a description of lumbar puncture, and Dr. Frank Taylor, for help in the revision of the proof sheets.

<div align="right">R. T. H.</div>

PREFACE

TO

THE FIRST EDITION

In this little book I have endeavoured to give a concise account of the preparation of, and the treatment of disease with, antitoxins and anti-sera, and various other substances, vaccines, &c., obtained from bacterial cultures and the like. In all cases brief directions are given for the making and testing of the various preparations; these are not in any way complete, since many small details, so conducive to success, can be learnt only by practical experience in the laboratory.

In order to render the subject-matter more complete, short descriptions of certain substances of a somewhat allied nature, such as the typhoid extract of Jez, cancroin, &c., together with blood-transfusion and saline infusion, have been included.

Further information upon immunity, and full details for the isolation and cultivation of the various micro-organisms mentioned, will be found in the writer's ' Manual of Bacteriology' (J. & A. Churchill, 2nd ed., 1902).

I have to thank Messrs. ALLEN & HANBURYS for the loan of block illustrating antitoxin syringes.

KING'S COLLEGE, LONDON,
   July 1903.

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SERUM THERAPY

CHAPTER I

INTRODUCTION—IMMUNITY—EHRLICH'S 'SIDE-CHAIN' THEORY—ANTITOXIN FORMATION—ANTI-MICROBIC SERA—HÆMOLYSIS

INTRODUCTION

The fascinating study of the production of immunity or insusceptibility to morbid conditions is one that dates back to remote times, though it is true that in the early and middle ages the insusceptibility aimed at was mainly against poison. It can hardly be doubted also that the ceremony of blood brotherhood, the history of which is lost in the mist of the past, was one in which by the interchange of the blood of two individuals something of their natures was supposed to be transferred one to the other so that they would in the future act together in harmony and to their common good. During the middle ages injections of blood were given for

various purposes ; among others, lamb's blood was used in the treatment of leprosy. It was not, however, until comparatively recent times that the study of the nature and production of immunity or insusceptibility to disease was systematically and scientifically pursued.

The first landmark which stands out pre-eminent above others was the discovery, by Jenner, more than a century ago, of the protective action of vaccinia against small-pox. The establishment of the truth of the germ theory of disease was almost a necessary preliminary to further advancement, and we have the remarkable work of Schwann, Tyndall, Davaine, Pasteur, Lister, and Koch in this direction. To Pasteur was reserved the honour of first artificially producing immunity to infective disease—namely, in anthrax, chicken cholera, and, best known of all, in rabies. In the Pasteurian method the *materies morbi* is artificially modified and weakened, and, on injection, causes a transient illness, which is soon recovered from, but which, for a limited period at least, protects against the disease. There has been, in recent years, a return to the Pasteurian method, as witness the cholera and plague vaccines of Haffkine and the typhoid vaccine of Wright. At first the Pasteurian

method was employed solely for prevention, and not as a means of cure; but more recently, mainly through the work of Wright and his collaborators, this kind of inoculation has been largely used in the treatment of infections, particularly when these are not acute. A new departure was made when Salmon and Smith in America found that the chemical products of the hog-cholera bacillus, freed from the micro-organisms themselves by filtration through porous porcelain, would produce immunity and protect an animal against injections of the living microbe. This fact, as is well known, was extended to diphtheria and tetanus through the researches of Roux, Vaillard, Behring, and others ; but it was reserved for Behring to show that the immunity produced by the injection of bacterial toxins could be transmitted to a second animal by injections of the blood-serum of the first or treated one, and that this blood-serum, termed antitoxin, could also be used as a curative agent. Although the practical application of antitoxin treatment is so largely due to Behring and Roux, no man has done more for the study and theory of immunity than Ehrlich, whose marvellous inductions, confirmed as they have been by most beautiful and masterly experimental methods, must place him

in the forefront of investigators in this difficult subject.

Immunity

In the first place, it is necessary to consider what is meant by immunity. Immunity is, briefly, insusceptibility to disease, generally to an infective disease. An infective disease is one which is caused by a living *materies morbi* or micro-organism, and is capable of being transmitted from one individual to another : it is an infection in contradistinction to an intoxication, in which the agent that causes the disease is a chemical substance, which is the product of the activity of a living organism or cell. Infective diseases include both the infectious and the contagious disorders so-called, between which there is no real distinction. It is true that in many disorders regarded as infective, no causative organism is known with certainty—for example, in small-pox, typhus fever, and chicken-pox ; but there can be little doubt that such are due to living organisms. Ptomaine poisoning proper, *e.g.* such as occurs from eating tinned foods, is an intoxication caused by toxic chemical substances elaborated by micro-organisms in the tinned food consumed.

It is also a striking and undoubted fact that some individuals are much more prone to the attacks of infective disease than are others. One individual will pass through life almost untouched, a second and less fortunate one seems to contract every possible disease. Immunity or insusceptibility to infective disease is not confined to man, but is a peculiar property of all living things, both animal and vegetable. Thus the white rat and Algerian sheep are not liable to anthrax infection ; glanders, which is common in the horse, is rare among cattle, and is unknown among swine. The monkey and ox suffer grievously from tuberculous disease, which in the dog and goat is hardly ever met with. Dealing with the vegetable kingdom, it is the aim of the horticulturist to produce varieties, especially of vegetables, insusceptible to the ravages of the various diseases, bacterial and fungoid, to which they are liable. Thus varieties of wheat and of the potato have been obtained by artificial selection which are far less liable to be attacked by the rust and by the dreaded potato disease respectively, due to fungi, than are others. Immunity or insusceptibility to disease is probably never absolute ; thus the fowl, which is almost insusceptible to the tetanus toxin, and can be given a dose

which would kill hundreds of guinea-pigs or rabbits, may be tetanised by massive doses of the toxin, and an individual who has passed unscathed through many epidemics of infective disease may ultimately become infected.

Many varieties of this immunity or insusceptibility may be distinguished. In the first place, there is a natural and there is an acquired immunity ; the former, natural immunity, is that which is pre-existent or inherent in the individual species ; acquired immunity, on the other hand, is that which is induced in some way. As regards natural immunity, this may be racial, appertaining to the race, for example : the black man is comparatively insusceptible to malaria and yellow fever, the result probably of the action of natural selection, and the Algerian sheep and white rat are immune to anthrax, while the European sheep and the brown rat are highly susceptible. Cases of individual immunity also occur ; a small proportion of persons of the white race, for instance, seem to be insusceptible to malaria. Natural immunity is doubtless due to a number of different factors, such, for example, as the body temperature, the degree of alkalinity of the blood and tissues, the presence of substances protein in nature, which exert an inhibitory

power on the development of, or a germicidal action upon, the specific micro-organisms, phagocytosis, and the inhibitory action of other micro-organisms. As regards the latter, Metchnikoff ascribes the immunity of animals to intestinal cholera as being largely due to the microbial flora of the intestine antagonising the action of the cholera microbes. Again, the sour-milk treatment now in vogue seeks by means of lactic-acid producing microbes to inhibit the development of undesirable microbes in the intestine. In the case of immunity to toxins, which, however, are relatively uncommon in Nature, on Ehrlich's 'side-chain' theory, to be discussed immediately, it may well be that the atomic groups which unite with the toxin molecule and so give rise to the toxic action are absent, and the cells are, therefore, immune.

It is, however, acquired immunity, and especially its artificial production, that is of chief interest. Acquired immunity is frequently produced as a result of an attack of disease ; it may be extremely marked and lasting, as in the case of small-pox, yellow fever, and scarlet fever, or is transient and ill-defined, as in diphtheria, erysipelas, influenza, and pneumonia, and in the two last, susceptibility may

even be increased shortly after the attack. That a transient immunity is produced in the last-named diseases seems certain, for it is difficult to conceive that the disease processes would otherwise come to an end.

There are also many artificial methods of producing an acquired immunity, the chief of which are the following.

1. By treatment with a modified and attenuated or less virulent form of the infective agent. This is the Pasteur system of vaccination, and is applied in anthrax and other diseases (*e.g.* cholera). The anthrax vaccine is prepared by growing the anthrax bacillus at a relatively high temperature (40–41° C.) or in the presence of small quantities of antiseptic, that is to say, under unfavourable circumstances, whereby its virulence is diminished to such an extent that a transient illness only and not death is produced on inoculation. The Pasteur system of inoculation for hydrophobia is probably based on the same principle, and it can hardly be doubted now that vaccinia is modified variola.

2. Secondly, immunity may be induced by cautious treatment with killed cultures, or with bacterial toxins. The typhoid and plague vaccines are killed cultures of the respective

microbes, while by treating an animal with gradually increasing doses of tetanus or diphtheria toxin, immunity to tetanus or diphtheria may be induced.

3. Thirdly, by injections of the blood-serum of an animal which has been treated by method 2, that is, with sterilised cultures or with bacterial toxins.

4. Fourthly, in rare instances by treatment with sterilised cultures or toxins of a different species ; thus sterilised cultures of the *Bacillus pyocyaneus* will protect against anthrax, and of the *Bacillus prodigiosus* against the *Bacillus coli*. Emmerich and Loew have isolated from cultures of the *Bacillus pyocyaneus* an enzyme-like body which possesses protective and curative properties against diphtheria.

Ehrlich by his classical experiments with abrin and ricin, two toxic proteins obtained from the jequirity and castor-oil beans respectively, concluded that acquired immunity is of two kinds, one ' active,' as he termed it, of long duration and resulting from an attack of the disease or vaccination with a modified virus and not transmissible to the fetus ; the other, ' passive immunity,' resulting from the inoculation of an animal with the blood-serum derived from another animal immunised by the injection

of bacterial toxins or of cultures. 'Passive immunity' is soon lost, but while present is transmitted to the fetus.

Anti-Sera

If an animal be treated for a long period of time with bacterial toxins or with bacterial cultures, the animal acquires a high degree of insusceptibility and its blood-serum may be used to confer immunity upon, or to cure disease in, a second animal. The blood-serum of an animal so treated and possessing these properties is termed generally an antitoxin or an anti-serum. There are two classes of curative sera, the one antagonising the bacterial toxins such as diphtheria and tetanus antitoxins, to which the term antitoxin is alone strictly applicable, the other antagonising the microbes, killing or otherwise disposing of them. This latter class may be termed anti-microbic sera ; such are anti-streptococcic and anti-plague sera. In all cases probably both anti-toxic and anti-microbic substances are present, but in an antitoxic serum the antitoxic constituent is relatively in very large excess, while in an anti-microbic serum it is almost negligible. The process of immunisation of the animal for the production

of anti-serum may be an extremely tedious one, extending over weeks or months. At first minute doses of the toxin or culture are administered, and as the animal becomes accustomed to the treatment, the dose which is administered, either subcutaneously or intravenously, is gradually and progressively increased. From time to time tests are made as to the protective power of the serum, and when this has reached a sufficiently high degree the animal is bled, the blood allowed to clot, and the serum bottled for use.

Nature and Formation of Anti-Bodies

What is the nature of these anti-bodies in the anti-sera ? how are they formed ? and how do they act ?

In order to answer fully these questions it will be preferable in the first place to discuss the mode of interaction which takes place between toxin and antitoxin, and for this purpose diphtheria toxin and diphtheria antitoxin may be taken as examples. Toxin and antitoxin antagonise each other, and at one time, especially under the influence of certain experiments by Büchner, it was believed that this interaction was a vital one, the antitoxin in

some way rendering the cells insusceptible to
the toxin. The quantitative experiments of
Ehrlich with diphtheria toxin, and the filtration
ones of Martin and Cherry with the same, and
also with snake venom, seem to prove that
the interaction between toxin and antitoxin is
a chemical combination analogous to the com-
bination of a strong acid with a strong base—
for example, hydrochloric acid and sodium
hydrate—an innocuous compound being formed,
and that the interaction follows the ordinary
laws of chemical combination. Thus cold
retards while concentration and warming hasten
the combination, and an interval of time is
required for the complete interaction to take
place. A mixture of toxin and antitoxin kept
in contact for a short time may still be toxic,
but after a longer time becomes non-toxic. If
a certain definite amount of diphtheria anti-
toxin, which may be termed one immunising
unit, be mixed with varying quantities of a
given diphtheria toxin, an amount of the toxin
which is exactly neutralised by this amount
of antitoxin, that is, by one immunising unit,
can always be determined. Ehrlich found, for
example, on using one-tenth of an immunising
unit of antitoxin, that the quantity of a cer-
tain toxin which was exactly neutralised was

0·24 c.c. On making an analogous determination with *ten* times the amount of antitoxin—that is, with one immunising unit—the maximum amount of toxin which could be given with it without producing any effect, that is was exactly neutralised, was found to be 2·4 c.c., namely, just ten times the previous amount. To put it algebraically, let AT=one unit of antitoxin, T=toxin, in cubic centimetres, and o=exact neutralisation, there being an excess neither of antitoxin nor of toxin, then in the first instance

$$(1) \quad \frac{AT}{10} + 0·24\ T = 0,$$

and in the second instance

$$(2) \quad AT + 2·4\ T = 0.$$

The amount of a toxin which when mixed with the unit of antitoxin is just neutralised is termed by Ehrlich the L_o dose (L=*limes*= boundary, *i.e.* between life and death).

It is also possible to mix antitoxin with an excess of toxin to the extent that a simple lethal dose of toxin remains unneutralised. This simple lethal dose of toxin which is thus left unneutralised is just sufficient to cause the death of a guinea-pig weighing 250 grams on the fourth or fifth day after inoculation ; the

amount can be experimentally determined with considerable accuracy, and has been termed by Ehrlich the L_+ dose. In the case of a certain toxin, on using one-tenth of an immunising unit of antitoxin Ehrlich found the L_+ dose was 0·037 c.c., but using ten times the amount of antitoxin, *i.e.* one immunising unit, the L_+ dose of toxin was only 0·26 c.c. and not 0·37 c.c. ten times the previous amount, as in the case of the L_o dose. Obviously in the last example ten times the amount or 0·37 c.c. would leave *ten* lethal guinea-pig doses free instead of *one*. Or, to express it algebraically, let AT=one unit of antitoxin, T=toxin, in cubic centimetres, and LD=the simple lethal dose, then in the first instance

$$(3) \quad \frac{AT}{10} + 0·037 \; T = 1 \; LD,$$

and in the second instance

$$(4) \quad AT + 0·26 \; T = 1 \; LD.$$

Evidently if ten times the amount of toxin had been used

$$(5) \quad AT + 0·37 \; T = 10 \; LD.$$

An example will make this clear. If ten equivalents of NaOH be mixed with eleven equivalents of HCl, one equivalent of HCl will remain unneutralised. If, however, the same

amount of acid is to remain free on using ten times the amount, or 100 equivalents, of alkali, there must be added not 10×11, or 110, but only 101 equivalents of acid, HCl. To express it in the form of an equation, in the first instance

$$(3') \quad 10 \text{ NaOH} + 11 \text{ HCl} = 10 \text{ NaCl} + 1 \text{ HCl left unneutralised};$$
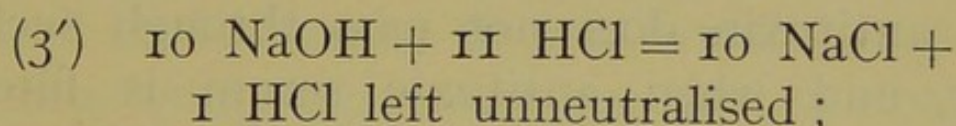
in the second instance if ten times the amount of alkali and of acid had been used

$$(4') \quad 100 \text{ NaOH} + 110 \text{ HCl} = 100 \text{ NaCl} + 10 \text{ HCl left unneutralised.}$$
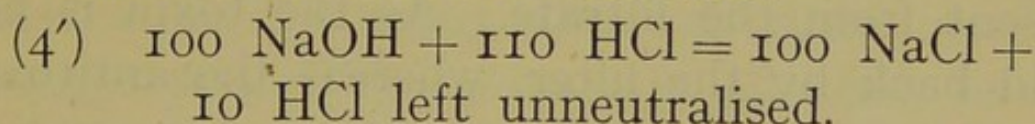
In order that only one equivalent of HCl shall be left unneutralised, the amounts to be used must evidently be

$$(5') \quad 100 \text{ NaOH} + 101 \text{ HCl} = 100 \text{ NaCl} + 1 \text{ HCl left unneutralised.}$$
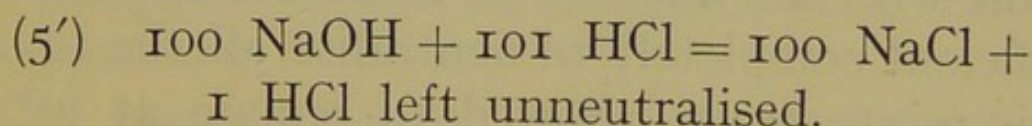
This shows that the neutralisation of toxin by antitoxin follows the laws of chemical combination.

In Martin and Cherry's experiments, mixtures of toxin and antitoxin were filtered through a gelatin-coated Chamberland filter after varying periods of contact. The gelatin-coated filter, devised by Martin, consists of a Chamberland porcelain filter which has been soaked in melted gelatin. This renders the pores very

fine, so fine indeed that albumin will not pass through, presumably because its molecule is too large to do so ; substances, however, which have a smaller molecule, such as sugar and also bacterial toxins, still pass through at a pressure of 50 atmospheres. Brodie showed that antitoxin does not pass through such a filter, and when antitoxic serum is filtered through gelatin the whole of the protein, and together with this all antitoxic virtue, is absent from the filtrate. As the toxin is not held back by the filter, whereas the antitoxin is, this provides a physical means of separating them, *provided they have not combined with each other*. Martin and Cherry mixed diphtheria toxin with an amount of antitoxin more than sufficient completely to neutralise all the toxin. This mixture was allowed to remain at 30° C. for two hours, and was then filtered through the gelatin filter : the filtrate was found to be quite innocuous. If the toxin had remained uncombined it presumably would have passed through the filter ; as it did not do so, the conclusion is that it had entered into some sort of chemical combination with the large antitoxin molecules. Another method was employed with snake venom. One of the toxic constituents of snake venom may be heated to 90° C. without injury,

whereas the snake-venom antitoxin, or anti-venin, is rendered inactive by heating to 68° C. for ten minutes. Martin and Cherry made mixtures of anti-venin and venom, pipetted off small portions at stated intervals, heated them at once to 68° C. to destroy the antitoxin, and injected into animals. It was found that when the anti-venin and venom are kept in contact for only a short time—two minutes to ten minutes, according to the amount of venom—death ensued ; whereas, when kept in contact for a longer period, the animals in all cases lived, showing that, as for all chemical combinations, time is an important factor. These experiments seem to prove that the neutralisation of toxin by antitoxin is due to a chemical union or combination.

The toxic action of toxin upon protoplasm would also seem to be due to a chemical union between the two ; and Ehrlich assumes that the toxin molecule possesses two different combining groups : one, which may be designated the ' haptophore ' group, conditions the union with the cells (and also with antitoxin), while the other, which may be designated the ' toxophore ' group, is the cause of the toxic action. The cause of toxic action is the presence of ' toxophile ' groups in the cells which unite with

2

the toxophore groups of the toxin. If toxophile groups be absent, the toxophore groups of the toxin are unable to act, and no toxic action ensues. A diagram may make this clearer. In fig. 1 the cell is shown with protuberances to represent the receptor and toxophile groups respectively, and the toxin with depressions
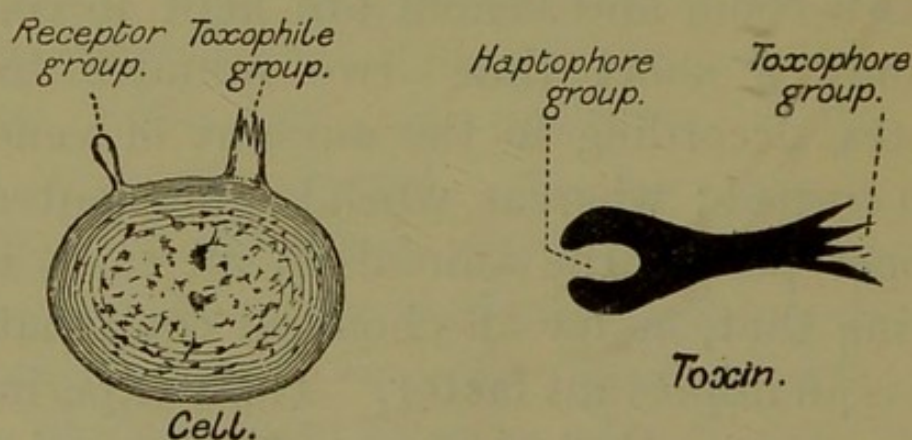
FIG. 1.—DIAGRAM TO REPRESENT THE COMBINING GROUPS OF THE CELL AND OF THE TOXIN RESPECTIVELY.

representing its haptophore and toxophore groups. It will be noted that the protuberances representing the receptor and toxophile groups of the cell are shaped so that they would exactly fit into the depressions representing the haptophore and toxophore groups respectively of the toxin—that is, the cell and the toxin could unite (see also fig. 3, p. 25). Ehrlich suggests that the receptor and toxophile groups subserve normal functions in the animal organism, and that they only incidentally, and

by pure chance, possess the capacity to unite with this or that toxin, for it is inconceivable that these atomic groups should exist simply for the purpose of fixing various toxins. Toxin may unite with the cells not only *in vivo*, but also *in vitro*. Thus tetanus is absorbed by fresh tissues *in vitro*, and so great is the affinity of fresh nervous tissues for it, that if an emulsion of tetanus toxin and fresh guinea-pig brain be prepared, the toxin unites so firmly with the nerve cells that the mixture is non-toxic, and can be injected into a guinea-pig without harm.

That toxin unites chemically with the cells seems, therefore, to be certain, whereas the alkaloids, anilin dyes, &c., form unstable combinations, if they combine at all, and can often be removed by the action of simple solvents such as alcohol and ether. The union of these substances with protoplasm seems to be of the nature of a ' solid solution ' analogous to that of the constituent metals in an alloy, or an ' adsorption phenomenon ' similar to the fixation of many dyes in the fibres of the fabric.

Ehrlich's 'Side-Chain' Theory

We are now in a position to formulate Ehrlich's ' side-chain ' theory. Ehrlich assumes

2 *

that the living matter, protoplasm or bioplasm, of the living cells of the animal body consists of a huge molecule or group of molecules with a number of atomic groupings, or, as they are termed by the organic chemist, side-chains, which are ready to enter into combination with other suitable atomic groups should the latter happen
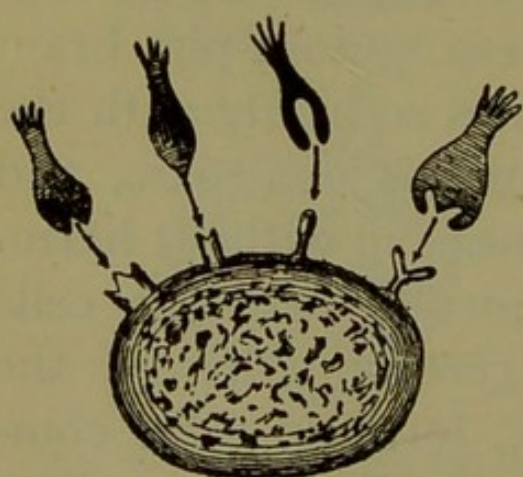
FIG. 2.—DIAGRAM TO REPRE-SENT THE CELL WITH ITS VARIOUS COMBINING GROUPS OR SIDE-CHAINS. (After EHRLICH.)

to be present, under the requisite conditions. Thus in fig. 2 a cell is depicted with protuber-ances of various shapes. These are the 'side-chains' which will only unite with other side-chains with which they have an affinity. The free bodies represent the latter, each will unite with the cell only by the protuberance, *i.e.* side-chain, to which it is apposed, as is indicated by the varying shapes. These free side-chains might belong to various food-stuffs, toxins, &c.

As a simple example of side-chains the chemical nature of the benzene ring may be considered. This is composed of 6 atoms of carbon and 6 atoms of hydrogen,

linked together to form a closed chain or ring, thus : [1]

$$
\begin{array}{c}
\text{H} \\
\text{C} \\
\text{HC} \quad \text{CH} \\
\text{HC} \quad \text{CH} \\
\text{C} \\
\text{H}
\end{array}
= \begin{array}{l} \text{Benzene,} \\ C_6H_6 \end{array}
$$
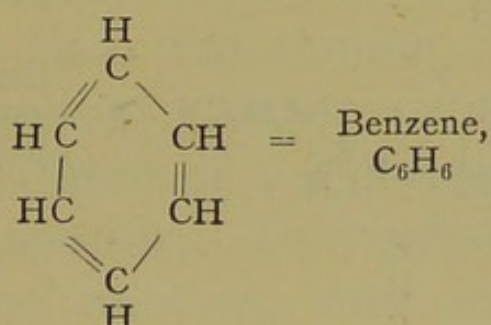
If into one of the CH groups composing this ring a methyl group is introduced by replacing the hydrogen H with methyl CH_3, the following grouping is obtained :

$$
\begin{array}{c}
\text{H} \\
\text{C} \\
\text{HC} \quad \text{C}-CH_3 \\
\text{HC} \quad \text{CH} \\
\text{C} \\
\text{H}
\end{array}
= \begin{array}{l} \text{Methyl} \\ \text{benzene} \\ \text{or toluene,} \\ C_7H_8 \end{array}
$$
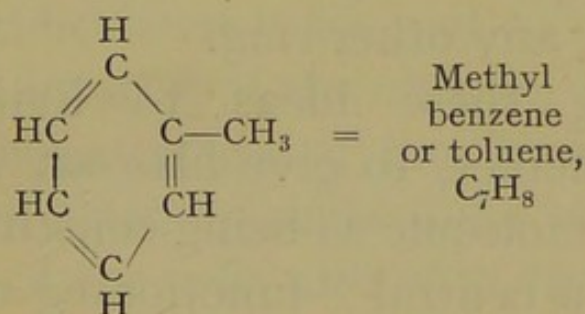
The methyl group CH_3 in this compound forms the side-chain and will readily unite with other atoms or atomic groups. If, for example, chlorine be allowed to act upon it, the compound C_7H_7Cl is formed, and on treating this with potassium cyanide a cyanogen group

[1] This is the orginal conception of the constitution of the benzene ring due to Kekulé, and suffices for our purpose, though it is to be noted that other constructions have been formulated.   See *Encyclopædia Britannica*, 10 ed., art. 'Chemistry.'

may be introduced, leaving all the rest intact, thus forming benzyl cyanide :

$$
\begin{array}{c}
\text{H} \\
\quad\text{C} \\
\text{HC} \qquad \text{C—CH}_2\text{CN} \\
\text{HC} \qquad \text{CH} \\
\quad\text{C} \\
\text{H}
\end{array}
= \;
\begin{array}{l}
\text{Benzyl} \\
\text{cyanide,} \\
\text{C}_7\text{H}_7\text{CN}
\end{array}
$$
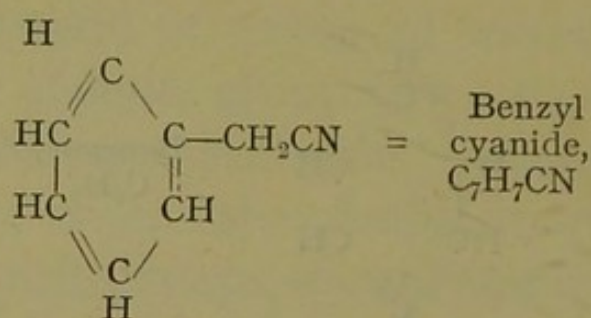
The side-chain may be defined as an atomic group, the carbon atom of which is itself attached to one of the carbon atoms of a compound having a ring structure, either the benzene ring, which has been taken as an example, or any other ring.

Extending these ideas to living matter, Ehrlich conceives, to give his own words, ' the protoplasm molecule as being constituted on the basis of (1) a central " functioning nucleus " of which the structure represents that adapted to discharge the specific functions characteristic of the cell, and (2) depending upon this nucleus, and providing for its sustenance certain " nutritive side-chains," which, in accordance with the food requirements, will be quantitatively and qualitatively different ; then the atomic grouping of these side-chains is so constituted that they are able to fix to themselves certain definite food-stuffs, important for the cell-life, and in

consequence these food-stuffs must on their part also contain atomic groups, possessed of a maximum affinity for the side-chains. The relationship of each functioning fixing group of the corresponding groups—*i.e.* those of the food-stuffs and those of the side-chains of the cell—must be specific. They must be exactly adapted to one another, and must have a relation to one another such as obtains, for example, with a male and a female screw (Pasteur), or with a lock and its proper key (Fischer). From this point of view we must contemplate the relation of the toxin to the cell. The relation between toxin and cell ceases to be shrouded in mystery if the view be adopted that the haptophore groups of the toxins are molecular groups fitted to unite alike both with the side-chains of the cells and with the antitoxins, and that it is by their agency that the toxins become anchored to the cell.'[1] Ehrlich then conceives that the toxins become anchored to the cells by means of their haptophore groups, and should the cells not possess side-chains or receptors[2] which 'fit' these haptophore groups, the toxophore groups cannot become attached to

[1] Croonian Lecture, Royal Society, London, 1900.
[2] Ritchie has pointed out that strictly it is incorrect to speak of the 'side-chains' of the cell; such side-chains belong to *molecules* within the cell. The term 'receptor' is a better one.

the cell, which therefore suffers no injury—that is, the organism is naturally immune. The haptophore groups would seem to act especially in bringing definite areas of the cell within the sphere of influence of the toxophore groups, and there is considerable difference in the behaviour of the haptophore and toxophore groups, as is shown by the following experiments of Dönitz and Heymans. If an animal be injected with suitable doses of diphtheria or of tetanus toxin there ensues an incubation period of some hours, perhaps of a day or two, during which the animal remains perfectly well and unaffected by the toxin. If this dose of toxin be injected into the circulation, and *immediately afterwards* a neutralising dose of antitoxin, no symptoms ensue, the neutralising dose of antitoxin is able to render all the toxin innocuous. If, however, the neutralising dose of antitoxin be injected not immediately but a few—seven or eight—minutes after the injection of the toxin, death occurs exactly as if no antitoxin had been given. If the animal be bled immediately after the injection of the toxin, its blood being replaced by fresh blood, it still dies, so rapid is the fixation of the toxin by the tissues. The toxin held fast in the tissues is, however, still able to be withdrawn from them if a *large* dose of

antitoxin be injected, and not the simple neutralising dose. The explanation of these phenomena is that the haptophore group comes into action *immediately after* injection into the organism and so anchors, but not indissolubly, the toxin molecule to the cell, while in every toxin, with the exception of snake venom, the
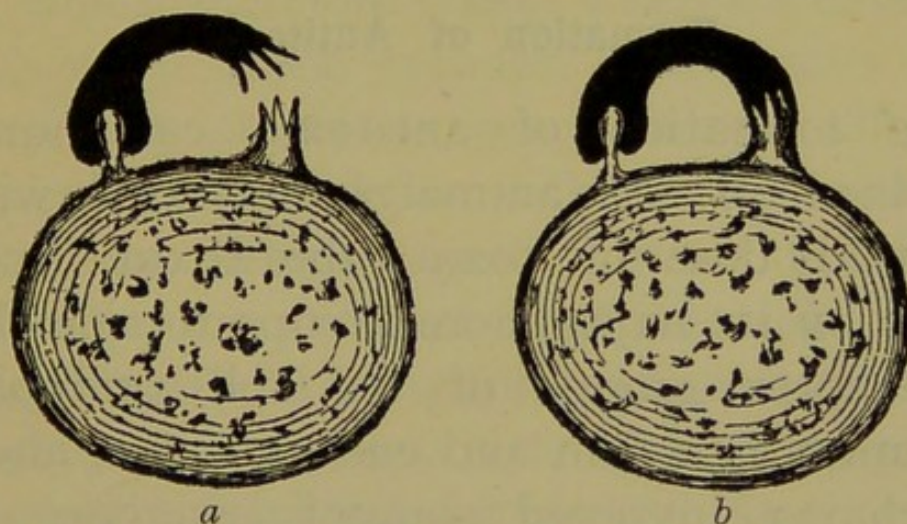
FIG. 3.—DIAGRAMMATIC SCHEME TO REPRESENT THE UNION OF TOXIN (BLACK) WITH THE CELL.

toxophore group does not come into activity until after the lapse of a longer or shorter incubation period.

This is shown diagrammatically in fig. 3, in which the union of toxin with the cell is depicted. In *a* the toxin is represented as anchored to the cell by the union of its haptophore group with the receptor group of the cell (*cf.* fig. 1, p. 18), the toxophore group being still unattached. This is the stage in which,

though the toxin has entered into union with the cells, there is as yet no toxic action, *i.e.* it is the incubation stage. In *b* the toxophore group of the toxin has now united with the toxophile group of the cell, and symptoms then ensue.

Formation of Antitoxin

The formation of antitoxin can now be explained. If an animal be injected with a sublethal dose of toxin, the toxin becomes united by its haptophore groups to side-chains or receptor groups of the cell protoplasm. The union is a firm and enduring one, and the side-chains involved cannot exercise their normal physiological functions while this union lasts. As Ehrlich puts it, they are shut out from participating in the physiological sense in the life of the cell, and a defect has thereby been created. Now Weigert enunciated the hypothesis that such a defect is replaced by regeneration. Therefore new receptors, similar to those which have been thrown out of action by the union with the toxin, are reproduced, and if more toxin be injected, again unite with it, and this union of receptors with toxin and regeneration of the receptors may be repeated

again and again, and the cells become educated, as it were, to reproduce the necessary receptors in ever-increasing quantity. This accounts for the immunity which may be induced by gradually increasing doses of toxin. Whereas at first the cells possess comparatively few of the side-chains in question, and a small amount of toxin would therefore create a serious defect or lesion, when these receptor side-chains have become very numerous, much more toxin may be injected without injury—that is, an immunity exists. But Weigert points out that simple replacement of a defect does not take place, more new material being formed than is necessary to replace the amount lost, and therefore the compensation proceeds far beyond the necessary limit—until at last the receptors are produced in such excess that the majority are no longer capable of remaining attached to the cells, but become free in the blood ; this excess of receptor side-chains in the blood is antitoxin. The antitoxin represents the side-chains reproduced in excess during regeneration, and therefore 'pushed off' from the protoplasm of the cells, and so coming to exist in a free state in the blood.

This secretory nature of the formation of antitoxin obtains additional support from

experiments of Salomonsen and Madsen, who showed that pilocarpine, which augments the secretion of most glands, produces in immunised animals a rapid increase in the amount of anti-toxin in the blood. In certain individuals, and in untreated horses also, a small amount
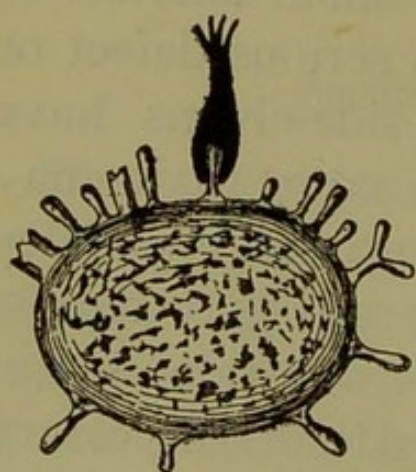
Fig. 4.—First Stage in Antitoxin Formation. (After Ehrlich.)

of diphtheria antitoxin seems to be *naturally* present, suggesting that antitoxin is a natural con-stituent of the body,[1] which becomes increased as the result of the treatment. The mode of action of antitoxin in neutralising toxin is also rendered clear. When the antitoxin is injected, it combines at once with the toxin by means of the haptophore groups of the latter, and so prevents the toxin from becoming attached to the cells, and from exerting its toxic action through the toxophore groups.

These stages in antitoxin formation are represented diagrammatically in the following

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[1] It has also been suggested that such small amounts of anti-toxin as occur naturally may be derived from the action of diphtheria bacilli present in the person or horse, but producing no symptoms. In the case of the horse at least, the explana-tion given in the text appears the more likely.

figures, and serve to visualise this difficult subject. In fig. 4 the first stage is shown.
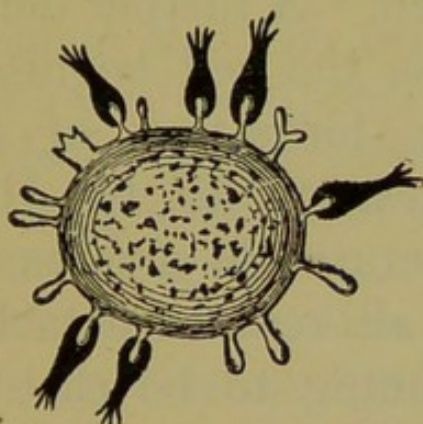
FIG. 5.—SECOND STAGE IN ANTITOXIN FORMATION. (After EHRLICH.)
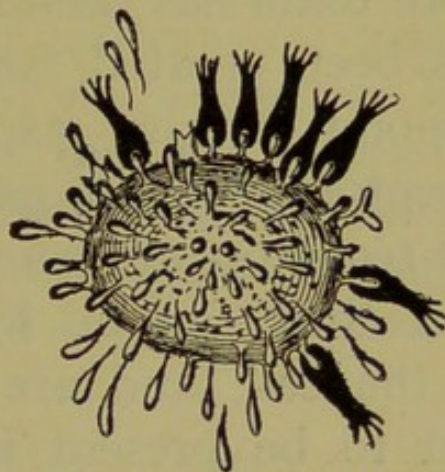
FIG. 6.—THIRD STAGE IN ANTITOXIN FORMATION. ANTITOXIN BEGINNING TO BE FORMED. (After EHRLICH.)
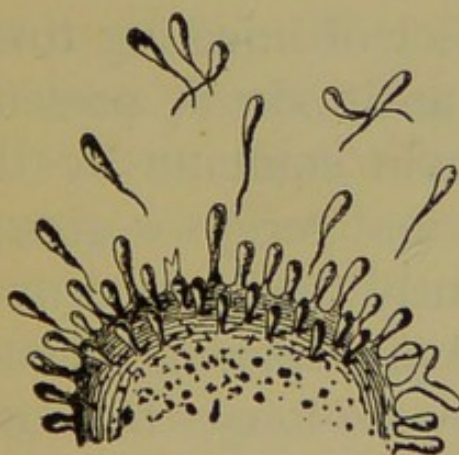
FIG. 7.—FOURTH STAGE IN ANTITOXIN FORMATION. ANTITOXIN FREE IN THE BLOOD. (After EHRLICH.)
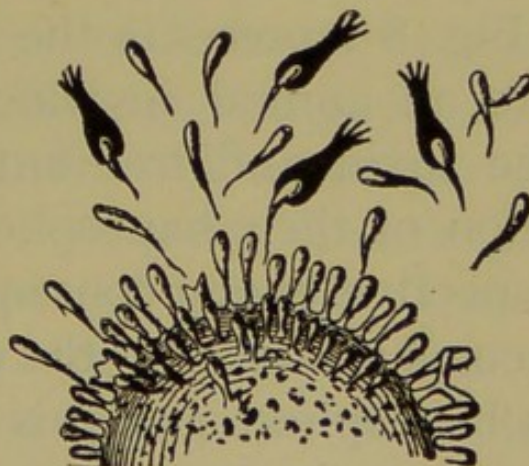
FIG. 8.—NEUTRALISATION OF TOXIN BY ANTITOXIN IN THE BLOOD. (After EHRLICH.)

Toxin has been injected, and has united with the cell protoplasm, thereby creating a defect. The cell responds to this by creating fresh

receptor side-chains of the same nature as those affected. Fig. 5 shows the condition when more toxin has been injected. A further defect has been created, and now the cell begins to respond by an excessive formation of the receptors affected, as is shown in fig. 6. Here the receptors are being generated in such excess that they can no longer all remain attached to the cell, and are beginning to be cast off and to be present in the blood. It is these free receptors which Ehrlich believes constitute antitoxin, and fig. 7 represents the condition of the blood of an animal which is freely producing antitoxin.

Fig. 8 represents the effect of injecting toxin into an animal in which antitoxin is present. The toxin and free antitoxin combine by the union of their haptophore and receptor groups respectively, and consequently the toxin cannot become attached to the cell protoplasm, because its haptophore group is now no longer free, and therefore the toxophore group is unable to exert its toxic action (*cf.* fig. 1, p. 18), because in order to do so it must first become attached to the cell protoplasm by its haptophore group.

Ehrlich's views on the toxin-antitoxin reaction and on the constitution of diphtheria toxin (see p. 107) have been much criticised, principally on physico-chemical

grounds. Whereas Ehrlich regards the toxin-antitoxin reaction as analogous to the reaction between a strong base and a strong acid, Arrhenius, Dreyer, and Madsen maintain that the avidity of antitoxin for toxin is feeble and corresponds to that of a feeble base for a feeble acid, and believe that this will explain the apparent presence of a multiplicity of substances in toxin having various affinities for antitoxin. (For further particulars, see Hewlett's *Manual of Bacteriology* (3rd ed. 1909), p. 153.)

Anti-Microbic Sera

These facts hold good for diphtheria and tetanus, which seem to be different from most of the other infective diseases, typhoid, cholera, plague, streptococcic and staphylococcic infections, Malta-fever, &c. The former are essentially ' intoxication ' diseases, diseases produced by the absorption of soluble chemical poisons, formed and excreted by the micro-organisms, while the micro-organisms themselves remain for the most part localised. In typhoid fever, plague, cholera, &c., on the other hand, the toxins are apparently to a large extent inherent to the protoplasm of the bacterial cells. If the diphtheria or tetanus bacillus be cultivated in nutrient-bouillon, and if, after the requisite time, the culture be filtered through a

Chamberland filter so as to remove the micro-organisms, the filtrate will be found to be highly toxic, a single drop of the diphtheria filtrate being sufficient to kill twenty guinea-pigs, while of the tetanus filtrate fifteen drops would destroy a thousand guinea-pigs. Carry out the same experiment with typhoid, plague, &c., and the filtrate will be found to be almost innocuous, even in doses of several cubic centimetres. An animal may be rendered highly immune or insusceptible to the typhoid bacillus or cholera vibrio by treating it with carefully graduated and increasing doses of cultures of these organisms, preferably commencing with killed cultures and afterwards employing the living organisms, yet its blood-serum (now termed 'immune serum') has only comparatively feeble *protective and curative properties.* It is true that a very small amount of the serum may protect against a single or two or three lethal doses of the organism, but the amount of the serum required to protect is not strictly proportional to the number of lethal doses of the organism, as is the case with toxin and anti-toxin. For example, if 0·005 c.c. of cholera serum, the serum of an animal immunised by repeated injections of the cholera vibrio, will just neutralise 5 milligrams of cholera culture,

injected into the peritoneal cavity of a guinea-pig, three times the amount of serum, or 0·015 c.c., will probably not protect against three times the lethal dose, or 15 milligrams of cholera culture, and, as stated above, when several lethal doses have been reached, it is impossible to save the animal, however much serum is administered. How can this extra-ordinary phenomenon be explained ? If about half an hour after the mixture of microbes and serum has been injected into the peritoneal cavity of the guinea-pig a microscopical preparation of the peritoneal exudate be made, it will be found that the microbes are in all stages of dissolution—that is to say, the serum causes the solution of the microbes and so destroys them. This is termed bacteriolysis, and the phenomenon is known as Pfeiffer's reaction, after its discoverer. If, however, the immune serum and the micro-organism be mixed together *in vitro* (in the test-tube), no *bacteriolysis occurs*, but Metchnikoff subsequently showed that the reaction would occur *in vitro* if to the mixture of microbes and immune serum some of the *fresh* peritoneal exudate of a *normal* guinea-pig were added. Bordet afterwards found that the reaction occurred *in vitro* in the mixture of microbes and immune serum

alone, provided that the serum were *perfectly fresh,* the immune serum becoming inactive *in vitro* after being kept for a few days.

Evidently therefore two substances at least are concerned in the reaction, one, a specific immunising body, relatively stable and therefore present even in an old immune serum, different for each microbe, and found in the serum only after treatment of an animal with the particular microbe, the other present in normal serum and exudates, but in small amount, and unstable, and rapidly undergoing destruction after the withdrawal of the blood from the animal. The former has been termed the immune body or amboceptor, the latter the complement,[1] addiment, or alexin. The following hypothesis has therefore been formulated to explain how it is that anti-typhoid or anti-cholera serum is only capable of neutralising at most a few lethal doses of the typhoid or cholera microbe, and possesses comparatively little curative power. In the immune serum only a *portion* of what is required is present, and the highly unstable complement is needed in addition to bring about the bacteriolysis or

[1] Probably there is a multiplicity of complements, but for our purpose it will suffice to consider that there is but one common complement. Complement also seems to consist of at least two portions,

solution and destruction of the microbes. This last is restricted in amount, so that there is a limit to bacteriolytic action. The manner in which the two constituents act is not known with certainty. The complement is the active agent in bringing about bacteriolysis, the solution of the microbes, and Ehrlich assumes that for the complement to exert its bacteriolytic action it is necessary for it to become attached, or to combine, as it were, with the bacterial protoplasm, but this it cannot do, presumably because the side-chains of the two do not correspond. The immune body, on the other hand, possesses side-chains which correspond, that is can

FIG. 9.—DIAGRAM TO SHOW THE UNION BETWEEN COMPLEMENT (BLACK) AND BIOPLASM OF CELL BY MEANS OF THE IMMUNE BODY (WHITE). (After EHRLICH.)

combine, with side-chains of both the complement and the bacterial protoplasm, and it serves therefore as a link, bringing about the union between complement and protoplasm. This is shown diagrammatically in fig. 9. The complement (black) is there depicted united to the cell-bioplasm by means of the intermediate link or ' amboceptor ' (white).

Different views, however, have been expressed as to the mode of action of the immune body and complement upon the micro-organism, and Gruber regards the immune body as in some manner preparing the way for the action of the complement, and terms it, therefore, the ' preparer.'

The beautiful experiments of Ehrlich and his pupils on hæmolysis or destruction of the red blood corpuscles are suggestive as to the truth of this hypothesis. If a guinea-pig be injected with defibrinated rabbit's blood, it will be found that the guinea-pig's blood-serum after this treatment possesses marked hæmolytic action (*i.e.* dissolves the red corpuscles) upon the red corpuscles of the rabbit, a power which it did not previously possess. If the hæmolytic serum be heated to 56° C., it is rendered inactive and does not hæmolyse, but it can be rendered active again by the addition of normal guinea-pig serum, and Ehrlich and Morgenroth explain the phenomenon of hæmolysis in this case as being due to the interaction of two substances, one specifically active and resistent, the immune body or amboceptor, and the other highly unstable, the addiment or complement. The phenomenon is exactly comparable to bacteriolysis.

The hæmolysins, as they are termed, are practically specific—that is, there is a different hæmolysin for every variety of erythrocyte. Specificity, in fact, is a salient feature with almost all anti-bodies and amboceptors, due, on Ehrlich's hypothesis, to the fact that the sub-stance injected which gives rise to the anti-body, the 'antigen' as it is termed, has an affinity only for the anti-body formed. The potentialities of proto-plasm seem to be endless, and Ehrlich says that ' the blood-serum is the carrier of substances in-numerable as yet little known or conceived of.'
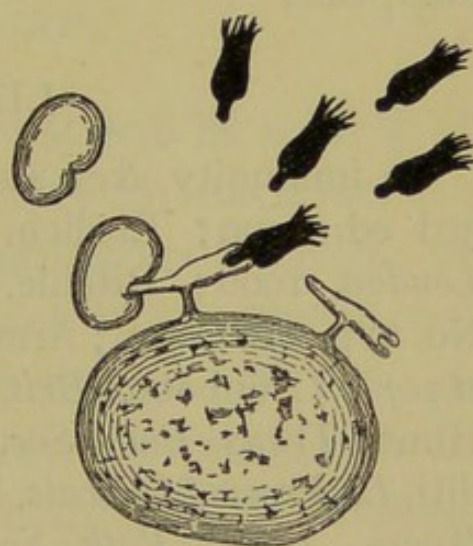
FIG. 10.—AN IMMUNE BODY OR CONNECTING - LINK, HAVING TWO AFFINITIES. (After EHRLICH.)

In some reactions with immune bodies, e.g. agglutination, the intermediate link (im-mune body) has two affinities (fig. 10), in others three.

There are, it is true, certain difficulties to be accounted for before Ehrlich's theory can be accepted in its entirety ; for example, it is difficult to understand why the injection of more toxin into a treated animal that has

already developed a considerable amount of antitoxin should be followed by the formation of more antitoxin, though it is perhaps explicable upon certain facts known in physical chemistry (see Ritchie). Nevertheless, Ehrlich's hypothesis has received a large amount of experimental support.

LITERATURE

On immunity, &c., see Hewlett, *Manual of Bacteriology*, 3rd ed. 1909; Ehrlich, *Croonian Lecture, Royal Society, London*, 1900; Ritchie, *Journ. of Hygiene*, vol. ii. 1902, No. 2, p. 215 *et seq.* ; Armit, *Brit. Med. Journ.* 1902, i. p. 784 *et seq.* ; Grünbaum, *Brit. Med. Journ.* 1903, i. p. 653 *et seq.* ; Hunt, *Trans. Path. Soc. Lond.* xlvi. 1895, p. 266 ; Levaditi, *La Presse Médicale*, No. 70, Aug. 31, 1901, p. 109 *et seq.* ; Myers, *Trans. Path. Soc. Lond.* li. pt. iii. 1900, p. 195 ; Ehrlich, *Collected Studies on Immunity* ; Wassermann, *Immune Sera* (Trans. by Boldnau), 1904 ; Emery, *Immunity and Specific Therapy*, 1909 ; Macfadyen, *The Cell as the Unit of Life*, 1908 ; Arrhenius, *Immuno-Chemistry*, 1907. There is a good popular account by Carl Snyder (' Physiological Immunity ') in *Harper's Monthly Magazine*, April 1903, p. 720.

CHAPTER II

GENERAL METHODS FOR THE PREPARATION OF THE ANTI-SERA

From the preceding chapter it will be seen that the anti-sera are specific—that is, each serum antagonises only its homologous toxin or microbe, *i.e.* the one with which it was prepared, therefore anti-diphtheria serum is of use in diphtheria alone, and anti-streptococcic serum in streptococcal infections. From this it follows that each serum must be obtained by injecting an animal with the pathogenic organism or its toxin for which the serum is to be an antidote. Various reports have been made of the production of antitoxin by the electrolysis of toxin, but the only practicable method of preparation is by the injection of an animal with toxins or cultures.

The Animal to be employed

As regards the choice of animal this depends upon circumstances. For experimental work in

the laboratory rabbits may be used ; but not more than about 20 c.c. of blood can be withdrawn from an animal at one time. A goat is a far more convenient animal, and 100 to 200 c.c. of blood may be withdrawn at each operation. For the preparation of the sera for therapeutic use larger animals of the equine species are always chosen—the ass, pony, mule, or horse. The ox might doubtless be used, but presents many disadvantages compared with equines ; it is prone to tuberculosis ; less tractable ; its blood does not coagulate so satisfactorily, and the yield of serum is less ; while horse serum seems to be the least toxic of any—an important consideration when large doses are being administered.[1]

Sound animals must, of course, be chosen, though such faults as roaring, broken knees, &c., are of no moment. Before use the animal must be carefully tested with mallein and tuberculin to exclude the possible presence of glanders and of tuberculosis, and should be kept isolated

[1] Horse serum seems to be practically non-toxic except in quantities far above the ordinary therapeutic doses. Salter found that administered subcutaneously a dose of 6 c.c. of bullock serum, 9 c.c. of dog, 12 c.c. of calf, 18 c.c. of sheep, 25 c.c. of ass, and 33 c.c. of horse serum, is the minimal lethal dose respectively per kilogram of body weight for the rabbit. For different animals the toxicity varies ; for the mouse and rat a quantity represented by half the body weight, for the guinea-pig $\frac{1}{25}$, and for the rabbit $\frac{1}{30}$.

under observation for a fortnight. On no account must a fresh animal be placed among the others under treatment until the absence of glanders or other infective disease has been proved, for the introduction of such a disease into the stable would be most disastrous ; and for the same reason those animals under treatment must be kept isolated from the outer world in their own premises. The animals should be stalled in a well-ventilated, but warm and light, stable during the period of reaction after inoculation, and for a day after bleeding, but otherwise may be kept during the daytime in favourable weather in the paddocks. The animals are fed and tended on general principles ; they are not subjected to any special treatment during the inoculations.[1]

'Homologous' Sera

In certain instances it has been found that an animal of the same species as, or nearly related to, the one which has to be treated will yield an anti-serum more active than that derived from an unrelated species. Thus Sorbenheim found that an anthrax serum obtained by immunising sheep protected sheep

[1] See Robertson, *Trans. Path. Soc. Lond.* xlvi. 1896, p. 297.

even in small quantities, but that for rabbits it had no protective action. Ehrlich explains this on the assumption that the ' immune body ' obtained by inoculating sheep does not meet with an appropriate ' complement ' in the rabbit, and suggests that the non-success hitherto obtained with the anti-typhoid and anti-cholera sera derived from the horse in the treatment of the human disease may be due to this factor. It is, however, difficult to see how this conception, even if true, will help matters, for the use of apes for the preparation of therapeutic sera for use in human diseases is hardly practicable. Therapeutic sera obtained by the treatment of a species nearly related to that for which they are to be used may be termed ' homologous sera.'

The Toxic Material for Inoculation

For those organisms which produce a powerful toxin, *e.g.* diphtheria and tetanus, *toxin broth* is employed for the most part for the inoculations, supplemented often by injections of the dead or living *cultures*, that is, the toxin broth together with the micro-organisms. For those organisms which do not produce any appreciable amount of toxin, the dead or living cultures are

injected. In either case a virulent organism should be employed, and it must be grown under conditions such as have been proved to yield a broth or culture of maximum toxicity ; these will be detailed when considering each organism.
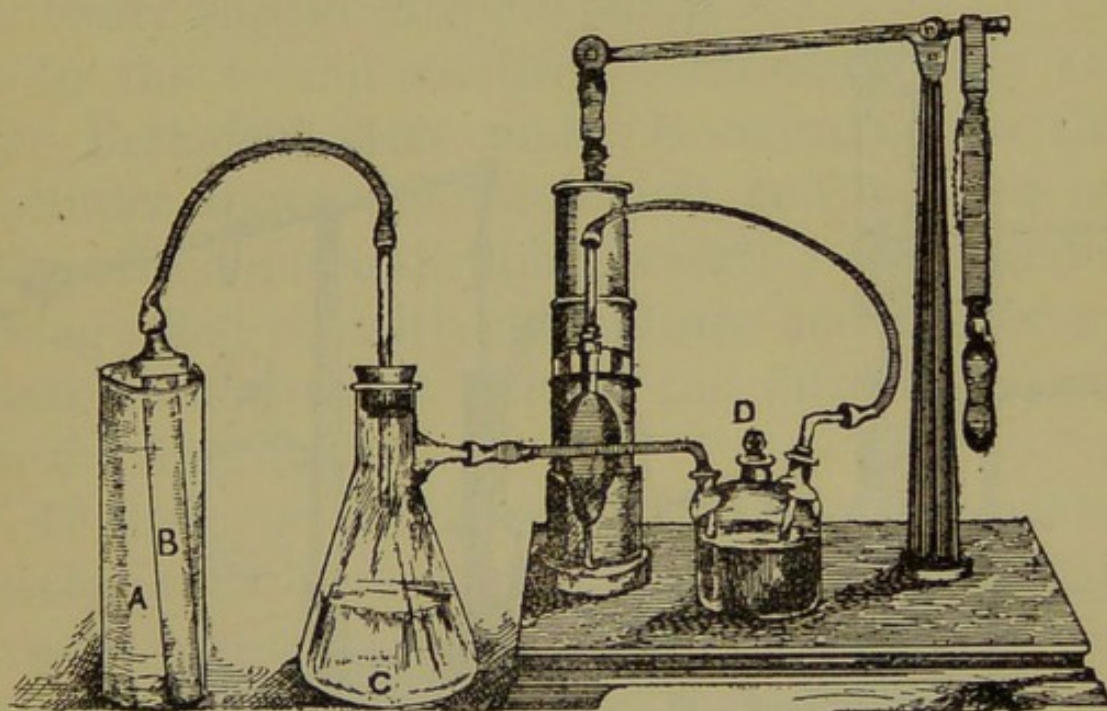
FIG. 11.—GERYK EXHAUST PUMP, ARRANGED FOR FILTRATION OF TOXIN.

In order to prepare the toxin for inoculation, the culture, after growing for the requisite time, is filtered aseptically through a porous porcelain filter, such as the Pasteur-Chamberland or Berkefeld. The latter, perhaps, is the more convenient, as the filtration is more rapid. The apparatus may be fitted up in two ways, and other modifications will suggest themselves in special instances. In the first (fig. 11) the

filter-candle (B) is attached by a piece of rubber *pressure* tubing to a short length of glass tubing, passing through a rubber cork, which stoppers the mouth of a thick glass filtering flask (C) ; the lower end of this glass tube should terminate
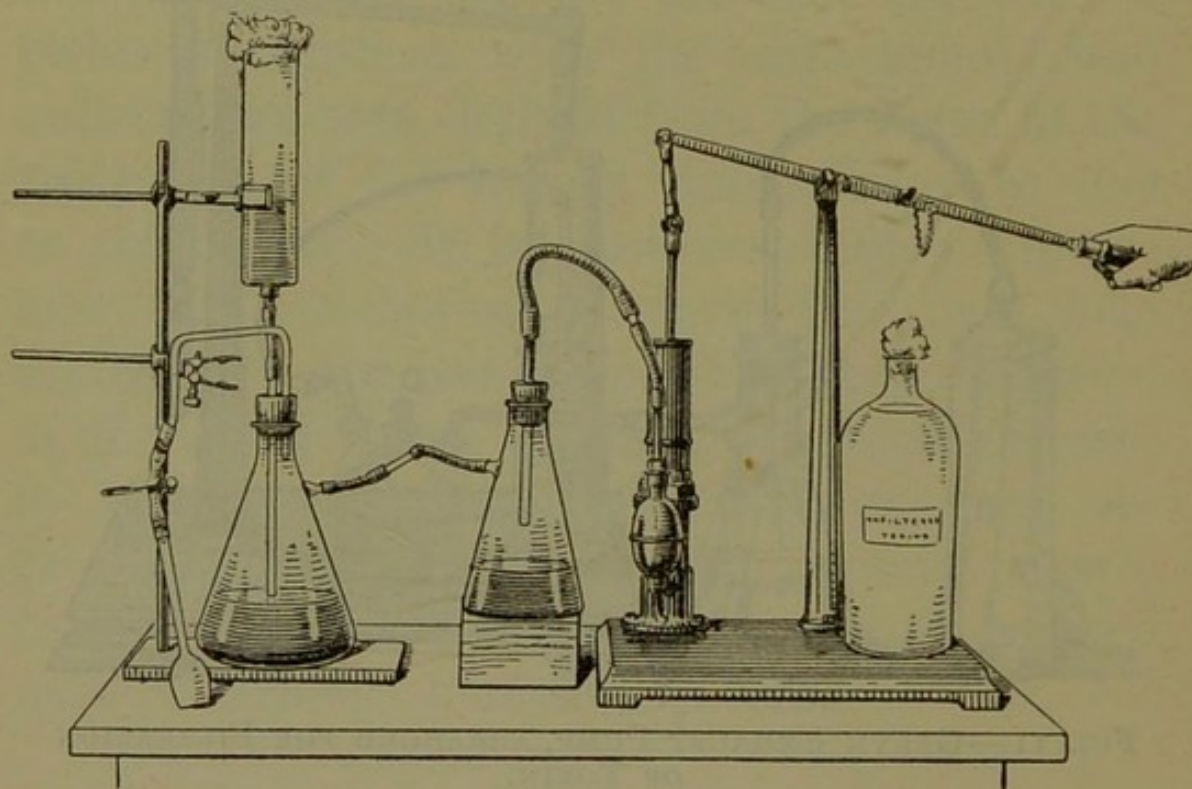
FIG. 12.—FILTRATION OF TOXIN.

just below the level of the lateral tubule of the flask. The candle is placed in a jar or cylinder (A) in which the culture is placed, the lateral tubule of the flask being connected to an exhaust pump, such as that figured, or to a water-pump. On a vacuum being created, the fluid passes through the filter-candle and

collects in the filter-flask (c). In the illustration
the pump is the Geryk pump, and the Wolf's
bottle (d) interposed between the flask and the
pump contains strong sulphuric acid, and is for
the purpose of preventing the passage of water
or water vapour into the pump.

In the second method of filtration (fig. 12)
the Berkefeld filter-candle is contained in the
cylinder above the filter-flask, and is fixed by
means of a nut and rubber washer ; the cylinder
being filled up with the culture and the filter-
flask exhausted as before, the filtrate collects
in the filter-flask.

If there be much suspended matter in the
toxin broth, it is advisable to filter it through
coarse paper previous to filtering through the
porcelain filter, in order to avoid blocking the
latter. The candle, rubber connections, and
filter-flask before use are sterilised by steaming
for some hours, and with care the filtrate may
be collected aseptically, and, by clamping the
rubber tube with screw clamps, may be preserved
without contamination until required. Or the
toxin may be preserved by the addition of
carbolic acid (0·5 per cent.) or of toluol. The
toxins after preparation undergo a gradual
diminution in strength, and so should be used
as soon as practicable after preparation. Until

used they ought to be kept in the dark in a cool place.

When the micro-organisms are to be injected broth-cultures may be employed, or emulsions of agar, serum, or other cultures are prepared with sterile physiological saline solution. In the latter instance it is as well to adopt some standard for the quantities of culture and saline solution employed, so that a rough approximation of the amount may be gauged for dosage ; for example, one standard platinum loopful (= I mgrm.) may be emulsified in I c.c., or the growth over the whole surface of an ordinary agar tube may be suspended in 10 c.c. of the saline solution, or that on the surface of the agar in a plate bottle (see Plague, p. 202) in 50 c.c., the emulsion being made by means of a platinum wire needle or platinum spatula. When the dead organisms are used for inoculating, heat is generally employed to destroy their vitality. For non-sporing forms a temperature of 65° C. for 10–20 minutes usually suffices, but care must be taken that the whole mass of fluid attains and is kept at this temperature for the requisite time. In certain instances the bacterial proteins derived from the bacterial cells are made use of instead of the organisms themselves. For this

purpose the organism is cultivated on a solid
medium such as agar, the growth is scraped off
and emulsified in distilled water.   To this is then
added caustic soda to the extent of 0·1–1·0 per
cent., and the mixture is then boiled and filtered.
To the filtrate dilute (1 per cent.) acetic or
hydrochloric acid is added ; this causes a
precipitate of the protein (probably a body of the
nature of a nucleo-protein), which is collected
on a filter-paper, washed first with slightly acid
water, and then with sterile distilled water, and
dried ; or it may be purified by re-solution in
alkali and precipitation with acid.   For injection
purposes it may be dissolved, with the aid of
heat if necessary, in a sterile 1 per cent. solution
of sodium carbonate.

During the preliminary stages of injection,
until some degree of immunity has been at-
tained, it is often advisable to diminish the
toxicity of the toxin when this is a very potent
one.   This can be done by heating the toxin for
a short time to 60°–70° C., or by mixing it with
one or two times its volume of iodine solution
(e.g. iodine 1 part, iodide of potassium 2 parts,
water 300 parts).

Or some antitoxin may be given at the same
time as the injection of toxin, sufficient to
partially neutralise the latter.

It seems to be a general rule that during the injections the antitoxic or anti-microbic properties of the blood gradually increase until they attain a maximum some months (3–6) after the commencement of the treatment ; they then remain at about this level for a further period of six to twelve months, but afterwards, in spite of continued injections of large doses of active toxin or culture, they gradually wane and finally almost disappear, although the animal still retains its immunity. After a period of rest, extending over a year or two, the same animal may again be employed for the production of the antitoxin. On Ehrlich's side-chain theory Weigert has explained this fall in antitoxic value as being due to damage done to the reparative portions of the cells which reproduce the antitoxic side-chains. As is so common in hyperplasia and hypertrophy generally, to which the over-production of the antitoxic side-chains has some analogy, the process after a time is apt to fail and degeneration to ensue. This may be the case with the antitoxic side-chains ; by continual stimulation the cells become exhausted, as it were, and fail to produce them ; while, since they are no longer present to combine with the toxin, immunity still persists. By resting the cells, provided they have not been too much

damaged, it may well be that they recover their functions, and subsequently behave as untreated cells.

Anti-Endotoxic Sera

As previously mentioned (p. 31), micro-organisms may be divided into two classes : (1) those that form an exo-toxin, excreted into the culture medium and separable from the organisms by filtration through a porcelain filter, *e.g.* the diphtheria and tetanus bacilli ; (2) those which form little or no excreted toxin, the toxin being intimately associated with the bacterial cells. This class includes the majority of pathogenic bacteria, *e.g.* the organisms of typhoid fever, cholera, plague, anthrax, &c. The therapeutic sera prepared for the last-named diseases are therefore anti-microbic sera, *i.e.* prepared by the injection of the micro-organisms themselves, and except in a few instances their use has proved disappointing.

The late Dr. Allan Macfadyen conceived that if the intra-cellular toxins (endo-toxins) of such organisms as the typhoid bacillus, cholera vibrio, &c., could be obtained free from the bacterial cells, it might be possible to prepare sera (anti-endotoxic sera) of much more therapeutic potency than the ordinary anti-microbic sera.

The disintegration of the bacterial cells in the presence of intense cold, to prevent chemical change in the bacterial juice obtained, was the method devised by Macfadyen to attain this end. With the aid of his colleagues, Mr. Rowland and Mr. Barnard, and of his laboratory assistants, Messrs. Burgess and Thompson, apparatus and methods were evolved to effect this.

By growing on the surface of agar or other suitable medium in plate-bottles (fig. 22, p. 202), scraping off the growth and suspending this in salt solution, centrifugalising at high speed, and collecting the bacterial cell mass on the walls of the centrifuge vessels, sufficient material is readily obtained to grind or triturate, and thus disintegrate the bacterial cells so as to liberate their contents. This is accomplished by means of the machine depicted in fig. 13.

A cone, *a*, mounted on the end of a spindle, is driven at high speed by a motor. The cone revolves in a metal pot, *b*, the bottom of which is so shaped as to fit the cone. The lower part of the spindle revolves in a socket with a flange at the end (shown just above *a*), and on to this flange the pot *b*, having received a weighed amount of the bacterial paste to be ground, is bolted. The joints are wound round with
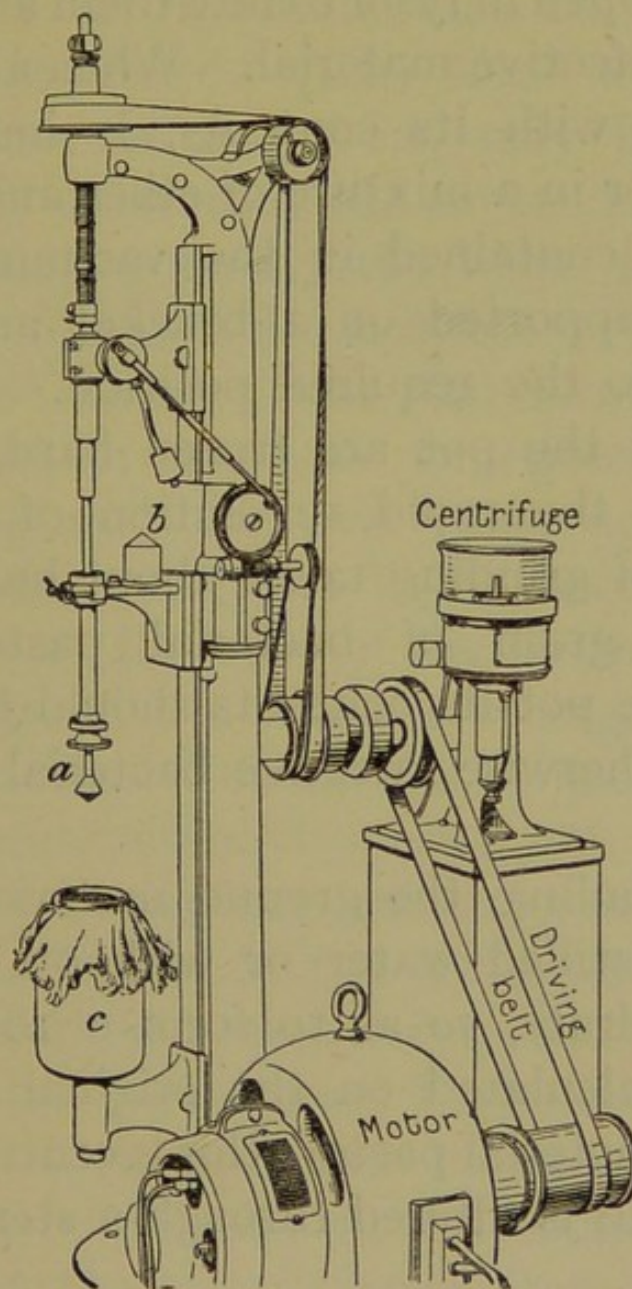
FIG. 13.—DIAGRAM OF THE MACHINE EMPLOYED BY
DR. MACFADYEN FOR TRITURATING BACTERIA.
a IS A CONE REVOLVING AT A HIGH SPEED IN THE
POT *b* (HERE DETACHED), IN WHICH THE BACTERIAL
PASTE TO BE GROUND IS PLACED. THIS IS COOLED IN
THE VESSEL OF LIQUID AIR, *c*, IN WHICH IT IS
IMMERSED. THE MACHINE IS DRIVEN BY THE MOTOR.
THE CENTRIFUGE IS FOR SEPARATING THE BACTERIAL
DÉBRIS FROM THE BACTERIAL CELL-JUICE AFTER
GRINDING.

4 *

bandage dipped in lysol to lute them and prevent escape of infective material. When all is ready, the pot *b*, with its contents, is immersed in liquid air, or in a mixture of ether and carbonic acid snow, contained in the vacuum vessel *c*, which is supported on a bracket and can be raised up to the required position. When the contents of the pot are frozen hard, they are ground by the rapid revolution of the cone. The time of grinding takes about half an hour for every gram of bacterial paste. Before opening the pot its contents should be allowed to thaw, otherwise infective bacterial dust may escape.

After grinding, the ground material is made up with distilled water or with 0·1 per cent. sodium hydrate, so as to form a 10 per cent. solution (calculated on the original weight of the moist bacterial paste) ; this is centrifugalised, and the fluid is filtered through a sterile Berkefeld filter.

The filtrate thus obtained is the endotoxin and is used to immunise horses and other animals, in the same manner as with any other toxin ; it should be used as fresh as possible. The amounts of a typhoid or cholera endotoxin employed for immunising must at first be small, 0·2–0·5 c.c., as it produces considerable

disturbance on injection, and the amount is gradually increased. After some weeks' treatment a dose of 20–30 c.c. may be injected. When tests show that the serum has attained the necessary potency, the horse is bled and the serum obtained and bottled. ·

There can be no doubt that serum prepared in this manner possesses experimentally high immunising and curative value against both the endotoxin and the living organism. Dr. Macfadyen at the time of his death had made experiments with a number of organisms, the typhoid bacillus, cholera vibrio, pneumococcus, streptococcus, staphylococcus, *M. melitensis*, *B. coli*, &c. ; but a great deal of work still remains to be done before it can be definitely stated that the method is superior to the injection of the unground organisms. Typhoid and cholera sera have been prepared by the method at the Wellcome Physiological Research Laboratories under the writer's supervision, and details of their use will be found in the sections dealing with these diseases. The writer believes that the endotoxins obtained by the Macfadyen method may also be employed as curative or prophylactic vaccines ; these are elsewhere referred to.

The method of preparing the endotoxin

as a vaccine is as follows : the endotoxin
having been obtained as detailed above, it is
standardised so as to obtain a definite weight
of endotoxin in a given volume. One to three
cubic centimetres of the filtered solution are
dried *in vacuo* over sulphuric acid and
weighed, so as to ascertain the weight of
material contained in the endotoxin solution.
This weight is regarded as the weight of endo-
toxin ; actually the endotoxin is slightly less
than is represented by this weight, in conse-
quence of the presence of traces of salts. The
amount of endotoxin having been thus ascer-
tained, sufficient sterile 0·8 per cent. sodium
chloride solution is added to the filtered solution
of endotoxin so as to form a 1 per mille
or any other solution. All the operations are
performed aseptically, in order to obtain a
sterile preparation.

LITERATURE

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Hewlett, *The Cell as the Unit of Life* (Churchill, 1908) ;

Hewlett, *Roy. Soc. Proc.*, B.vol. lxxxi. 1909, p. 325 ; *Proc. Roy. Soc. Med.* iii. 1909–1910 (Pathological Section), p. 165.

'Polyvalent' Sera

Different strains or races of the same species of microbe undoubtedly vary somewhat in their pathogenic action. Thus the local reaction at the seat of inoculation may be much more marked with one diphtheria bacillus than with another, and it has been found that an anti-pneumonic serum prepared with one strain may not immunise against another strain (p. 191), and the same probably holds good for the streptococcus (p. 183). In view of this the writer has for years advocated the use of several strains of the microbe for the preparation of an anti-serum, believing that such a serum would be found to be more generally efficacious than one prepared with a single strain. A serum prepared with several strains or races of the microbe is termed a ' polyvalent ' serum.[1]

Injection of the Toxin

Toxin, in the early stages of immunisation, is usually administered by subcutaneous injections. An ordinary syringe, with glass barrel

[1] Grünbaum, *Practitioner*, Nov. 1902, p. 620.

and asbestos packing for the piston, is employed
of suitable capacity—5, 10, 20, 50, or 100 c.c.—
according to the dose, and graduation marks on
the barrel are an advantage. The syringe is
rendered aseptic by boiling in water and is
completely filled with the toxin solution, which
is injected into the loose subcutaneous tissue
at the base of the neck just in front of the
shoulder. The skin at the seat of injection
should be previously prepared by shaving an
area four to six inches square, washing with soap
and water, and then with lysol. The needle
of the syringe should be kept bright and sharp,
and it may be attached to the syringe by a
short length of rubber tubing, if the horse is a
restive one. The injections are preferably made
alternately on the right and on the left side,
the skin being pinched up and the needle pushed
through the skin with a quick plunge.

A second injection should not be adminis-
tered until the reaction caused by the previous
one has subsided, but in the later stages, when
the animal ceases to react, the injections may
be given once or twice a week. The animal
does not usually need further restraint than
that secured by a halter, but a twitch may be
necessary ; more elaborate restraint in a frame-
work with straps, &c., is only exceptionally

required. In all operations the eyes of the animal should be covered with a cloth.

The temperature of the animal should be taken previous to the injection, and at intervals of a few hours after the injection, until the temperature attains and remains at the normal (for the horse = 100° F.). The effect of the injections, in the earlier stages at any rate, is to cause some fever and general malaise, during which time the animal should be kept in the stable. The injection must not be repeated until the animal recovers from its indisposition, until the temperature has remained at the normal for a clear day, and until any acute local irritation at the seat of inoculation has subsided. The second injection may equal in amount the first, and the dose should not be increased until the reaction produced becomes slight. The injections should be given in different places in order to avoid setting up too much local irritation, with perhaps cellulitis or suppuration. When large doses have been reached by subcutaneous inoculation, intravenous injection may be substituted. The needle of the syringe is passed into the jugular vein in the same manner as that described for bleeding (see below), but pointing posteriorly, *i.e.* in the direction of the blood-stream. For injecting large amounts,

administration by gravitation may be employed. The needle is attached by a short length of rubber tubing to a funnel or cylinder containing the requisite dose of toxin or other material, which is then allowed to run slowly into the vein. Care must be taken to avoid injecting any air, and the toxin should be injected slowly. For intravenous injection, the toxin should always be warmed to about 37° C.

Bleeding the Animal

The serum of the treated animal having acquired the requisite potency, the animal must be bled. The best time for withdrawing blood, in relation to the injections of toxin or cultures, varies in different circumstances, and will be referred to when discussing the preparation of the individual anti-sera. Bleeding is a comparatively simple matter in the horse, in which the jugular vein runs superficially in the groove on either side towards the lower aspect of the neck. If pressure be made at the base of the neck over the vein, this will fill up and stand out and be quite evident. The skin is shaved over the course of the vein at about its middle, and disinfected by well scrubbing with soap and water and then with lysol (2 to 3 per cent.).

The apparatus required are a large pointed cannula, about 6 inches in length and of $\frac{1}{16}$ to $\frac{1}{8}$ in. bore, several large filtering flasks of two to three litres capacity, glass tubing, rubber corks, &c. The cannula is carefully sterilised by boiling, and may be kept for use in a sterile glass tube plugged with cotton-wool. The filtering flasks are fitted up as follows: the neck is plugged with a rubber cork pierced with one hole, through which a short length of glass tubing passes. Attached to the free end of the glass tube is a short length of rubber tubing, of such a size that it will fit the cannula, which is clamped at its proximal end with a screw clamp, and plugged with cotton-wool at its distal free extremity. The side tubule of the filtering flask is also plugged with cotton-wool, and the whole is carefully sterilised for several hours in the steam steriliser or autoclave. For an ordinary bleeding about six of these flasks will be required. The horse does not usually require more restraint than that employed for injection of the toxin.

The position of the vein having been ascertained by applying pressure at the base of the neck, and observing the filling up of the vein, a small longitudinal incision is made through the skin with a sharp scalpel in the prepared area.

The vein being rendered prominent by pressure on its course below the seat of operation, the sterile cannula is pushed into the vein in the direction of its long axis and anteriorly—*i.e.* towards the head. When the cannula is properly in the vein the blood will run freely from it, and it is then connected to the sterile flask by the rubber tube, the wool plug being removed and the clamp unscrewed. When the flask is about two-thirds filled it is replaced by another, the rubber tube being clamped and plugged with sterile wool. Six to twelve litres of blood, according to the size of the animal, may usually be withdrawn without inducing any symptoms, and all the operations must be carried out as aseptically as possible. *The animal must not be bled too soon after the last inoculation of toxin or culture, or the serum may possess toxic qualities.*

Some makers allow the blood to run into a strong solution of an oxalate or citrate, containing the requisite quantity of the salt to prevent coagulation. The flasks are allowed to stand until the red corpuscles have settled, the plasma is then pipetted off, the amount of calcium chloride necessary to neutralise the oxalate or citrate is added, the clot allowed to form and contract, and the serum is finally pipetted off. By this

means any tendency to hæmolysis is prevented, an unstained serum is obtained, and the yield of serum is greater.

Preservation of the Anti-Serum

After the blood has been collected, the flasks are allowed to stand in a cool place for two or three hours until clotting has occurred. It is a good plan then to give each flask a few sharp twists so as to detach the clot from the sides of the flask. The flasks are then allowed to stand in a dark, cool place for twenty-four to forty-eight hours, until the serum has thoroughly separated, and the serum is then decanted off through the side tubule into sterilised bottles, in which it is kept until required. It is better to mix the serum of several horses if possible, as the serum is then less likely to produce rashes, &c. (p. 85). A small amount of an antiseptic is generally added—*e.g.* 0·2 per cent. carbolic acid or, preferably, 0·3 per cent. trikresol. Camphor, previously flamed to sterilise it, has also been used, but it is only a feeble antiseptic.

As a slight precipitate is apt to form in the anti-serum after it has been kept for a short time, rendering it turbid, it is usual to filter the serum after it has stood for a few days, and

before bottling, through a Berkefeld filter, arranged as for the filtration of toxin (p. 43).

For distribution the anti-serum must be bottled aseptically. The bottles are of varying capacity and may be made of a non-actinic glass—*e.g.* orange or green, and corks, if used, are preferably of india-rubber. Both must be carefully sterilised by steaming and be kept in covered metal or glass jars until required. Nowadays, phials of the form depicted in fig. 14 are made use of. These have open mouths, which are plugged with cotton-wool, and the plugged phials are sterilised in the hot-air steriliser. After filling, the mouths are sealed in the blow-pipe flame. Just where the stem joins the bulb a scratch is made so that the stem may be broken off. Generally speaking, a single therapeutic dose only is contained in a bottle or phial, so that there shall be no risk of contamination by the repeated opening of a bottle.

FIG. 14.— PHIAL OF ANTITOXIN, SEALED, AS SENT OUT BY THE MAKER.

For bottling various devices may be adopted. In the one figured (fig. 15), the whole apparatus is carefully sterilised by steaming, and the large bulb *a* filled with the serum from the

stock-bottles. *b* is a two-way cock, so that the graduated side tube *c* may be filled with the serum by turning the cock one way, and by turning it the other, any requisite measured amount may be run out into the bottles, the hood *d* tending to prevent aërial contamination. Each bottle or phial as filled with the required amount is handed to an assistant who corks or seals it. If bottles are used, after corking, the tops may be dipped into melted paraffin-wax to seal the bottles hermetically. Each batch as finished is at once labelled with the appro-

FIG. 15.—SIMPLE APPARATUS FOR BOTTLING ANTITOXIN.

priate labels, so that no mistake can occur, and the bottles are then stored in a cool chamber in the dark. Machines have also been devised for the process of bottling. The whole of the manipulations must be carried out as expeditiously as possible, and with the most

scrupulous aseptic precautions. The bottling chamber should be used for nothing else, the walls should be tiled and the tables and floors of cement, so that the whole may be sprayed with an antiseptic. Double doors should be provided, and the ventilating inlets and outlets screened with cotton-wool, so that filtered air only is admitted. The operators should wear sterilised blouses or mackintosh ones, so that these may be swabbed down with an antiseptic, and their hands must, of course, be disinfected.

When the anti-serum has to be kept for any length of time, to be exported, and especially for use in hot climates, it is preferable to evaporate to dryness and to send out the solid dried serum. The evaporation is carried out *in vacuo* over sulphuric acid, and a simple apparatus for this purpose was devised by the writer (fig. 16). A large stout bell-jar with ground rim and two tubules, one on the top into which a mercurial gauge is inserted through a rubber cork, the other at the side near the base, which is plugged with a rubber cork, through which a glass tube with stopcock passes, ending flush with the inside and the outer end connected by means of thick rubber *pressure* tubing to an exhaust pump. The bell-jar stands on a stout piece of ground glass ($\frac{5}{8}$ in. thick), and a metal

framework with shelves is placed inside.   The
shelves support shallow ($\frac{3}{4}$–1 in.) glass dishes,
and there is just sufficient distance between them
to admit the glass dishes.

The dishes containing strong commercial
sulphuric acid and serum alternately are stacked
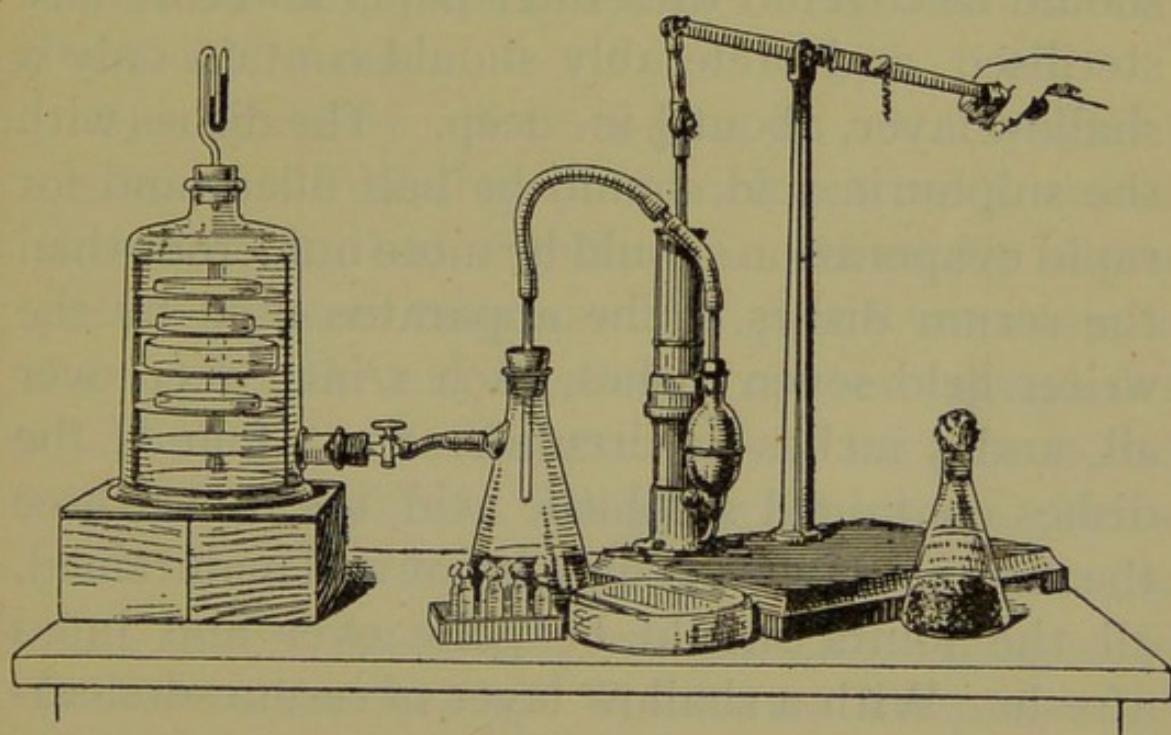
FIG. 16.—APPARATUS FOR DESICCATING ANTITOXIN.

upon the shelves, the bell-jar is placed over the
whole and is then exhausted.   The gauge will
show the amount of the vacuum, and the
exhaustion should be stopped and the stopcock
closed as soon as the serum commences to
bubble.   All joints of the apparatus must be
made tight with a luting composed either of the
resin ointment of the Pharmaopcœia or of a

stiff paste of beeswax and Geryk pump oil mixed by gentle warming. Not only should the ground edge of the bell-jar be smeared with the luting, but when pressed home the angle between the rim and the ground-glass plate should be plastered with it. The dishes for the serum should be covered with filter-paper and carefully sterilised, and preferably should contain only a shallow layer, about $\frac{1}{4}$ in. deep. The dishes with the sulphuric acid should be half filled, and for rapid evaporation should be more numerous than the serum dishes. The apparatus used by the writer held seven dishes, each 1 inch deep over all, and 5 inches in circumference ; four of the dishes contained sulphuric acid, the other three the serum. If the vacuum is not well maintained, all the joints should be gone over and luted afresh. With a shallow layer of serum, desiccation will be complete in 24–48 hours ; but if the dishes be nearly filled, it will take longer, and the sulphuric acid may have to be renewed once. Warmth assists the evaporation, and the apparatus may be kept in a warm room or be placed in a warm (37°–40° C.) incubator. Special pieces of apparatus have been devised to carry out the desiccation at 40° C.

The dried serum is chipped off the dish with a sterile spatula, and is put up in sterile glass

tubes.   If the layer of fluid serum is shallow, the dry substance will form thin scales very suitable for solution ;   but if a deep layer be evaporated, the dry mass will need pulverising in a sterile mortar before bottling.   One gram or $15\frac{1}{2}$ grains of the dry substance corresponds to about 10 cubic centimetres or $2\frac{3}{4}$ drachms of the fluid serum.

The stability of anti-sera is an important question.   Probably the antitoxic sera retain their activity but slightly unimpaired for a long period.   Some tests on this point were made for the Royal Army Medical Corps[1] on (1) samples of diphtheria antitoxin which had been on charge in military hospitals at home for a year ;   and (2) samples a year old, which during that period had been one or two voyages to India on transports.   The latter samples were kept in the refrigerating chamber during the voyages.

The table (on next page) shows the results of the tests.

It may therefore be concluded that diphtheria antitoxin retains its activity for a long time.

As regards the anti-microbic and anti-endotoxic sera, however, there is evidence to show that their activity rapidly deteriorates.

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[1] *Journ. Roy. Army Med. Corps*, May 1904.

Date of issue	Strength at date of issue	Laboratory at which tested	Date of test	Strength at date of test	Where kept
December 29, 1902	400 antitoxic units per c.c.	Conjoint laboratories of R.C.P. & R.C.S., London	March 1904	400 antitoxic units per c.c.	Home Hospital
January 15, 1903	500 antitoxic units per c.c.	Ditto	Ditto	475 antitoxic units per c.c.	Home and two voyages to India on transports
January 1, 1903	333·3 antitoxic units per c.c.	Lister Institute	March 4, 1904	300 antitoxic units per c.c.	Home Hospital
January 21, 1903	700 antitoxic units per c.c.	Ditto	Ditto	700 antitoxic units per c.c.	Home and two voyages to India
January 1903 (3 samples)	666 antitoxic units per c.c.	Messrs. Burroughs & Wellcome's laboratories	March 14, 1904	Not less than 600 antitoxic units per c.c.	All these samples had been to India on transport
January 15, 1903	550 antitoxic units per c.c.	Ditto	Ditto	450 antitoxic units per c.c.	Two voyages to India

The serum was in each case obtained from Messrs. Burroughs & Wellcome's laboratories.

Concentration of Antitoxin

Antitoxin seems to be of the nature of a globulin, or at least is associated with the globulin content of the serum, being carried down with the precipitate on saturation with magnesium sulphate. Moreover, the globulin

content of the serum of antitoxin horses tends to be increased above normal. By partial saturation of antitoxic serum with ammonium sulphate, the antitoxin is carried down with the second precipitate, that is with the pseudo-globulin fraction. It is thus possible to concentrate antitoxic serum, and to make use of a weak serum, which would otherwise be inconvenient on account of the volume necessary to inject, to introduce the requisite amount of antitoxin. For this purpose various salts have been employed for saturation, ammonium sulphate (Pick and others), magnesium sulphate (Dieudonné), mixtures of sodium and potassium chlorides (Atkinson), &c.

Dzerzgowski and Predtétchensky have elaborated a very exact method by which they state that the whole of the antitoxin can be concentrated and recovered from a comparatively weak serum. A solution of ammonium sulphate is prepared by adding 750 grams of the salt to 1 litre of water at 80° C., and agitating until the salt is completely dissolved. The solution is filtered hot, cooled to 19° C., and water at the same temperature is added until a specific gravity of 1,245 is attained. The solution now contains 53·57 grams of ammonium sulphate per 100 c.c. All the manipulations are carried

out at 19° C., in cupboards containing basins of
water so as to prevent evaporation, and by
means of special burettes. To every 100 c.c.
of serum 42·5 c.c. of the ammonium sulphate
are added, the mixture is filtered through
ordinary paper, and then to every 100 c.c. of the
filtrate 22·5 c.c. of the ammonium sulphate are
added. This second precipitate contains the
whole of the antitoxin ; it is collected by
filtration through a fine filter-paper (Schleicher
& Schül, No. 575). The precipitate is pressed
to express fluid, dried in a current of dry air
at 40°–50° C., placed in a dialyser and dialysed
for three days in a stream of water. The
contents of the dialyser are somewhat thick
and filter badly. In order to filter easily a
solution is made containing 7 grams of sodium
chloride, 3 grams of sodium carbonate, and 0·5
gram of phenol per 100 c.c.; of this 10 c.c.
are added to every 90 c.c. of the dialysate,
and the mixture is filtered aseptically
through a sterilised Pasteur-Chamberland
filter. The filtrate forms the concentrated
antitoxin.

It is probably necessary, however, to
work out for every serum the exact amount
of ammonium sulphate required to obtain a
maximum concentration of antitoxin.

LITERATURE ON THE CONCENTRATION OF ANTITOXIN

Pick, *Beitrage zur chemisch. Physiolog. u. Patholog.* (Hofmeister), Bd. i. p. 351 ; Dieudonné, *Arbeit. a. d. Kaiserl. Gesundheitsamte,* Bd. xiii. 1897 ; Atkinson, *Journ. Experiment. Med.* vol. iv. 1899, p. 649 ; Ledingham, *Journ. of Hygiene,* vii. 1907, p. 65 ; Dzerzgowski & Predtétchensky, *Archives des Sciences Biologiques,* T. xvi. Nos. 1 and 2 ; Banzhaf, Gibson & Collins, *Collected Studies from the Research Lab. Dept. of Health, City of New York,* iii. 1907.

CHAPTER III

General Principles of Treatment with Anti-sera

It should be clearly understood and recognised that the phrase ' cure of disease ' is, in an active sense, a misnomer. The actual ' healing ' of a damaged tissue or restoration of function of a disordered organ is brought about by the reparative powers of the vital activities of the living matter, bioplasm, or protoplasm of the body, the *vis medicatrix naturæ*. Strictly speaking, the physician or surgeon, by his art and science, does not ' cure ' in the sense of repairing actual damage ; all that he does is, so far as possible, to bring about a condition of things most favourable for the exercise of its healing functions by the living matter. First of all, so far as possible, he removes the cause of the disordered condition, and then, for example,

he compels rest, and applies cold or heat to an inflamed part ; he administers drugs which are known by experience or experiment to modify the activities of the living matter in a particular direction, and so on. It therefore follows that if tissue damage has occurred through the action of mechanical injuries, toxic substances, and such like, the administration of drugs and similar substances will not directly repair the damage, but will only assist the curative action of the tissues. *Tissue damage is repaired by cellular action alone.*

The antitoxins and anti-sera are able to neutralise the toxins or activities of the bacteria which produce disease, and if they be administered early enough, they will prevent the tissue damage to which the morbid symptoms are due. Antitoxin has no more power than any other drug to repair tissue damage if this has already occurred.

Early treatment is, therefore, of paramount importance.

As will be mentioned later, the mortality from diphtheria is practically nil when the disease is treated with antitoxin from the first day, and steadily rises on each succeeding day that the treatment is delayed.

Secondly, as has been shown in Chapter I,

the neutralisation of toxin by antitoxin is strictly quantitative—that is, a given quantity of toxin requires an equivalent amount of anti-toxin to neutralise it. Moreover, as toxin seems to combine with the tissues, and since the longer the toxin acts the more stable this combination appears to become, a relatively larger amount of antitoxin should be administered if the disease has lasted for some time than if it has only just shown itself. Probably a *massive* dose of the anti-body is able to displace the toxin from its combination with the tissues in the earlier stages.

The anti-serum must be regarded as a solution of the antidotal substance in blood-serum, and in different sera the same volume of serum may contain very different amounts of the antidotal substance. The anti-serum should therefore be standardised, and the prescribed dose given.

A sufficient amount of anti-serum must always be administered. This does not depend upon its volume, but upon the amount of antidotal substance it contains.

Certain toxic symptoms may develop owing to the serum being a 'foreign' one, but these, though unpleasant, do not seem to be dangerous, unless a previous dose of the same kind of serum has been given more than a fortnight previously

(see pp. 83, 89). Salter considers that if human beings were as susceptible as the most susceptible animals, quantities up to 200–250 c.c. of horse serum for a dose might be given to an infant without overstepping the limits of safety.

Use of Normal Serum with Anti-microbic Sera 'Blocking' of Complement

There does not seem to be any risk of injecting too much of an antitoxic serum, but as regards an anti-microbic serum it may be useless, possibly dangerous, to inject more than a certain amount. However, data are at present wanting to guide us in this matter. Wassermann suggested that the efficacy of an anti-microbic serum would be much enhanced by the simultaneous injection of a *perfectly fresh* normal serum. The anti-microbic sera act by the union (?) of two substances, the 'immune body' of the anti-microbic serum, and the 'complement' present in the blood of the individual and in freshly drawn blood. The amount of complement being limited (see p. 34), the injection of *fresh* serum, together with the anti-microbic serum, should supply additional 'complement' to increase the action of the anti-microbic serum. This method seems

hardly to be a practicable one, and there is, of course, the difficulty of obtaining *perfectly fresh* serum.

The possibility of danger from the administration of too large an amount of an anti-microbic serum is suggested by the fact that, experimentally, it has been found that, while a certain amount, for example, of anti-cholera serum will protect against a certain amount of living cholera culture, a *larger* amount of the serum does not protect against the *same* dose of culture (deflexion, blocking, or deviation of complement : Neisser-Wechsberg phenomenon).
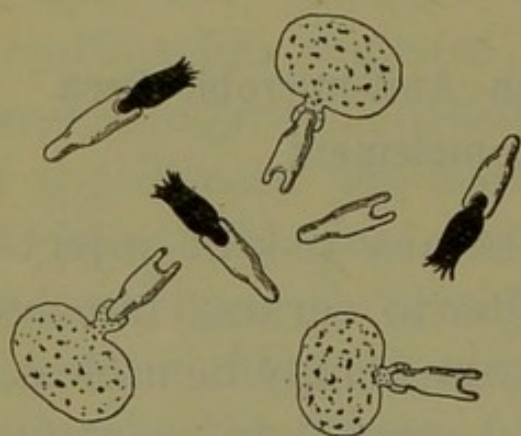
FIG. 17.—DIAGRAM TO REPRESENT THE CONDITION OF THE BLOOD IN WHICH THERE IS AN EXCESS OF AMBOCEPTORS. THE AMBOCEPTORS (WHITE) UNITE WITH BOTH COMPLEMENT (BLACK) AND RECEPTORS (DOTTED), SO THAT THE COMPLEMENT CANNOT COMBINE WITH THE AMBOCEPTOR-RECEPTOR GROUPS.

This has been explained by supposing that if amboceptor is in large excess it may unite with both the bacterium and the complement, and thus prevent the latter from exerting its action on the bacteria, and, consequently, bacteriolysis does not take place. This action is represented diagrammatically

in fig. 17, which will render the explanation
clearer.[1]

Administration of Anti-Sera

The antitoxins and anti-sera are usually ad-
ministered by subcutaneous injection. Various
statements have been made and reports are
frequently published in the medical journals of
the successful administration of anti-sera by
the mouth or rectum. In some experiments
made by the writer [2] it was found, however, that
using guinea-pigs and rabbits, both diphtheria
and tetanus antitoxins were completely unab-
sorbed when given by the mouth or rectum, and
therefore this mode of administration must be
regarded as inadvisable, particularly if a rapid
action be desired.

The syringe employed for the injection usually
has a capacity of ten cubic centimetres, the barrel
being made of glass and the piston of asbestos,
so that the whole may be sterilised by boiling
(figs. 18 and 19.) Other forms of syringe are
also to be had ; one is made entirely of glass,
barrel and piston, and from the aseptic point
of view is a most desirable form, but is of course
more liable to breakage.

[1] Other explanations of this remarkable phenomenon have
been offered.
[2] *Trans. Path. Soc. Lond.* liii. 1900, pt. ii. p. 220.
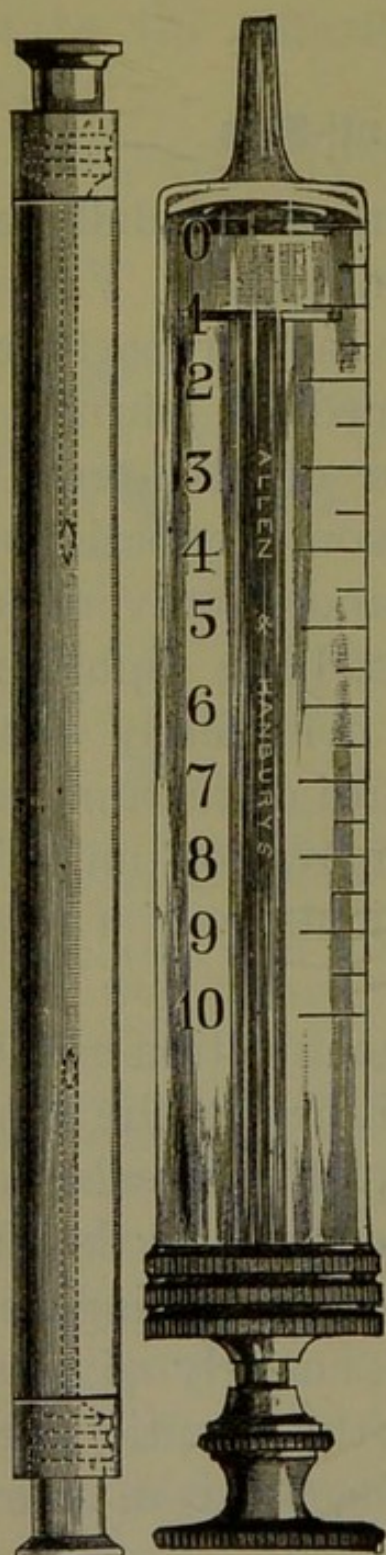
FIG. 18.—ANTITOXIN SYRINGE.
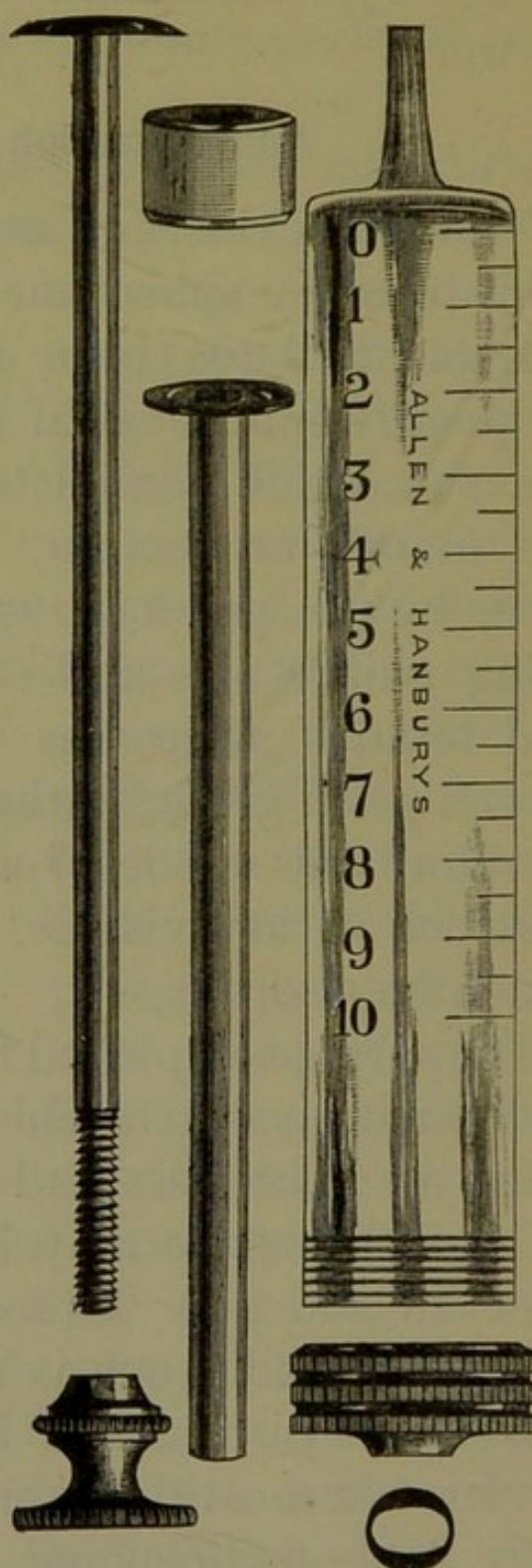
FIG. 19.—ANTITOXIN SYRINGE, SHOWING COMPONENT PARTS.

In a new syringe with asbestos piston, or one which has not been used for some time, the piston may work loose owing to contraction of the asbestos, but the latter will soon swell when placed in water or during sterilisation. There is usually a small nut at the end of the piston rod, just below the handle, which can be screwed up, and by compressing the asbestos plug enlarges its diameter, and by this means the tightness of the piston may be adjusted and the proper fit obtained.

For sterilising the syringe and needle, boiling should always be employed, though, if desired, a previous soaking in an antiseptic may be given; but this should always be followed by boiling, or at least by rinsing in sterile water, as the presence of any antiseptic is undesirable owing to the tendency of most antiseptic substances to precipitate blood-serum. For boiling, an enamelled iron dish heated on a tripod over a bunsen or spirit-lamp flame is best, but in the household any small saucepan or kettle may be used. Ordinary tap-water may be used, preferably some that has already been boiled in a kettle in order to ensure sterility, and also to diminish the deposit of salts which takes place when tap-water is boiled. The needle should be detached from the syringe, the

syringe filled with the water, the nut on the piston rod loosened, and then the syringe and needle should be boiled for at least five minutes. The syringe is then fitted to the needle, handling the needle only by the socket, the nut on the piston rod is screwed up so as to obtain a good fit, and the whole, after being rinsed out two or three times with the boiling water, is placed aside to cool, care being taken that the needle touches nothing. The bottle of serum is then taken and the neck may with advantage be wiped round with a pledget of wool soaked in 1–20 carbolic or absolute alcohol to remove any dust that may have collected. If the serum is in a phial which has been sealed, a nick is made with a small triangular file in the neck, so that this may be broken off (this is now generally done by the manufacturer).

The syringe having cooled may then be filled with the serum, the proper dose as directed in the instructions issued with the particular brand of serum being used. The requisite amount having been sucked up into the syringe, the latter is held upright, needle upwards, and the air expelled, and then the injection may be given.

The preferable site for the injection is the flank or between the scapulæ. The skin should

be disinfected by rubbing with a pledget of wool soaked in 2 per cent. lysol or other efficient antiseptic, and the needle is plunged well into the subcutaneous tissue. No dressing need be used, or a little wool and collodion may be applied over the puncture. Not more than 40 c.c. should be given in one place, and when injections are being repeated, they should be administered on alternate sides of the body and in different situations to avoid local irritation so far as possible. In the case of children with whom struggling may be expected, the needle may be attached to the syringe by a short length of rubber tubing (also boiled) to diminish the risk of breakage. The absorption of an anti-serum when this is injected subcutaneously is a comparatively slow process, a 10 c.c. dose is probably not completely absorbed and distributed over the body under about thirty-six hours. This, therefore, may entail serious loss of time, and when a rapid action is desired intravenous injection should be adopted. This may be done into one of the superficial veins of the forearm or back of the hand. A ligature is placed round the upper arm sufficiently tight to obstruct the venous but not the arterial circulation, so as to render the veins prominent, as in the operation of venesection. The antitoxin

6

should be warmed by standing the bottle in hot water (40° C. or 105° F.) for a quarter of an hour. The serum should be carefully examined after shaking the bottle to ascertain whether it is turbid or no ; if particles are present, it must be strained through some fine muslin which has been boiled for a few minutes into a small cup or other receptacle that has been washed with an antiseptic and afterwards rinsed with some boiling water. The syringe detached from the needle is filled and all air carefully expelled from it. The needle is then inserted obliquely into the vein in the direction of the shoulder, so that its point lies free in the lumen of the vein ; when this is the case, the blood drips from it, and the syringe is then attached and the serum slowly injected. There is no danger whatever in this procedure provided the simple precautions detailed above are observed. Intracerebral inoculation, which is employed in tetanus, will be described in the section devoted to tetanus antitoxin (p. 155).

Intravenous administration should always be adopted if a rapid action is desired, e.g. in a case of diphtheria which comes under treatment at a late stage, in plague, snake-bite, &c. It may, however, be difficult, especially in

children, to adopt intravenous injection. In that case deep intramuscular injection may take its place, for Henderson Smith has found that absorption is more rapid by this mode of administration than by either subcutaneous intrapleural or intraperitoneal injection.

Local inoculation near the site of a lesion is sometimes desirable (see p. 216).

After use, the syringe should be rinsed out with *cold* water, the needle carefully wiped and a wire inserted in it. The needles may with advantage be kept in a stoppered bottle in absolute alcohol.

Complications and Sequelæ of Serum Treatment

A. *Complications not peculiar to the Treatment. Abscess.*—Abscess at the seat of inoculation is seldom met with, and, with the observance of proper antiseptic or aseptic precautions, should be practically unknown unless the serum has become contaminated. The serum should always be carefully scrutinised before use and any suspicious bottle rejected and returned to the maker. Abscess did not occur in 500 cases treated by Stanley.

Septic Infection.—This is almost unknown,

6 *

and could hardly happen unless the serum were contaminated.

Hæmorrhage.—Slight hæmorrhage sometimes occurs at the seat of inoculation, but does not seem to give rise to any trouble.

Albuminuria.—In diphtheria, albuminuria has been stated to occur as the result of the injection of antitoxin. As a foreign albumin, such as egg-albumin, is excreted by the kidney on injection, it is possible that antitoxin (which is a foreign serum) might be similarly excreted. But the general consensus of opinion is that albuminuria is *less* frequent in diphtheria under antitoxin treatment than formerly. It may be confidently stated that antitoxin does not give rise to any kidney trouble.

B. *Concomitants and Sequelæ peculiar to the Treatment.*—There are certain concomitants and sequelæ which are very commonly met with in patients who have received injections of anti-sera ; they are (*a*) various rashes, (*b*) swelling and pain in the joints, and (*c*) pyrexia with or without rash or joint pains. These effects are best known in diphtheria, since the cases of this disease treated with antitoxin are vastly more numerous than those of any other disease, and the statistics given below refer to it. These

effects are solely due to the injection of a foreign serum, such as antitoxin is, and are not due to the antitoxic or anti-microbic constituent, and their frequency and severity, therefore, depend to some extent upon the *quantity* of serum injected, and were more often met with in the early days of antitoxin treatment, when the dose of serum was larger, than at present when the dose is smaller, in consequence of the preparation of more potent sera. But they also depend somewhat upon the source of the serum, and some horses yield a serum which gives rise to these effects to a greater extent than other horses.

That it is the serum and not its antitoxic constituent which produces these rashes, &c., is proved by the fact that *normal* serum—*i.e.* that from an untreated animal—gives rise to these effects to the same extent as antitoxic serum, and if antitoxic serum be heated to 59·5°–60° C. for half an hour, it loses these properties without impairing its antitoxic value.[1] According to Brodie [2] the rash-producing constituent is soluble in acetone, which does not dissolve the antitoxin.

In the Report of the Metropolitan Asylums

[1] Béclère, *Ann. de l'Inst. Pasteur*, x. 1896, p. 567.
[2] *Journ. of Path. and Bact.* iv. 1897, No. 4, p. 460.

Board upon antitoxin treatment in 1896 the following statistical table is given of the occurrence of these complications :—

Number of cases treated, 483		
Complications	Number of cases	Percentage of total cases
Rash	183	37·8
Joint pains	55	11·3
Pyrexia, with or without rash or pains	83	17·1
Abscess at site of injection . .	6	1·2

In Stanley's series of 500 cases reported in 1902 rashes were met with in 112 cases, or rather more than 20 per cent. Carrière estimates that rashes occur in 14 per cent. of the cases.

Pyrexia.—This sometimes occurs after antitoxin treatment, usually between one and three weeks after the commencement, and is generally accompanied by joint affections and rashes.

Rashes.—The appearance of rashes is a very common sequela of antitoxin injection. The rashes are of varied character, and usually develop some time after the inoculation of the serum, but occasionally early at the seat of inoculation. Stanley gives the following illustrative tables :—

	Cases
Cases of diphtheria receiving antitoxin . .	500
Antitoxin eruptions	112
Erythemata	58
Erythemata + urticaria	15
Urticaria	30
Scarlatiniform	6
Morbilliform	3
Transient early erythema and urticaria (usually at seat of injection)	17

Average day of onset of eruption—

	Day
Erythemata (varied from 4th to 29th day)	12·2
Urticaria (varied from 4th to 19th day) .	9·2
All eruptions	10·8

A *polymorphous erythema* is the commonest of all the skin eruptions. It occurs especially upon the extensor surfaces, commencing usually upon the trunk, sometimes on the face, and spreading to the limbs ; it may be from vivid scarlet to dusky red in colour, lasts two to three days, may be accompanied by some swelling, and followed by slight desquamation. In size it varies from small spots to large patches, or may cover a large area, and is generally more or less circular in outline. Some general symptoms accompany it—viz. slight malaise, a rise of temperature of 3° F., and sometimes joint pains.

An *urticarial* eruption comes next in frequency, and may sometimes pass into an erythema. It lasts usually about two days.

A *morbilliform erythema* is less frequent than the two preceding and may appear upon the face and trunk. Stanley describes it as being pinker than that of measles, and more nearly resembling rötheln. There may be some rise in temperature and redness of the throat, but no coryza.

A *scarlatiniform erythema* is also not an infrequent eruption, and generally occurs late, with some rise in temperature and general symptoms. It may, in some instances, be difficult to distinguish it from scarlatina, which not unfrequently is complicated with diphtheria. The points of distinction are the mildness of the symptoms (neither convulsions nor vomiting, and fever slight), the throat and tongue have not the appearance of scarlatina, and the rash disappears within forty-eight hours.

Stanley states that he has seen a case with a measly rash on the face, a scarlatiniform one on the trunk, and a circinate erythema on the extremities.

Purpuric and *pruriginous* eruptions have also been met with, but are very rare. Transient early erythema and urticaria occasionally appear near the seat of inoculation.

These eruptions do not seem in any way to add to the gravity of the case ; they are usually

mild and pass rapidly away. Treatment must be on general principles ; in the urticaria calcium chloride may be given.

Joint Pains.—Swelling and pain in the joints frequently follow the injection of antitoxin. They usually are of small moment and soon subside.[1]

The Serum Disease

While the injection of an anti-serum usually produces no ill effect other than the rashes, joint pains, and pyrexia already mentioned, even if large amounts of the serum be given extending over days or even two or three weeks, a second injection of serum, given after a first injection with an interval of twelve days or more between the two injections, is liable to be followed by effects which may be more or less serious, constituting the so-called ' serum disease,' or immediate or accelerated reactions, ' supersensitation,' may ensue.

The symptoms of the serum disease are nausea and vomiting, small and rapid pulse, faintness or more serious heart failure, dyspnœa with rapid and shallow respiration and feeling of suffocation, collapse, rigors, convulsions, and

[1] See Stanley, *Brit. Med. Journ.* 1902, i. p. 386 ; Carrière, *Le Nord Médical,* November 1, 1902, p. 241.

even coma. The severity of the symptoms varies in different cases, and the symptoms usually pass off in the course of an hour or two; but a few fatal cases have been recorded.

In the immediate reaction, rash, pyrexia, joint pains, vomiting, rigors, and occasionally convulsions and collapse occur generally within six hours after the second injection of serum. In the accelerated reaction, these phenomena appear between the eighteenth hour and the fifth day after the second injection of serum.

The immediate and accelerated reactions may occur a long time after the first course of serum treatment if more serum be given. Goodall records one case in which over four years elapsed between serum treatment for first and second attacks of diphtheria, an accelerated reaction occurring after the reinoculation for the second attack.

The amount of serum given does not definitely influence the result. The remarkable features of the phenomena are: (1) they do not occur unless an interval of about twelve days or more elapses between the two injections of serum; (2) the long time which may intervene between the two injections of serum and be followed by the symptoms; (3) the serious nature of the symptoms in some instances,

The explanation of the phenomena is difficult. Undoubtedly the symptoms are due to some substance in the serum which has a toxic action, and have nothing to do with the anti-toxic constituent, for *normal* serum produces the same effects.

The phenomenon has been studied in animals, and the condition of supersensitation induced by the primary injection of serum is known as 'anaphylaxis' (*i.e.* the opposite of 'prophy-laxis'). Proteins other than those of serum, *e.g.* egg-white, will produce it. In some cases, also, toxins, such as tetanus toxin, instead of producing immunity as is usually the case with appropriate doses, may also induce this hyper-sensitive condition, so that after a few doses of toxin have been given without ill effect, a relatively smaller dose may lead to a fatal tetanus. In experimental anaphylaxis produced in animals by the injection of normal serum, it is found that the condition only occurs if the two doses of serum are separated by an interval of about twelve days or more. Moreover, the two injections must be of the same serum or other protein ; thus a first injection of horse serum followed by a second injection of rabbit serum would not produce it. Extremely small doses of serum will also bring it about ; and

lastly, anæsthetisation, when the second dose of serum is given, prevents the development of the symptoms, a very extraordinary result.

The Arthus phenomenon occurs when a guinea-pig receives at intervals of some days several doses of normal horse serum. Another injection of horse serum then causes an œdematous mass, an aseptic abscess, or an area of necrosis at the site of the new inoculation, which may be far removed from the region of the previous inoculations, and the animal becomes cachectic and dies.

The Theobald Smith phenomenon occurs when a guinea-pig has been sensitised by a very small single dose of normal horse serum, 0·01 c.c., 0·001 c.c., or even 0·000001 c.c. ; if, then, after an interval of 12–14 days a somewhat larger dose of serum, 0·1 c.c., be given, the serious symptoms of hypersensitiveness develop within a few minutes, viz. respiratory failure, paralysis, clonic spasms, and frequently death. The symptoms are generally much more serious when the primary dose of serum is minute than when it is larger, e.g. one or more cubic centimetres.

Various hypotheses have been advanced to account for anaphylaxis.

Besredka believes that anaphylaxis is caused by the presence of two substances in the serum,

one thermostable and having the properties of an antigen (see p. 37), which he terms 'sensibilisogen,' and which on injection produces its anti-body, ' sensibilisin.' The other substance is thermolabile and is termed ' anti-sensibilisin,' and combines with sensibilisin wherever it meets with the latter. Sensibilisin is particularly fixed by the cells of the nervous system, and, according to Besredka, it is the violent reaction between antisensibilisin and sensibilisin in the nerve tissues which causes the serious disturbance characteristic of anaphylaxis. When, therefore, a small dose of serum ($\frac{1}{100}$–$\frac{1}{50}$ c.c.) is administered the sensibilisogen slowly forms sensibilisin. If a second dose of serum is given twelve days or more after the first injection, the antisensibilisin in it combines with the sensibilisin formed by the first injection and disturbance results. The reason why a large primary injection of serum (*e.g.* 3–5 c.c.) gives rise to much less disturbance with the second dose of serum than a small primary injection does, is that the large amount of antisensibilisin present in the serum combines gradually with the sensibilisin as this is in process of being formed (*i.e.* in the pre-anaphylactic stage), and, therefore, there is little or no sensibilisin left for the antisensibilisin present

in the second dose of serum to combine with, hence the disturbance caused is much less.

The reason why anæsthetisation with ether when the second injection is given prevents the symptoms of anaphylaxis developing is, according to Besredka, that the anæsthetic renders the nerve cells insensitive to the reaction between the sensibilisin and antisensibilisin.

Anaphylaxis, supersensitation, or hypersensitation may be of considerable importance in serum treatment.

If, for example, a case of diphtheria has a redevelopment of the disease, or if the disease be contracted a second time, or if serum treatment is employed for some other disease after treatment with serum for a previous disease, e.g. first diphtheria and afterwards erysipelas, since the *serum* is of the same kind, viz. derived from the horse, the condition is very liable to occur.

The prophylactic use of a serum must similarly entail the same risk. If, for example, a patient has received a prophylactic dose of diphtheria antitoxin and then contracts diphtheria a fortnight or more later, treatment with diphtheria antitoxin will be liable to be followed by the anaphylactic symptoms.

In Goodall's series of ninety cases, which were re-injected with antitoxin for a relapse

or for a second attack of diphtheria, 39 or 43'4 per cent. suffered from an immediate or an accelerated reaction; nine had an immediate reaction, twenty-two an accelerated reaction, and eight had both. In seven of the cases of immediate reaction the symptoms were severe.

Besredka suggests that an anti-anaphylactic state may be induced by various procedures and the anaphylactic symptoms thus prevented. If the serum be heated to 56° C. for an hour on four successive days, its therapeutic potency will be little impaired, but its power to produce anaphylaxis after a previous injection will be almost destroyed. Narcosis with ether or alcohol at the time of the second injection will also prevent anaphylaxis. Very small doses of serum (0'02–0'1 c.c.) subcutaneously vaccinate in a few hours, and intravenous inoculation seems to be without harm.

LITERATURE ON THE SERUM DISEASE, SUPER-SENSITATION, AND ANAPHYLAXIS

Rosenau & Anderson, *Journ. Amer. Med. Assoc.* 1906, p. 1007; Von Pirquet & Schick, *Die Serum-Krankheit*, 1905; Richet, *Ann. de l'Inst. Pasteur*, xxi. p. 497; Currie, *Journ, of Hygiene*, vii. 1907, pp. 35, 61, and viii. 1908, p. 457; Goodall, *Journ. of Hygiene*, vii. 1907, p. 607; Besredka, *Ann. de l'Inst. Pasteur*, xxi. 1908; *ib., Bull de l'Inst. Pasteur*, vii. 1909, p. 721.

The Prophylactic Use of Anti-sera

Experimentally, the anti-sera are found to possess immunising properties in a high degree both towards toxin and towards the living organism. A guinea-pig inoculated with a small amount of diphtheria antitoxin is thereby rendered insusceptible to many times the fatal dose of diphtheria toxin or of diphtheria culture. This insusceptibility is rapidly acquired—within a few hours—but gradually passes off, so that at the end of a month hardly any trace of it will be left. This immunising property of diphtheria antitoxin has been extensively applied practically for prophylactic purposes. Tetanus antitoxin and the anti-streptococcic and anti-plague sera have also been used as preventives.

The objection to the use of sera as prophylactics is that the anaphylactic state will be induced thereby, and if subsequent serum treatment becomes necessary serious symptoms may be caused (see *ante*).

The prophylactic use of the anti-sera will be referred to in the sections dealing with each serum.

CHAPTER IV

THE ANTITOXIC SERA

DIPHTHERIA ANTITOXIN—TETANUS ANTI-TOXIN—ANTI-VENIN

Diphtheria Antitoxin

Anti-diphtheritic serum or diphtheria anti-toxin stands foremost among the antitoxic sera on account of its extensive use, and of the excellent results obtained therefrom. Its mode of preparation is briefly as follows. A virulent diphtheria bacillus is grown in a special broth for ten to fourteen days in the incubator at 37° C. ; it is then filtered through a Pasteur-Chamberland or Berkefeld filter to remove the diphtheria bacilli, and the filtrate is employed for injecting the horses, commencing with a dose of 0·01–0·1 c.c. The injections are given subcutaneously about twice a week, the dose being gradually increased until the serum attains a sufficient degree of potency, as is ascertained by testing the serum in the manner

hereafter to be described (p. 109). The treatment will probably extend over a period of five or six months before the serum of the horses attains sufficient antitoxic power, and the dose of
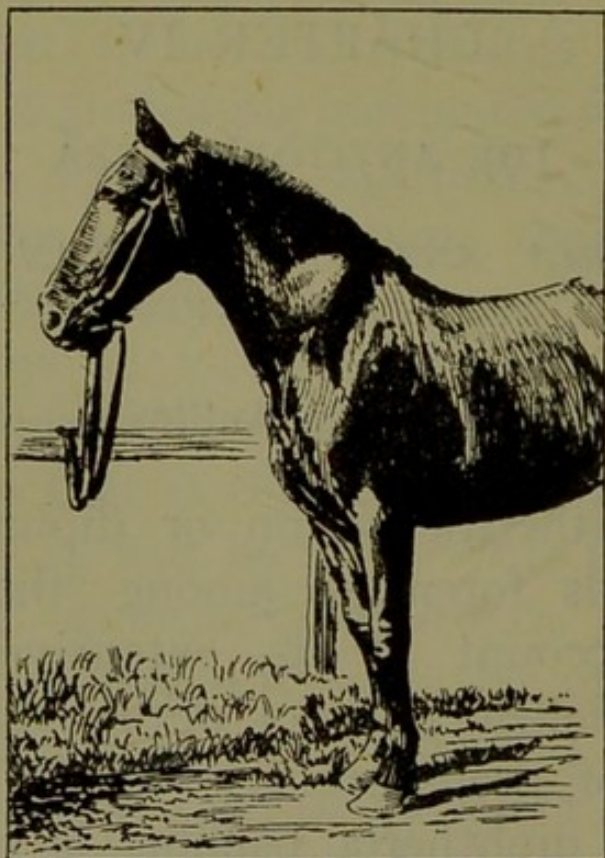
FIG. 20.—LOCAL SWELLING AT SEAT OF INOCULATION OF TOXIN DURING EARLY STAGES OF IMMUNISATION FOR THE PREPARATION OF DIPHTHERIA ANTITOXIN.

toxin will ultimately reach as much as $\frac{1}{2}$ to 1 litre. At first the injections give rise to considerable constitutional disturbance, with swelling at the seat of inoculation (fig. 20) ; later, very little disturbance occurs. It is inconvenient to employ a serum the strength of which is below about 400 units per cubic centimetre. The

diphtheria bacillus employed must be a highly virulent one ; few strains, in fact, possess the necessary virulence, and most of the diphtheria antitoxin is obtained by the use of two or three strains, notably one obtained by Behring and a second isolated by Park in America ; in the latter case $\frac{1}{200}$, $\frac{1}{400}$, or even $\frac{1}{500}$ of a cubic centimetre of the toxin is sufficient to kill a guinea-pig weighing 250 grams.

Subcutaneous inoculation seems to be preferable to intravenous inoculation for antitoxin production.

The culture-medium may be ordinary beef-peptone-bouillon, alkalised by the addition of 5–7 c.c. of normal caustic soda solution per litre after neutralisation. Spronck has recommended the use of meat which has been kept for some days until incipient putrefaction has taken place in order to destroy the muscle sugar which he believes interferes with the toxin production. Formerly the bacillus was grown for four or five weeks in special flasks, so arranged that a current of air could be continuously aspirated through the culture ; but this method has now been given up, the medium being distributed in ordinary Erlenmeyer flasks each containing about half a litre, and being grown for about eight to twelve days. It is important to obtain

a growth upon the *surface* of the medium.
With ordinary strains of diphtheria bacilli this
rarely occurs spontaneously; in order to obtain
it, a serum culture may be rubbed up with some
sterilised cork raspings, which are then added
to the flasks of culture-medium. The cork
particles with attached bacilli float, and from
them a growth starts and spreads over the
surface. Once having obtained the surface
growth, it is transferred to the fresh flasks of
culture-medium. The American bacillus shows
a greater tendency to produce this surface
growth.

L. Martin prepares the bouillon for the
diphtheria toxin from the stomach of the pig.
The pig's stomach (muscular and mucous coats)
is minced fine, and of this 200 grams are placed
in 1,000 c.c. of water acidified with 10 c.c. of
pure hydrochloric acid. The mixture is kept at
50° C. for twelve to twenty-four hours, then
heated to 100° C. for a few minutes, filtered
through cotton-wool, heated again at 80° C., and
rendered alkaline; filtered through paper, heated
again to 120° C. in the autoclave, filtered once
more through paper, and filled into flasks, which
are then sterilised by heating to 115° C. for
fifteen minutes. This method is still employed
at the Pasteur Institute. Spronck has also

employed a yeast-water medium for the cultivation of the organism.

Cartwright Wood has devised a method for immunising the horses by which he claims that the period of treatment may be much shortened. It consists in cultivating the diphtheria bacillus in ordinary broth, to which has been added ten to twenty per cent. of blood-serum, at a temperature of 37° C. for at least three or four weeks ; for use this is heated to 65° C. for an hour and then filtered through a Pasteur-Chamberland filter. Of this heated serum-toxin two or three injections of 100 to 200 c.c. are administered at intervals of some days, after which the serum-toxin plus ordinary toxin, or ordinary toxin alone, is given. The effect of the serum-toxin seems to be to produce a rapid immunity, so that considerable doses of ordinary toxin can soon be given and the tedious preliminary stage of the treatment much shortened. Wood also found that the serum used for the culture-medium must be homologous with the animal that is to be treated—*i.e.* if the horse is to be treated, horse serum must be employed ; if the rabbit, rabbit serum, and so on. Other sera, such as ox or sheep, added to the culture-medium, had for the horse little or no effect in producing rapid immunity.

Dean recommends a rapid immunisation with pure toxin. The commencing dose is about 0·01 c.c. with a toxin of which 0·0025 c.c. is the minimal lethal dose for a guinea-pig. The dose of toxin is doubled for each injection and given every third day.

Modified toxins, *e.g.* modified by heat or iodine, or the administration of antitoxin as well as toxin in the earlier stages of immunisation have also been advocated, but are not considered by Dean to give such good results as toxin alone. Some horses are decidedly more susceptible than others, and, generally speaking, probably a susceptible horse is likely to yield a more potent antitoxin than one which is not so susceptible. Certain horses cannot be made to yield a potent antitoxic serum ; no matter how large the doses of toxin, and for how long the treatment is prolonged, their serum does not acquire a high antitoxic value, although their immunity to the toxin may be very marked. It is not possible to say upon what factors these differences depend.

The best times for injecting the toxin and for bleeding the animal are of some importance and have been elucidated through the work of Salomonsen and Madsen. These observers found that shortly after injecting a large volume

of toxin a fall in the antitoxic content of the blood takes place. This, however, is soon recovered from ; the antitoxic content begins to rise again about the third day, and reaches a maximum about the ninth or tenth day, being then higher than before the injection. The proper time for bleeding the animal is therefore about the tenth to the fourteenth day after the last injection. With regard to the best time for repeating the injections of the toxin, Dean believes this to be on the third day after the last injection. The fact that an animal, after yielding antitoxin for a shorter or longer period, becomes exhausted, as it were, and its serum progressively diminishes in antitoxic value, has been referred to above (p. 48). This generally occurs with animals yielding diphtheria antitoxin.

LITERATURE

Dean, *Trans. Path. Soc. Lond.* li. pt. i. 1900, p. 15, and in *The Bacteriology of Diphtheria ;* Martin, *Ann. de l'Inst. Pasteur*, xi. 1898, p. 32 ; Park and Williams, *Journ. of Exper. Med.* i. 1896, No. 1 ; Spronck, *Ann. de l'Inst. Pasteur*, ix. 1895, p. 758 ; Cartwright Wood, *Proc. Roy. Soc. Lond.* lix. 1896, p. 290 ; *Lancet*, 1896, i. p. 980 ; *Centr. f. Bakt.*, Abt. I., March 3, 1902 ; Rosenau, *Bull*, No. 21, Hyg. Lab. U.S. Pub. Health and Mar.-Hosp. Serv. Wash. 1905.

The Standardisation of Diphtheria Antitoxin

Various methods have been adopted for the standardisation of diphtheria antitoxin. In Roux's method the lethal dose of the toxin having been ascertained for a 450 to 500 gram guinea-pig, the number of grams of guinea-pig which can be immunised by 1 c.c. of antitoxin is calculated. For example, if 0·01 c.c. of antitoxin will neutralise the lethal dose of toxin when injected simultaneously into a 500 gram guinea-pig, the immunising value of the serum is said to be 50,000 ($=500 \times 100$). But this method has many objections ; in using a single lethal dose of toxin, it is evident that if only a small fraction of the toxin be neutralised death may not ensue, and therefore the method may fail to give the actual immunising value of the antitoxin. Thus, in the above example it is quite possible that only three-quarters of the minimal lethal dose might be neutralised, and therefore the true immunising value of the serum would be not 50,000, but $\frac{3}{4} \times 50,000 = 37,500$—a very great difference. It is therefore preferable and customary when Roux's method is employed to work with a multiple of the minimal lethal dose, generally ten lethal doses (Behring's

standard), as in the case of tetanus antitoxin, and of anti-venin ; but for diphtheria antitoxin a far more accurate method has been devised by Ehrlich.

The strength of the diphtheria antitoxin is estimated in 'units,' the 'unit' being an arbitrary standard. It was arrived at originally by ascertaining the quantity of antitoxic serum which was necessary just to neutralise ten times the minimal lethal dose of diphtheria toxin when the mixture is injected subcutaneously into a guinea-pig of 250 grams weight. This amount of antitoxic serum was regarded as containing $\frac{1}{10}$ of an antitoxic unit, and one unit was therefore the equivalent of 100 lethal doses of toxin.

The unit refers to the *strength* or the neutralising power of the serum towards the toxin, and not to the *amount* of the serum ; in fact, one unit may be contained in 0·1 c.c., 0·01 c.c., 0·005 c.c., or even less of the serum. In this method of testing it was at first taken for granted that 100 minimal fatal doses of any toxin would be neutralised by one unit of anti-toxin. But in 1896 Ehrlich in a profound study of the diphtheria toxin found that this was not the case, and that the same amount of antitoxin might neutralise anything from 16 to

136 minimal fatal doses of various toxins (see T. Smith, *Journ. Boston Soc. of Med. Science*, vol. v. No. 1, 1900, p. 1).

In order to explain this it will be necessary briefly to consider the constitution of the diphtheria toxin broth.

Constitution of Diphtheria Toxin.—The constitution of diphtheria toxin is much more complex than was at one time supposed. If in the case of three toxins the minimal lethal dose for a guinea-pig is 0·5 c.c. for the first, 0·03 c.c. for the second, and 0·002 c.c. for the third, and if the toxin broth in each case is a solution of one and the same substance, the toxin, characterised always by having the same relative toxicity and affinity for antitoxin, it follows that these three toxin broths are merely solutions of toxin of varying strength, and that therefore the same amount of toxin must be present in each lethal dose, that is one 'lethal guinea-pig toxin equivalent.' On this assumption the same amount of antitoxin ought to render the lethal doses of each of the three toxin broths innocuous, but such is not the case. It is found with various toxin broths that very different amounts of the toxin are neutralised by a uniform amount of antitoxin, an amount which may be termed ' one

immunising unit.' Ehrlich found that the L_o dose of various toxins—that is, the amount of toxin which is exactly neutralised by 1 immunising unit of antitoxin (see p. 13)—varied from 16 to 136 lethal guinea-pig doses; moreover, a toxin broth in course of time gradually diminishes in toxicity, but at the same time there may be little alteration in its combining power with antitoxin ; that is, the L_o dose of the old toxin may be almost the same as that of the fresh toxin. It follows from this that there are present in the toxin broth substances which, though non-toxic, are capable of combining with antitoxin, and therefore of destroying its neutralising power towards toxin : to these substances the names of toxoids and toxones are given. The toxoids are bodies which are regarded by Ehrlich as being formed by alterations in the toxin, more or less of which are always present in the toxin broth, but the toxones he considers to be primary secretory products of the diphtheria bacillus, formed together with toxin during its growth. The toxones (formerly termed epitoxoids) possess less affinity for the antitoxin than toxoids and toxins, so that in the course of the partial neutralisation of toxin broth with antitoxin, the order of combination with antitoxin is first some of the toxoids, then

the toxin and remaining toxoids, and lastly the toxones. There are at least three kinds of toxoid : proto-, deutero-, and trito-toxoid : of which proto-toxoid has the greatest affinity for antitoxin, combining with it before anything else. The toxoids seem to be comparatively innocuous, but the toxones, while not possessing any acute toxic action, have the power of producing a certain amount of induration at the seat of inoculation, but not in so marked a degree as toxin, and of inducing the slowly developing diphtheritic paralyses. Since by neutralisation is meant that there are not only no general symptoms of intoxication but also no local ones, such as swelling and induration at the seat of inoculation, all these constituents have to be taken into account in the process of standardisation (see also p. 117).

Ehrlich's views on the constitution of diphtheria toxin have of late been much criticised, chiefly on physico-chemical grounds, and particularly by Arrhenius and Madsen. Danysz observed that if diphtheria toxin and antitoxin are brought into contact, the degree of neutralisation depends to some extent on the manner of mixing. If an excess of toxin be added to the antitoxin in two fractions with a considerable interval between the two additions, the mixture contains more free toxin than if the whole

(and same) amount of toxin be added at once to the antitoxin. This phenomenon is known as the 'Danysz effect,' and seems inexplicable on the supposition that toxin and antitoxin have relations the same as a strong acid and a strong base, which is Elhrich's view.

Arrhenius and Madsen maintain that toxin and antitoxin react like a *weak* acid and a weak base, *e.g.* boric acid and ammonia. They suggest that the avidity of the toxin for the antitoxin decreases as antitoxin is added, so that the reaction becomes slower and slower, and that the successive fractions of the toxin require more and more antitoxin to neutralise them. On these grounds they consider that it is not necessary to regard the diphtheria toxin broth as containing a mixture of several substances having different affinities for antitoxin. On Ehrlich's views, the 'curve' representing the combination of toxin and antitoxin would be a straight line, but on Arrhenius and Madsen's hypothesis, although the greater part of the toxicity of toxin is neutralised by a proper addition of antitoxin, the latter must be added in large excess before the toxicity is completely neutralised, and the course of neutralisation is represented by a hyperbolic curve.

Process of Standardisation.—Antitoxin has been made the standard for testing purposes, and not toxin, on account of its better keeping qualities. Standard antitoxin is supplied by the 'Serumprüfungs-Institut,' Frankfort-on-Maine.

It is dried over phosphoric anhydride and is kept *in vacuo* in sealed tubes. The dry serum in these tubes is standardised so that one gram contains 1,700 units of antitoxin.

Standard Antitoxin Solution. — With the standard antitoxin the laboratory toxin is first standardised, and then with this standardised toxin any antitoxin may in its turn be standardised. One gram of the antitoxin is dissolved in 100 c.c. of a solution consisting of equal parts of glycerin and 10 per cent. sodium chloride solution. Each cubic centimetre of this solution contains therefore 17 units of antitoxin ; this forms the *stock* solution, and it retains its potency unimpaired for three months at least, if kept in a cool and dark place. Solutions of antitoxin are also sent out by the ' Serumprüfungs-Institut ' for testing purposes ; their strength varies and is marked on the bottle. For use 1 c.c. of the stock solution is measured out and mixed with 16 c.c. of tap-water, or of physiological salt solution. This forms the *test* solution and contains one unit in every cubic centimetre. For measuring out the solution of antitoxin, a pipette graduated for *glycerin* should be used, or, failing this, it must be run out slowly from a 1 c.c. pipette and a single drop in excess of 1 c.c. allowed to

compensate for the fluid which clings to the glass of the pipette. The operations must be carried out with the greatest care and delicacy, for the process is comparable in accuracy to that of a volumetric chemical analysis.

The Test Toxin.—The toxin broth should be an active one. It is filtered through a porous porcelain filter, and the filtered toxin broth or ' toxin ' is preserved by the addition of some pure toluol which should form a layer a quarter of an inch in thickness on the surface of the toxin. The toxin must be kept in the dark in a cool place, preferably upon ice, and in large bulk, and should be re-standardised about every month.

Standardising the Toxin.—First of all the minimal lethal dose of the toxin is ascertained approximately by injecting various amounts into guinea-pigs weighing 250 grams. In order to attain greater accuracy, if the toxin is an active one it should be diluted ten to one hundred times with tap-water, so that errors introduced by the measurement of small quantities may be reduced to a minimum.

The smallest amount of toxin which will kill with certainty on the fourth or fifth day after injection of the toxin is the minimal lethal dose. For testing purposes there is no need to

ascertain the minimal lethal dose with great accuracy. Ehrlich's normal diphtheria toxin (DTN 1) is one in which the minimal lethal dose is 0·01 c.c., a toxin in which the minimal lethal dose is 0·005 c.c. is DTN 2, one in which it is 0·02 is DTN ·5, and so on.

The following table (Table I.) illustrates the method of testing :

TABLE I.—THE MINIMAL LETHAL DOSE OF A TEST TOXIN

Dose of Toxin	Weight of guinea-pigs in grams					
	—	24 hours	48 hours	72 hours	Fourth day	Fifth day
0·01 c.c. .	275	235 *l*	dead			
0·005 c.c. .	265	233 *l*	200	dead		
0·0033 c.c. .	263	230 *l*	230	235	220	dead
0·0025 c.c. .	260	235 *l*	215	225	210	died on ninth day

l = local reaction.

From this test, therefore, the acute lethal dose is about 0·003 c.c.

Next, the maximum amount of the toxin which is just neutralised by one unit of standard antitoxin, and the amount of toxin which when mixed with one unit of standard antitoxin is unneutralised to the extent that death is caused on the fourth or fifth day, are ascertained by

injecting varying amounts of toxin mixed with one unit of standard antitoxin into guinea-pigs. The former, the maximum amount of toxin which is just neutralised by one unit of standard antitoxin, is termed the L_o dose and need be ascertained only approximately ; the latter, the amount of toxin which when mixed with one unit of standard antitoxin causes death on the fourth or fifth day, is termed the L_+ dose, is the more important, and must be estimated with the greatest accuracy. In standardising, only the L_+ dose is used.

The method of ascertaining the L_o and the L_+ doses is as follows. Into each of several small conical glasses, termed test glasses, 1 c.c. of the test solution of the standard antitoxin is measured, i.e. one unit, and with this are mixed different amounts of toxin varying from a little below up to considerably above one hundred times the minimal lethal dose of toxin. Each mixture is made up to a volume of about 4 c.c. with tap-water, and is injected subcutaneously into a guinea-pig. The guinea-pigs used for all the processes of testing must weigh as nearly 250 grams as possible, the lighter weights being used for the mixtures containing the smaller amounts of toxin, and the animals must be kept so far as possible under the same conditions.

The test glasses hold about 5 c.c., are made from glass tubing of $\frac{1}{2}$ inch internal diameter, and are 1 inch deep, the upper $\frac{1}{2}$ inch being cylindrical, the lower $\frac{1}{2}$ inch conical and tapering to a point. They are supported in a stand consisting of a block of wood drilled with suitable holes, or may be made with flat glass 'feet,' like a squat wine-glass. The object of the conical shape is to ensure the whole of the mixture being drawn up into the syringe, and the mixture is diluted with tap-water in order that the loss of toxin caused by the small amount that adheres to the glass may be reduced to a minimum. The mixture must be injected, by means of a sterile 5 c.c. syringe, entirely subcutaneously. The animals are weighed at the time of injection and daily afterwards at about the same hour. If the toxin is unneutralised and is exerting its toxic action, the weight of the animal steadily falls; the more acute the intoxication, the more rapid the fall in weight. The seat of inoculation must also be examined daily; when the toxin is not completely neutralised, more or less local reaction is present in the form of swelling and induration, and sometimes even of necrosis. In this manner, first of all the L_+ dose of the toxin is found between two limits, and when

this has been done a further series of experiments have to be performed with amounts between these limits in order to obtain the exact amount correct to the second decimal place. Coincidently, the amount of the L_o dose will be approximately ascertained ; it is required to be known only to exclude an unsuitable toxin (see below). The following tables (Tables II. and III.) illustrate the method of testing :

TABLE II.—TESTS MADE TO ASCERTAIN THE L_+ DOSE
OF A TOXIN

The minimal lethal dose of the toxin was 0·002 c.c.

Amount of the Toxin mixed with one unit of Antitoxin	Weight of guinea-pigs in grams					
	—	24 hours	48 hours	72 hours	96 hours	120 hours
0·1 c.c.	248	245	250	253	260	268
0·2 c.c.	250	245	259	255	270	278
0·3 c.c.	250	225	dead			
0·4 c.c.	255	dead				
0·5 c.c.	255	dead				

From this series of experiments the L_+ dose of this toxin lies between 0·2 and 0·3 c.c. ; 0·2 c.c. is too little and 0·3 c.c. is too much, and further experiments have to be performed in the same manner to ascertain the exact amount to the second decimal place. The L_o dose is very nearly 0·2 c.c.

TABLE III.—THE L$_+$ DOSE OF A TOXIN (*continued*)

The L$_+$ dose from Table II. lies between 0·2 and 0·3 c.c.

Amount of the Toxin mixed with one unit of Antitoxin	Weight of guinea-pigs in grams				
	—	24 hours	48 hours	72 hours	—
0·24 c.c. . .	273	256 *t*	277 *s*	285	lived
0·26 c.c. . .	230	195 *t*	210 *sl*	220	lived
0·28 c.c. . .	255	228 *sl*	236 *sl*	242	lived
0·30 c.c. . .	248	243	dead		
0·31 c.c. . .	250	248	dead		

t = trace of, and *sl* = slight, local reaction.

From this second series of experiments it follows that the L$_+$ dose of the toxin lies between 0·28 and 0·3 c.c., and further experiments have to be performed to arrive at the exact amount. The standardisation of a test toxin may thus entail the sacrifice of a large number of animals, but this is unavoidable.

In a satisfactory toxin for testing purposes the L$_+$ dose should not exceed 1 c.c., and the difference between the L$_+$ dose and the L$_o$ dose (L$_+$ − L$_o$) should not exceed about 15 minimal lethal doses. If toxin broth contained toxin only, and no other substances which have an affinity for antitoxin, the difference between the L$_+$ dose and the L$_o$ dose would be equal to the minimal lethal dose (L$_+$ − L$_o$ = 1 MLD); but since

other substances having an affinity for anti-
toxin are always present, the difference between
the two is always a multiple of the minimal
lethal dose $(L_+ - L_o = x\,MLD)$. The L_+ dose
therefore usually corresponds to about 110–115
minimal lethal doses, but Ehrlich has found that
it varies from 16 to 136 minimal lethal doses as
extremes.

In fresh toxin broth the amount of toxoids
is small, and it is owing to the toxone that there
is this difference between the L_+ and the L_o doses.
The influence of the toxoids is negligible, but
that of the toxones is very different. If to a
toxin broth antitoxin is added so as to form
a physiologically neutral mixture, the antitoxin
will have combined with the toxin and toxone.
On adding more toxin broth, the toxin in the
addition will displace the toxone from the toxone-
antitoxin complex, because the toxin has a
greater affinity for antitoxin than the toxone
has, and this liberated antitoxin will then be
free to combine with some of the added toxin.
The effect of this is seen from the following
considerations.

Suppose a certain amount of a toxin broth
contains 90 units of toxin and 10 units of toxone,
and to this amount 100 units of antitoxin
are added so as to form a physiologically neutral

mixture. The combination which occurs is shown by the following ' equation ' : 90 toxin-antitoxin + 10 toxone-antitoxin = L_o. If an amount of the toxin broth be now added, corresponding to 11 units of toxin, the effect will be as though only *one* unit of toxin had been added, as is shown by the following ' equation ' : 90 toxin-antitoxin + 10 toxone-antitoxin + 11 toxin = 100 toxin-antitoxin + 10 toxone (free) + 1 toxin (free) = L_+. Thus, although the equivalent of eleven minimal lethal doses of toxin has been added to the physiologically neutral mixture of toxin broth and antitoxin, only *one* minimal lethal dose of toxin remains free and active, because ten toxin units displace the ten toxone units from the toxone-antitoxin complex and are neutralised by the antitoxin thus set free.

If toxin broth contained toxin only, and no toxone, Ehrlich conceives that the L_+ dose and the L_o dose would be 201 and 200 minimal lethal doses respectively. The standard unit of antitoxin can now be defined : it is that amount of antitoxin which would exactly neutralise 200 minimal lethal doses for the guinea-pig of a hypothetical toxin broth containing toxin only. But inasmuch as such a toxin broth has not yet been prepared, it corresponds approximately to

100 minimal lethal guinea-pig doses of the ordinary toxin broth. For ordinary purposes the unit of diphtheria antitoxin may be defined as that amount of antitoxin which will neutralise about 100 lethal guinea-pig doses of diphtheria toxin broth.

Standardisation of Antitoxin.—A suitable diphtheria toxin having been prepared and having been standardised with standard diphtheria antitoxin as described above, and the L+ dose having been ascertained with accuracy, this standardised toxin in its turn is employed to standardise any diphtheria antitoxin that may have been prepared. The method of procedure is as follows: First, the antitoxic serum to be tested must be considerably diluted; if it is believed to contain at least 100 units per c.c., as is usually the case, it is diluted one hundredfold, if less, fifty-, twenty-, or tenfold. In the first case one cubic centimetre of the serum is accurately measured out by means of a 1 c.c. pipette and run into a 150 c.c. flask; 99 c.c. of tap-water are then added from a 100 c.c. burette, the water being run slowly out of the burette so as to allow time for the water that adheres to the glass to run down. The water and serum are thoroughly mixed, but, to avoid air bubbles, should not be shaken, and the mixture may with advantage be

allowed to stand for half an hour to allow the froth to subside. The serum is thus diluted so as to form a one per cent. solution. Varying amounts of this diluted serum are then placed in the test glasses; to each is added the L_+ dose of the standardised toxin, the mixture is made up to about 4 c.c. with tap-water, and the whole is injected subcutaneously into a 250 gram guinea-pig.

The following example will render this description clearer:

TABLE IV.—TESTING THE ANTITOXIN

An antitoxic serum to be tested for 100, 200, 300, 400, *and* 500 *units per c.c.*

Of the dilute solution of antitoxic serum (1 c.c.=0·01 c.c. of serum), 1 c.c., 0·5 c.c., 0·33 c.c., 0·25 c.c., and 0·20 c.c. are placed in five test glasses respectively, to each is added the L_+ dose of the test toxin (in this instance 0·4 c.c.), and each is made up approximately to 4 c.c. by the addition of tap-water. Each mixture is then injected into a guinea-pig.

Number of units tested for	Weight of guinea-pigs in grams				
	—	24 hours	48 hours	72 hours	Fifth day
100	250	260	260	268	280
200	250	293 *t*	305 *t*	310 *t*	320 *t*
300	255	225 *sl*	275 *sl*	295 *t*	305 *t*
400	260	290 *l*	275 *l*	268 *l*	232 *n*
500	265	273	dead		

t=trace of, *sl*=slight, *l*=large, local reaction; *n*=necrosis at seat of inoculation.

From these experiments it follows that this

specimen of antitoxic serum contained about 300 units of antitoxin per cubic centimetre.

This, then, is the method by which diphtheria antitoxin is tested and standarised, and it will be seen that it is by no means an easy one and can only be carried out by an expert.

Some doubt has of late been cast on the designation of a serum in Ehrlich units as indicating its therapeutic value. Steinhardt and Banzhaf, however, find experimentally that, both preventively and curatively in guinea-pigs (using living culture), the potency of an anti-diphtheritic serum depends on the number of anti-toxin units present, and conclude that the present method of standardising antidiphtheritic serum accurately measures its therapeutic value.

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Therapeutic Use of Diphtheria Antitoxin

Value of Antitoxin.—The immense value of diphtheria antitoxin in the treatment of diphtheria cannot be doubted by any unbiassed

observer. In the hospitals of the Metropolitan
Asylums Board there has been a progressive fall in
the case mortality since the introduction of anti-
toxic treatment, as the following table shows
(*Metrop. Asylums Board Rep.* 1908):

CASES OF DIPHTHERIA IN THE HOSPITALS OF THE METRO-
POLITAN ASYLUMS BOARD

Year	Cases	Deaths	Case Mortality, per cent.
1890	942	316	33·5
1891	1,312	397	30·6
1892	2,009	583	29·3
1893	2,848	865	30·4
1894 *	3,666	1,035	29·3
1895	3,635	820	22·8
1896	4,508	948	21·2
1897	5,673	987	17·7
1898	6,566	991	15·4
1899	8,676	1,182	13·9
1900	7,873	988	12·3
1901	7,622	849	11·1
1902	6,520	739	11·0
1903	5,072	504	9·7
1904	4,687	469	10·0
1905	4,148	347	8·3
1906	5,218	445	8·8
1907	5,744	544	9·6
1908	5,230	507	9·7

* 1894, the year in which antitoxic treatment was commenced.

The statistics published by the Chicago
Health Department, although some years old,
are so striking as to be worthy of attention.
During the five years preceding antitoxic treat-
ment there had been an aggregate of 7,411

deaths from diphtheria and croup, an annual average of 1,482 deaths and an annual death rate of 11·23 per 10,000 of population. Antitoxin treatment was commenced on October 5, 1895, and during the five years ending December 31, 1900, there was an aggregate of 4,309 deaths from diphtheria and croup, giving an annual average of 862 deaths and an annual mortality rate of 5·45 per 10,000 of population. These figures show a reduction upon the five-year pre-antitoxin period of nearly forty-two (41·96) per cent. in the actual numbers and of nearly fifty-two (51·72) per cent. in the mortality rate. The case mortality of diagnosed diphtheria during the years 1891–1895 averaged about 35 per cent. During the 63 consecutive months between October 1895 and December 1900 the case-mortality rate was less than seven (6·79) per cent.—a reduction of 80 per cent. upon the pre-antitoxin rate.

So far as possible the inspectors of the Health Department visit every case of sore throat ; if it be suspicious, antitoxin is used without waiting for a bacteriological examination, hence a large proportion of the cases are treated within the first day or two of the disease. Moreover, every case of ' sore throat ' is examined bacteriologically.

The record of 5,727 cases shows two deaths in 476 treated on the first day of the disease—a mortality rate of 0·42 per cent. ; 22 deaths in 1,426 cases first treated on the second day—a mortality rate of 1·54 per cent. ; 73 deaths in 2,034 cases first treated on the third day—a mortality rate of 3·59 per cent. ; while there were 118 deaths in 1,037 cases first treated on the fourth day of the disease—a mortality rate of 11·38 per cent. ; and 174 deaths in 754 cases treated later than the fourth day, or over 23 per cent. These figures show strikingly the value and importance of early treatment, which are still more manifest from the following considerations. In 3,936 cases of diphtheria treated with full doses of antitoxin on the third day of the disease or earlier, there were 97 deaths—a mortality rate of less than *two and a half* (2·46) per cent. In the remaining 1,491 cases first treated on the fourth day or later there were 292 deaths—a mortality of more than 19·5 per cent.

For these statistics, diphtheria and all croup, including catarrhal, cases are grouped together to eliminate error that might otherwise be caused by changes in diagnosis and nomenclature. Estimated on the money basis of the value of a human life, the saving of life during

the antitoxin period in 1900 represented a saving of nearly a million and a half pounds! Two other interesting and complete summaries of the influence of antitoxin in reducing the death rate from diphtheria in a number of large towns, &c., are given by Cobbett and by Rosenthal.

With regard to the clinical use of antitoxin in diphtheria, Burrows published an elaborate study based upon 2,093 cases treated by him with antitoxin in the Boston City Hospital. Of this number 131 proved to have a mixed infection and 1,962 were uncomplicated. Of the uncomplicated cases 240 died, giving a death rate of 12·23 per cent. But of the 240 fatal cases, 69 were moribund and died within twenty-four hours of admission, and if these be deducted the death rate so modified would be 9 per cent. The 131 cases of mixed infection were coincident scarlatina and diphtheria.

Goodall gives an exhaustive report upon the value of antitoxin in the treatment of diphtheria, and shows how the case mortality has fallen under the treatment. In cases treated with antitoxin, the extension of existing and formation of fresh membrane are stopped, and that already present clears off rapidly. When the nasal passages are affected, the foul discharge that is so often present quickly ceases, greatly

to the comfort of the patient. The lessening of the faucial inflammation allows the patient to breathe and to take nourishment without discomfort. The enlargement of the cervical glands and the inflammation of the cellular tissues of the neck subside. There is a visible improvement in the condition of the patient, pulse rate and temperature fall, appetite returns, and convalescence is soon established.

But the value of antitoxin is perhaps most strikingly seen in laryngeal cases. From the large mass of statistics collected by Goodall and others, it will be found that about 33 per cent. only, of all laryngeal cases, whether operated upon or not, recovered before the introduction of antitoxin ; while of the cases not operated upon about 47 per cent., and of tracheotomy cases not more than about 29 per cent., recovered. Since the use of antitoxin, of all laryngeal cases about 72 per cent. recover, while of the cases not operated upon about 80 per cent., and of the tracheotomy cases about 63 per cent., recover.

The *Report of the Metropolitan Asylums Board* for 1908 shows that in laryngeal cases the case mortality has fallen from 62·0 per cent. in 1893 to 17·0 per cent. in 1908. In tracheotomy cases the case mortality has fallen from 66·2 per cent. in 1890–1893 and 70·5 per cent. in 1894 to

36·6 per cent. in 1906, and 27·8 per cent. in 1908.

Among the 1,962 cases of diphtheria studied by Burrows, there were 337 cases with laryngeal stenosis. Of these, 213 were intubed, but the remaining 124 responded promptly to the use of antitoxin and were relieved without the necessity for intubation. Of the 213 intubations, 96 died ; reintubation was necessary in many cases, in one as many as thirteen times. Of the intubations a subsequent tracheotomy was required in three. Burrows remarks that the experience gained in these cases ' leads to the overwhelming conviction that primary tracheotomy no longer has a place in the treatment of simple diphtheritic laryngeal stenosis.' He concludes his valuable paper with the remark that ' there can be no disease more fascinating than such a one over which science and the skill of the clinician have so nearly gained complete control ' (through antitoxin).

Shurley also believes in intubation rather than tracheotomy, and has reported 200 cases treated with intubation and antitoxin with 149 recoveries. He advocates the free and early use of antitoxin.

It is to be remarked that the American physicians are greatly in favour of intubation

rather than tracheotomy. Tracheotomy is doubtless a much easier procedure for those who have no experience of intubation, but when the experience can be obtained, intubation would seem to be worthy of a more extended trial than appears to have been given it in this country.

With regard to the incidence of paralysis, this seems to have increased slightly since the introduction of antitoxin treatment (see Woollacott). The explanation given to account for this is that more cases, and especially the bad ones, recover now than formerly, and consequently more cases of paralysis are met with, and this is doubtless correct. Ransom made an *experimental* study of the occurrence of paralysis after the injection of toxin and antitoxin, and formulates the following conclusions :—

1. Paralysis may certainly be expected after intoxication with not less than one-fourth of the minimal fatal dose of toxin ; it may occur with doses between one-fourth and one-eighth, but not when the dose is below one-eighth.

2. Antitoxin given fifteen to twenty-two hours after intoxication with not more than the lethal dose of toxin, exercises in large doses a mollifying influence on the subsequent paralysis.

Small doses of antitoxin have no evident effect in diminishing the paralysis.

3. Transferring these results to practice among human beings, we may expect liberal doses of antitoxin, given early in the illness, to influence favourably the subsequent paralysis, and this beneficial influence is likely to manifest itself not so much on the local paralyses (soft palate, &c.) as on such symptoms as failure of the heart. Severe cases are, however, likely to be followed by some paralysis in spite even of large doses of antitoxin.

Dosage.—The amount of antitoxin to be administered has been much discussed. In the early days of the treatment, the doses given were undoubtedly too small. Woollacott considers that 4,000 units should be the ordinary minimum dose. Anderson thinks that a single dose of 4,000 units may suffice for a mild case ; in severe cases he recommends 4,000 units every three hours for three or four doses, repeated the next day if it appears necessary. He prefers to give 4,000 units repeated frequently rather than 8,000–12,000 units at a single injection, and remarks that the appearance of the membrane is the chief criterion determining the repetition of the dose. If nasal discharge is present, and has entirely ceased, the injections may be

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discontinued. If no nasal discharge is present, but the membrane appears sodden and has a well-marked loosened edge, not much more antitoxin will be required. The course of the temperature is not much guide, though there is generally a rapid fall in uncomplicated cases.

Burrows points out that there is unfortunately no way of estimating the amount of toxin that may have been absorbed by a given patient ; the amount of membrane is an uncertain guide, and the number of bacilli, their virulence, and the susceptibility of the patient are unknown quantities. He gives as a rule 4,000 units, which dose is repeated every four hours so long as may be necessary. In some bad cases 4,000 units were given every two hours, and in others 8,000 units every four hours for a time. Beggs states that in practice the dose will probably range from a minimum of 3,000 units to a maximum of 24,000 units. To sum up, the dose should probably range from 3,000–4,000 units to 8,000 units. If more be given, it would be preferable, in my opinion, to administer in smaller amounts at short intervals rather than in a single massive dose. The injections should be repeated until the disease has manifestly subsided.

Early treatment is of paramount importance ;

in cases treated within the first twenty-four hours or so the mortality is practically *nil*. Every hour's delay lessens the chance of successful treatment. In any case of suspected diphtheria, *antitoxin should be given at once,* without waiting for the result of the bacteriological examination.

As regards the day of disease on which the treatment is commenced, the following table from the *Report of the Metropolitan Asylums Board* for 1908 shows the importance of early treatment :

Day of Disease on which Treatment began										
	1st		2nd		3rd		4th		5th and later	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
	202	6	1,076	70	1,182	125	822	106	1,249	185
Percentage Mortality .	3·0		6·5		10·6		12·9		14·8	

No matter how mild the case may seem to be, if there is a reasonable suspicion that the disease is diphtheritic, or if the bacteriological examination shows the presence of diphtheria bacilli in an otherwise unsuspected case, a

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single dose of 3,000–4,000 units should be given : it can do no harm.

In a case coming under observation late, *i.e.* after the second or third day, the first dose of antitoxin may with advantage be administered intravenously. Valuable time is thereby saved (Cairns). Berghaus states that antitoxin injected intravenously is 500 times as potent as when injected subcutaneously. General medical treatment should also be employed in addition to antitoxin. A chlorine gargle or spray is useful, but should not be used if there is struggling ; pyocyanase has also been extolled as a local application (see p. 326). Careful feeding must be the rule. Burrows remarks that digitalis should be used with caution, if at all, on account of the weakened state of the cardiac muscle. Hot-packs may be used with great benefit when the urinary secretion is diminished, and drachm doses of a saturated solution of magnesium sulphate given every hour prove a valuable diuretic in children. Vomiting may be treated with rectal feeding. The recumbent position must be absolutely maintained, and the greatest caution is required in allowing patients to sit up. The heart is usually a reliable guide, and if not affected by a short time out of bed, the

time up each day may be gradually lengthened. Patients should sit quietly in a chair for fifteen minutes on the first day out of bed, and they are not allowed to walk to and from the chair.

Albuminuria, so far from being increased by antitoxin, is often not met with in those patients treated early and with large doses of antitoxin ; water, lemonade, and magnesium sulphate were the only diuretics employed by Burrows.

In the foul sloughing throats sometimes met with in diphtheria the streptococcus seems to be associated with the diphtheria bacillus, and it has therefore been suggested that injections of anti-streptococcic serum should be given in addition to diphtheria antitoxin. This combined treatment has not, however, found much favour, but it might prove useful in certain instances and should be borne in mind.

Diphtheria antitoxin has also been used in conditions other than diphtheria (see p. 197).

LITERATURE

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The Prophylactic Use of Diphtheria Antitoxin

The prophylactic use of diphtheria antitoxin is especially indicated when a case or cases of diphtheria occur among susceptible individuals who are more or less closely associated, as for example in families, schools, and institutions. Under such conditions, as soon as the primary case is recognised, all those who in any way may have come in contact with it, or, better still, all the susceptible individuals in the institution, may be injected with a dose of diphtheria antitoxin. Many records have now been published as to the efficacy of prophylactic injections as a preventive. Biggs, the Director of the New York Health Department, states that of 3,100 individuals known to have been exposed to the infection of diphtheria, and injected with a prophylactic dose of antitoxin, only 9 contracted the disease, and these in a mild form. The immunising dose (150 units) was however too small, and we now give more than this (*e.g.* 500 units, see p. 137).

In England several examples have been

published of the prophylactic value of anti-toxin.

Porter gives some interesting details of an epidemic which occurred in the combined rural districts of Chelmsford and Maldon. There were 24 families in which cases of diphtheria had occurred. The remaining unaffected members of these families comprised 144 individuals, and to 136 of these prophylactic injections of diphtheria antitoxin were given, and among them a single doubtful case of diphtheria occurred. Of the 8 uninjected individuals, 3 subsequently developed diphtheria. In another series of 24 families, no member of which was injected, of 125 individuals, 21 subsequently developed diphtheria.

In a convalescent home containing 38 children, three consecutive cases of diphtheria occurred. The remaining 35 children were each injected with diphtheria antitoxin (334 units each), and no further case developed (P. B. Blake).

A serious outbreak of diphtheria occurred in the districts of Cambridge and Chesterton in the autumn of 1900, but by energetic measures of isolation and prophylactic injection the epidemic was stamped out (L. Cobbett).

Another instance of the prophylactic use of diphtheria antitoxin, in which the writer was

called in in consultation, is recorded by Norton. The outbreak occurred in a Poor-law school accommodating about 300 children. Cases of sore-throat and follicular tonsillitis had occurred with an unusual frequency, and on January 6 two children were found to be suffering from diphtheria, and on bacteriological examination of the whole of the inmates, by January 12, 20 cases of diphtheria had been notified. Although inspection and bacteriological examination were persisted in and suspicious cases isolated, by February 10, 66 cases had been notified. On February 11–13 every child now remaining (in all 251) received 500 units of antitoxin (16 isolated and suspicious cases received 1000 units each). Further cases of diphtheria occurred on February 21 (one), February 27 (one), and March 2 (two). A second injection of antitoxin was given on March 6–8. No further cases occurred until April 9 (one), April 12 (one), and May 10 (two). With those cases the epidemic ended. Thus 8 cases of diphtheria arose after the use of antitoxin, but two of these occurred a month, and two more than two months, after the second injection.

Jump discusses the immunity produced by the injection of diphtheria antitoxin for pro-

phylactic purposes, and quotes the views of various authors. He considers that infants may have a longer artificial immunity, just as they are less susceptible to the disease. His own practice has been to isolate the sick child, disinfect the rooms he has occupied, and remove the unaffected children from the house when possible, at the same time giving an immunising dose. The amounts used by various observers for this purpose vary from 100 to 500 units. Jump concludes :

1. That as diphtheria antitoxin is practically harmless, all exposed persons should receive an immunising dose in proportion to age.

2. That 250 units should be given to children under two years, and 500 to all others.

3. That the immunity will last for at least three weeks, provided a reliable antitoxin be used.

4. That all exposed persons should be removed from infected surroundings, either by thorough disinfection of their own quarters or by removal to other places.

With regard to the amount of diphtheria antitoxin required for prophylactic purposes, probably *as a minimum*, 300 units should be given to children and 500 units to adults : it would be better to administer in all cases at least 500 units. Although the immunity

induced by the injection is rapidly acquired, probably within a few hours, it slowly passes off, and cannot be regarded as lasting for more than three weeks.

The one and only objection to the prophylactic use of diphtheria antitoxin is the production of the anaphylactic condition, and the development of serious symptoms should it be necessary subsequently to treat the person with serum, either for diphtheria or for any other disease. The writer believes that it will be possible to use a diphtheria endotoxin prepared by triturating washed diphtheria bacilli (see p. 50) as a prophylactic vaccine. The diphtheria endotoxin possesses powerful immunising properties experimentally and seems to be quite harmless to guinea-pigs.

LITERATURE

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Tetanus Antitoxin

The writer was the first in this country to prepare tetanus antitoxin, the most potent of the antitoxins, by immunising a horse with

tetanus toxin. It had previously been prepared by Roux and Vaillard and by Tizzoni and Cattani. The method of immunisation employed is similar to that already described for diphtheria antitoxin, the horse being injected with increasing doses of tetanus toxin instead of diphtheria toxin.

Preparation of the Tetanus Toxin

The tetanus bacillus being a strict anaërobe, special means have to be taken to cultivate it, either by replacing the air in the culture flask with hydrogen, or, more convenient still, by cultivating in sodium sulphindigotate broth. For the former, a simple method devised by the writer may be employed. Glass flasks of various sizes up to a litre have a lateral branch one to two inches in length passing from the middle of their necks, formed by fusing in horizontally a piece of glass tubing, and turning it down at the distal extremity for an inch or so. For the smaller sizes 'yeast flasks' do excellently (fig. 21).

The neck of the flask is corked with a perforated rubber cork, through which a narrow glass tube passes vertically to the bottom of the flask, projecting two inches above the cork.

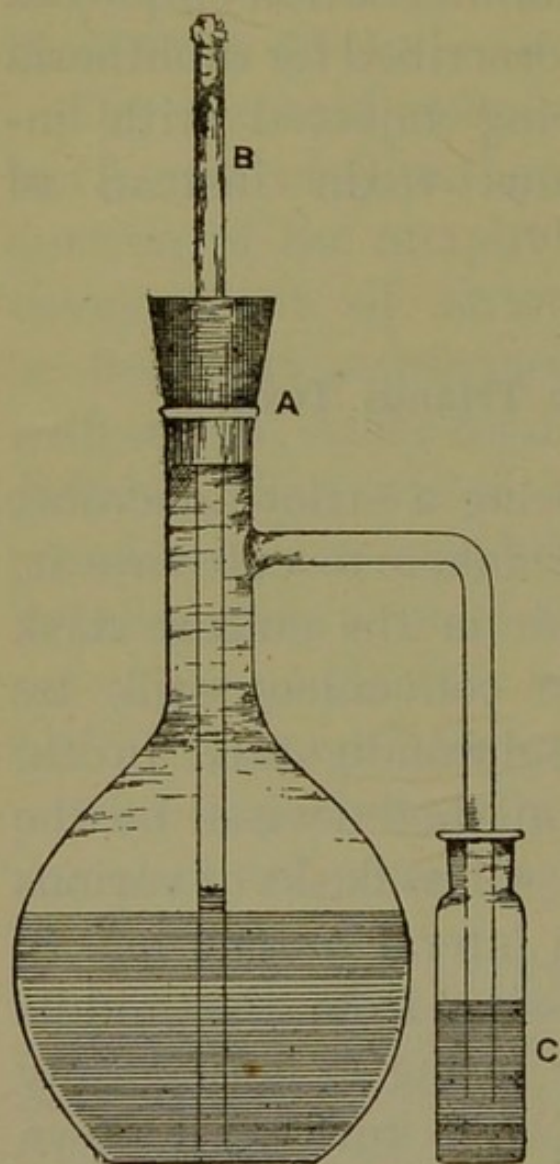This projecting portion of the tube and also the end of the lateral tube are plugged with cotton-wool, care being taken that the plugs are loose enough to allow gas to pass freely through them. The flasks are filled with a 1 per cent. grape - sugar bouillon, sterilised, and inoculated after momentarily withdrawing the rubber cork and tube. The air is expelled from the flask and replaced by hydrogen by connecting the vertical glass tube with a Kipp's hydrogen apparatus ; the gas bubbles through the bouillon and escapes by the lateral tube.

FIG. 21.—YEAST FLASK ARRANGED FOR ANAËROBIC CULTIVATION OF THE TETANUS BACILLUS.

After the hydrogen has been passing for half an hour, a small capsule or tiny phial containing mercury is applied to the free turned-down end of the lateral tube, and tied on, so that the

open extremity of the tube just dips beneath the surface of the mercury. The projecting end of the tube which passes through the rubber cork is then sealed off in the blowpipe flame. The flask is thus filled with hydrogen, and air cannot enter on account of the mercurial valve, but if any gas be formed during the growth of the organism, it can escape. The flask, with the mercurial valve *in situ*, is placed in an incubator at 37° C. After a lapse of two days the bouillon becomes turbid, and gradually a small precipitate, consisting chiefly of bacilli and spores, falls to the bottom ; a surface film never forms. The culture can be used after it has been growing for three weeks, but preferably for a month : after five weeks' growth the virulence seems to diminish.

By the sulphindigotate method, ordinary stoppered bottles filled completely full with the medium may be used, but there is some risk of breakage owing to gas formation, and it is preferable to employ a special bottle such as that devised by Dean. In this form, a glass gutter, which can be filled with mercury, is moulded on to the neck of the bottle, and a glass cap fits loosely over the mouth of the bottle and dips into the mercury. In this way a mercury valve is formed, and any gas developing during

incubation can escape by lifting up the cap. The medium consists of ordinary bouillon to which 0·3 per cent. of sodium sulphindigotate has been added. The bottle being filled as full as possible, the medium is inoculated and incubated, and the tetanus bacillus will be found to grow well and to produce an excellent toxin. The toxin broth is filtered through a porcelain filter and preserved.

Immunisation of the Horse

Tetanus toxin being extremely active and the horse being very susceptible, extreme caution must be observed during the earlier stages of immunisation. The toxin may be weakened by heating or by mixing with some agent such as iodine; Gram's iodine solution may be employed.[1]

The following illustrates the dosage employed for a pony immunised by the writer.

To commence with, the toxin was weakened by the addition of an equal volume of Gram's iodine solution; of this mixture gradually increasing doses were injected subcutaneously in the neck or shoulder, starting with 0·5 c.c. and going up to 8 or 10 c.c. This took from May 2

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[1] Iodine 1 part, potassium iodide 2 parts, water 300 parts.

to June 22, three injections being given weekly. The next two doses were 4 c.c. and 8 c.c. of a mixture of 2 parts of toxin and 1 part of Gram's iodine solution. After this, commencing July 2, the pure toxin was injected, the dose being 1 c.c., which was increased by degrees until 22 c.c. were injected on July 23. On July 25 4 c.c. were injected into the jugular vein, followed by rather alarming symptoms half an hour after, the animal falling prostrate, with legs extended, laboured respiration, and rapid small pulse. These symptoms, however, lasted only about ten minutes, after which the animal seemed to recover completely. On three other occasions somewhat similar attacks occurred after intravascular injections.

In spite of the apparent risk, the injections into the jugular vein were continued in gradually increasing amounts, and on August 30 the animal received a dose of 70 c.c. without ill-effect. This was in the early days of the preparation of antitoxins, and I do not think that there is any particular advantage in giving the smaller doses by intravenous injection. When a high immunity has been attained, a large dose of toxin may be given once every week intravenously. Just as in diphtheria, the anti-toxic value of the serum, having attained a

maximum, remains at this for a time, then begins to sink, and ultimately becomes almost extinguished. This is the writer's experience, though Salter has stated the contrary.

Standardisation of the Tetanus Antitoxin

Various units of potency and methods of standardising tetanus antitoxin have been adopted.

The potency of the Pasteur Institute serum is stated to be 1,000,000,000 ; *i.e.* to protect a mouse against the minimal lethal dose of toxin, it is sufficient to inject it with $\frac{1}{1,000,000,000}$ of its weight of serum.

At the Bacteriological Institute of Lyons several guinea-pigs are injected subcutaneously with amounts of serum corresponding to $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, $\frac{1}{10,000,000}$, and $\frac{1}{100,000,000}$ of their weight. Twelve to twenty-four hours afterwards they, as well as controls, are inoculated subcutaneously with a single fatal dose of tetanus toxin. The potency of the serum is then stated as that amount which protected the guinea-pig receiving it from the dose of toxin fatal for the control. Tizzoni in Italy adopts as a unit the amount of serum which, injected simultaneously with the toxin, will protect a

rabbit weighing 1 kilogram from a dose of toxin fatal in four or five days. The toxin and antitoxin are allowed to interact for half an hour *in vitro* and the mixture is then injected into the posterior part of the thigh. The Tizzoni tetanus antitoxin has a potency of 80,000 ; *i.e.* 1 c.c. contains sufficient antitoxin to neutralise 80,000 such toxin units.

Behring devised a system of testing analogous to that employed for diphtheria antitoxin, and this is now the official German method of testing. Both the antitoxin and the toxin for testing can be obtained from the ' Höchst ' chemical works. The test toxin (described as ' Testgift No. V.') is of such a strength that 1 c.c. would kill after a lapse of four to five days 4,000,000 grams of living mice, 50,000 grams of living guinea-pigs, and 100,000 grams of *young* rabbits. The test antitoxin (A T) contains 100 units in one gram, the unit being such that

$$\frac{1}{1000} \text{ unit A T} + 0\cdot01 \text{ c.c. test toxin}$$
$$\text{dissolved in } 0\cdot4 \text{ c.c. distilled water}$$
$$= L_0 \text{ dose for the mouse}$$
$$(L_0 = \text{exact neutralisation, see pp. 13, 113}).$$

The exact method of testing is as follows :
0·1 gram of test antitoxin is dissolved in 100 c.c. of 0·3 per cent. aqueous carbolic ; this

is the 'antitoxin test solution,' and each c.c. contains $\frac{1}{10}$ unit. The following mixture is then made in an Erlenmeyer flask, viz. :

> 1 c.c. of the antitoxin test solution,
> 1 c.c. of the test toxin (No. V.),
> 38 c.c. of distilled water.

The mixture is allowed to stand for thirty minutes, and 0·4 c.c. is then injected sub-cutaneously into a mouse weighing about twelve grams (0·1 c.c. for every three grams of body weight) ; no tetanic symptoms should ensue.

A second mixture is then made, viz. :

> 1 c.c. of the antitoxin test solution,
> 1·1 c.c. of the test toxin (No. V.),
> 37·9 c.c. of distilled water.

This, mixed and injected into a mouse in the same way, should cause some tetanic symptoms, but not death.

The third mixture :

> 1 c.c. of the antitoxin test solution,
> 1·2 c.c. of the test toxin (No. V.),
> 37·8 c.c. of distilled water,

should cause the death of the mouse in $3\frac{1}{2}$–5 days.

Behring expresses these results as follows :

1. $\frac{1}{1000}$ antitoxin unit + } L_o (= neutrali-
0·010 c.c. test toxin No. V. } sation)

2. $\frac{1}{1000}$ antitoxin unit + } L (= symp-
0·011 c.c. test toxin No. V. } toms)

3. $\frac{1}{1000}$ antitoxin unit + } L_+ (= death)
0·012 c.c. test toxin No. V. }

Having controlled the test toxin with the standard antitoxin in this way, the antitoxin to be tested is substituted for the standard anti-toxin and the tests made in the same manner.

This is carried out as follows. One cubic centimetre of the fluid antitoxin is mixed with 99 c.c. of distilled water in an Erlenmeyer flask. Of this dilution, 1 c.c. is mixed with 38 c.c. of distilled water in another Erlenmeyer flask, and to it is added 1 c.c. of the test toxin No. V. In the case of another toxin, an amount equivalent (in lethal doses) to 1 c.c. of test toxin No. V. is added to the 1 c.c. of antitoxin solution, and the whole is made up to 40 c.c. by the addition of distilled water. Of the mixture, 0·4 c.c. is injected into a 12-gram mouse. If the mouse remains unaffected, then the serum contains at least ten antitoxin units per c.c. ; if it becomes tetanic, the serum contains less than ten units per c.c., and further tests have to be made,

using stronger dilutions of the serum, until the exact neutralisation point is hit off.

The American unit is defined as follows :

' The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of anti-tetanic serum necessary to save the life of a 350-gram guinea-pig for 96 hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service.'

The work of Marx showed that dried tetanus toxin kept *in vacuo* in glass tubes is fairly stable, and the American standard toxin is such that 0·0006 gram contains 100 minimal lethal doses for guinea-pigs weighing 350 grams ; this is the L_+ dose of the toxin (analogous to the L_+ dose of diphtheria toxin) and is used for testing.

In the actual test, the toxin is dissolved in sterile salt solution (0·85 per cent.) in the proportion of 0·1 gram in 166·666 c.c. of the salt solution ; 1 c.c. of the solution is the test dose, and contains 100 minimal lethal doses. With a test dose of toxin various quantities of serum are mixed, allowed to remain *in vitro* for one hour at room temperature in diffused light, and the mixtures are then injected subcutaneously in the region of the umbilicus into guinea-pigs

weighing approximately 350 grams. The following table is an example of a test :

| No. of guinea-pig | Weight of guinea-pig in grams | Subcutaneous injection of a mixture of | | Time of death |
		Toxin (test dose)	Antitoxin	
1	360	0·006	0·001 c.c.	2 days 4 hours
2	350	,,	· 0·0015 c.c.	4 days 1 hour
3	350	,,	0·002 c.c.	symptoms
4	360	,,	0·0025 c.c.	slight symptoms
5	350	,,	0·003 c.c.	no symptoms

From this test, guinea-pig No. 2, which received 0·0015 c.c. of serum, lived beyond the death time limit (96 hours), and therefore this dose of serum contains one-tenth of an immunity unit, as the unit is defined as ten times the least amount of serum necessary to save the life of a 350-gram guinea-pig for at least 96 hours against the official test dose of toxin. This serum would, therefore, contain 66 units per cubic centimetre ($\frac{1·0}{0·015} = 66·6$).

Therapeutic Use of Tetanus Antitoxin

The therapeutic use of tetanus antitoxin, in spite of its great potency experimentally, has not been attended with results so striking as with diphtheria antitoxin. Two factors help

to explain this somewhat disappointing result. In the first place, tetanus toxin is extremely active ; in fact it is by far the most active toxic substance that is known, and minute doses are sufficient to cause irreparable tissue damage. Secondly, tetanus is not recognised until the nerve centres have been attacked by the toxin ; spasm of the facial muscles is the first indication of the onset of the disease, and this may be overlooked or be unnoticed until definite tetanic seizures announce that the central nervous system is gravely implicated. As previously stated, antitoxin cannot repair damage already done, and if this be severe the disease must run its course. In diphtheria, happily, the disease is usually localised and recognisable by the local lesions (membrane, &c.) at an early stage, before any great absorption of toxin, with consequent tissue damage, has taken place.

There is some difficulty in arriving at a just estimate of the value of antitoxin in tetanus, for there is a considerable difference in the mortality in different cases, some being comparatively mild, and tending to recover spontaneously. Generally speaking, though not always, the severity of the attack is proportional to the duration of the incubation period : the shorter the latter, the more severe the disease.

The so-called idiopathic cases [1] are also usually less severe than the manifestly traumatic ones.

The case mortality from tetanus has been variously estimated. Gowers estimates the mortality in traumatic tetanus at 90 per cent. and in idiopathic tetanus at 50 per cent. Lambert thinks that a fair estimate is 88 per cent. for acute cases, and 40 per cent. for the sub-acute and chronic ones. He has collected details of a number of cases treated with anti-toxin and gives the following summary : ' We have a total of 279 cases with a mortality of 44·08 per cent., but of these we must rule out 17 cases, made up of 4 deaths from intercurrent diseases, 8 deaths in cases in which the antitoxin was given but a few hours before death, and 5 recoveries in which antitoxin was not given until after the twelfth day, as they probably would have recovered without it. We have left 262 cases with 151 recoveries and 111 deaths, a mortality of 42·36 per cent. Dividing the cases into acute and chronic, we have 124 acute cases, with 35 recoveries and 89 deaths, a mortality of 71·77 per cent., and 128 chronic cases with 116 recoveries and 22 deaths, a mortality

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[1] All the forms of tetanus, whether traumatic, idiopathic, or ' rheumatic,' are due to the *B. tetani* of Nicolaier. In the two last named, the bacillus finds a nidus in some minute wound which escapes notice.

of 15·94 per cent. In interpreting critically these statistics we see that in acute cases the mortality is but slightly reduced, being but 72 per cent. instead of 88 per cent. But in the less acute cases there is a decided improvement, from 40 per cent. to 16 per cent.'

The writer some years ago collected details of 50 cases treated with antitoxin. Only 16 deaths occurred, which gives a mortality of 32 per cent. One of the cases was an idiopathic one, and ended in recovery ; another was a case of tetanus neonatorum and ended fatally. The remaining 48 cases were all traumatic, though in two or three instances the incubation period was abnormally long. The details of 5 of these cases were incomplete. Of the remainder (43), 24 had an incubation period of eleven days or under, with 9 deaths ; while 19 had an incubation period of more than eleven days with 3 deaths. Of the acute cases having an incubation period of eleven days or under, this would give a case mortality of 37.5 per cent.

Leyden states that he has never known recovery to take place when the temperature has risen to 105° F.

Dosage and Mode of Administration.—In no condition is early treatment of such paramount importance as in tetanus. Immediately there

is the slightest suspicion of tetanus, antitoxin should be given ; if tetanus does not ensue, no harm will have been done.

If the case be seen immediately upon the development of the premonitory symptoms (stiffness, &c., of the facial muscles), 20–30 c.c. of the serum may be injected subcutaneously, followed by an injection of 10 c.c. every eight hours so long as the symptoms last. If any time has elapsed since the development of the premonitory symptoms, 10 c.c. should be administered intravenously and 20 c.c. subcutaneously, followed by 10 c.c. subcutaneously every eight hours as before. From the work of Marie and Morax and of Ransom and Meyer, it has been found that the tetanus toxin is absorbed by the nerve trunks. Apparently the toxin enters by the motor end plates and then travels along the axis cylinders of the nerves. This fact explains the occasional utility of amputation in tetanus, and Ransom and Meyer recommended in traumatic tetanus that if the site of the wound permit, antitoxin should be injected into the course of the nerves above the wound. If the case has lasted any length of time, especially if spasms have already occurred, the antitoxin should be given by intracerebral, or intraspinous, inoculation without delay.

Tetanus toxin has an especial affinity for the nerve centres, and if injected into the blood stream rapidly (within a few seconds) disappears and becomes ' anchored ' to the tissues of the central nervous system. By intracerebral injection, therefore, the tetanus antitoxin is at once enabled to attack or counteract the tetanus toxin *in situ*. This is very important; for, as already stated, antitoxin is comparatively slowly absorbed from the subcutaneous tissues, and it may be many hours before a subcutaneous dose is completely absorbed. Intravenous injection is more efficacious, but intracerebral inoculation is obviously the one which gives the best hope of success, and in tetanus every moment's delay adds to the risk of failure. Nocard and Roux have shown experimentally that in the treatment of tetanus intracerebral is far more potent than subcutaneous inoculation of the antitoxin. The antitoxin may be injected subdurally, or into the substance of the cerebral hemispheres, or preferably into the lateral ventricles. Barker describes a successful case of the first mode of injection; the skull was trephined with a $\frac{1}{4}$-inch trephine and 7·5 c.c. of the antitoxin injected, while on each of the four succeeding days 20 c.c. was injected subcutaneously in the flanks. Semple records

a case treated by the second method with recovery.

Intracerebral inoculation is performed as follows : An imaginary line is taken over the scalp from one auditory meatus to the other, a second line is taken from the base of the nose to cross the first at right angles on the top of the head, and a third one from the outer angle of the orbit to the point of intersection of the first two lines. The centre of the last line is the seat of operation. A small incision is made through the soft tissues down to the bone and the edges of the wound are held open. Then a hole slightly larger than the needle of the syringe is drilled through the bone by means of an archimedean drill having a movable collar so that the depth to which the drill should penetrate may be regulated. The needle should be about two inches long and should have a blunt rounded point to avoid the risk of wounding a blood-vessel, and may be attached to the syringe by a short length of rubber tubing. If such a needle be not available, the ordinary syringe needle may be converted into one by filing or rubbing it down on a stone after heating it to redness in a spirit lamp or gas flame, and slowly cooling in order to soften the steel. The needle is then passed into the

brain perpendicular to its surface and 2·5 c.c. of the antitoxin injected *very slowly*. The injection should occupy at least ten minutes, in order that absorption may take place and the brain tissue be undisturbed. A similar dose should then be injected in the same manner on the other side; a total dose of 5 c.c. of the antitoxin is thus given. It is preferable to use a *concentrated* antitoxin, so that as much as possible may be administered. For this purpose the *desiccated* preparation may be used and should be dissolved in *half* the usual quantity of sterile water, viz. 1 gram in 5 c.c. Only a single administration is given intracerebrally; a subcutaneous dose of 20 c.c. is given at the same time, and this is followed by 10 c.c. thrice daily so long as severe symptoms are present.

For injection into the lateral ventricle the following method may be adopted. A point is taken $1\frac{1}{4}$ inches behind and $1\frac{1}{4}$ inches above the centre of the auditory meatus. Here the bone is drilled after incision of the soft tissues as for intracerebral injection, and the needle is passed into the brain for 5–6 centimetres (2–$2\frac{1}{2}$ inches) in a direction pointing to the tip of the auricle of the opposite side. The antitoxin is then injected as for intra-cerebral inoculation. Letour records five cases

of tetanus in which recovery followed intra-
cerebral administration of the antitoxin. The
serum was used freely, as the following details
of the dosage show :

	Case I.	II.	III.	IV.	V	
Subcutaneous	110	60	130	—	—	c.c.
Intracerebral	20	34	48	13	19	c.c.

(quoted by Grünbaum, *Practitioner*, Nov. 1902,
p. 621). Bates also records a case of recovery
following the administration of 5 c.c. into the
lateral ventricles and 35 c.c. subcutaneously
(*Lancet*, 1902, i. p. 227).

As a modification of intracerebral inoculation,
intraspinous inoculation may be mentioned, the
antitoxin being injected into the subarachnoid
space of the spinal cord. This may be done by
means of lumbar puncture ; 10 c.c. of the cerebro-
spinal fluid is drawn off, and a similar amount of
antitoxin injected. Leyden has reported a case
successfully treated by this method. D'Ancona
has also treated two severe cases in this way ;
four injections were given in each case, in the
first on the 13th, 15th, 19th, and 22nd days,
and in the second on the 5th, 8th, 9th, and 12th
days.

Directions for the Performance of Lumbar Puncture

(By LAWRIE McGAVIN, F.R.C.S.)

Lumbar puncture for the purpose of obtaining cerebro-spinal fluid for examination, or of injecting various sera into the spinal theca, is a very simple, and in most cases a perfectly painless, operation, requiring neither general nor local anæsthesia, since the skin of the middle line of the back is peculiarly insensitive.

The operation may be carried out by means of an ordinary exploring needle, which should not be less than three inches in length. Since, however, such needles are of very narrow calibre, have no stylet, and are of brittle material, it is wiser (though by no means essential) to use a Barker's spinal analgesia needle. This is wider in the bore, less liable to snap, more obliquely bevelled at the point, and has a closely fitting stylet and cannula. It is therefore less likely to be blocked by clot, or obstructed by contact with nerves of the cauda equina. The object of the cannula is to facilitate the injection of fluids into the theca without fear of their partial loss in the extra-dural space, and this is effected by the projection of the point of the cannula about one millimetre beyond the point of the needle. Thus, even if the bevelled portion of the needle is only halfway through the dural wall of the theca, the point *only* lying within it, the mouth of the cannula when *in situ* must be wholly within the theca.

The method of puncture is as follows :

The patient is placed either in the sitting or in the right lateral position, according to his condition. In

the former, he should be seated on the edge of a table, with his forearms on his knees, and leaning well forwards : in the latter, his knees should be well drawn up towards his chin, and his head well flexed towards his knees. Thus the spinous processes are as widely separated and caused to stand out as prominently as possible. The landmark required is a line joining the highest points of the iliac crests, and this passes between the third and fourth lumbar spines. The second lumbar spine is thus found and is marked by the tip of the index finger of the left hand. The puncture is made usually in the first lumbar interspace, but any of these interspaces, or even the dorsi-lumbar interspace, will do. The needle, held like a pen, is entered accurately in the middle line of the back, just above the point of the left index finger, and is pushed *directly forwards* without any sudden jerking, until, the theca being punctured, the cerebro-spinal fluid begins to appear either in drops or as a steady stream. Should the needle point encounter bone, it should be slightly withdrawn, its direction in the *vertical plane*[1] slightly altered, and again pushed forward. If no fluid is obtained after one or two attempts, it is better to try another space than to alter the direction of the needle in the *horizontal plane*. A sharp pain down one or other limb indicates contact with one of the nerves of the cauda equina of that side, and will serve to show the error in direction of the needle. The pain is momentary and no damage is done.

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[1] The words ' vertical ' and ' horizontal ' are here used in the anatomical sense.

On completion of the operation the needle is with-drawn, along with the cannula when Barker's needle is employed : there is no need to seal off the puncture wound in any way ; in fact, it is safer to simply sponge it over with some antiseptic and leave it alone.

The following points should be remembered :

1. With patients in the lateral position, especially women, the spine is apt to sag slightly, so that in cases where the spinous processes are not easily felt, allowances must be made for this.

2. The middle line should be closely adhered to owing to the presence of the posterior longi-tudinal veins on either side within the extra-dural space ; they are in little danger of injury if this rule is adhered to.

3. The spinal cord terminates opposite the body of the first lumbar vertebra, and therefore, al-though the puncture *can* be made at any point higher than this, the proceeding is neither so simple nor so free from danger ; it should there-fore be left in the hands of an expert if for any reason it cannot be done at the usual level.

4. When it is proposed to administer sera, the syringe should be capable of containing the entire amount to be injected, since the removal and refilling of it involves the risk of losing some of the serum through the needle or cannula, as well as of the displacement of the needle point from its position in the theca, during the reattachment of the syringe.

5. Not more than half an ounce of cerebro-spinal

fluid should be removed from an adult, nor more than two drams from a child, at one operation.
6. The most rigid asepsis is, of course, a *sine qua non.*

In all cases of tetanus, in addition to antitoxin, general treatment should be adopted. The wound, if there be one, should be excised, if the situation permit, or opened up and scraped and cleansed, and it might be useful to thoroughly swab it out with a solution of iodine (*e.g.* Gram's solution, p. 142), which destroys the tetanus toxin. The dry, finely powdered antitoxic serum may also with advantage be dusted over the wound. It is doubtful whether so severe a procedure as the amputation of the limb in which the wound is situated is of any use, though from experiments by Marie and Morax tetanus toxin seems to spread along the nerve trunks.

A full dose of chloral and potassium bromide (gr. 30 of the former and gr. 40 of the latter) should be given at once and repeated as occasion requires. Tartar emetic and morphine may also be given, gr. $\frac{1}{8}$–$\frac{1}{6}$ of each every two hours. Chloroform anæsthesia should be used if deglutition induces spasm. Amyl nitrite is valuable for reducing the dangerous spasm of the glottis and respiratory muscles ; capsules should be kept by the bedside and a drop or two applied on a

handkerchief to the nose. The patient should be kept in a quiet darkened room and should have abundant fluid nutriment ; plenty of fluid is indicated in order to promote the elimination of the tetanus toxin.

Prophylactic Use of Tetanus Antitoxin

Many lives might be saved if the prophylactic use of tetanus antitoxin were more general. In any wound likely to be followed by tetanus, *e.g.* lacerated and contused wounds soiled with earth, and especially if neglected, foul and suppurating, and in ' tetanus districts,'[1] injections of tetanus antitoxin may be given with the certainty of preventing the onset of tetanus. This procedure has recently been strongly advocated by Cheatle.

Since antitoxin does not immunise for more than three weeks, and since the incubation period of tetanus may be as long as one month, at least two injections of the antitoxin would be necessary to immunise for this period, viz. 10 c.c. immediately and 10 c.c. ten days later. A longer interval than ten days between the two doses should be avoided, owing to the risk of inducing anaphylactic symptoms.

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[1] In certain districts, as in the West Indies and West Coast of Africa, tetanus is extremely frequent.

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Anti-Venin (Anti-venomous Serum)

Venomous snakes belong to the two families *Colubridæ* and *Viperidæ*. The chief poisonous snakes are, in Europe, three species of viper, one (*Vipera berus*) occurring in England; in Asia, the cobra (*Naja tripudians*), king cobra (*N. bungarus*), common krait (*Bungarus cœruleus*), banded krait (*B. fasciatus*), daboia or Russell's viper (*Vipera Russellii*), phoorsa (*Echis carinatus*), and the common sea snake (*Enhydrina valakadien*); and in Japan, the habu (*Trimeresurus riukiuanus*). In North Africa a species of

cobra (*N. haje*) and two vipers (*Cerastes cornutus* and *Echis carinatus*) are common; and in South Africa a cobra (*N. flava*), the puff adder (*Bitis arietans*), and *Causus rhombeatus* are met with.

In America several rattlesnakes (*Crotalus*), the copperhead (*Ancistrodon contortrix*), and moccasin (*A. piscivorus*), and species of *Lachesis* occur. In Australia the deadly snakes are the tiger snake (*Notechis scutatus*), the black snake (*N. pseudechis*), the death adder (*Acanthophis antarcticus*), and others.

The active constituents of snake venom are partly protein in nature, consisting of a globulin-like body and of a peptone-like body, the relative proportions of which differ very much in different venoms. Cobra venom, for example, contains 2 per cent. of the globulin and 98 per cent. of the peptone, whereas rattlesnake venom contains 25 per cent. of the globulin and 75 per cent. of the peptone. The exact nature and mode of action of the poisonous constituents of snake venom are still matters of controversy. Calmette believed that all venoms have the same physiological action and differ only in their degree of toxicity. Martin stated that snake venoms probably contain two or more poisonous proteins, and that varying proportions of these constituents give rise to the different

effects of the various venoms. He thought that proteins coaguable by heat act on the blood and heart, while proteins non-coagulable by heat act on the central nervous system. Lamb, however, found that the hæmolysing constituent of cobra venom is not coagulable by heat, and that there is great variation in the physiological effects of various venoms ; thus cobra and daboia venoms have a marked hæmolytic action, cobra venom never produces intravascular clotting, daboia venom produces extensive intravascular clotting, banded krait venom induces only slight hæmolysis and moderate intravascular clotting. The poisonous constituents of snake venoms may be classified under three heads : (1) neurotoxins, which combine with nerve cells and render them inactive ; (2) cytolysins, acting on various cells, e.g. blood corpuscles, endothelium, &c. ; and (3) fibrin-ferments, which induce intravascular clotting. The potency of the various venoms —that is, the smallest amount which can be relied upon to cause the death of the rabbit when injected subcutaneously—is as follows :

Tiger-snake venom 0·00005 gram per kilo of rabbit.
Death-adder   ,,   0·0002   ,,   ,,   ,,
Cobra   ,,   0·00025   ,,   ,,   ,,
Rattlesnake   ,,   0·004   ,,   ,,   ,,
Viper   ,,   0·004   ,,   ,,   ,,

It is to be noted that the susceptibility of different animals varies very considerably ; thus, Fraser found that the minimal lethal dose of cobra venom per kilogram of body weight was for the guinea-pig 0·00018 gram, for the rabbit 0·000245 gram, for the white rat 0·00025 gram, for the cat somewhat less than 0·005 gram, but for a six-weeks-old kitten 0·002 gram, and for the grass snake 0·03 gram. The mongoose is perhaps relatively slightly less susceptible than are other animals, but it owes its immunity when attacking the cobra to its agility, whereby it escapes being bitten. The amount and activity of a snake's venom vary somewhat at different seasons and under different conditions. Thus, with one specimen of cobra venom Lamb found the minimal lethal dose for the rat to be 0·04 mgrm., with another specimen it was 0·07 mgrm. After biting a few times in rapid succession the venom becomes exhausted.

For the preparation of the anti-venin the horse is the animal chosen, and is immunised by the cautious injection of repeated doses of venom. The venom is for the most part collected from snakes that have been killed, being expressed from the poison gland through the fangs and collected on a dial glass. Or the venom glands may be dissected out, the venom dissolved from

them by means of distilled water, and the solution evaporated to dryness *in vacuo* over sulphuric acid.

From living snakes kept in captivity the venom can be obtained by seizing the snake by the head with a long pair of tongs with flattened extremities covered with india-rubber and thus lifting it from the cage without risk. It is then grasped with the left hand immediately behind the head, so that it cannot bite, and a shallow dish or dial glass, preferably covered with rubber, being presented to it, the snake strikes, and the venom is caught on the vessel ; this procedure may be repeated two or three times, and a further quantity may be expressed from the glands by applying pressure ('milking'). The venom should then be dried in a desiccator over strong sulphuric acid, and in the dry state may be kept almost indefinitely without diminution in toxicity. For use a weighed quantity is dissolved in sterile distilled water.

The immunisation of the horse is an extremely tedious process : in the earlier stages because the dose must be increased very slowly and cautiously ; in the later ones, when a large dose has been attained, on account of the difficulty of procuring a sufficient supply of the venom. In the earlier stages, the process of immunisation

may be accelerated by giving some preliminary injections of anti-venin.

The dosage of venom for immunising may be illustrated by the following example given by Tidswell for that of the Australian tiger snake :

The treatment was commenced on June 7, 1898, by the subcutaneous injection of ·0005 gram of the venom. This was repeated in a week, and a week later the dose was increased to ·00075 gram. Increments of ·00025 at each dose were maintained during the first six months of the treatment, but after that they were greater, viz. ·0005 (January 1899), ·01 (March 1899), ·05 (May 1899), and ·1 (January 1901), gram. The increments were pretty regularly given, the same dose being repeated only on the rare occasions when the reaction was more than usually pronounced. Between October 1899 and May 1900 the pressure of other work interfered with regular treatment, but otherwise the horse was injected once a week (June 1898 to April 1899) ; once a fortnight (May 1899 to May 1900) ; and once a month (July 1900 to January 1902). The lengthening of the intervals was due to the difficulty of collecting the larger amounts of venom required as the dose increased. This same difficulty limited the maximum dosage to 0·6 gram, which was reached in April

1901, and which it was not possible to maintain more than approximately. During the period of three-and-a-half years covered by the treatment the horse received a total quantity of about 10 grams of pure tiger-snake venom. The dose which the horse could finally bear without effect (0·6 gram) was about equal to the aggregate yield of twenty-one or twenty-two average snakes, and the total amount received by the horse during the treatment was about equal to the amount which would be yielded by 333 average snakes.

Calmette commences immunisation with 1 milligram of cobra venom.

Standardising the Anti-venin

Calmette points out that neither the method of Behring nor that of Roux employed for testing diphtheria and tetanus antitoxins is applicable in the case of anti-venin, because (1) the sensibility of various animals to snake venom is very different, (2) the toxicity of the venom is different for each species of venomous serpent, and for the same species varies with the time of collection, and (3) the amount of anti-venin required to immunise different animals varies inversely as their resistance. Thus the amount

of serum required to immunise a guinea-pig of 500 grams weight against the minimal lethal dose of venom is about twelve times greater than is required to immunise a rabbit weighing 2,000 grams. The fowl is one of the most sensitive of animals to the venom. Calmette therefore suggested and adopts the following method of standardisation :

1. The amount of dry venom which is certainly lethal in fifteen to twenty minutes for the rabbit is determined, the dose being dissolved in sterile water and injected into the marginal vein of the ear. This varies from 0·5 milligram (*Bungarus cœruleus*) to 6 milligrams (viper).

2. A series of rabbits, each of about 2 kilograms in weight, is injected intravenously (by the aural vein) with increasing amounts of the anti-venin, 0·5 c.c., 1 c.c., 2 c.c., 3 c.c., &c.

3. A quarter of an hour after the injection of the serum, the lethal dose of venom is injected into the other aural vein. If 1 c.c. of the serum suffices to keep alive a rabbit weighing 2,000 grams, the serum is said to contain 2,000 units per c.c. or 20,000 units in 10 c.c.

Semple and Lamb raise the following objections to Calmette's method, viz. : (1) there is no direct estimation of the amount of venom which a given quantity of serum will neutralise ;

(2) no estimation is made of the amount of venom the injection of which an untreated animal is capable of surviving ; and (3) Calmette's test dose for the rabbit amounts to about three lethal doses for that animal.

Calmette apparently uses both for immunising and for standardising a mixture of venoms heated to 73° C. for half an hour and then filtered. Myers pointed out that it would be preferable, in order to obtain a correct estimate of the curative value of the serum, to use an unheated venom, and he found that mice would do quite as well as rabbits for testing purposes. He also showed that more accurate results are obtained by mixing the venom and serum *in vitro* than by Calmette's method. Lamb and Hanna employ rats weighing about 115–120 grams for testing purposes. They are larger and more easily managed than mice and are very susceptible, the minimal lethal dose of cobra venom being at the rate of 0·33 milligram per kilogram of body weight. The venom for testing is obtained by ' milking ' the snakes and drying in a desiccator. For use it is powdered, again dried over sulphuric acid, and weighed out, a 0·1 per cent. solution being made in physiological salt solution. The certain minimal lethal dose for rats of the above weight was found to be 0·04

milligram, and 0·035 milligram was the maximum non-lethal dose, and for testing ten times the certain lethal dose was used, viz. 0·4 milligram. This test-dose of venom was mixed *in vitro* with varying amounts of the serum to be tested, allowed to stand for not less than half an hour at the laboratory temperature (25° C.), and then injected subcutaneously.

The following table (Table V.) given by Lamb and Hanna illustrates the method and the results obtained :

TABLE V.—EXPERIMENTS TO ASCERTAIN THE AMOUNT OF VENOM WHICH ONE CUBIC CENTIMETRE OF COMPARATIVELY FRESH ANTI-VENOMOUS SERUM WAS CAPABLE OF NEUTRALISING

Animal	Amount of dried venom in milligrams	Amount of serum in cubic centimetres	Result
Rat 1	0·4	0·2	Died in 2 hours
2	0·4	0·3	,, ,, ,,
3	0·4	0·4	Died within 42 hours
4	0·4	0·5	,, ,, ,,
5	0·4	0·5	Died within 46 hours
6	0·4	0·6	Ill for one day ; recovered
7	0·4	0·6	No symptoms
8	0·4	0·7	,, ,,
9	0·4	0·7	,, ,,
10	0·4	0·8	,, ,,

From this testing it is seen that 0·5 c.c. of this serum failed to neutralise 0·40 − ·035 = 0·0365 milligram, while 0·6 c.c. neutralised at least this

amount ; therefore 1 c.c. would neutralise 0·61 milligram of cobra venom.

Specificity of Anti-venin

The specificity or otherwise of anti-venin is of considerable importance. Calmette for a long time maintained that anti-venin is not specific ; that is, cobra anti-venomous serum, though acting most potently against cobra venom, would also antagonise other venoms. Some experiments by Martin threw considerable doubt upon this, and the further ones of Tidswell conclusively showed that Calmette's anti-venin, which is prepared mainly with cobra venom, though small quantities of other venoms are also used, has little or no neutralising power against tiger-snake venom, and that his own tiger-snake anti-venin does not neutralise the venoms of other Australian snakes, viz. those of the black snake, brown snake, and death adder.

These results have been extended by Lamb, who found that tiger-snake anti-venin has no neutralising action against the venoms of the cobra, banded krait, and Russell's viper, and that cobra anti-venin (Calmette's) does not antagonise the venoms of the banded krait, Russell's viper, and hamadryad.

Therapeutic Use of Anti-venin

The action of snake venom is so rapid that unless the case be seen soon after the bite, the chances of successful treatment are remote. A ligature should at once be applied above the bite, if the locality permit. The wound should be opened up and thoroughly washed out with a solution of chromic acid (1 per cent.), or, still better, a solution of chloride of gold (1 per cent.), a freshly prepared solution of chloride of lime (1 in 60), or a strong solution of potassium permanganate. The two last-named substances are most efficacious in destroying any venom that may not have been absorbed, and Fayrer and Brunton have devised a hollow lancet by which powdered potassium permanganate can be introduced into the deeper parts of the wound.

Without delay an injection of anti-venin should be given intravenously into a superficial vein. Calmette recommends that from 10 c.c. to 20 c.c. should be given. But Lamb and Hanna calculate from their experiments that about 37 c.c. of Calmette's serum would be required to neutralise the full dose of venom which a cobra could inject at a bite, assuming man to be as susceptible as the most susceptible

animal, and Martin and Lamb regard 100 c.c. or even 200 c.c. as probably the minimal neutralising dose for a full dose of cobra venom.

Fraser and Calmette, however, consider that the lethal dose for man approximates probably to that for the cat or dog, rather than to that for the vegetable feeders, the rabbit and guinea-pig, and this may account for the many cases of snake-bite reported to have been cured with comparatively small amounts of anti-venin. But to be on the safe side, at least 30 or 40 c.c. of the anti-venin should be injected in every case of cobra-bite, and a still larger dose if symptoms have already ensued. Symptomatic treatment should also be employed, rest, warmth, stimulants, such as alcohol (in moderate amount, so as to avoid any narcotic effect), ether, ammonia, strong coffee, with strychnine hypodermically, and electricity if respiratory paralysis threatens.

A number of cases of snake-bite successfully treated by anti-venin have been reported in India and elsewhere.

In Japan 118 cases of 'habu' bite were treated with the anti-habu serum, with five deaths, a mortality of about 3–4 per cent., the total amount used for a case being 40–80 c.c. Of 2,028 cases bitten and not treated with the

serum, 263 died, a mortality of 12·9 per cent. So far it has not been found practicable to prepare a polyvalent serum, which is necessary if any snake-bite is to be treated with success.

The experiments of Martin and of Tidswell mentioned above show that the anti-venin prepared by Calmette at Lille, which is practically the only serum on the market, can hardly be relied upon as an antidote to any venom other than that of the cobra.

N.B.—The anti-venin must be as fresh as possible, for Lamb and Hanna have found that it undergoes a progressive and fairly rapid deterioration when stored in hot climates, and that this deterioration is greater and more rapid the higher the mean temperature to which it is subjected.

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CHAPTER V

*THE ANTI-MICROBIC SERA — ANTI-STREPTO-
COCCIC SERUM—ANTI-PNEUMOCOCCIC SERUM
— ANTI - MENINGOCOCCIC SERUM — ANTI -
PLAGUE SERUM—INJECTION OF ANTI-STREP-
TOCOCCIC AND ANTI-PLAGUE SERA—ANTI-
TYPHOID SERA — ANTI-TYPHOID EXTRACT OF
JEZ—OTHER SERA*

In the previous chapter the chief antitoxic
sera have been dealt with. We now have to
consider those diseases the specific micro-
organisms of which produce little or no toxin,
and the sera for which are mainly anti-microbic
in nature. The chief of these are streptococcic
infections, pneumonia, plague, and enteric or
typhoid fever.

Anti-streptococcic Serum

Marmorek was the first to prepare an anti-
serum for streptococcic infection. The *Strepto-
coccus pyogenes* or *erysipelatis* (the two forms
are generally regarded as being identical) is the
organism met with in erysipelas and in certain

forms of pyæmia and spreading septic infections. It forms but little toxin in cultivations, and immunisation has to be carried out by means of the cultures. Recent work shows that there are a number of different strains of streptococci differing in their virulence, the length of their chains, their fermentation reactions, and production of hæmolysins.

Preparation of the Streptococcus Cultures

Enhancing the Virulence.—In the first place, the virulence of the streptococcus has to be raised by a succession of passages through a susceptible animal, the rabbit being that generally employed. The mouse may also be used for the earlier inoculations, and when a fair degree of virulence has been attained the rabbit is substituted. Of an ordinary streptococcus just isolated 0·5 c.c. of a thirty-hours' broth culture, injected subcutaneously, may be required to kill a mouse, or 1 c.c. intravenously a rabbit. When the animal dies, the heart-blood, obtained in a glass pipette, is injected into a second animal, and so on. The inoculations may be carried on from animal to animal, without cultivation on artificial media, or the heart-blood may be inoculated into the

special serum broth (see below), and cultivated for forty-eight hours, and this culture is then inoculated into the second animal, and this process is repeated again and again. Aronson found that by inoculating rabbits with broth cultures of feebly virulent streptococci, together with a small amount of diphtheria toxin, the animals died, and the subcultures from them were then found to have attained sufficient virulence to kill without the aid of the toxin, and the organisms were further increased in virulence by a succession of passages. Bulloch recommends cultivating for eighteen hours, inoculating after the first two or three passages subcutaneously in the abdomen, and sub-culturing from the liver. The number of passages required varies from about twenty to thirty, at the end of which time the virulence does not become exalted by further passages ; the culture may therefore be termed a ' fixed virus.' The virulence has now reached an extraordinary pitch ; according to Marmorek, one thousand-millionth ($\frac{1}{1,000,000,000}$) of a cubic centimetre of broth culture will kill a large proportion of the rabbits inoculated with it, while one hundred-millionth ($\frac{1}{100,000,000}$) of a cubic centimetre is invariably fatal. If the dose be larger, e.g. 0·1 c.c., the animal dies within a few hours. The

culture needs to be continuously passed through rabbits in order to keep up the virulence. Bokenham states that he is by no means sure that extreme virulence is necessary, and considers that the conditions of cultivation and of the culture-medium may perhaps be of greater importance.

Preparation of the Cultures.—Ordinary broth is not a suitable culture-medium, as in it the virulence of the streptococcus is rapidly lost. The best medium of all consists of human blood-serum 2 parts, and ordinary peptone beef broth 1 part. Since human blood-serum is difficult to obtain, ascitic or pleuritic fluid may be substituted, 1 part of this and 2 parts of beef broth. Failing this, ass's or horse's blood-serum may be used, preferably the former, 2 parts, beef broth 1 part. Veal broth may be substituted for beef broth with advantage. As a culture-medium, Aronson uses a bouillon made from horse-flesh and containing 0·5 per cent. sodium chloride, 0·5 per cent. peptone, and 0·1 per cent. glucose, the reaction being neutral to phenol-phthalein, but alkaline to litmus. The cultures are grown for from two to three weeks, and then the whole, *i.e.* unfiltered, cultures, containing both microbes and toxin, are used for the inoculations. Bordet grows the

cultures for twenty-four hours only. For the earlier injections the cultures are killed by heating to 58°–60° C. in a water-bath for one to three hours, care being taken that the cultures are thoroughly mixed during the sterilisation, so as to ensure all parts being raised to the lethal temperature. Subsequently, when some immunity has been attained, the living cultures may be employed, and are injected intravenously to avoid the formation of abscesses, commencing with small doses.

Besredka recommends cultivating on a solid medium. Plate bottles (p. 202) are prepared with agar, and an hour or so before insemination 1–1·5 c.c. of horse serum previously heated to 56° C. for half an hour is spread over the surface. On this medium an abundant growth is obtained in twenty-four hours ; the growth for injection is suspended in 100 c.c. of salt solution.

Immunisation

The horse, mule, or ass may be employed ; the horse, however, is much less susceptible than the ass to the toxic action of the cultures. With the horse, the treatment may be commenced by an injection of 3–5 c.c. of the killed culture. Since horses vary very much in

sensitiveness it may be well first to give a single cubic centimetre in order to gain some idea of the reaction that will occur. This induces a rise of temperature of 2–4 degrees. A second injection is given when all reaction produced by the first one has disappeared. The dose is gradually increased, the aim being to produce some amount of reaction with each injection.

The process of immunisation is a tedious one and several months' treatment is necessary before a sufficiently active serum is obtained. Ultimately a dose of 100–200 c.c. of culture is administered.

The period of bleeding the horse is important, since Marmorek found the serum to be toxic up to fifteen days after the last inoculation ; Bulloch, however, did not find it toxic for a longer period than twenty-four hours after the inoculation. Perhaps the period may differ with different strains of the organism, and it would be well to test this point in all cases, by inoculating rabbits subcutaneously with 5 c.c. of the serum, taken at varying intervals after the last inoculation.

Aronson has raised the question whether a serum obtained by means of streptococci isolated from man and passed through animals is

active against a virulent human streptococcus not passed through animals ; he finds that it is.

Streptococci virulent for animals are occasionally obtained from man, and may be readily cultivated without loss of virulence in a broth somewhat more alkaline than usual and subcultured every fortnight. Aronson is of opinion that a serum prepared with such non-passaged cultures is more active than one obtained with cultures passed through animals.

Aronson also found that the serum of treated horses protects monkeys from streptococcal infection ; a fact on which some doubt had been thrown by experiments by Zangemeister, who concluded that horse anti-streptococcic serum was inactive against streptococcal infection in monkeys.

Polyvalent Serum

While Marmorek and others have affirmed the unity of streptococci obtained from various sources, the general opinion now is that there are a number of different strains, if not species, and Piorkowski has found that one streptococcus may not protect against another. It is therefore essential to conduct the immunisation with several strains of the streptococcus isolated from different sources, as has been done by

Aronson, by Tavel, and by Besredka, and is now generally adopted.

Standardising the Serum

A mouse is inoculated subcutaneously with 10 minimal lethal doses of a serum-broth culture (probably $\frac{1}{1,600,000}$ c.c.), and 18–24 hours afterwards 0·001 c.c. of the serum injected intra-peritoneally should protect. A rabbit injected with 100 lethal doses (probably $\frac{1}{40,000}$ c.c. of culture) should be protected by 1·5–2·0 c.c. of the serum injected two hours later into the veins or peritoneal cavity.

Therapeutic Use of Anti-streptococcic Serum

The therapeutic use of streptococcic serum in septic and puerperal infections, it must be con-fessed, has not been followed by that benefit which was at one time anticipated. This is due partly to the fact that these conditions are not solely due to streptococci, but may be dependent upon infection with a number of other organisms, e.g. various staphylococci, the *Micrococcus tetra-genus*, pneumococcus, *Bacillus coli*, gonococcus, &c. An anti-serum being specific, anti-strepto-coccic serum will act only in a streptococcic

infection, and, as mentioned above, an anti-serum for one strain of streptococcus may not be active towards another strain.   Lastly, it has to be remembered that an anti-microbic serum, such as anti-streptococcic serum, is never as potent as an antitoxic serum.   Nevertheless, in any septic infection the use of anti-strepto-coccic serum should be tried ; it will do no harm, and three or four doses will prove whether it will do good.   A dose of 10 to 20 c.c. should be given twice daily, and if any decided effect upon the temperature and general condition ensues, it should be persevered with (see also p. 216). Some continental authorities have suggested giving far larger doses (up to 150 c.c.) than those here suggested. *The serum should be obtained as fresh as possible ; it probably rapidly deteriorates with keeping.*

It is in cutaneous erysipelas perhaps that the best results are obtained with anti-streptococcic serum : a dose of 10 c.c. given twice daily usually cuts short the attack within two or three days. The writer has obtained excellent results with the serum treatment in this disease.   As regards puerperal infection, a Committee of the American Gynæcological Society in 1899 remarked that, if any benefit is to be derived from the use of anti-streptococcic serum in a given case of

infection, it will respond to the injection of 20–30 c.c., and from 30–50 c.c. will control responsive cases if treatment be commenced early.

Diphtheria.—Treatment with anti-streptococcic serum may be combined with antitoxin treatment when the throat is foul and sloughing.

Scarlatina.—As streptococci are generally found associated with scarlatina, Moser, Escherich, Bujwid and Gertler and others have employed anti-streptococcic serum in the treatment of this disease. There seems little evidence, however, that the disease is beneficially influenced thereby, but in some cases in which a bad septic throat is a prominent symptom anti-streptococcic serum may do good.

Acute Rheumatism.—A serum prepared with the coccus (*D. rheumaticus*) met with in acute rheumatism has been employed in the treatment of the disease.

Gonorrhœa.—Soltau Fenwick recommends the rectal administration of polyvalent anti-streptococcic serum in gonorrhœal pyæmia and arthritis.

Another and promising method of treating streptococcic infections is by means of vaccines

Prophylactic Use of Anti-streptococcic Serum

The prophylactic use of anti-streptococcic serum has been suggested by Watson Cheyne and others before operations upon the mouth and throat to prevent the septic pneumonia which so frequently develops after these procedures ; also in operations upon the rectum. A dose of 10 c.c. should be given on each of the three days preceding the day of operation.

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Anti-pneumococcic Serum

G. and F. Klemperer first immunised rabbits against the pneumococcus by the intravenous injection of a broth culture, the vitality of which had been destroyed by heating to 60° C. for one to two hours, and subsequently inoculating

the animals with living cultures. During this treatment many animals may succumb, but those which recover are rendered immune to subsequent inoculation. This is probably the best method, but others may be used; for example : injection with filtered cultures, or of a glycerin extract of blood from a case of pneumococcic infection, or by inoculation with attenuated cultures. Washbourn was the first in this country to immunise a horse against the pneumococcus. The pony was injected with broth cultivations that had been heated to 60° C. for one hour, commencing with about 50 c.c. injected subcutaneously in the shoulder, the injections being repeated so soon as all reaction caused by the preceding injection had passed off. Afterwards living broth cultivations and emulsions of living agar cultures were employed. The following data given by Washbourn indicate the method of procedure and the effect upon the animal :

November 21, 1895. 70 c.c. broth cultivation heated to 60° C. for one hour. The temperature rose next morning to 103·5° F., but soon subsided.

November 30. 150 c.c. broth cultivation heated to 60° C. for one hour. No pyrexia and no local reaction.

December 7. A small loopful of a living agar cultivation. No local reaction.

December 13. The whole of a living agar cultivation. No local reaction.

December 19. Two living agar cultivations. No local reaction.

January 4, 1896. Six living agar cultivations. A swelling appeared at the seat of injection and lasted five days.

January 13. 50 c.c. living broth cultivation. A swelling of the size of an orange appeared at the seat of injection and lasted a few days.

January 21. 82 c.c. living broth cultivation. A large swelling appeared at the seat of inoculation and lasted nine days.

February 7. 150 c.c. living broth cultivation. A diffuse swelling appeared, but soon subsided. The temperature reached 104·2° F.

February 21. 100 c.c. living broth cultivation injected into both sides of the neck. A swelling appeared on both sides of the neck, more marked on the right side. This subsided in a few days. The temperature reached 103° F.

March 3. 112 c.c. living broth cultivation injected into both sides of the neck. The animal appeared ill for twenty-four hours, and there was some swelling at the seat of injection.

March 14. 150 c.c. broth cultivation injected

into both sides of the neck. Some swelling occurred on both sides, but soon disappeared. Animal ill for twenty-four hours.

March 27. 200 c.c. broth cultivation injected into both sides of the neck. Marked swelling occurred on both sides, and lasted two days.

The animal then developed an abscess in the near foreleg, due to streptococcic infection, and the injections were discontinued, but five months afterwards it was found that 0·03 c.c. of the serum protected against ten lethal doses of the pneumococcus.

Pane has in a similar manner prepared a highly active anti-pneumococcic serum which has been found by Washbourn and Eyre to possess the protective and immunising powers claimed for it. Another active anti-pneumococcic serum is prepared by Römer, but is somewhat expensive.

Different strains of the pneumococcus are found to vary considerably in virulence, and it is necessary to employ a virulent one, which may be obtained by passage through a succession of rabbits (8–12). The virulence is easily lost on artificial cultivation and must be kept up by an occasional passage through two or three rabbits, and the culture-medium for subculturing should

always be agar smeared with blood, preferably rabbit's blood.

Further, distinct varieties of the pneumococcus exist—thus Foa found that animals immunised against one variety of pneumococcus were not necessarily immunised against another, and Washbourn and Eyre that, whereas Pane's serum protected against four out of five varieties of the pneumococcus examined, it possessed no protective power against the fifth, which was a typical pneumococcus obtained from a fatal case of pneumonia. The serum should therefore be a ' polyvalent ' one.

The horse having been treated for a long period of time, five to nine months, the strength of the serum must be estimated. This is done by obtaining a pneumococcus of fixed virulence —that is, one the virulence of which, after a succession of passages through rabbits, is not increased by further passages. The pneumococcus of fixed virulence having thus been obtained, a special culture-medium is employed, so that the virulence may be preserved unaltered. This is a nutrient agar having a definite reaction smeared with sterile rabbit's blood, and the cultures are carefully capped and preserved in the incubator at a temperature of 37·5° C. The nutrient agar during its preparation is never heated above

100° C., and after it is made it is neutralised with caustic soda solution, rosolic acid being used as the indicator. After neutralisation 4 c.c. of normal caustic soda solution are added to each litre of the agar. Cultivations twenty-four hours old are used, and platinum loopfuls of the growth are made into an emulsion with a known quantity of sterile broth, so that by dilution any fraction of the contents of the loop can be obtained. The same-sized loop is used throughout, and should contain 0·5 milligram of the growth. First of all, the minimal fatal dose of the culture is ascertained by injecting varying fractions of a loopful into the peritoneal cavity of rabbits. Having done this, measured quantities of the serum to be tested are mixed with *ten times* the minimal fatal dose of the culture, and the mixtures immediately after being made are injected into the peritoneal cavities of rabbits. The smallest quantity of serum which protects the animal in these circumstances Washbourn terms a unit. For instance, if 0·03 c.c. of the serum mixed with ten times the minimal fatal dose protects, then each cubic centimetre of the serum contains approximately 33 units. Usually 0·000001 of a loop represented the minimal fatal dose, therefore 0·00001 of a loop was the quantity mixed with the serum.

Occasionally the minimal fatal dose was one-tenth of that stated, viz. 0·0000001 loop, as was the case in the following testing given by Washbourn :

	grms.		
Rabbit O	3,000	0·0000001 loop (control)	Died in 36 hrs.
,, P	3,600	0·000001 ,, ,,	,, 60 ,,
,, Q	3,000	0·000001 ,, + 0·03 c.c. serum . .	Not affected
,, R	1,600	0·000001 ,, + 0·015 c.c. serum . .	Died in 36 hrs.

Minimal fatal dose = 0·0000001 loop.

With the same serum it was found that 66 units protected against ten minimal lethal doses of the pneumococcus when injected five to six hours, but only delayed the fatal event when injected eight or twelve hours, after the infection.

Landman [1] has prepared a polyvalent anti-pneumococcic serum by the injection of a number of strains of the pneumococcus into horses, oxen, and sheep and mixing these sera for use. Experimentally the serum appeared to be very active.

Therapeutic Use of Anti-pneumococcic Serum

Anti-pneumococcic serum must necessarily have a limited therapeutic use, though if it can

[1] *Deut. med. Woch.*, Nov. 26, 1908.

be obtained it might be employed in elderly, debilitated, or otherwise unhealthy subjects in whom the disease is likely to run a serious course. Pane's or Römer's serum seems to be the most potent. Probably a dose of 20 to 30 c.c. should be given subcutaneously twice daily until convalescence is established. Serum treatment may also be combined with vaccine treatment.

Wilson describes the treatment of 18 cases of acute croupous pneumonia with the anti-pneumococcic serum. The serum was not used to the exclusion of other treatment, which consisted in the systematic administration of Dover's powder, ice-bags to the affected region, the use of calomel, strychnine, alcohol, and inhalation of oxygen when necessary, and other symptomatic treatment as seemed judicious. Of the 18 cases four died—a mortality of 22·2 per cent. In two of the fatal cases no improvement followed the administration of the serum ; in the other two, slight improvement followed the earlier doses, but the later injections were without effect. The defervescence was by crisis or rapid lysis. The duration of the attack varied between five and fourteen days, the majority of the cases coming to an end on the sixth, seventh, or eighth day.

The summary of the dosages is interesting.

In the earlier cases the serum was administered somewhat timidly, and at considerable intervals, the effect being closely watched ; later it was given freely and repeatedly. In the first four cases the age of the serum—that is, the period that had elapsed from the time the serum was drawn from the animal until its employment therapeutically—was not ascertained. In the subsequent cases this was known and recorded. The serum in all the cases was administered hypodermically, and the total quantity given varied from 20 c.c. to 460 c.c., three cases each receiving 400 c.c. or more. The administration extended over a period varying from six hours to eight days. The age of the serum, as above stated, varied from seven to fifty-three days. The immediate effects were more favourable and more marked in recently drawn serum than in that which had been drawn for longer periods. They consisted, in general, in lowering of the temperature and pulse frequency, mitigation of pain and of the tendency to drowsiness. Several of the patients expressed themselves as feeling better after the injection, and seemed to be anxious for the time when it should be repeated. The average duration of the stay in hospital of 13 out of the 14 cases that recovered was twenty and a half days. Of 20 patients

admitted into another hospital during the same period, and treated in the ordinary manner without serum, four died—a mortality of 20 per cent.—so that it cannot be said that the mortality was lessened by the use of the serum.

Six cases of pneumonia treated with anti-pneumococcic serum with one death are described by Tyler. He found toxæmic symptoms to be completely absent in cases treated with serum ; but it was doubtful if the serum had any effect upon the condition of the affected lung, though it seemed to prevent the involvement of fresh areas. He gave 20 c.c. of the serum every six to eight hours until the temperature reached the normal.

Tyler has also collected the records of 141 cases of pneumonia treated with anti-pneumo-coccic serum, with 121 recoveries and 20 deaths—a mortality of 14·18 per cent. Excluding several alcoholic, cardiac, and other cases, 127 are left with six deaths—a mortality of only 4·7 per cent.

Knauth treated seven severe cases of pneumonia with Römer's serum. All recovered and he considered the serum to be very beneficial. Pässler also treated twenty-four cases of pneumonia with Römer's serum with four deaths ; of the four fatal cases, two were moribund and

died a few hours after injection, a third had an ulcerative pneumococcic endocarditis. In the cases which recovered the serum seemed to help recovery, having a decided beneficial effect on the respiratory and circulatory symptoms.

On the whole anti-pneumococcic serum cannot be said to have been very successful, and vaccine treatment has in this country practically superseded it (see p. 300), though there is no reason why serum and vaccine treatment should not be combined.

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Diphtheria Antitoxin in Pneumonia and in Asthma

Talamon treated 50 cases of acute pneumonia with injections of diphtheria antitoxin with only seven deaths, or a mortality of 14 per cent. Under similar conditions, but with ordinary treatment, the mortality during the preceding year had been 37 per cent. The cases were

unselected, of all ages and of all degrees of severity, including eight alcoholic ones. Twenty-five of the patients came under treatment from the second to the fifth day, and among these the mortality was only 4 per cent. Usually two or three injections of 20 c.c. each are necessary for patients under fifty years of age and four or five for those above this age. The course of the temperature is the guide as to the frequency of the injections. Usually each injection is followed the next morning by a lowering of the temperature ; should the temperature continue to fall towards evening, an injection is not necessary ; should it tend to rise, then the injection should be repeated. The total amount of the serum used in a patient coming under treatment early should not exceed 20, 40, or 60 c.c., according to the age. In grave cases there should be no hesitation in injecting 20 c.c. morning and evening, to be repeated the following day should the temperature not fall.[1]

Legros has not been able to observe any beneficial result from the use of diphtheria antitoxin in mice infected with cultures of the pneumococcus.[2] It is to be noted that experimental infection is probably very different from the pulmonary infection or pneumonia in man.

O'Malley and Paton have also used diphtheria antitoxin in various infective conditions, and in bronchopneumonia of children.[3] It may be of some use, and if so probably acts by stimulating phagocytosis and improving nutrition.

[1] *La Sem. Méd.*, February 27, 1901, p. 69.
[2] *Ibid.* May 8, 1901, p. 158.
[3] See ref. in *Treatment*, March 1903, p. 36.

Diphtheria antitoxin has also been given in asthma, 1–3 c.c., injected subcutaneously on two or three successive days, with, it is stated, much benefit.

Correspondents in the *Brit. Med. Journ.* (1909, ii. pp. 300, 356) refer to an injection of diphtheria antitoxin *inducing* (1) an attack of asthma, (2) an attack of fatal dyspnœa in an asthmatic subject.

Aikman[1] has found a dose of 1000 units of diphtheria antitoxin, repeated at intervals, of considerable service in asthma.

Duke[2] notes that Haffkine's prophylactic appeared to cure several patients suffering from asthma.

Anti-meningococcic Serum

Epidemic cerebro-spinal meningitis or spotted fever is caused by the *Diplococcus meningitidis* of Weichselbaum. An anti-serum has been prepared by Plesner, Kolle and Wassermann, Dopter, Flexner, Jobling, and others by cultivating the organism on blood-agar, emulsifying the growth, and injecting the killed and then the living culture into horses.

The serum for use is warmed to the body temperature by standing in water at about 40° C., and is then injected intraspinously by lumbar puncture (p. 158) after withdrawal of

[1] *Brit. Med. Journ.* 1909, ii. p. 1016.
[2] *Ibid.* p. 580.

an equivalent amount of cerebro-spinal fluid.
The dose at one injection is 20–30 c.c., and
is repeated on three or four successive days
or even longer. The injection should be done
slowly so as to avoid compression, particularly if
it has only been possible to withdraw a volume
of cerebro-spinal fluid smaller than the volume
of serum injected.

Although Horder and Gordon were unable to
find any evidence experimentally of protective
influence exerted by anti-meningococcic sera,
the statistics of the serum treatment of the
disease in man indicate that it is of very decided
value, the mortality being reduced from 60–70
per cent. to 30 per cent.

In a survey of the value of serum treatment,
de Meric gives the following table of the mor-
tality without and with serum treatment :

MORTALITY PER 100

	Without Treatment	With Treatment
Philadelphia, 1907 . . .	—	20
Boston, 1908	75	20
Porterville	95	25
Scotland and Ireland . . .	70	26
Belfort	72	30
Cleveland	80	32
New York	90	34
Edinburgh	79·5	43

The influence of early treatment is also evident, for of 123 cases treated from the first to the third day of the disease there were 16 deaths, a mortality of 16·5 per cent. ; of 126 cases treated from the third to the seventh day there were 40 deaths, a mortality of 23·8 per cent. ; of 112 cases treated after the seventh day there were 39 deaths, a mortality of 35 per cent.

Epidemic cerebro-spinal meningitis has also been treated by vaccine therapy (see p. 314).

See Kolle and Wassermann, *Deutch. med. Woch.* 1906, Nos. 16 and 22 ; Flexner, *Journ. Exper. Med.*, March 1907 ; Flexner and Jobling, *Studies from the Rockefeller Institute for Med. Research*, ix. 1909, p. 690 ; Horder and Gordon, *Rep. Med. Officer Loc. Gov. Board.* 1907–1908, p. 341 ; de Meric, *Med. Press and Circular*, March 9, 1910, p. 250.

Anti-plague Serum

An anti-plague serum was first prepared by Yersin in 1895, since when several modifications have been introduced by different workers.

Preparation of the Anti-plague Serum

In Yersin's method increasing quantities of living agar cultures were injected intravenously into a horse. The method now employed at the

Pasteur Institute is as follows : A virulent plague bacillus is made use of, and it is well to employ many strains of the bacillus isolated from different epidemics. The organism must not be passed through animals, or its virulence, though enhanced for one species, may be diminished for another. The virulence may be maintained unimpaired by cultivating on surface agar ; a tube is inoculated and incubated at 37° C. for twenty-four hours, and is then kept at room temperature, and a fresh subculture is made every week.
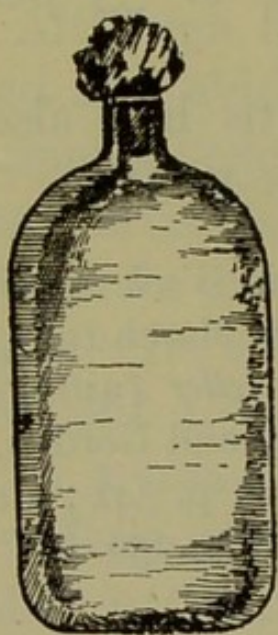
FIG. 22.
'PLATE' BOTTLE.

The material for inoculating the horses is obtained by growing the bacillus on the surface of agar in flat bottle-shaped flasks (fig. 22), the broad side of which has an area of 20 × 10 centimetres and is coated with nutrient agar. An ordinary surface agar tube culture of 18–24 hours' growth is used for inoculating. A little sterile broth or physiological salt solution is introduced into the tube, and an emulsion of the growth prepared, and 1 c.c. of this emulsion is sprayed over the surface of the agar in one of the flat flasks by means of a sterile glass

pipette, and the flask, laid flat, is incubated at 37° C. for from thirty-six hours to three days. A good growth having been obtained, 20 c.c. of physiological salt solution are introduced into the flask, and an emulsion of the growth is made. The emulsion is then filtered through a layer of sterile cotton-wool to remove particles. .

In the earlier stages of the inoculation this emulsion is heated to 65° C. for one hour, in order to kill the bacilli, care being taken that the whole of the fluid attains and is kept at this temperature for this time. The first dose is a little less than 1 c.c. of this killed emulsion, and about a week is usually allowed to elapse between the injections. The dose is gradually increased until, at the end of about three months, the bactericidal power of the blood will have increased to such a degree that if living bacilli be injected they will be almost immediately destroyed. Then the living cultures are injected, one dose every week, until at the end of about six months the whole growth from one flask may be injected without causing symptoms.

Both the dead and the living cultures are inoculated intravenously ; subcutaneous injection gives rise to abscesses.

An interval of a fortnight is allowed to elapse between the last dose and the bleeding, which is carried out in the usual way.

Testing the Anti-plague Serum

The protective power of the serum may be tested by injecting $\frac{1}{20}$ c.c. of the serum into a mouse, and twenty-four hours afterwards pricking it in the hind foot with a needle dipped in a plague culture. This amount of serum should suffice to protect the animal completely.

The curative power of the serum is tested by pricking a mouse in the hind foot with a needle dipped in a plague culture, and sixteen hours afterwards injecting $\frac{1}{4}$ c.c. of the serum ; this should be sufficient to save the animal's life.

The preparation of the cultures, inoculation of the horses, and testing of the serum must be carried out with all precautions to avoid the dissemination of infection. The access of flies to, and the escape of fleas from, the test animals should be prevented by the use of wire gauze.

Lustig's Anti-plague Serum

Lustig and Galeotti devised an anti-plague vaccine, and by repeated inoculation of a horse

with this vaccine, an anti-serum can be obtained. The vaccine fluid for inoculation is prepared by growing the bacillus on the surface of agar in dishes for three days, scraping off the growth, and treating with 1 per cent. caustic soda solution. The fluid is then filtered through paper and precipitated with very dilute acetic or hydrochloric acid, or by saturation with ammonium sulphate. The precipitate is dissolved in a 0·5 per cent. solution of sodium carbonate, and filtered through a Chamberland filter. This forms the vaccine fluid, which has the chemical characters of a solution of nucleo-proteins.

Therapeutic Use of Anti-plague Serum

It is difficult to estimate with any approach to accuracy the value of anti-plague serum, on account of the variation of the mortality of the disease in the different clinical varieties, in different epidemics, and in different races, and the influence of sex and age.

The following details are largely taken from papers by Choksy, who is the chief exponent of the serum treatment of plague.

The following table illustrates the frequency of the various forms of plague in India :

Types	Frequency
Bubonic	92·8 per cent.
Cellulo-cutaneous . . .	3·7 ,,
Septicæmic	2·4 ,,
Pneumonic	1·0 ,,
Pestis ambulans	0·1 ,,

The following table gives the number of cases and mortality under ordinary expectant treatment (India) :

Types	No. of cases	Died	Re-covered	Case Mortality per cent.
Bubonic . . .	12,080	8,947	3,133	74·06
Cellulo-cutaneous .	497	317	180	63·77
Septicæmic . . .	312	306	6	98·07
Pneumonic . . .	134	133	1	99·25

The average mortality in plague varies. During the epidemic of 1896–97 it stood at about 61·53 per cent. at the Arthur Road Hospital, and 64·5 per cent. and 68·28 per cent. at the Government House and Grant Road Hospitals, respectively. The second epidemic of 1897–98 showed a higher rate, from 78·55 per cent. at the Arthur Road Hospital to 79·26 per cent. at the Grant Road Hospital. The third epidemic of 1898–99 gave a still higher rate, the lowest being 78·97 per cent. at Arthur Road Hospital,

and the highest 81·40 per cent. at the Modikhana Hospital.   The average mortality in 5,836 cases treated at the Modikhana, Maratha, and Arthur Road Hospitals during 1898–99 was 80·39 per cent.   During the fourth epidemic of 1899–1900 the Maratha Hospital shows a mortality of 80·95 per cent. in 2,599 cases, and the non-serum cases at the Arthur Road Hospital have a mortality of 79·54 per cent.   So that, for all practical purposes, the normal mortality rate of plague in *public hospitals* may safely be put down at 70–80 per cent.   The influence of race, age, and sex may be gathered from the following tables :

INFLUENCE OF RACE

Races	Number	Died	Recovered	Case Mortality per cent.
Hindus . . .	10,315	7,899	2,416	76·57
Mahomedans . .	1,450	998	452	68·82
Native Christians .	1,088	716	372	65·80
Parsees . . .	126	63	63	50·00
Beni-Israelites .	38	26	12	68·42
Eurasians . .	3	—	3	—
Japanese . .	2	—	2	—
Chinese . . .	1	1	—	100·00
Total .	13,023	9,703	3,320	74·50

Among Europeans the case mortality is 30–40 per cent.

INFLUENCE OF SEX

Sex	Number	Died	Recovered	Case Mortality per cent.
Males . . .	9,126	6,908	2,218	75·69
Females . .	2,636	1,967	669	74·62
Children . .	1,261	828	433	65·66
Total .	13,023	9,703	3,320	74·50

The mortality in males of all the races is the highest, and in children the lowest, the latter having a lower mortality rate by nearly 10 per cent.

The value of serum treatment in plague must now be considered.

Symmers prepared anti-plague serum, which he believed to be as potent as Yersin's but experimentally it possessed little immunising or curative power. On the other hand, Calmette and Salimbeni reported favourably upon the use of Roux's serum in the epidemic of plague at Oporto. Of 63 cases treated before serum was available, 18 went into hospital and 45 were treated at home; of the former 7 died, of the latter 21 died, giving a case mortality of 39·0 and of 46·6 per cent. respectively. From September 3 to November 18, 142 cases were treated with the serum in hospital, of whom only 21 died, a case mortality of 14·8 per cent.

During the same period, of 72 cases in their own homes, and not treated with the serum, 46 died, a case mortality of 63·7 per cent.

Clemow used both Yersin's and Lustig's sera, but did not obtain favourable results with either. But the cases were few and the dosage small.

The first Indian Plague Commission also did not report favourably on serum treatment.

Haffkine treated cases with the Yersin-Roux serum and came to the conclusion that while the serum failed to reduce the mortality, the serum treatment prolonged life for at least a day longer than expectant treatment.

Choksy gives the following table showing the results of the treatment of plague with various serums in the Arthur Road and Maratha Hospitals, Bombay :

RESULTS OF TREATMENT OF PLAGUE WITH VARIOUS SERUMS

Serum	Number	Died	Recovered	Case Mortality per cent.
Lustig's (1899–1901)	1,089	715	374	65·6
Yersin-Roux (1905–1907)	449	273	176	60·8
,, (1904)	80	55	25	68·7
Brazil's	50	41	9	82·0
Tavel's	28	18	10	64·2
Terni's	16	12	4	75·0
Haffkine's	15	11	4	73·3
Paltauf's	8	4	4	50·0
Japanese	4	1	3	25·0
Total .	1,739	1,130	609	64·9

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Among these cases, therefore, the case mortality was reduced by at least 10 per cent. by serum treatment. In the above series of cases treated by Choksy with the Yersin-Roux serum during 1905–1907, all cases which appeared unlikely to benefit by serum treatment were rejected, as well as convalescent or semi-convalescent cases, and those in whom the illness had lasted for six days or more, for these are either too advanced for treatment or are beginning to improve spontaneously. In a later series of 400 cases the above rules were observed, and alternate cases were treated with the serum, the others acting as controls, and the following results were obtained :

Treatment	Number	Died	Re-covered	Case mortality per cent.
Control cases . . .	200	148	52	74·0
Serum cases . . .	200	127	73	63·5

The above figures indicate that while under ordinary treatment only 26 cases out of 100 recover, with serum treatment nearly 37 cases out of 100 recover ; there is a reduction in case mortality of 10·5 per cent. with serum treatment. All the cases, control as well as serum, were of

the bubonic type, and all received the same drug and other treatment.

Dosage and Administration.—Anti - plague serum does not seem to be very potent, and the non-success of the treatment recorded by many observers is probably to some extent due to the use of too small an amount. Calmette lays down the following rules for the administration of the serum : All patients suffering from the bubonic or pulmonary form of plague should be treated as soon as possible, and should receive at once 20 c.c. by *intravenous* inoculation, followed by two subcutaneous injections of *at least* 40 c.c. each within the first twenty-four hours. Afterwards 10 c.c., 20 c.c., or 40 c.c., according to the condition of the patient, should be given daily subcutaneously until the temperature has become normal.

Choksy lays down the following rules for the administration of Lustig's serum :

' I. As soon as the diagnosis has been made, inject 60 to 80 to 100 c.c. in adults ; for children under twelve, half the dose ; for infants, 10 c.c. *The patient must be injected as early as possible. Much valuable time is lost in waiting.'*

It would be well to give a first injection of 20 to 30 c.c. intravenously as recommended by Calmette.

The importance of early treatment is seen from Choksy's statistics as given in the following table :

MORTALITY OF CASES TREATED WITH SERUM ACCORDING TO DURATION OF ILLNESS

Duration of illness	Number	Died	Recovered	Case Mortality per cent.
First day . . .	323	98	225	30·3
Second day . . .	311	164	147	52·7
Third day . . .	248	155	93	62·5
Fourth day . . .	106	60	46	56·6
Fifth day . . .	52	32	20	61·5
Sixth day . . .	14	8	6	57·1
Seventh day . . .	4	4	—	100·0

It will thus be seen that of those cases treated on the first day the case mortality is only about 30 per cent., afterwards with some irregularities it averages about 59 per cent.

' 2. Injections should be given in the morning and repeated after twenty-four hours. The patient, if seen for the first time in the afternoon or evening, should be injected at once, and the next injection should be given on the following morning.

' 3. The quantity to be injected on subsequent occasions should depend upon the range of the temperature on the previous evening and the

general condition of the patient. If the temperature is the same as on the evening of the day of the first injection, 40 to 60 c.c. may again be injected ; if lower, then 30 c.c. or less.

' 4. The quantity of the serum injected should be gradually decreased day after day, as above, until the temperature reaches to normal in the morning.

' 5. There is a drop in temperature of one to three degrees or more during the course of plague, and it may occur on any day from the second to the seventh. The injections should not be discontinued when this happens.

' 6. Injections in the evening are not necessary unless secondary buboes develop, or the temperature suddenly goes up higher than on the previous evening ; 30 to 40 c.c. may be injected in these circumstances.

' 7. If the temperature on any evening is found to be lower than in the morning, it is a favourable indication, and the quantity of serum injected the following morning may be safely reduced.

' 8. Six to eight injections may be required to effect a cure.

' 9. The total quantity required for a cure may vary from 150 to 300 c.c., depending upon the severity of the case, complications, &c., and

the strength of the serum used.' In the last series of hospital cases treated by Choksy the amount of serum injected averaged 285 c.c. per patient.

In one very bad case Choksy states that he administered 240 c.c. in four doses of 60 c.c., each within thirty-six hours, with the happiest results. In a few cases the amount of serum given was no less than 400–800 c.c. In a case of accidental infection during the epidemic at San Francisco, with the development of plague pneumonia, which was treated with anti-plague serum, 60 c.c. were injected subcutaneously, and the same quantity intravenously, the whole amount being given within twenty-four hours. Within a few hours after the last injection the temperature dropped to 100°, and before the end of the third day reached the normal.

Martini, as the result of an experimental study of plague, concludes that 100 c.c. of anti-plague serum should be given subcutaneously for a dose. In plague pneumonia, 50 c.c. should be administered intravenously, and 50 c.c. sub-cutaneously.

Cairns gives the following as his conclusions as to the value of anti-plague serum based on cases treated during the Glasgow epidemic of 1901 : (1) Yersin's serum is a remedy of the

greatest value in the treatment of bubonic plague ; (2) its action is bactericidal as well as antitoxic ; (3) this double action of the serum is best secured by its early administration in large doses, both subcutaneously into the lymphatic area which drains towards the bubo and also intravenously ; (4) in mild cases the former will suffice, but in severe cases the combined method should be employed. For the latter the initial combined dose should be 150–200 c.c.

Ferrari at Rio de Janerio administers the serum in massive doses (100–350 c.c.) intra-venously, and in 69 cases so treated the mortality was only 7·2 per cent.

The writer would therefore urge that, if serum treatment be adopted in plague, the serum should be administered in large doses and the primary dose by intravenous inoculation.

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Injection of Anti-streptococcic and Anti-plague Sera

Denys and Tartakowsky state that the efficacy of anti-streptococcic serum is considerably enhanced if it be injected into the neighbourhood of the infective focus, in such a manner that the serum is carried by the lymphatic vessels towards this focus.

The same seems to hold good for anti plague serum. If a guinea-pig be infected with plague intraperitoneally, and if the exudate be examined a short time afterwards, no phagocytosis will be observed and the animal ultimately succumbs. The same is the case if the animal be injected subcutaneously with anti-plague serum, but if the serum be injected intraperitoneally phagocytosis will be observed to take place and the animal survives. In this manner, 0·1 c.c. of the serum injected intraperitoneally may save the animal, whereas 10 c.c. subcutaneously may fail. Similarly, if the guinea-pig be infected in the foot, a great difference is observed whether the serum be injected in the foot or in

the back. In the first instance the local lesion becomes much less pronounced and the buboes are limited. The authors consider that these results are applicable to treatment in man.[1]

Enteric Fever

The typhoid bacillus under ordinary conditions produces little or no toxin in artificial culture media, and all attempts to produce an anti-toxic serum by injection of the dead or living microbes have hitherto proved failures. Chantemesse, however, by a special method of cultivation, claims to have been able to prepare a soluble typhoid toxin, and with this, by inoculating horses, to have obtained a curative serum. The culture-medium consists of a maceration of spleen and bone marrow with some defibrinated human blood.

Shallow flasks (see fig. 32, p. 363) of this medium are inoculated with a virulent typhoid culture. After forty-eight hours' incubation, the growth forms a thin film on the surface of the medium and a toxin is formed. At the end of seven days' incubation, the culture is distributed into small flasks, which are heated to 55° C. and their contents then centrifugalised. The fluid is decanted and used for the inoculation

[1] *La Semaine Médicale*, January 31, 1900, p. 40.

of the horses. The injections into the horses are so spaced that a considerable elevation of temperature results after every injection. Those horses which have given the best curative serum have been immunised for several years. About twenty days after injection the serum possesses its maximum activity.

For treatment the serum is injected subcutaneously into the fore-arm. The action of the serum persists for ten days and it is rarely necessary to give a second injection.

The amount of serum given at the primary injection is now 5 drops (formerly it was 10–12 c.c.). If after 10–12 days little or no amelioration has occurred, a second injection of 2–3 drops is given (formerly it was 4–10 c.c.).

These extremely small doses are quite contrary to the supposition that the serum acts as an antitoxin. Wright therefore suggested that probably the serum contains a toxin and that it really acts as a vaccine, and Chantemesse has adopted this explanation, and finds that the injection causes a marked rise in the opsonic index in favourable cases.

Chantemesse states that the effect of the serum is to cause a decided increase in the size of the spleen soon after the injection, a fall in temperature, a decrease in the rapidity of the

pulse and increase in blood-pressure, and general improvement in the condition of the patient.

As regards the results of the treatment, Chantemesse states that in the Paris Hospitals from April 1, 1901, to July 31, 1907, 5,621 cases of typhoid fever were admitted, and, under ordinary treatment, of these 960 died, a case mortality of 17 per cent. During the same period, 1,000 cases were treated with the Chantemesse serum, of whom 43 died, a case mortality of 4·3 per cent.

The ordinary anti-typhoid sera on the market are anti-microbic sera obtained by treating a horse first with killed and afterwards with living cultures of a virulent typhoid bacillus, just as for the anti-plague serum. This anti-typhoid serum has given disappointing results. Thus 18 cases of enteric fever were treated by Newall with anti-typhoid serum procured from ' a reliable source.' The dose used was either 5 c.c. or 10 c.c. injected into the iliac region ; in some cases only one injection was given, in others two, three, or four were administered. Some cases were treated with serum alone, others had intestinal antiseptics, and symptomatic treatment of complications was adopted. It may be said that in none of these cases (nor in 13 others subsequently treated) did the use of the serum

appear to have any beneficial effect. In four cases the temperature fell markedly after the first dose for a short time, but with subsequent doses not at all.

Meyer and Bergell tried various methods for obtaining a typhoid toxin. Using a peptone meat broth, prepared with ox-spleen, they obtained a toxin of some potency with which animals were immunised. Of the serum so obtained 0·005 c.c. protected mice against twenty lethal doses of culture.

Anti-endotoxic Serum.—The late Dr. Macfadyen found that typhoid cell juice obtained by the cold-grinding method (p. 50) possessed powerful immunising properties when injected into animals, and at the time of his death was immunising a horse with a view to preparing an anti-endotoxic typhoid serum for treatment. This work has been continued by the writer at the Wellcome Research Laboratories and a serum was prepared. Experimentally, the serum was very potent, 0·001 c.c. protecting a guinea-pig from an intraperitoneal injection of ten or more lethal doses of typhoid culture injected simultaneously. The writer treated nine cases of typhoid fever with this serum. In two of the cases the progress of the disease appeared to be cut short by the serum, five of the

remainder seemed undoubtedly to have been more or less benefited by the serum, and only one case failed to react. All the cases recovered, the last-named alone having relapses.

Chart I. shows the course of the temperature in a case (Case I.) treated with Macfadyen's

CHART I.
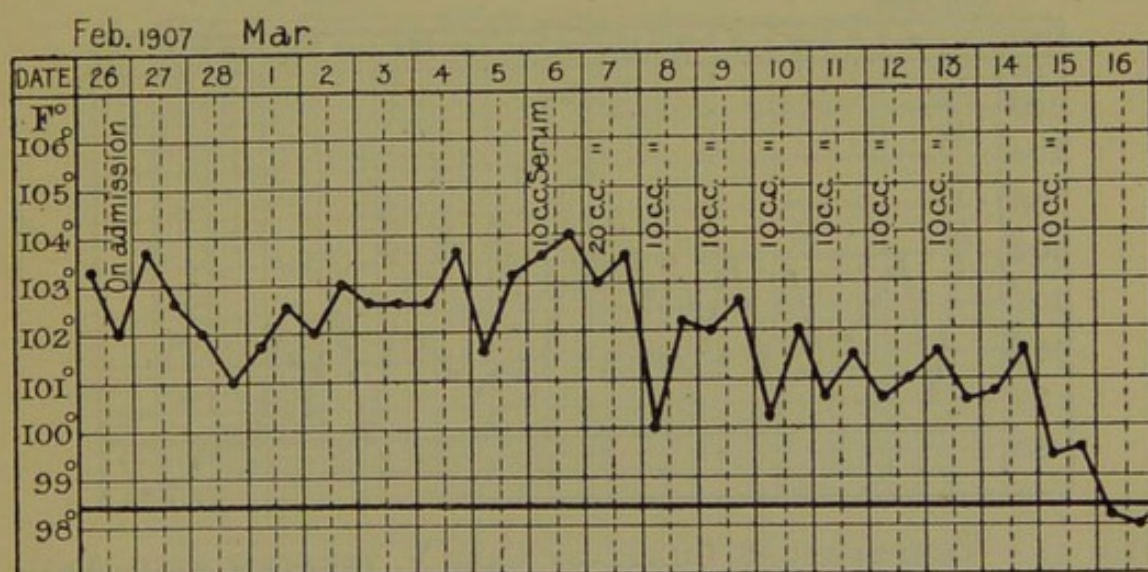
FIG. 23.—TEMPERATURE CHART OF CASE I. TREATED WITH ANTI-ENDOTOXIC SERUM. (Macfadyen's.)

serum. The patient, aged eighteen years, was admitted in the course of the third week of the disease, and was very ill. It will be seen that the temperature was tending to rise until the serum was given, when there was an immediate response.

Chart II. is that of a case (Case V.) treated with the writer's serum. The patient had been

ill eight days and the temperature was tending to rise until the serum was given, when there was an immediate fall.

Chart III. is that of a case (Case VI.) also treated with the writer's serum. The disease was probably well within the first week, and the

CHART II.
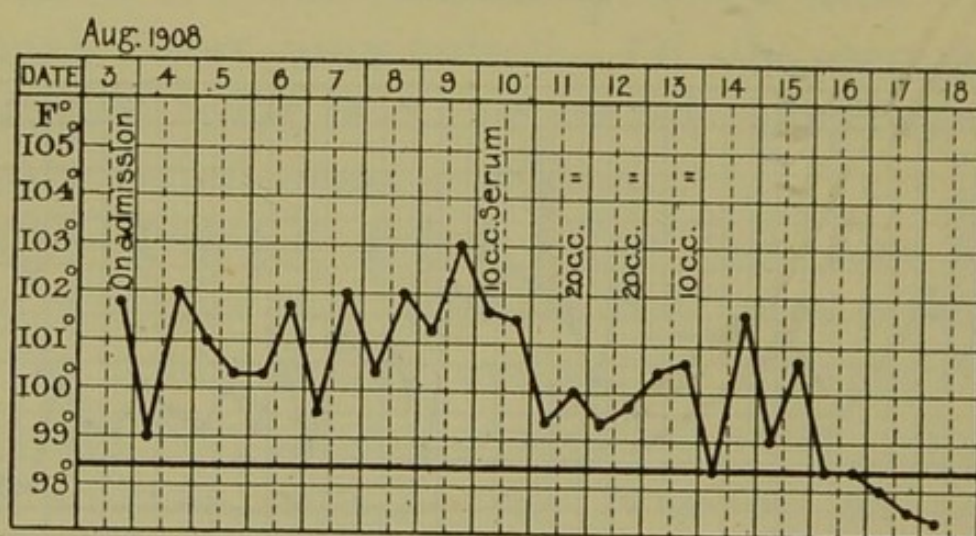
FIG. 24.—TEMPERATURE CHART OF CASE V. TREATED WITH ANTI-ENDOTOXIC SERUM.

temperature became normal about a week after the commencement of serum treatment.

The serum was usually given daily, on a few occasions both morning and evening, in doses of 10–20 c.c., the number of doses varying from four to eleven.

Goodall also treated twenty-six cases with the anti-endotoxic serum, the dose varying from 20 c.c. (one injection) to 200 c.c. given in

eight injections spread over eleven days. Seven
of the cases appeared to be benefited by the
serum, and in two of these the fall of temperature
was abrupt after the use of the serum. Two
other cases may also have benefited by the serum.
In none of the remaining seventeen cases was

CHART III.
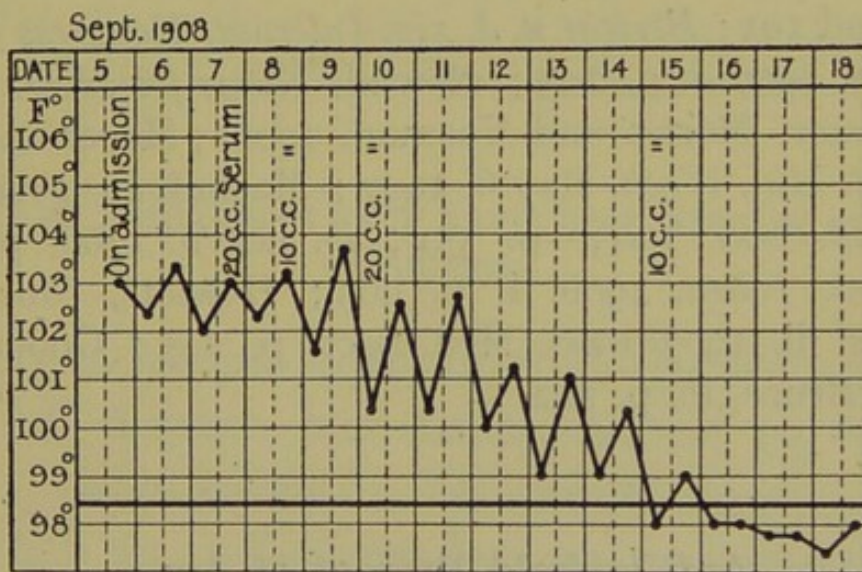
FIG. 25.—TEMPERATURE CHART OF CASE VI. TREATED WITH ANTI-
ENDOTOXIC SERUM.

any effect apparent. In ten only of the cases
had the patients been ill for less than a fortnight
and only in five for less than ten days. Bruce
also treated five cases with the same serum, and
his impression is that at least two were benefited
by the serum, and one other case would probably
have run a more severe and prolonged course
but for the serum. The results so far obtained
are distinctly encouraging. Early treatment

(as with other antitoxic sera), however, is very important, but unfortunately is the exception in typhoid fever, as cases do not usually come under observation until after the lapse of a week from the onset.

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Anti-typhoid Extract of Jez

This is prepared by injecting rabbits with typhoid culture and so immunising them, killing the animals, and then extracting the minced-up spleen, brain and spinal cord, bone-marrow, and thymus gland with a solution consisting of sodium chloride, glycerine, and alcohol, with a little carbolic acid. It forms a dark, reddish-yellow fluid of alkaline reaction which is administered by the mouth. According to the severity of the case, a dessert-spoonful is give every one or two hours until the temperature becomes remittent, then every three hours until the morning temperature does not exceed 100·5° F. The total amount of the extract

required by a patient averages 17 fluid ounces. Under the treatment the general condition is said to improve, the pulse becomes slower, and the temperature generally falls considerably, and the morning remissions become more marked. Eichhorst used Jez's preparation in 12 severe cases with success ; on the other hand, Pometta in 10 cases was unable to observe any benefit.

See Jez, *Wien. klin. Woch.* January 24, 1901. Ref. in *Brit. Med. Journ.* Epit. 1901, i. p. 51, No. 212; *ib.* 1902, i. p. 27, No. 108 ; and *ib.* 1904, i. p. 64, No. 239.

Other Diseases which have been treated with Anti-sera

There is reason to think that the serum treatment of disease has been carried to excess ; in fact, there seems to be hardly any condition for which an anti-serum has not at some time or other been tried.

Care must be taken not to confuse the various vaccines (anti-typhoid, anti-cholera, anti-plague, &c.) with the anti-sera.

The following are some instances of other diseases, arranged in alphabetical order, in which anti-sera have been employed.

Anthrax

Sclavo, Marchoux, Sorbenheim (see p. 41), and others have prepared anti-sera against anthrax.

The general method of preparation is to vaccinate a donkey with a modified living virus (the Pasteur method) and subsequently to treat the animal with increasing doses of anthrax culture ; Legge gives full particulars of the mode of preparation and experimental tests of Sclavo's serum.

Lazaretti reports 23 cases of anthrax in man treated with Sclavo's serum with only 1 death.

Lockwood and Andrewes record a case of cutaneous anthrax with recovery treated only with Sclavo's serum (40 c.c. in one dose), and Bowlby and Andrewes give particulars of a second similar case treated successfully in the same way.

San Felice has immunised dogs against anthrax, and with their serum has treated a case of anthrax in man.

See Lazaretti, *Brit. Med. Journ.* Epit. 1902, ii. p. 64 ; Marchoux, *Ann. de l'Inst. Pasteur*, ix. 1895, p. 785 ; San Felice, *Centr. f. Bakt.* Abt. I. *Originale*, xxxiii. 1903, p. 61 ; Sorbenheim, *Brit. Med. Journ.* Epit. 1902, ii. p. 90 ; Legge, *Brit. Med. Journ.* 1905, i. p. 591 ; Lockwood and Andrewes, *ib.* p. 16 ; Bowlby and Andrewes, *ib.* p. 296.

Botulism (Meat and Sausage Poisoning)

One form of poisoning, arising from the ingestion of unwholesome meat and sausages, is due to infection

with an anaërobic bacillus, the *B. botulinus* of Van Ermengem. This organism produces a powerful toxin with which animals may be immunised, and a truly anti-toxic serum obtained.

See Van Ermengem, *Ann. de Micrographie,* viii. 1896, p. 66 ; Kempner, *Zeitschr. f. Hyg.* xxvi. p. 481 and xxvii. p. 213

Cholera Asiatica

No satisfactory anti-cholera serum has yet been prepared. A serum obtained by injection of animals with increasing doses of cholera cultures is of little or no value. Metchnikoff, Roux, and Salimbeni, by growing the cholera vibrio in a gelatine peptone-water salt solution in a shallow layer for a few days, obtained a feeble toxin, with which they were able to immunise animals and to obtain an anti-toxin which experimentally possessed curative properties.

Salimbeni, in the recent epidemic of cholera in Russia, has employed this serum, and considers that it undoubtedly is of value. The dose given was 50–100 c.c. in saline solution (150–500 c.c.) injected intravenously.

Bran and Denier have modified the above method by making use of a medium consisting of horse serum to which is added 10 per cent. of

defibrinated horse blood. For use, the medium is heated to 60° C. for three hours, inoculated with a considerable amount of culture, and the cultivation after seven days' incubation at 39° C. filtered to obtain the toxin.

Anti-endotoxic Serum.—The late Dr. Macfadyen found that by trituration of cholera cultures (see p. 50) an endotoxic poison is obtained which produces an anti-serum on inoculation into animals.

The writer has immunised a horse with the cholera endotoxin prepared in this way at the Wellcome Research Laboratories. The serum so obtained is very active experimentally, 0·0001 c.c. in some cases protecting against ten lethal doses of cholera culture injected simultaneously intraperitoneally into guinea-pigs. The serum has been used for the treatment of a few cases of cholera in St. Petersburg, but the results were indefinite.

See Metchnikoff, *Ann. de l'Inst. Pasteur*, x. 1896, p. 257; Bran and Denier, *ib.* xx. 1906; Salimbeni, *ib.* xxiv. 1910, p. 34; Macfadyen, *Lancet*, 1906, ii. p. 494.

Infection by the Bacillus Coli

The *Bacillus coli* plays an important rôle in human pathology, causing the peritonitis following perforation

and obstruction of the bowel, certain forms of puerperal infection, ischio-rectal abscess, cystitis and pyelitis, &c. A barran and Moser have prepared an anti-serum by injecting animals with cultures, and suggest that it might be used in infections of the urinary tract, or before operations upon the urinary organs, to prevent subsequent infection. Pearson[1] records cases of empyema and sub-diaphragmatic abscess due to *B. coli* treated with an anti-coli serum administered by the *mouth* (25 c.c. doses) with apparently considerable benefit.

Vaccine treatment has now superseded serum treatment in *B. coli* infections (see p. 307).

Dysentery

There are two chief types of dysentery, the amœbic and the bacillary. It is the last-named for which an anti-serum has been prepared.

Bacillary dysentery is caused by a group of organisms classed as the *Bacillus dysenteriæ* which includes a number of varieties ; of these there are two main types, the Shiga-Kruse, which has no action on mannitol, and the Flexner-Harris, which produces acid from mannitol.

The serum, which should be polyvalent, is a combined antitoxic and anti-microbic one, and is prepared by injecting horses alternately with toxin and with living cultures at weekly intervals.

[1] *Brit. Med. Journ.* 1909, ii. p. 78.

The toxin used is a filtered broth culture. Todd found that if the *B. dysenteriæ* is cultivated for four to six weeks in an alkaline broth (broth just alkaline to litmus to which 7 c.c. of normal solution of NaOH per litre is added), the filtered broth is markedly toxic, 0·1 c.c. being a fatal dose for a large rabbit.

The horse is very susceptible both to the culture and to the toxin, and the doses given are 0·25 c.c., 0·5 c.c., 1·0 c.c., 2 c.c., 3 c.c., &c. of a twenty-four hour broth culture, and the same for the toxin. The maximal quantity of culture and of toxin will not exceed 50 c.c., and the treatment must be conducted cautiously and gradually. The serum should be a polyvalent one, a number of different strains being used for the immunisation.

For testing the serum large rabbits (1800–2000 grams weight) are used ; doses of 0·25–0·5 c.c. of serum should protect against a subcutaneous dose of living culture lethal in three or four days, and 1 c.c. of serum should protect against a dose of toxin of which 0·25 c.c. is fatal in 12–16 hours when injected intravenously or 1·0 c.c. is fatal in 3–4 days when injected subcutaneously. Also 1–2 c.c. of serum injected 24 hours *after* a dose of culture fatal in 3–4 days should protect.

Ruffer and Willmore make use of digested cultures of the dysentery bacillus for immunising. A suspension of agar cultures is killed by heating to 57° C. for one hour and is then digested with pepsin and hydrochloric acid for twelve hours. The mixture is then neutralised with alkali and used for immunising the animals. The authors claim that by this method immunisation is rapid and produces very little disturbance in the inoculated animals.

The effect of the serum in doses of 10–30 c.c. subcutaneously in cases of dysentery is to reduce the number of stools markedly, and after two or three doses on successive days to cure.

The serum may also be used as a prophylactic (see also p. 360). If the condition becomes chronic, vaccine treatment, with or without serum, is indicated (see p. 308).

Rosenthal in Moscow found that without serum treatment the mortality from dysentery was 11–12 per cent., with serum treatment it was 4·5 per cent.

See Todd, *Journ. of Hygiene*, iv. 1904, p. 480 ; Rosenthal, *Deutsche med. Woch.* 1903 ; Kruse, *ib.* 1903 ; Ludke, *Centralbl. f. Bakt.*, Abt. i. (*Originale*), xxxix. 1905, xl. 1906 ; Coyne and Auché, *Rev. de Médecine*, 1907 ; Ruffer and Willmore, *Brit. Med. Journ.* 1908, ii. p. 1176.

Anti-thyroid Serum for Exophthalmic Goitre

It has been suggested that the secretion of the normal thyroid gland neutralises a toxin present in the body, and that an over-production of this neutralising secretion causes an intoxication and the condition known as exophthalmic goitre. Ballet and Enriquez have used in the treatment of this disease the serum of dogs whose thyroids had been previously extirpated. This serum, therefore, on the above supposition, would contain the normal body toxin, which on injection should neutralise the excess of thyroid secretion. Many cases have now been reported as benefited by this treatment. The serum termed 'anti-thyroidin' is supplied by Messrs. Merck.

Stradiotti has employed a thyrotoxic serum in exophthalmic goitre, with some, though not marked, benefit. The serum was prepared by injecting sheep with glycerin extracts of extirpated human thyroids treated with chloroform so as to preserve them.

See Ballet and Enriquez ; Schultes, *Münch. med. Woch.* May 20, 1902, and *Brit. Med. Journ.* Epit. 1902, ii. p. 71 ; *Lancet*, 1902, i. ; Stradiotti, *Brit. Med. Journ.* Epit. 1907, i. No. 324.

Hay Fever

Hay fever, which is universally admitted to be produced by the action of the pollen of various species of *Graminaceæ* (and to some extent by

that of certain other plants), from the experiments of Dunbar seems to be caused not by the mechanical irritation of the pollen grains, but by a toxic substance contained in them. By extracting this toxic substance, which possesses intensely irritating properties upon the nasal mucous membrane, by means of ether, and injecting the ethereal extract into horses, Dunbar has obtained an anti-serum, termed ' Pollantin,' which, from his own experiments and those of Semon and others, immediately causes the disappearance of the subjective symptoms produced by applying the toxin to the conjunctiva, and seems to be of considerable service in the treatment of this troublesome complaint. For the eye condition, instillation of a drop of the fluid pollantin is used ; for the nasal catarrh the serum is best applied in the powdered dry form, a little being sniffed up each nostril.

See Semon, *Brit. Med. Journ.* 1903, i. p. 713 ; Glegg, *Journ. of Hygiene,* iv. 1904, p. 369 (Bibliog.) ; Somers, *Med. News* (N.Y.), April 23, 1904 ; Prausnitz in *Handbuch der Technik und Methodik der Immunitätsforschung.*

Hydrophobia

Tizzoni and Centanni have prepared an anti-rabic serum by immunising sheep with gradually increasing doses of anti-rabic vaccine (prepared according to the

Pasteurian method). The serum is stated to be both protective and curative. The ordinary method of inoculating for rabies or hydrophobia is useless if the disease has declared itself : it is only a preventive.

See *Lancet*, 1895, ii. p. 659 *et seq.*

Leprosy

Carasquilla prepared a serum for leprosy by injecting asses with the blood derived from advanced cases of the disease. Herman and Abraham immunised horses with the juice obtained from the leprous nodules emulsified in saline solution. Anti-venin has also been extolled in the treatment of this disease by Dyer, of New Orleans, but in the writer's hands utterly failed to produce any improvement in a well-marked case, as did also diphtheria antitoxin (as was expected).

Vaccine treatment has also been tried for leprosy (see p. 293).

Malignant Disease

An attack of erysipelas having a beneficial action in cases of carcinoma, it was proposed to employ an anti-erysipelatous serum in the treatment of this condition, and at one time encouraging reports were published of this mode of treatment. No lasting good, however, seems to result, and the treatment has fallen into

disrepute, and it is difficult to conceive that it should have had any effect.

Doyen having isolated a micrococcus (*M. neoformans*) from carcinomatous growths, both serum and vaccine prepared with it have been tried in cancer, but without any result. Schmidt's serum[1] also seems useless, and no form of successful serum or vaccine treatment, with the possible exception of Coley's, has yet been devised for malignant disease (see also Coley's Fluid, p. 322 and p. 326).

Mediterranean or Malta Fever

Wright first immunised goats and subsequently horses by the subcutaneous injection of killed cultures of the *M. melitensis*. A case of the disease in man was treated by Wright and Semple in 1899 and other cases by Aldridge in 1898, by whom good effects were ascribed to the serum treatment.

Eyre in 1905 immunised a horse with cultures, using first dead and then living ones, but experimentally the results were by no means encouraging.

Vaccine treatment has also been used (see p. 310).

[1] See *Lancet*, 1903, ii. pp. 1374 and 1381.

See Aldridge, *Lancet*, 1898, i. p. 1394 ; Wright and Semple, *ib.* 1899, i. p. 1204 ; Eyre, *Rep. Mediterranean Fever Commission*, pt. v. 1907, p. 42.

Pernicious Anæmia

Hunter has treated cases of pernicious anæmia with anti-streptococcic serum, apparently with benefit.

See *Lancet*, 1901, i. p. 473.

Anti-staphylococcic Serum

Attempts have been made to prepare a serum which will antagonise the effects of the *Micrococcus pyogenes aureus*, the commonest of the pyogenic cocci, but so far without much success. Doyen claims to have obtained a satisfactory serum with which he has treated cases of furuncle, phlebitis, &c. with benefit. The amount injected was 5 c.c. The serum was active against the *M. pyogenes aureus*, but not against the streptococcus. Paltchikowsky has also immunised horses by means of the *M. pyogenes aureus*, and has obtained a serum which would protect against the *M. aureus* or *albus*. Pröscher points out that therapeutically it is important to know whether the various strains of staphylococci are identical. By the agglutination test, it was found that staphylococci of pus were agglutinated by the serum of the patient, but those from the air, skin, and vaccine lymph were not so agglutinated, pointing to a difference between the races. By inoculating rabbits subcutaneously for a month a serum was obtained of which 1 c.c. would protect against 5–7 times the minimal fatal dose.

The researches of Gordon and of Andrewes and Gordon also show that there are many different staphylococci.

Vaccine treatment has now quite superseded anti-staphylococcic serum for staphylococcic infections (see p. 295).

Trypanosomiasis

Laveran has found that in certain cases human serum has an inhibitory action in cases of nagana (tsetse fly disease), and it has been suggested, therefore, that horse serum might be of use in cases of human trypanoso-miasis. Attempts to immunise against trypanosomata have proved failures.

Tuberculosis

Numerous attempts have been made to pre-pare a serum or antitoxin for the treatment of that scourge of civilisation—tuberculosis. The best-known sera are those of Paul Paquin in America, of Maragliano in Italy, and of Marmorek in France. Maragliano's serum is prepared by injecting cows with an aqueous tuberculin, and with a bacillary pulp made by triturating tubercle bacilli, emulsifying in water and filtering through a porcelain filter. The cow is injected subcutaneously with the two preparations in increasing doses, commencing

with 5 c.c. of each, until a dose of 20 c.c. is reached, the frequency of the injections being determined by the reaction produced. Marmorek's serum is prepared by growing tubercle bacilli in a special medium consisting of glycerin liver bouillon with a leucotoxic calf serum (obtained by injecting calves with leucocytes) and injecting the filtered culture into horses. It cannot be said that the published results of the serum treatment are very promising. Mircoli published the statistics of the treatment of 2,899 cases of pulmonary tuberculosis in various stages with Maragliano's serum—1 c.c. on alternate days. Of these cases, over 14 per cent. are stated to have been cured, 50 per cent. improved, and in only 18 per cent. did the disease tend to progress during treatment. Hemsted records a case of disseminated tuberculosis treated with twenty-eight 5 c.c. doses of Marmorek's serum with marked benefit.

A number of different tuberculins and tubercle anti-sera are referred to by Mitulescu.

The various tuberculins are now usually employed for the treatment of tuberculosis

See Marmorek, *Bull. de l'Inst. Pasteur*, i. 1903, p. 851; Hemsted, *Brit. Med. Journ.* 1909, ii. p. 1337; Mitulescu, *Berl. klin. Woch.* Aug. 9 and 16, 1909.

CHAPTER VI

Transfusion of Blood

The transfusion of blood has of late years fallen into discredit, for the success of the method is recognised to be due not to the corpuscular elements, but to the fluid introduced. The corpuscles of one species of animal are rapidly destroyed when introduced into another species, hence it is useless to transfuse the blood of a lower animal.

Direct or immediate transfusion, in which the blood of the donor is introduced directly into the veins of the recipient, is now never practised.

Indirect or mediate transfusion consists in bleeding a healthy or healthy individuals, receiving the blood into a bowl kept warm by standing in hot water (100° F.), whipping the

blood to remove the fibrin, straining through fine muslin which has been treated with boiling water or boiled to sterilise it, after which the defibrinated blood is injected intravenously into the patient in the manner detailed at p. 241.

Saline infusion has now almost entirely taken the place of blood transfusion. Blood transfusion should be employed only in cases of grave anæmia, and then is of doubtful value. The loss of the red corpuscles in hæmorrhage may be largely compensated for by oxygen inhalation.

Transfusion of Animal Blood as a Therapeutic Measure

The transfusion of blood from one animal into another or into man is frequently followed by dangerous symptoms, due to toxic or to hæmolytic action or to both. These are usually much more marked when the blood is heterologous, *i.e.* is derived from a species different from that into which it is injected.

The symptoms are rigors and fever, sweating, albuminuria, and hæmoglobinuria, agglutination and solution of the blood corpuscles, and enlargement of the spleen.

Bier suggested that the disturbance thus induced might be made use of as a therapeutic measure, and has employed defibrinated lamb's blood in cases of tuberculous disease and cancer. The amount injected by transfusion ranged from 4 c.c. to 20 c.c., and the transfusion was repeated only at intervals of a week or ten days. Four cases of lupus showed marked improvement, and in a case of sacro-iliac tuberculosis after twelve injections the suppuration in the pelvis had nearly ceased, the fistulæ were almost healed, the patient had gained in weight and was able to be up. The injections were followed by marked reaction. The technique of the injection is simple. An assistant lightly grasps the arm above the elbow, so as to produce venous engorgement and swelling of the superficial veins. The operator takes a swollen superficial vein near the elbow between the finger and thumb of his left hand, and, the skin having been cleansed, introduces into the vein the sterile needle of a Pravaz syringe. When the needle is in the lumen of the vein, which is known by the blood welling out, the assistant attaches the filled syringe and slowly injects the defibrinated blood.[1]

[1] *Münchener med. Wochenschr.* 1901, No. 15, April 9, p. 569.

Serum Therapy of Nephritis

In uræmic conditions associated with nephritis injections of goat serum, derived from blood obtained from the *renal vein*, have been recommended by Teissier and Lawis. The blood is obtained by laparotomy and ligature of the renal vein after anæsthetisation of the animal.

Saline Infusion

The infusion of saline solutions is a most valuable method of treating severe hæmorrhage, shock, and collapse, and for this purpose almost entirely replaces blood transfusion.

Various formulæ have been devised for the preparation of saline infusions, and tablets or cachets can be obtained containing the requisite constituents for preparing a given amount of solution, and are very convenient, the object being to imitate the saline constituents of the blood. Hare gives the following formula :

Calcium chloride	. .	0·25 gram or	2 grains
Potassium chloride	. .	0·10 ,,	1 grain
Sodium chloride	. .	9·0 grams or	70 grains
Sterilised water .	. .	1000·0 ,,	1 pint

A solution of 1 drachm of common salt to the

pint of water does perfectly well for ordinary purposes.

If there be time, the solution may be prepared and then sterilised by boiling ; but if not, the salts should be placed in a vessel which has been scalded out, and an ounce or two of *boiling* water added to dissolve and sterilise them, the solution being then made up to the right amount by the addition of *boiled* water at a temperature of about 120° F. A small funnel is attached by a short length (18 in.) of rubber tubing to a glass or metal cannula, and the whole is sterilised by thorough rinsing with boiling water, or with an antiseptic followed by boiled water. The rubber tubing should be closed by a spring or screw clip.

The median basilic is one of the most convenient veins to expose. In opening the vein surgical cleanliness must be carried out.

After exposing the vein and incising it, a sterilised cannula is inserted into the opening, and is held in position by means of a catgut ligature.

Care must be taken that air is not admitted with the solution. This danger has been exaggerated ; a bubble of air does no harm, the quantity to cause death must be considerable. To avoid the injection of air with the solution,

allow the fluid to run through the cannula while the latter is being inserted into the vein.

In hæmorrhage or shock from other causes the amount of fluid to be injected in an adult should never be less than a pint, frequently two to four pints are required. Of course, the quantity is determined by the patient's general condition.

The fluid should be injected at the rate of a pint in fifteen minutes, and at a temperature ranging from 105° to 110° F. It is remarkable how rapidly a solution at this temperature will stimulate a flagging heart. When the pulse comes down to 120, or thereabouts, the cannula may be removed, the vein ligatured and the incision closed. If it is necessary to repeat the dose, a hot saline rectal injection, as a rule, will give good results, or the solution may be injected into the cellular tissue of the axilla or under the mamma. In collapse,[1] e.g. in severe vomiting or diarrhœa or in cholera, large amounts of fluid must be injected.

The injection of saline fluid has recently been advocated in the treatment of cholera.

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[1] It is to be noted that the pathology of shock and of collapse are different. In shock the vessels in the splanchnic area—i.e. the abdominal veins—are enormously dilated, and the patient bleeds, as it were, into his own vessels. In collapse there is withdrawal of fluid from the blood and tissues.

Leonard Rogers[1] finds that the effect is better if the salt solution be hypertonic, and consist of 2 drachms of sodium chloride and 3 grains of calcium chloride to the pint of water. Three to four pints of this solution should be given intravenously if possible, but if owing to the collapsed state of the veins this is not feasible, the fluid may be given intraperitoneally, a cannula being inserted half an inch below the navel, and the fluid allowed to run in by gravity by attaching a rubber tube and reservoir, the time occupied being about 10 minutes.

These saline solutions must always be sterilised before use, as they possess no inhibitory properties against the development and multiplication of micro-organisms. To remedy this Tavel suggested the addition of a small quantity of sodium carbonate (0·1 per cent.) ; but Baisch[2] has found that this induces gangrene when injected subcutaneously.

Barker[3] recommends the subcutaneous injection into the axillary region of 500 c.c. of a solution composed of 5 grams of glucose dissolved in 100 c.c. of 0·6 per cent. salt solution twice daily in exhausted patients for some days preceding and following operation.

[1] *Philippine Journ. of Science*, iv. 1909, p. 99.
[2] *Brit. Med. Journ.* Epit. 1902, ii. p. 67, No. 276.
[3] *Brit. Med. Journ.* 1902, i. p. 770.

'Serum' of Trunecek

This is a saline solution which is inoculated subcutaneously and is stated to be of great benefit in arteriosclerosis. The salts employed are

Sodium chloride	10 grams
Sodium sulphate	1 gram
Calcium phosphate	0·75 gram
Magnesium phosphate . . .	0·75 ,,
Sodium carbonate	0·40 ,,
Sodium phosphate	0·30 ,,

One gram of this mixture is dissolved in 15 c.c. of sterile distilled water. The treatment is commenced by injecting hypodermically, preferably into the region of the buttock, 2 c.c. of this sterile solution, and the injection is repeated every other day, being increased in amount by 1 c.c. on each occasion, until a dose of 8 c.c. is reached. In special cases the dose may be increased to 12 c.c. The mixture has also been given *per rectum* and by the mouth.

See *La Presse Médicale*, January 15, 1902, and June 18, 1902 ; *Le Nord Médical*, November 1, 1902, p. lxxxi. Also *Treatment*, vol. vi. 1902, pp. 267 and 417.

Normal Blood-serum for Alimentation and Treatment

Sterile blood-serum, preferably horse serum that has been heated to 60°–62° C. for half an hour to destroy the rash- and fever-producing

constituents, may be administered by sub-cutaneous injection in cases in which food cannot be given by the stomach on account of vomiting, obstruction, &c., or it may be used to supplement gastric or rectal feeding.

Salter has found that rats and mice may be kept alive for weeks by means of injections of serum alone without any other food.

The doses given must, however, be adequate : to young children 30–50 c.c., to adults 100–150 c.c. may be given daily. In a case of gastric car-cinoma the writer supplemented gastric feeding with injections of serum with excellent results.

Kirton has used subcutaneous injections of sterile horse serum when mouth, nasal, and rectal methods of alimentation failed, and speaks favourably of them. He recommends 20–40 c.c. subcutaneously, daily.

Injections of ox serum have been used in the treatment of chorea.

Hort finds that normal horse serum may be given by the mouth in daily doses of 30–40 c.c. for weeks with impunity. It should be fresh and sterile and be taken directly after food.

It is useful in many cases of so-called simple anæmia, in hæmoptysis, purpura, hæmophilia, and the hæmorrhage of typhoid ulceration.

In conditions such as chronic gastritis, gastric

and duodenal ulcer and ulcerative colitis, serum feeding is generally of considerable value, and the serum applied locally as a dressing in varicose ulcer, tuberculous and staphylococcic infections gives excellent results.

See Salter, *Guy's Hospital Reports*, liii.; Kirton, *Lancet*, 1901, i. p. 1666; *Brit. Med. Journ.* Epit. 1902, i. p. 36, No. 147 (Chorea); Hort, *Proc. Roy. Soc. Med.* i. 1907–1908, Med. Sect. p. 241.

CHAPTER VII

VACCINE THERAPY

RÔLE OF THE SERUM IN PHAGOCYTOSIS—OPSONINS AND THEIR NATURE—THE OPSONIC INDEX AND METHOD OF DETERMINING IT—PRINCIPLES OF VACCINE THERAPY—PREPARATION OF THERAPEUTIC VACCINES—RÉSUMÉ OF VACCINE THERAPY OF VARIOUS DISEASES—ANTI-RABIC INOCULATION—COLEY'S FLUID—CANCROIN—YEAST, &c.

SECTION I

By a series of classical experiments Metchnikoff showed that in a local bacterial infection, if not too intense, leucocytes migrate into the region of infection and ingest and ultimately remove the infecting bacteria. This action he termed ' phagocytosis ' (see fig. 26) and the cells performing it ' phagocytes,' the principal phagocytes being the polymorphonuclear and large mononuclear leucocytes, endothelial cells, and to some extent, perhaps, certain wandering cells of the tissues. On this fact he based his

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phagocytic theory of immunity. He further showed that in the case of a virulent organism, phagocytosis might be practically absent, *e.g.* in the case of anthrax, and a fatal infection therefore ensues. But if the animal be first immunised with anthrax vaccine, it is found that injection of virulent anthrax *is* followed by phagocytosis of the virulent bacilli. At first Metchnikoff ascribed this alteration of the behaviour of the phagocytes after immunisation to an 'education' of the leucocytes and other phagocytic cells. But it has been found that if the serum of the immunised animal be injected into a non-immunised one, the phagocytes in the latter are then able to ingest virulent organisms; they behave in fact in the same manner as do the phagocytes in the immunised one. The effect of the vaccination must, therefore, in part at least, be due to something generated in the plasma or serum, and Metchnikoff ascribed the action to substances, 'stimulins'

FIG. 26.—PHAGOCYTOSIS. *a*, A POLYNUCLEAR LEUCOCYTE WITH ITS NUCLEUS, *b*, WHICH HAS INGESTED SEVERAL MICROCOCCI, *c*; *d*, RED BLOOD CORPUSCLES.

which heighten the activity of the leucocytes and other phagocytic cells. Whether stimulins exist or no is a vexed question, but after Leishman had devised an ingenious method for estimating phagocytosis,[1] Wright and Douglas[2] showed that the serum has a marked influence in inducing phagocytosis. Thus washed leucocytes without serum are non-phagocytic (or practically so), but become phagocytic on the addition of normal serum. If, however, the serum be heated to 60° C. for fifteen minutes it loses its capacity for inducing phagocytosis, therefore the substance causing phagocytosis is readily destroyed by heat, *i.e.* is ' thermolabile.'

Moreover, various experiments show that the action of the serum in inducing phagocytosis is exerted on the bacteria and not on the leucocytes[3] (or but to a slight extent on the leucocytes). Thus, if the unheated serum is mixed with bacteria, the mixture kept at blood heat (37° C.) for fifteen minutes, and then heated to 60° C. for a further fifteen minutes, which, as previously stated, destroys the capacity of the serum for

[1] *Brit. Med. Journ.* 1902, i. p. 73 (see p. 260).
[2] See various papers in the *Lancet* and *Brit. Med. Journ.* 1903–1908, in the *Proc. Roy. Soc. Lond.* B. lxxii.–lxxvii., and Wright, *Studies in Immunisation,* 1909.
[3] On the rôle of the serum in phagocytosis, see Dean, *Brit. Med. Journ.* 1907, ii. p. 1409 (Bibliog.).

inducing phagocytosis, on the addition of leu-
cocytes to the mixture phagocytosis takes place
as well as if the serum had not been heated.
Again, if a mixture of serum and bacteria is
prepared, and kept at 37° C. for fifteen minutes,
and if the bacteria are then washed free from
serum by repeated centrifugalisation with salt
solution, phagocytosis occurs if washed leucocytes
(*i.e.* leucocytes free from serum) are added to the
washed bacteria and the mixture is incubated
at 37° C. This demonstrates that the serum
acts in some way on the bacteria, rendering
them suitable prey for the phagocytes. The
substances in the serum which thus prepare
the bacteria for phagocytosis are termed by
Wright and Douglas ' opsonins.'

The term ' opsonin ' is derived from a word
meaning ' I cater for,' ' I prepare a feast for '
(Gr. ὀψωνέω ; Latin, *obsōno* or *opsōno* = I buy
fish or victuals, hence ' I cater for ').

Wright and Douglas further showed that there
is considerable variation in certain circumstances
in the capacity of the serum for inducing
phagocytosis, that is the opsonising action of the
serum is variable. While there is little variation
in the opsonising action of the serum of normal
individuals, active immunisation (see pp. 8, 9),
with a bacterial vaccine, or a natural infection,

alters the opsonising action of the serum towards the organism injected or of the infection. This is ascertained by determining the ' opsonic index ' (see p. 260), which in principle consists in making a mixture containing the serum of the injected or infected person, washed leucocytes, and a suspension of the micro-organism in question, incubating at blood heat for from five to fifteen minutes, and then preparing stained films. A control experiment is made at the same time, using the serum of a *normal* individual and the same leucocytes and bacterial suspension, and similarly preparing stained films. The films are then examined microscopically and the number of micro-organisms ingested by 50 or 100 polymorphonuclear leucocytes is counted in each of the two films. The ratio of the number of organisms in the film prepared with the *abnormal* serum (*i.e.* that of the injected or infected person) to that in the film prepared with the *normal* serum gives a figure, the opsonic index, and if the number of organisms in the normal serum film be taken as unity, the index will generally be a figure above or below unity. For example, if a healthy person be injected with a vaccine consisting of a killed culture of the *Micrococcus pyogenes aureus*, an amount say of 500 million cocci, and if the

serum be examined in this way five to seven days later, the film preparation might show say 300 cocci ingested by 100 polymorpho-nuclear leucocytes, while the film prepared under similar conditions with normal serum (*i.e.* the serum of a healthy and uninjected person) might show only 200 cocci ingested by the same number of leucocytes. The ratio is therefore 300 : 200 or $\frac{300}{200}$, and if the normal be taken as unity this gives the figure $\frac{300}{200} \times 1 = \frac{3}{2} = 1\cdot5$, which is the opsonic index of the injected person, that is the capacity of his serum to induce phagocytosis, compared with that of a normal individual, taken as unity. In this instance increased phagocytosis is induced by the serum of the injected indi-vidual, his opsonic index is above normal, or is raised.

In the case of a person suffering from acute boils, generally caused by infection with the *M. pyogenes aureus*, a similar procedure would probably give a film containing perhaps 120 cocci ingested by 100 leucocytes, the film prepared with normal serum containing 200 cocci in the same number of leucocytes. The ratio in this case is therefore 120 : 200, or $\frac{120}{200}$, and taking the normal as unity as before, the figure for the opsonic index becomes $\frac{120}{200} \times 1 = \frac{3}{5} = 0\cdot6$. In this instance, therefore,

phagocytosis is less active with the serum of the infected person than with that of a normal person, in other words the opsonic index is below normal, or is depressed. Other facts may also be brought out by experiments of this kind. Thus, if the serum of the person injected or infected with the *M. pyogenes aureus* be tested in the same manner as to its capacity to induce phagocytosis, not towards the *M. pyogenes aureus*, but towards some other organism, *e.g.* the *Bacillus coli* or the tubercle bacillus, it will probably be found that the opsonic index is unaltered compared with normal serum, the number of organisms in the same number of leucocytes in the films prepared with the abnormal and the normal serum will probably be approximately the same, and the ratio in the two specimens will therefore be approximately $x : x$, that is the index will be 1·0, or normal and unaltered. From this it is to be inferred that in normal serum opsonins are present which act on a number of different organisms, *i.e.* are 'common.' In the serum after vaccination ('immune serum') opsonins are developed which act only on the particular organism employed, *i.e.* are 'specific.'

The nature of opsonins has not yet been thoroughly worked out. The opsonin of normal

serum is highly thermolabile, *i.e.* is destroyed by heating to 55° C. for half to one hour, or to 60° C. for five minutes. In infections and after vaccination, however, thermostable opsonins, *i.e.* opsonins not destroyed by this degree of heat, may be present.

Bulloch came to the conclusion that the blood contains a number of specific opsonins, one for tubercle, another for *M. pyogenes*, and so on. Simon, Lamar, and Bispham, however, from a number of carefully devised experiments conclude that specificity of opsonins does not exist, and suggest that opsonins may be a constant quantity, and that the number of organisms taken up by the leucocytes is influenced by a second unknown and variable factor.

Russell also concludes that in *normal* serum the opsonins are 'common' and not specific, and can be removed by a number of bodies. In immune serum, on the other hand, both 'common' and 'immune' opsonins are present, the latter being quite specific; that is to say, in the process of immunisation specific opsonins are formed, and the increase of opsonins following injection of a vaccine is probably due to the formation of immune opsonins which react specifically.

Muir and Martin[1] as a result of their work formulate the following conclusions :

1. The thermolabile opsonin of a normal serum and the thermostable opsonin of an immune serum are two distinct classes of substances. In addition to differing markedly as regards their resistance to heat, they differ in their combining relationships.

2. The thermostable opsonin of the anti-serum investigated is a true anti-substance, and possesses the comparatively specific characters of anti-substances in general ; it is left undetermined whether it has the constitution of an agglutinin or of an immune body, though certain facts point in favour of the former.

3. Emulsions of organisms other than the organism used in immunisation (*Micrococcus aureus*) do not absorb the immune opsonin ; on the other hand, they absorb large amounts of the normal complement-like opsonin.

4. Powerful complement absorbers—red corpuscles or bacteria treated with immune body or serum precipitate — have no effect on the thermostable immune opsonin, whereas they remove almost completely the labile opsonin

[1] *Proc. Roy. Soc. Lond.* B. lxxix. 1903, p. 187 ; Muir, *Studies on Immunity*, 1909, pp. 184 and 190.

of the normal and the immune serum alike. They further state that their general conclusions are that in the case of normal sera the opsonic effect is generally due to the labile non-specific complement ; it may act with or without a natural immune body.

In the case of immune sera the opsonic effect may be increased by immune bodies leading to the union of more complement. There is in addition, however, the formation of immune opsonin (bacterio-tropin), which produces an opsonic effect by itself, and this substance accordingly has the constitution of an agglutinin.

Wright considers the opsonins to be substances distinct from all others ; Metchnikoff, Dean, and other observers suggest that they are identical with the ' substance sensibilisatrice ' (p. 34).

Emery concludes that amboceptor appears to have the power of sensitising bacteria to phagocytosis, and this power seems to be increased by the concurrent action of complement. Further, there is no sufficient evidence of the existence of a thermostable opsonin apart from amboceptor. As regards the thermolabile opsonin there is an equally close resemblance between it and complement,

and we are forced to the conclusion that complements may play the part of opsonins. It may be suggested that the increased phagocytosis brought about by immunisation may be due to the fact that the presence of amboceptor produced by the immunisation enables the bacterium to be prepared for phagocytosis by the concurrent action of many complements which otherwise would be able to attack it only with great difficulty.[1]

Pfeiffer[2] is of opinion that the opsonins are identical with the bacteriolysins, the extracellular digestion of the bacteria by the bacteriolysins being masked by the rapid phagocytosis which occurs.

As regards the source of opsonins little is known. If thermolabile opsonin is identical with complement, it is probably derived from the polymorphonuclear leucocytes. Opsonin does not seem to be formed in the blood ; according to Allen it is probably derived from the muscular tissues.

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[1] See Emery, *Immunity and Specific Therapy*, pp. 282–288.
[2] Harben Lectures, *Journ. Roy. Inst. of Public Health*, xvii. 1909, Nos. 7, 8, and 9.

SECTION II

METHOD OF DETERMINING THE OPSONIC INDEX

This is based on Leishman's method for estimating the activity of phagocytosis, which is carried out as follows :

A thin suspension of some micro-organism, *e.g. M. pyogenes*, is mixed with an equal volume of blood from the finger, a droplet of this mixture is placed on a clean slide, and covered with a cover-glass, and the preparation is at once placed in a moist chamber in the incubator at 37° C. for half an hour. At the end of this time it is taken out, the cover-glass slipped off, and the films on slide and cover-glass are dried, fixed, stained and examined microscopically, and the number of microbes ingested by the polymorphonuclear leucocytes is counted.

For the determination of the opsonic index by Wright's method the following requisites are necessary : (1) the serum of the patient, (2) the serum of a healthy person for a control, (3) an emulsion of the organism for which the determination is to be made, (4) a suspension of living leucocytes, (5) several Wright pipettes, with india-rubber teats or nipples.

1 and 2. *The Sera.*—These two specimens should be taken at about the same time, and the determination

should be made as soon as possible, preferably within a few hours of taking the samples. (If the tubes be sealed up, Wright states that no alteration takes place for two to three days.)

The blood is preferably collected in a Wright's capsule (*d*, fig. 27). Both ends of the pipette are broken off, and the blood is collected by immersing the *bent* end in the blood as it runs from a prick in the ear or finger. The
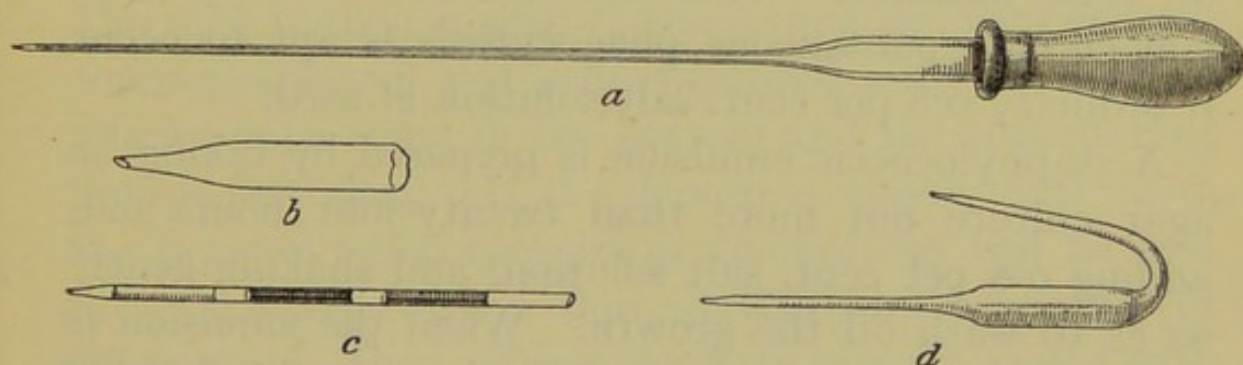
FIG. 27.—*a*, GLASS PIPETTE, WITH INDIA-RUBBER TEAT FOR OPSONIC DETERMINATIONS, &c.; *b* SHOWS (ENLARGED) THE CONTRACTED EXTREMITY OF THE PIPETTE; *c* SHOWS THE STEM OF THE PIPETTE, CONTAINING THE EQUAL VOLUMES OF SERUM, LEUCOCYTIC SUSPENSION, AND BACTERIAL EMULSION, BEFORE MIXING; *d* IS WRIGHT'S CAPSULE FOR COLLECTING BLOOD.

capsule should be at least one-third filled. For pricking, a flat-pointed needle of the Hagedorn type is preferable; a prick with an ordinary needle does not yield sufficient blood. After filling, the capsule is sealed in the flame, the dry or straight end being sealed first. After coagulation the capsule is centrifugalised to obtain clear serum; for this purpose the capsule is hung by the curved end in the centrifuge.

3. *Emulsion of the Organism.*—In the case of tubercle, suitable dead cultures can be purchased. To

prepare the emulsion from this, take a small portion (about as big as a grain of rice) and place it in a small agate mortar and grind it up with the pestle ; then add 1·5 per cent. salt solution drop by drop until about 2 c.c. have been added, continuing to grind meanwhile. This gives an emulsion which contains isolated bacilli as well as clumps. These latter must be got rid of, and to do this it is necessary to centrifugalise for three or four minutes. With the tubercle bacillus and gonococcus spontaneous phagocytosis is apt to occur if ordinary 0·8 per cent. salt solution is used.

A staphylococcic emulsion is prepared by taking an agar culture not more than twenty-four hours old, adding 0·8 per cent. salt solution, and shaking gently so as to wash off the growth. When the emulsion is made it must be pipetted off into a small tube and centrifugalised for a few minutes. The emulsion must not be too thick, otherwise the leucocytes will take up an uncountable number of cocci ; the emulsion should be only faintly opalescent, but the proper density can be judged by experience alone. The aim is to obtain an average of about two organisms per leucocyte with *normal* serum. Emulsions of pneumococci and other organisms are made in the same way. Variations in the number of bacteria ingested may occur according as recently isolated or old strains are employed.

Instead of centrifugalising, the emulsion may be filtered through a double thickness of filter-paper.

4. *Suspension of Living Leucocytes.*—To prepare this, take about 10 c.c. of physiological saline solution containing ½ per cent. of sodium citrate, to prevent the

coagulation of the blood. This must be freshly pre-
pared (or kept sterile, which is inconvenient), and the
simplest method is to use ' soloids ' prepared for the
purpose by Burroughs & Wellcome ; one of these dis-
solved in 10 c.c. of water will yield the solution re-
quired. This is put into a centrifugalising tube and
warmed to blood-heat. A healthy person is then
pricked in the ear or finger, and his blood is allowed to
drop into the fluid until 1 c.c. or more has been collected.
The tube is then placed in the centrifuge, very exactly
counterbalanced, and centrifugalised until all the cor-
puscles have come to the bottom and the supernatant
fluid is left clear. If the deposit is closely examined,
the layer of red corpuscles at the bottom will be seen
to be covered with a thin whitish film consisting of
leucocytes. Then, with a capillary pipette armed with
an india-rubber nipple, or with a syringe, the whole of
the clear fluid is pipetted off as close as possible to the
leucocyte layer, but without disturbing the latter. The
tube is then filled with saline solution, the blood and
fluid are mixed, the mixture is centrifugalised, and the
clear fluid again pipetted off, and this process of wash-
ing is repeated. Or the leucocyte layer with the
underlying red corpuscles may be first pipetted off,
introduced into a second tube, and washed twice.
Next, the leucocyte layer with the upper layer of red
corpuscles (which also contains leucocytes) is pipetted
off into a small tube, and the whole is thoroughly mixed
by repeatedly sucking into and expelling from the
pipette. The result is a suspension of the living
leucocytes mixed with red corpuscles.

5. *Wright's Pipettes with India-rubber Teats.*—These are made of glass tubing drawn out in the blowpipe flame into the form shown in *a*, fig. 27, which is two-thirds full size. The end of the fine extremity should be contracted as shown in *b*. Glass tubing must be chosen which properly fits the teats.

The Process.—(1) Prepare a pipette by placing an india-rubber teat on the thick end. Then, with a grease pencil or with pen and ink, make a transverse line about an inch from the pointed end. The volume of fluid contained in the tube between the point and this mark is spoken of as the unit.

(2) Having the patient's serum and the suspensions of the leucocytes and of the bacteria ready to hand, take the pipette between the index finger and thumb of the right hand and compress the nipple. Immerse the point beneath the surface of the suspension of bacilli, and relax the pressure on the nipple until the emulsion has risen exactly to the mark so that one unit has been drawn up ; then remove the point from the fluid and relax the pressure again, so that a small volume of air is sucked up. This will be quite easy if the point is a good one, otherwise it will be difficult or impossible, as the column of fluid will either refuse to stir or will oscillate violently. Next immerse the point in the suspension of leucocytes and draw up one unit. This will be separated from the emulsion of bacteria by the bubble of air. Remove the point from the emulsion and draw up a second volume of air.

Lastly, draw up one unit of serum. There will now be in the pipette (counting from the nipple towards the

point) one unit of bacterial emulsion, a bubble of air, a unit of leucocytes, a bubble of air, and, lastly, a unit of serum (*c*, fig. 27).

(3) Put the point of the pipette on to a clean hollow ground slide or an artist's porcelain sunk palette, and express the whole of its contents, and mix well together, aspirating them repeatedly into the pipette, and expelling without causing bubbles. If bubbles form, a hot wire brought near will quickly dispel them. When thoroughly mixed aspirate the mixture into the pipette, suck up a short volume of air, and seal the tip in the flame.

Then place the pipette point downwards in the incubator, or better into a water-bath, at 35° to 37° C., noting the time exactly, and proceed to prepare a second pipette in precisely the same way, using the same suspensions of bacteria and leucocytes, but the control serum instead of the patient's. Place this in the incubator or water-bath, by the side of the other, noting the time at which this is done. When each pipette has been incubated for a quarter of an hour it is removed from the incubator or water-bath, the end broken off and the nipple fitted to the thick end, then the contents are expelled on to a hollow slide or porcelain palette and mixed thoroughly together. Films are then prepared. This may be done by depositing a drop in the middle of a large cover-glass (1 inch square, No. 2), dropping on to it another cover-glass and drawing the two apart. Or films may be made on slides, or which Wright recommends roughing the slides with emery paper and spreading the drop with

the sharp edge of a slide broken in half after scratching with a file or glass knife. The films then have to be stained. For staphylococci, streptococci, pneumococci, *B. coli*, &c., the films may be fixed with formalin and stained with carbol-thionine blue, or they may be stained without previous fixing with Leishman stain. For tubercle, the films may be fixed with mercuric chloride solution (one to two minutes), stained in warm carbol-fuchsin, decolorised with $2\frac{1}{2}$ per cent. sulphuric acid in methylated spirit, and counter-stained with methylene blue.

Lastly, the films are examined with the oil-immersion lens, preferably with the aid of the mechanical stage, and the number of organisms contained in not less than fifty polymorphonuclear leucocytes counted. Parts of the film in which the cells are broken down or not well stained, or cells containing obvious clumps of organisms should be avoided.

Wright now uses the whole blood instead of the leucocyte layer only. After the blood has been drawn into the citrated salt solution it is centrifugalised, washed twice with salt solution, and finally the corpuscles are well mixed. With the blood the various mixtures are made and incubated as previously described. Then, in order to make the film for staining and counting, the contents of the pipette are discharged on to one end of a slide roughened with emery paper, and the mixture is spread by means of a slide which has been broken across after notching the sides with a file or glass cutter. The object is to get a broken edge having a very slight concavity, and many slides may have to be sacrificed

before exactly the right edge is obtained. When an edge of the proper sort is used and the film is spread by drawing (not pushing) along, the leucocytes adhere to the edge, and finally are deposited mostly at the end of the preparation, the red corpuscles being left behind.

There has been much criticism of the reliability of opsonic determinations.

Thus Hort [1] gave portions of the same samples of serum to various skilled observers for estimation of the opsonic indexes to tubercle. In some cases the indexes obtained by the various observers were very close, in others there was considerable difference, *e.g.* as much as 1·40 (Observer A = 2·20, B = 0·82).

Many other criticisms might be quoted, and Shattock and Dudgeon maintain that not only does the serum vary in its power of inducing phagocytosis, but that the capacity of the leucocytes for phagocytosis also varies, and consider that *both* these factors should be taken into account.

On the whole, however, in skilled hands, with careful technique and much practice, the results are of some value.

[1] *Brit. Med. Journ.* 1909, i. p. 400.

SECTION III

PRINCIPLES OF VACCINE THERAPY [1]

The immediate result of an infection is to cause a lowered opsonic content of the blood towards the particular organism of the infection. Could the opsonic content be estimated in the pre-infective stage, it would probably be found that the fall in opsonic content is really antecedent to the clinical manifestations of the infection, that is to say, during the incubation stage there is a fall in the amount of opsonin, and when this has developed the infection becomes manifest. Obviously, however, the opportunity for observing this course of events must rarely occur, though it has occasionally been noticed, and it has been demonstrated experimentally.

As regards the opsonic content of the serum in infective diseases, much depends on the period at which the disease comes under observation. In acute and recent infections, the opsonic index as a rule will be low. In subacute and chronic local infections the index

[1] See Allen, *Vaccine Therapy*, 1908 ; Wright, *Studies on Immunisation*, 1909. Also Discussion, *Proc. Roy. Soc. Med.* iii. 1909–10.

is also usually low, but if the condition has lasted for some time it may be higher than normal. In chronic infections which are not strictly localised, *e.g.* tuberculosis, the index will sometimes be low, sometimes high. A low index generally indicates an infection, or a low power of resistance to the particular organism. A high index usually indicates that the person has had an infection but has overcome it, or is tending, or making an effort, to overcome it. With good technique, it is found that the normal index for healthy persons varies only within narrow limits—from about 0·8 to 1·2 as extremes ; an index above or below these values is therefore probably abnormal and pathological.

Moreover, the disappearance, the elimination of an infection is generally accompanied by a rise of the index. It may be expected, therefore, that if in an infection the index can be artificially raised, the tendency will be for the infection to terminate and the condition to recover. Sir A. E. Wright, finding that an appropriate dose of a vaccine usually raises the index for the organism of the vaccine, conceived that the same principle might be applied to cases of infection with various organisms, and found that in many instances the injection of

an appropriate dose of a vaccine, consisting of a killed culture of the infecting organism, does raise the opsonic index, and, coincident with this, improvement or cure results. This is well seen in cases of boils or of pustular acne caused by the *Micrococcus pyogenes aureus*. The opsonic index for this organism in these cases is generally low, and by administering appropriate doses of a vaccine, consisting of a killed culture of the organism, at proper intervals, a cure follows. The number of doses required to bring about this result varies in different cases, it may be one, two, three, or more. In chronic infections like tuberculosis, the doses of vaccine may need to be numerous and to be administered over a long period.

The first effect, however, of the administration of a dose of vaccine is generally to depress the index somewhat,[1] and if the dose be large a marked and perhaps long continued depression of the opsonic index may result. This effect is termed the 'negative phase' by Wright, and is illustrated in the following chart (Chart I.).

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[1] It is, of course, not strictly correct to speak of a vaccine causing a depression or an elevation of the opsonic *index*. What it does do is to alter the opsonic content of the serum, which is indicated by an alteration in the index. The meaning, however, is sufficiently obvious.

From this chart it will be seen that the first effect of the vaccine is to depress the opsonic index from 1·1 to 0·7 (the negative phase),

the depression lasting for two days. After this there is a marked rise in the index to 1·8 (positive phase), after which the index falls, and on the seventh day after inoculation has become only 1·3, and three weeks after inoculation the index is normal once more. Occasionally a slight rise in the index occurs before the development of the negative phase—this is the 'false rise' of Wright.

With a larger dose of vaccine, the negative phase is still more marked. In Chart II. the effect is shown of injecting a healthy rabbit with a dose of staphylococcic vaccine half as large

CHART I.

FIG. 28.—EFFECT ON THE OPSONIC INDEX OF A HEALTHY RABBIT PRO- DUCED BY THE INOCU- LATION OF 1 C.C. OF A STAPHYLOCOCCIC VAC- CINE   (1,000,000,000 COCCI).

again as the dose given in the previous instance.

From this chart it will be seen that the first

effect of the vaccine is to cause a pronounced fall in the opsonic index, from 1·15 to 0·4. This is recovered from in forty-eight hours, but the

CHART II. CHART III.
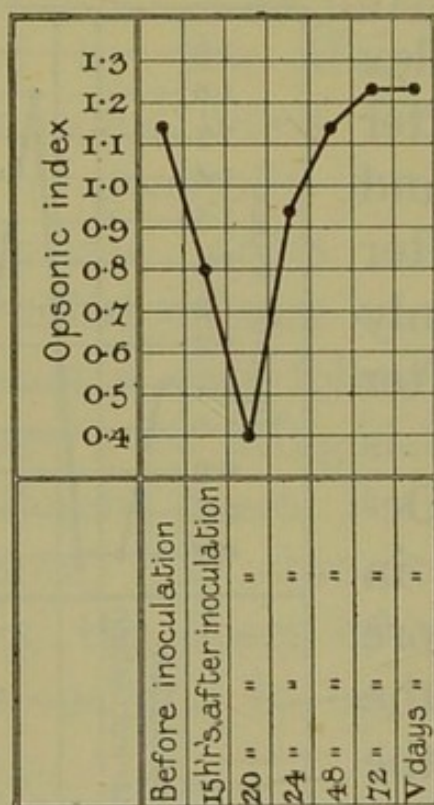
FIG. 29.—EFFECT ON THE OPSONIC INDEX OF A HEALTHY RABBIT PRODUCED BY THE INOCULATION OF 1·5 C.C. OF A STAPHYLOCOCCIC VACCINE (1,500,000,000 COCCI).
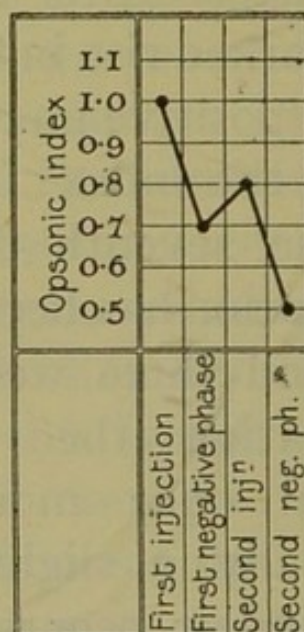
FIG. 30.—DIAGRAM TO ILLUSTRATE SUMMATION OF NEGATIVE PHASES PRODUCED BY A SECOND DOSE OF A VACCINE GIVEN DURING OR IMMEDIATELY AFTER THE NEGATIVE PHASE PRODUCED BY THE FIRST DOSE.

subsequent rise in the index is much less than in the previous instance with the smaller dose of vaccine, viz. to 1·25 only, or hardly above normal.

It may be concluded, therefore, that while moderate doses of a vaccine produce a slight negative phase followed by a marked rise of the index, a large dose of vaccine will produce a marked negative phase and may be followed by little or no subsequent rise above the normal. The aim, therefore, in treatment by vaccine is so to adjust the dose that the preliminary fall of the index or negative phase may not be excessive and that this may be followed by a positive phase or rise of the index.

Another consideration has also been emphasised by Wright. It is probable, and this is borne out by experience, that if a second dose of vaccine be given during the negative phase produced by the first dose, a further depression of the index will be produced ; in other words, a summation of negative phases and an aggravation of the condition occurs. This is illustrated in Chart III. Similarly, if the doses are given too close together, though not close enough to produce a summation of negative phases, little or no rise in the index may follow.

On the other hand, if the doses of vaccine be so spaced that they are given when the index is well on the rise, or better still, is at the summit of the rise produced by the previous injection, a further rise of the index will be produced

with greater amelioration of the condition. This is illustrated in Chart IV.

These then should be the aims in treatment with bacterial vaccines, viz. : (1) to give such doses that the opsonic index is not unduly depressed ; (2) so to space the doses that a summation of negative phases is avoided, and that successive doses are given when the index is well on the rise, or, better still, when the maximum effect of the previous dose of vaccine has been attained.

It must, however, be clearly understood that it is not possible to raise the index indefinitely ; to raise the index to 2·0 or 2·5 is quite exceptional; generally one is content if the index is raised. to 1·5– 1·8, according to the point at which it starts. After the preliminary two or three or more doses of vaccine have well raised the index, the aim is that subsequent doses shall *maintain* the raised index and not allow it to drop. Generally it is easier to raise

CHART IV.

Fig. 31. — Diagram to illustrate the Progressive Rise in the Index by properly spaced Doses of Vaccine.

the index when this is below, than when it is above, the normal. As a rule, if an interval of seven to fourteen days is allowed between successive doses, summation of negative phases will be avoided, the negative phase generally being of short duration, but occasionally it may last as long as three weeks. Hence opsonic determinations, especially at the commencement of treatment, may be very valuable in guarding against untoward effects.

Although, as a rule, amelioration or cure of the condition coincides with a rise in the opsonic index, occasionally patients improve while the index remains persistently low, *e.g.* in tuberculosis, and sometimes the disease progressively advances while the index is raised, *e.g.* in meningococcal cases. The proper dosage of vaccines has been arrived at by experience controlled by opsonic determinations. In dealing with cases the golden rule is to commence treatment with *small* doses, subsequently increasing the amount according to the clinical aspect of the case and the effect on the opsonic index.

Again, as the effect produced by the infecting organism on the opsonising action of the serum is very specific, in many instances a vaccine prepared with the particular strain of organism of

the infection will act better than a vaccine prepared from a stock laboratory strain of the organism. This is especially seen with strepto-cocci and pneumococci. Lastly, since a period of at least twenty-four hours is necessary for a dose of a bacterial vaccine to influence the opsonic index in an upward direction, the treatment by vaccines of very acute infections which run a course of from a few hours to two or three days, *e.g.* cholera and some streptococcic and pneumococcic infections, at present seems rather hopeless.

Stimulating and suggestive as the opsonic theory has been, it must be recognised that it does not contain the whole truth as regards immunity and the cure of infective disease. Watson Cheyne, discussing the defensive arrange-ments of the body as illustrated by the incidence of disease in children and adults, shows how remarkably the incidence of infective disease (tuberculosis, pneumococcic, coli, and other infections) differs at different age periods, and in the two sexes, and remarks : [1]

At the present time, it is the fashion to look on the opsonic content of the blood as the essential agent concerned in bringing about immunity and cure in the case of infective diseases. I have searched the

[1] *The Wightman Lecture* for 1908.

literature, inquired among investigators, and had a few observations made for me without finding any marked differences in the opsonic index to different organisms at various periods of life corresponding to the variations in the incidence of disease, and in their course at different ages ; nor has any difference in the opsonic index to various infective organisms been pointed out as regards sex, and yet, as we have seen, there are noticeable variations in the incidence and severity of these diseases in the two sexes. . . . The only investigations which I have come across (bearing on this point) are some described by Dr. J. H. Wells in the May number of the *Practitioner*. He has made observations on the opsonic index in infants under one year old, with the result that he finds marked differences in young babies as compared with older people. He comes to several conclusions, of which I may mention the following : ' A low opsonic index is not diagnostic in children under one year old.' ' In infants a low opsonic index is not inconsistent with health, and the child may be thriving well with a declining index.' ' The anti-bacterial defense in children cannot depend upon the opsonic content of the serum.' These are very important conclusions, and if found to apply to a sufficiently large number of cases, they raise the question whether the significance of the opsonic index and opsonins generally is being properly interpreted. Although infective troubles, when they do occur in infants are, as a rule, more acute than in older children—as already instanced in the case of tuberculosis, erysipelas, &c.—nevertheless

infants do not develop these infections so rapidly as children do after the first or second year of life—in general terms, their defensive power is greater. But, if it is a fact that the anti-bacterial defense in infants does not depend on the opsonic content of the serum, and yet at that period their defensive powers are high, is it right to assume that at a later period of life the anti-bacterial defense does depend on the opsonic content of the serum ? May there not be some other interpretation of the variations in the opsonic index than that which is put forward by Wright and which is being so extensively translated into practice ?

I must confess that while there is much that is interesting and important in the extensive work which is at present being done on opsonins, it would be more convincing to me if the theories on this matter were less complete and simple, and if the writings were tinged with a little philosophic doubt. Unfortunately, when we come to study the results of the application of these theories to practice, they do not work out as they ought to do. In some cases good apparently does result, while in others it is an open question whether the benefit which follows would not have occurred to an equal extent without the vaccination ; in many instances, however—and my experience especially concerns tubercle—one cannot convince oneself that the slightest benefit has resulted—in some, indeed, the condition seems to have become worse. . . . I think that all we can say about these matters at present is that they are highly interesting

from a scientific point of view, and that their continued study is highly desirable, but that it is very doubtful if the opsonin theory is the complete solution of the problem.

With these views of Sir Watson Cheyne the writer is in complete agreement, and he still believes that serum therapy is the ideal method for treating infections.

Preparation of the Vaccine

The best results are generally obtained when the vaccine is prepared with the particular strain of organism of the patient's infection. In many cases, therefore, the isolation of the organism from the patient should at once be commenced.   This, however, may be im-practicable in some cases, *e.g.* in tuberculosis, or may entail such delay that valuable time may be lost, *e.g.* in pneumonia. In the case of tuberculosis, one of the tuberculins is therefore usually employed (see later) ; in the case of an acute condition like pneumonia treatment may be commenced with a stock pneumococcic vaccine, until the particular strain of organism of the infection has been isolated. In other infections, such as those produced by the

staphylococcus or gonococcus, stock vaccines generally act well ; if they do not, however, recourse may then be had to isolation of the patient's own strain and the preparation of a vaccine therewith.

The vaccine is prepared by growing the organism under appropriate conditions, the staphylococcus on agar, the streptococcus, pneumococcus and gonococcus on blood-agar, &c. The growth is then emulsified by adding a few drops of sterile 0·1 per cent. sodium chloride solution and well rubbing up with a sterile glass or aluminium rod. Two or three tubes are treated in this way ; the emulsion is poured into a small sterile Erlenmeyer flask of stout glass, the tubes are rinsed out with a little more of the salt solution and the washings added to the contents of the flask, two or three sterile glass beads are added and the flask is shaken vigorously for some minutes thoroughly to break up the masses of organisms. The contents of the flask, which should measure 5 c.c. or thereabouts, are then centrifugalised for some minutes and the emulsion is poured off from the deposit into a second sterile flask and is now ready for standardisation.

Standardisation is carried out by Wright's method. Two or three volumes of citrate

solution are sucked up into a pipette such as that used for opsonic determinations, the finger is pricked and one volume of blood is taken up in the pipette, separated from the citrate solution by an air bubble, and finally one volume of the bacterial emulsion, also separated from the blood by an air bubble, is taken up. The whole contents of the pipette are then well mixed by expelling on to a clean slide and sucking up three or four times. About one-third of the mixture is then transferred to each of three clean slides and the drops spread by smearing with the edge of a slide so as to obtain thin uniform smears. These are allowed to dry, stained with Leishman's stain, and the number of red corpuscles and bacteria counted in a number of microscopical fields. Counting is much facilitated by introducing a diaphragm with a square hole of appropriate size into the eyepiece, so that a few corpuscles only are in view in the field. Assuming that there are 5,000,000 red cells in a cubic millimetre of blood, it is easy to calculate approximately the number of bacteria contained in the emulsion. Suppose that 500 red cells have been counted and with these 1,500 bacteria are admixed. Since equal volumes of blood and emulsion have been taken, one cubic millimetre of bacterial emulsion will

contain $\dfrac{5,000,000 \times 1,500}{500} = 15,000,000$ bacteria.

But one cubic centimetre contains 1,000 cubic millimetres, therefore the emulsion contains $15,000,000 \times 1,000 = 15,000,000,000$ bacteria per cubic centimetre, and by appropriate dilution any bacterial content of the emulsion may be obtained. Thus, if 1,000,000,000 organisms per cubic centimetre are desired, 1 c.c. of the emulsion must be diluted with 14 c.c. of salt solution. To the prepared dilution of the bacterial emulsion 0·5 per cent. of carbolic acid or 0·2 per cent. of trikresol is added, and the flask is placed in a water-bath at 56°–60° C. for one or one and a half hours, according to the resistance of the organism. The stock solution may subsequently be introduced into small sterile glass serum phials of 1–2 c.c. capacity, and the phials after sealing and standing for twenty-four hours may again be sterilised for an hour at 60° C. to ensure that the organisms have been killed ; cultures may be made from the sterilised vaccine to make certain that this is the case (the sterility test). The lower the temperature and the less the heating, consistent with sterilisation, the more active will be the vaccine.

In large clinics where numerous doses of

certain stock vaccines are being given daily, the stock vaccine may be preserved in squat bottles or flasks capped with a rubber cap, the needle of the syringe being passed through the rubber in order to take up the required amount of vaccine.

Dosage of Vaccines

The table on p. 284 summarises the doses for adults of some of the chief vaccines which are employed,[1] but these will vary in individual cases and be modified according to circumstances. For children the doses must be proportionately reduced. Further particulars will also be found in the sections which follow dealing with individual diseases.

The smaller doses are given at the commencement of the treatment, and the doses are gradually increased. The clinical effects produced by the vaccine are noted ; too large a dose may be followed by an aggravation of the condition or symptoms. In the earlier stages, at least, opsonic determinations are very useful, but in some conditions, *e.g.* acne, they are not necessary. At the same time it is well to bear in mind the limitations of the opsonic technique

[1] See Harris, *Practitioner*, May 1908, p. 647.

and its indications, viz. : (1) the skill requisite
for a reliable opsonic determination ; (2) a

DOSAGE OF VACCINES

Vaccine	Relative Toxicity	Doses	Frequency of Inoculation
Tuberculin, TR and Bacillary Emulsion	Very toxic	$\frac{1}{100,000} - \frac{1}{10,000} - \frac{1}{2000}$ milligram	Every 10–14 days
B. coli	Very toxic	10–50 millions	Every 2, 5, or 10 days
Pneumococcic	Less toxic than B. coli	20–50 millions	Every 36–48 hours in acute pneumonia; every 10 days in chronic infections
Streptococcic	More toxic than pneumococcic, less so than B. coli	5–60 millions	Every 7–14 days
Staphylococcic	Less toxic than streptococcic	100–1000 millions	Every 10 days
M. melitensis	—	$\frac{1}{10}$ sq. cm. of surface growth of a 48-hour agar culture [1]	Every 7–14 days
Gonococcic	Slightly toxic	100–500 millions	Every 7–14 days

high index is not necessarily an indication of
immunity, nor that the lesion is undergoing
cure ; (3) a low index is not necessarily an

[1] This dosage is adopted because it is very difficult to count the organisms.

indication of lack of immunity, nor that a patient will not improve.

The vaccine is generally administered by subcutaneous inoculation in the back, flank, or arm. Occasionally it seems preferable to inject in the neighbourhood of the lesion, e.g. in tuberculous glands.

The vaccine is administered with a sterile syringe, the skin being cleansed with a little lysol. Latham, Spitta, and Inman [1] give the results of the treatment of a number of cases of various infections with the corresponding vaccines and tuberculin administered by the mouth in saline solution or normal horse serum. Much the same dosage was given as by hypodermic injection. Clinical effects and alterations of the opsonic index were obtained as by the hypodermic method of administration. The general opinion is, however, that administration by the mouth is a very uncertain method on account of the variations in the rate and amount of absorption by the gastric or intestinal mucous membrane. If absorption from the intestine be desired, the vaccine must be put up in capsules coated with keratin or some other material so that they may escape gastric digestion and pass through the stomach into the bowel.

[1] *Proc. Roy. Soc. Med.* i. 1907–1908, Med. Sect. p. 195.

Experiments by Noon [1] indicate that the subcutaneous method of inoculation is preferable to either intravenous or intraperitoneal inoculation.

Auto-Inoculation

Auto-inoculation by the products produced by the infecting organism necessarily occurs in an infective disease, and it is doubtless this auto-inoculation which gives rise to the abnormal opsonic index in cases of disease.

Auto-inoculation can also be demonstrated in other ways. Thus movement or massage of a tuberculous joint may modify and alter the opsonic index towards the tubercle bacillus.

The various methods of local treatment of infective lesions—fomentations, poultices, stupes, setons, blisters, and Bier's methods of inducing hyperæmia—as well as the treatment of pulmonary tuberculosis by graduated exercise, probably all act partly by producing auto-inoculation.

Hort [2] maintains that the response to infection includes not only response to bacterial products, but also response to the products of cellular

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[1] *Brit. Med. Journ.* 1909, ii. p. 530.
[2] See *Rational Immunisation in the Treatment of Pulmonary Tuberculosis and other Diseases,* 1909.

and tissue disintegration. Cellular and tissue degeneration and disintegration necessarily occur in any infective condition and such products are themselves unquestionably toxic.

Hort therefore maintains that by the use of bacterial vaccines only the organismal, and not the somatic, part of the infection is attacked, and claims that by auto-inoculation, by massage, exercise, and other means, the benefits of response both to the organismal and to the somatic parts of the infection are obtained and results ensue, particularly in pulmonary tuberculosis, better than by the use of vaccines alone.

Endotoxins as Vaccines

The writer has suggested the use of endotoxins obtained by the Macfadyen process (see p. 50) instead of sterilised cultures. He has found that the endotoxins of the *M. pyogenes aureus* and of the tubercle bacillus have a marked effect on the opsonic index.[1] The staphylococcus endotoxin vaccine has been used with good results in some cases of acne. From the experimental results, the writer is inclined to think that the endotoxin vaccine acts more

[1] *Proc. Roy. Soc. Lond.* B. vol. lxxxi. 1909, p. 325, and *Proc. Roy. Soc. of Med.* iii. April 1910 (Pathological Section).

rapidly, is more potent, and produces less negative phase than a bacterial vaccine.

The vaccine is prepared by standardisation of the endotoxin after filtration by evaporating a given volume to dryness *in vacuo* and weighing, and so estimating the content of solid matter and making up the bulk with salt solution so that it contains a definite weight of solid matter per cubic centimetre. The dose that has so far been given is 0·2–1·0 milligram of solid matter.

Sensitised Vaccines

Besredka has suggested for *prophylactic* vaccination the use of bacterial cultures, the organisms of which have been submitted to the action of the homologous anti-serum (see p. 333). The micro-organisms take up and fix the immune body (amboceptor) of the immune (anti-) serum and are then spoken of as being ' sensitised.' Such ' sensitised ' vaccines are stated to produce no reaction, local or general, on injection, and it would seem desirable that such sensitised vaccines should be tried for *therapeutic* use. They might produce no negative phase and rapidly induce a rise in the opsonic content of the serum, and if so would be better adapted than the ordinary vaccines for the treatment of disease.

SECTION IV

VACCINE THERAPY IN VARIOUS DISEASES

Tuberculosis

The numerous researches conducted during the last ten years show that there are at least two types of mammalian tubercle bacilli, the bovine and the human, met with in man. The bovine type is more frequent in abdominal lesions, particularly in children.

The vaccine treatment is conducted by means of one of the tuberculins, those usually employed being Tuberculin T R, and Tuberculin B E (bacillary emulsion). Their nature and preparation are described at p. 362, where directions for preparing the dilutions will be found.

Although good results are obtained with a tuberculin prepared with one type of bacillus, *e.g.* the human, Allen believes that the best results follow the use of mixed human and bovine tuberculins, *i.e.* a mixture of a tuberculin prepared from the human bacillus with a tuberculin prepared from the bovine bacillus. In the earlier stages of treatment, at least, opsonic determinations are of considerable value in estimating the effect produced, the appropriate

dosage, and the proper spacing of the doses. At first the doses should not be repeated at a less interval than 17–21 days, later an interval of 14 days, and finally one of 10–12 days may be allowed between the injections. The clinical condition should be carefully followed and the effect on the temperature watched.

In active pulmonary tuberculosis the initial dose of tuberculin, T R or B E, may be $\frac{1}{100,000}$ mgrm., cautiously increased according to the effect produced until a dose of $\frac{1}{10,000} - \frac{1}{5,000}$ mgrm. is reached.

In chronic pulmonary tuberculosis, the initial dose may be $\frac{1}{20,000} - \frac{1}{10,000}$ mgrm. increased until a dose of $\frac{1}{1,000}$ mgrm. is attained.

In tuberculosis of glands and joints, the initial dose may be $\frac{1}{10,000} - \frac{1}{5,000}$ mgrm.[1] The treatment may be very tedious, unless the disease is first treated surgically. Genito-urinary tuberculosis in many instances does well under tuberculin treatment. The initial dose in renal and vesical tuberculosis should be small.

In lupus discordant results have been recorded with tuberculin treatment. According to Wright the dry and scaly variety is but little benefited, the ulcerating form, on the other hand,

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[1] All dosage is stated for adults : for children the doses must be proportionately reduced.

often reacting well. Whitfield obtained disappointing results in lupus, but tuberculous ulcers did better. The old tuberculin often produces marvellous results in lupus, but the cases generally relapse. Treatment with the old tuberculin, combined with surgical, X-ray, or Finsen light treatment, and followed by injections of Tuberculin T R or B E to attempt to produce immunity, may be tried in lupus. The initial dose of the old tuberculin may be 0·0001 to 0·001 c.c. The dose is gradually increased by the same amount and given every third or fourth day (but never repeated until all reaction caused by the previous dose has subsided) until 0·005 c.c. is reached, after which the increase may be by 0·002 c.c. until 0·01 c.c., then the increase may be more rapid until 0·1 c.c. is reached.

Carmalt-Jones [1] administers the bacillary emulsion by subcutaneous injection, the doses being repeated at intervals of ten days, commencing with a minimal dose of $\frac{1}{15,000}$ or $\frac{1}{20,000}$ mgrm. The maximal dose for children under five was $\frac{1}{10,000}$ mgrm., for older children and adults $\frac{1}{4,000}$ mgrm. was rarely exceeded. In phthisis and pleurisy the initial dose given was small, $\frac{1}{50,000}-\frac{1}{25,000}$ mgrm.

[1] *Brit. Med. Journ.* 1909, ii. p. 531.

Allen recommends the combined use of tuberculin R with the Denys' tuberculin (see p. 368) in cases in which toxæmia is an important factor. The initial dose of the Denys' tuberculin is very small, 0·0000001 c.c., then 0·0000002 c.c., 0·0000003 c.c., 0·0000006 c.c., 0·000001 c.c., 0·000002 c.c., and so on until a dose of 0·0005–0·001 c.c. is attained in combination with 0·0001–0·0002 c.c. of T R. The writer[1] has found that tubercle endotoxin prepared by the trituration of tubercle bacilli has a marked effect on the opsonic index of healthy rabbits, an effect more marked than that produced by any of the tuberculins. A tubercle endotoxin vaccine may therefore be more potent than the tuberculins in the treatment of tuberculosis.

Although usually given by subcutaneous injection, tuberculin may be administered by other channels. Thus Moeller[2] has found that it may be absorbed by inhalation, by application to the nasal mucous membrane, or by the rectum from suppositories or enemata. It may also be given by the stomach. Moeller found that if protected from the action of the gastric juice by enclosure in some form of capsule,

[1] *Proc. Roy. Soc. Med.* iii. April, 1910 (Pathological Section).
[2] *Münch. med. Wochr.* Nov. 10, 1908.

tuberculin given by the mouth produces its full effect. The limitations of this method of administration are referred to at p. 285.

In determining the opsonic index for tubercle, the emulsions of bacilli are prepared in 1·5 per cent. salt solution. A moist killed or living culture of tubercle bacilli is employed, and it requires prolonged grinding up in an agate mortar in order to obtain a uniform emulsion.

Leprosy

Rost,[1] by using a special culture-medium free from chlorides, claimed to have cultivated the leprosy bacillus, and these cultures concentrated by evaporation he introduced under the name of 'leprolin' as a curative agent for leprosy: it does not, however, appear to be of any value.

More recently, by the use of other culture-media, Rost believes he has succeeded in cultivating the leprosy bacillus, and has prepared a vaccine. Details of the treatment are at present wanting.

Deycke[2] obtained cultures of an acid-fast streptothrix from leprosy tissue. By treatment

[1] *Brit. Med. Journ.* 1905, i. p. 294.   [2] *Ibid.* 1908, i. p. 802.

with benzoyl chloride the fatty acid-fast substance is dissolved out and the solution under the name of 'nastin' has been employed in treatment.

Brinckerhoff and Wayson [1] report on the treatment of six cases of leprosy with nastin; two of the cases gave slightly encouraging results, the other four were quite unaffected, even after treatment for a year.

Ashburton Thompson [2] similarly obtained no benefit from the use of various preparations of nastin (Nastin B 1, Nastin B 2, and ketin).

Others, however, have reported favourably on the treatment of leprosy with this substance. Thus Raschid [3] treated three cases of leprosy with nastin, apparently with some benefit.

The old tuberculin gives a marked reaction in leprosy, and some of the cases treated with it have remained permanently quiescent.

Dr. Nicholls at the Seamen's Hospital treated two or three cases of leprosy with a vaccine prepared by emulsifying fragments of the nodules excised from the patient in salt solution and sterilising, with apparent benefit.

[1] *Studies on Leprosy*, v., Public Health and Marine Hospital Service, U.S.A. Washington, 1909.
[2] *Brit. Med. Journ.* 1910, i. p. 565 (Refs.).
[3] *Ibid.* 1909, ii. p. 1343.

Staphylococcic Infections

It was in staphylococcic infections that vaccine treatment was first applied, and the results are generally excellent. These infections include boils, abscesses, carbuncle, pustular acne, impetigo, and sycosis.

The infecting organism is usually the *Micrococcus* (*Staphylococcus*) *pyogenes aureus*, sometimes the *M. pyogenes albus* or *citreus*, occasionally other cocci, and sometimes admixtures of two or more species.

Infective endocarditis, suppurative periosteitis and osteomyelitis, peritonitis, pleurisy, meningitis, septicæmia and pyæmia may also be caused by staphylococci, which may also secondarily infect in other conditions.

Acne.—The index is generally low. A suitable initial dose is 250,000,000 cocci. The first effect is usually to render the condition somewhat worse (during the negative phase), followed by amelioration.

The treatment must be persisted in, and in obstinate cases repeated increasing doses administered, up to 1,000,000,000 or even 2,000,000,000 cocci. An autogenous vaccine may act when a stock one fails (see also p. 275).

Boils and Carbuncles.—These cases also usually do well. The initial dose may be from 100,000,000 to 250,000,000 cocci.

Sycosis is often very obstinate and local treatment should be combined with the vaccine. An autogenous vaccine is often necessary and relapses are liable to occur.

Septicæmia and Pyæmia.—Few cases of these have yet been recorded. The initial dose may be 10,000,000–15,000,000 cocci, and an autogenous vaccine should be prepared as soon as possible by means of cultures from the blood.

Secondary infections with staphylococci occur in tuberculous, *B. coli*, and other infections, and combined treatment with staphylococcic vaccine and the vaccine of the infection often does much good.

The results of the vaccine treatment of fifteen cases of staphylococcic infections are detailed by Clarke Begg.[1] He concludes that vaccine treatment is of the greatest service, that as a rule there is no need for control by opsonic determinations, and that autogenous vaccines often act when stock vaccines fail.

The writer has employed staphylococcic endotoxin as a vaccine with success (see p. 287).

[1] *Brit. Med. Journ.* 1910, i. p. 186.

Acne

Fleming, having shown that in the early stage of acne before pustulation there is present in the seborrhœic plugs a peculiar small Gram-positive bacillus, treats the cases with a vaccine prepared with this bacillus, the best results being obtained with doses ranging from 5,000,000 to 10,000,000 organisms, the inoculations being repeated about once a week. This vaccine is usually employed in combination with a staphylococcus vaccine (200,000,000 – 500,000,000), and the results obtained are claimed to be better than when the staphylococcus vaccine is used alone.[1]

The acne bacillus grows only on special media : Fleming using ordinary agar with 1–5 per cent. of oleic acid added, Südmersen and Thompson [2] a 3 per cent. agar of reaction + 40 to which is added one-third of its volume of blood serum.

Whitfield [3] believes that while the acne bacillus may be the cause of the comedo, pustulation is usually associated with the presence of staphylococci.

[1] *Brit. Med. Journ.* 1909, ii. p. 533.
[2] *Journ. Pathol. and Bacteriol.* 1909.
[3] *Proc. Roy. Soc. Med.* iii. April, 1910 (Pathological Section).

Streptococcic Infections

Streptococci are the cause of many infections—erysipelas, abscess, cellulitis, periosteitis, septicæmia, pyæmia, puerperal fever, infective endocarditis, &c.

The streptococci include a number of races, and hence an autogenous, or a polyvalent, vaccine is generally necessary.

Some brilliant results have been obtained in infective endocarditis, as in a case recorded by Barr and Douglas.[1]

The initial dose may be 5,000,000 to 10,000,000 cocci.

A streptococcic vaccine has also been found of service in scarlatina, particularly when the original condition is severe.[2] The vaccine should be an autogenous one.

Gonococcal Infections

The gonococcus may cause septicæmia, infective endocarditis, cerebro-spinal meningitis, peritonitis, and arthritis, in addition to the ordinary gonorrheal affections, urethritis, &c.,

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[1] See Wright, *Lancet*, August 24, 1907.
[2] See Gabritschewsky, *Centr. f. Bakt.* Abt. I (Originale), xli. 1906, p. 844, and Langowoy, *ib.* xlii. 1906, pp. 362 and 463.

endometritis, salpingitis, and conjunctivitis. Vaccine treatment is very serviceable in the chronic infections, persistent gleet, arthritis, &c. The initial dose should be 40,000,000 to 50,000,000 cocci. The negative phase is sometimes lengthy and the doses should not be repeated at less than fortnightly intervals.

Catarrhal Affections of the Respiratory Tract

These may be caused by a variety of organisms —the *M. catarrhalis*, *B. septus* (*coryzæ segmentosus*), Friedländer's bacillus, *B. influenzæ*, or a mixture of two of these.

Both the acute and chronic affections may be treated with the appropriate vaccine, the initial dose of any of these organisms being about 200,000,000 cocci.

Benham [1] reports on the bacteriology and vaccine treatment of ' common colds ' and winter cough. The prevailing organisms were the *M. catarrhalis*, *M. paratetragenus*, and *B. septus* (*B. coryzæ segmentosus* of Cautley). A dose of 250,000,000–500,000,000 of these organisms, either singly or mixed, was given apparently with beneficial results. In some cases a mixed vaccine was prepared by planting

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[1] *Brit. Med. Journ.* 1909, ii. p. 1338.

sputum on a 'nasgar' (nutrose ascitic-fluid agar) plate, growing, and washing off the mixed growth. The author concludes :

1. That in cases of catarrh caused by the *M. catarrhalis* marked fluctuations of the opsonic index occur.

2. That a fall in the index corresponds with an increase, a rise in the index with a decrease, in the severity of the symptoms.

3. That a suitable dose of vaccine given at an appropriate time will cause a rise of the opsonic index, with beneficial results.

Acute Pneumonia and Pneumococcic Infections

Acute croupous pneumonia, running as it does an acute and rapid course, must be treated early and as soon as possible if a favourable result is to be attained.

Willcox and Parry Morgan [1] recommend that so soon as a diagnosis is made a dose of a stock pneumococcal vaccine should be immediately administered (anti-pneumococcic serum may be given as well) and steps taken at once to prepare an autogenous vaccine. The procedures they recommend are as follows :

Method 1.—The sputum is carefully examined and cultures are made on serum or blood agar.

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[1] *Brit. Med. Journ.* 1909, ii. p. 1050.

It is usually necessary to make subcultures in order to obtain a pure pneumococcal growth. This of course involves delay, so that as a rule thirty-six to forty-eight hours elapse before the autogenous vaccine is ready.

Method 2.—A blood culture may be made as follows: About 10 c.cm. of blood are taken from one of the veins of the arm with the usual antiseptic precautions. The blood is added in equal parts to four tubes of ordinary broth and these are incubated. Sometimes after twelve hours a good growth may be found, but at other times the clot encloses the organisms, which have to be shaken out, and it may then be necessary to make subcultures in order to have sufficient growth to prepare a vaccine, which of course involves delay. Frequently the tubes of broth after admixture with the blood and incubation remain sterile.

Method 3.—The third method employed is that of puncture of the chest wall in the region where the signs of consolidation are most marked. A 1 c.cm. syringe with a fine needle is used. About $\frac{1}{4}$ c.cm. of sterile broth is aspirated into the syringe and the needle is passed into the pleural cavities or surface of the consolidated lung. A small quantity of the contained broth is then injected and immediately aspiration is

performed by withdrawal of the piston. The needle is now gradually drawn out of the chest, the position of the piston being such that there is a negative pressure within the syringe. In this manner a mixture of blood plasma and broth is obtained, which is added to two culture tubes containing sterile broth. This forms a good medium for the growth of the organisms. The tubes are now incubated and occasionally shaken. Within twelve hours it is often possible to obtain a growth containing 1,000,000,000 organisms per cubic centimetre. The contents of the tubes are then sterilised at 60 C. for one hour and are diluted with $\frac{1}{2}$ per cent. carbolic acid solution to a suitable strength, such as 100,000,000 per cubic centimetre. By this procedure it is sometimes possible to inoculate with an autogenous vaccine in twelve hours from the time of making the cultures. It should be added that not infrequently the fluid withdrawn from the chest by aspiration as described above will prove sterile.

As regards the advantages and disadvantages of the three methods, *Method* 1 causes no discomfort to the patient, but is usually inapplicable in the case of children, who rarely cough up sputum. In the most favourable circumstances it involves delay. *Method* 2 also

may involve delay and may be inapplicable in the case of children, owing to the small size of the veins. *Method* 3 is the most rapid and certain. Moreover, to whatever causative organism the pneumonia may be due it will probably be isolated.[1] There is a small risk of hæmorrhage and of introducing air into the pleural cavity.

Methods 2 and 3 also necessarily involve some pain, which may be objected to.

If opsonic determinations are made, difficulties arise. The pneumococcus obtained may not be opsonised by normal serum, and it may be agglutinated by the patient's serum. The opsonic power of the serum of healthy persons for the pneumococcus also varies considerably, and the control serum should therefore be the mixed serum of several individuals.

Willcox and Morgan recommend an initial dose of 20,000,000–30,000,000 cocci, and after the first dose a second dose of 30,000,000–50,000,000 is given 24–48 hours after the first, and further doses may be given at suitable intervals if necessary. Generally speaking, there was a definite improvement in the clinical symptoms

[1] Croupous pneumonia in 95 per cent. of the cases is due to the pneumococcus. Broncho-pneumonia occurring idiopathically or in the course of other diseases may be caused by many different organisms.

of the disease, and in some protracted cases the beneficial effects of the vaccine were most striking.

Boellke[1] has also employed the mixed vaccine obtained by direct inoculation of the sputum into broth, and claims successful results.

Butler Harris[2] describes several cases of pneumonia and pneumococcal infections treated with pneumococcal vaccine with, as a rule, good results. The dose given was 20,000,000 to 50,000,000 cocci.

Chronic pneumococcic infections of the frontal sinus, antrum, lung, joints, &c. generally do well under injections of pneumococcic vaccine.

Betham Robinson[3] reports a case of primary diffuse pneumococcic peritonitis treated by drainage followed on the fifth day after operation by pneumococcic vaccine. Eight injections of vaccine were given at intervals of 5–6 days, the dose ranging from 11,600,000 to 23,000,000 cocci. Betham Robinson says, ' It must be considered, I think, that the use of vaccine contributed in a large degree to the successful issue of the case.'

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[1] *Deutsche med. Wochr.* 1907, No. 33, p. 1487.
[2] *Brit. Med. Journ.* 1909, i. p. 1530 (Refs.).
[3] *Ibid.* 1909, i. p. 651.

Bronchial Asthma

Carmalt-Jones points out [1] that persons the subjects of chronic bronchitis frequently suffer from attacks of spasmodic dyspnœa, which are often termed ' bronchial asthma.' Having isolated from certain of these cases a bacillus possessing peculiar characters and having ascertained that the opsonic index to this organism was low, he considered that the bacillus might be causally related to the disease and determined to vaccinate with it. Fifty-two cases were treated with doses of from 25,000,000 to 100,000,000 bacilli, the dose in some cases being repeated two or three times at intervals. Of these, 31 presented some degree of improvement in the frequency, and 39 in the severity, of their attacks ; 26 improved in their powers of taking exercise, and 29 slept better ; in some cases improvement was slight, in others temporary. In four cases no improvement resulted. The organism is a short bacillus with rounded ends, Gram-negative, non-sporing and non-motile. It does not liquefy gelatin or curdle milk, nor form acid or gas from glucose, lactose, sucrose, dulcite, or mannite. It grows freely on agar at 37° C., forming a greyish creamy growth.

[1] *Brit. Med. Journ.* 1909, ii. p. 1049.

Whooping Cough

The specific microbe of this disease seems to have been isolated and identified by Bordet and Gengou.   It is a somewhat influenza-like bacillus and grows only on a special medium.[1]

Bordet gave twenty children who were in contact with whooping cough prophylactic doses of sterilised cultures.   These children subsequently contracted the disease in a severe form, probably, Freeman suggests, because a profound negative phase had been induced by the dose employed.

Freeman[2] has treated children (number not stated) with a vaccine prepared with the Bordet-Gengou bacillus, the dose being 5,000,000 to 20,000,000.   The following results (in percentages) were obtained :

Class	Vaccine Cases	Control Cases
Much better . . .	31·0	21·55
Better	37·1	34·55
Unchanged . . .	15·7	23·9
Worse	15·2	18·15
Much worse . . .	1·0	1·85

[1] For characters of the organism, &c., see Bordet, *Brit. Med. Journ.* 1909, ii. p. 1062.

[2] *Brit. Med. Journ.* 1909, ii. p. 1064.

Bacillus coli Infections

The *B. coli* causes cholangitis and cholecystitis, cystitis, urethritis and pyelitis, enteritis, peritonitis and appendix abscess, ischio-rectal abscess, endometritis and septicæmia.

Urinary and hepatic infections frequently react well to vaccine treatment.

Probably an initial dose of 40,000,000–50,000,000 should not be exceeded, and the vaccine should be an autogenous one.

In carrying out opsonic determinations for *B. coli*, the time of incubation of the mixture should be six, instead of fifteen, minutes.

Typhoid Infections

The chronic infections produced by typhoid and paratyphoid bacilli, cholecystitis, cystitis, periosteitis, &c., lend themselves to vaccine treatment, an initial dose of 250,000,000 being suitable.

Pescarolo and Quadrone have treated typhoid fever by injections of living but attenuated typhoid culture. Fifteen- to twenty-days-old agar cultures were heated to 45°–50° C. for some hours. One to three platinum loopfuls of the

culture were suspended in 5 c.c. of sterile physio-
logical salt solution, and of this suspension
0·5–1·0 c.c was injected subcutaneously ; the in-
jections were repeated if necessary after intervals
of four to seven days, the dose being gradually
increased until 5 c.c. were given. They report
favourable results by this method (see *Brit.
Med. Journ.* (Epit.), 1908, ii. No. 292).

Attempts are now being made to adapt
vaccine therapy to the treatment of enteric and
paratyphoid fevers.

The application of vaccine therapy to the
elimination of bacilli from chronic carriers
does not, so far, seem to have met with success.

(See also p. 217 for the use of the Chantemesse
serum, which apparently is really a vaccine.)

Bacillary Dysentery

One form of dysentery, the bacillary, is caused
by various organisms belonging to the group
B. dysenteriæ.

Dysenteries which are becoming chronic are
suitable cases for treatment with vaccines,
which should be autogenous. Forster [1] has

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[1] *Ind. Med. Gazette,* June 1907, p. 201. Also Newman,
Lancet, 1908, i. p. 1410.

applied the treatment with success. He stan-dardises his vaccine so that the minimal lethal dose for a rabbit of 1200–1400 grams weight is not less than 0·4 c.c. Of such a vaccine 0·1 c.c. forms the initial dose, and the subsequent doses are 0·1 c.c. more than the preceding ones, and are given at intervals of ten days.

Forster used a stock vaccine of the Shiga bacillus. Taylor and Hare have isolated Flexner's bacillus from a case of chronic dysentery contracted in England, and prepared an autogenous vaccine which was used with success. (Private communication.)

Glanders

Zieler[1] has successfully treated a case of extensive chronic glanders with a vaccine prepared with the organism isolated from the patient. The vaccine was made with one normal loopful of a forty-eight hours' culture on glycerin agar emulsified in each cubic centimetre of salt solution and sterilised. A dose was given on each of two consecutive days followed by an interval of six to eight days.

Vaccine treatment is deserving of extended trial in this intractable and fatal disease.

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[1] *Wien. med. Klin.*, May 2, 1909.

Mediterranean (Malta) Fever

Staff-Surgeon Reid, R.N., in 1905,[1] treated a small number of cases of Mediterranean fever with vaccine with successful results.

Fleet-Surgeon Bassett Smith, R.N., in 1906,[2] gave the method an extensive trial.

The vaccine was prepared by emulsifying ten-day-old agar cultures prepared from a recently isolated strain of the *M. melitensis* in distilled water, heating to 60° C. for half an hour and adding 0·5 per cent. carbolic acid. The opacity of the emulsion should be such that a layer 1·5 c.m. deep should just obscure 0·5 Snellen type ; of this, 0·5 c.c. formed the dose, which was repeated at intervals of ten days. The general results were that in a certain number of cases, vaccine treatment exerted a beneficial effect, the severity of the symptoms being diminished, the general condition improved, and the duration of the disease curtailed. Many cases, however, failed to react favourably, and in the more acute type, with high fever and evidence of severe intoxication, the treatment appeared to have a deleterious, instead of a favourable, action. Allen suggests that a

[1] *Trans. Path. Soc. Lond.* lvii. p. 463.
[2] *Journ. of Hygiene,* vii. 1907, p. 115.

modification of the treatment in the direction of smaller doses at more frequent intervals might secure more favourable results.

Pyorrhœa Alveolaris

A number of organisms are present in the pus in this affection. Goadby [1] has found in various cases, streptococci, the pneumococcus, *M. pyogenes aureus*, *M. citreus granulatus* (Freund, a Gram-negative coccus), organisms of the *M. catarrhalis* type, diphtheroid bacilli, and *Saccharomyces neoformans ;* all these organisms, judged by the opsonic index and agglutination reaction, are causative. Many other bacteria were also isolated, but were probably not causative.

Goadby investigates the cases by making agar streaks from the pus, isolation of the organisms, and determination of the opsonic index of the patient for the organisms present. If the opsonic index of the patient's serum shows a marked deviation from the normal with an organism or organisms, a vaccine is prepared and employed. The results recorded by Goadby are very encouraging in this otherwise intractable disease.

[1] *Lancet,* March 9, 1907 ; *ib.* December 25, 1909 ; *Brit. Med. Journ.* 1908, ii. p. 477.

Eyre and Payne [1] cultivate on blood agar by inoculating several tubes in series, and after isolation in pure culture of the various cocci present, opsonic determinations were made with each. That organism or organisms to which the index was low or high they regarded as responsible for the infection. The following organisms, regarded as the responsible ones, were isolated :

Micrococcus pyogenes aureus .	.	2 cases
Micrococcus catarrhalis .	. .	9 ,,
Streptococcus pyogenes longus .	.	7 ,,
M. catarrhalis and *S. pyogenes longus*	11	,,
Diplococcus pneumoniæ	. .	4 ,,

Autogenous vaccines were prepared and employed systematically. The number of doses of vaccine ranged from 4 to 19 in different cases, given at intervals of 5–21 days : full details are given in the original paper. Of the cases treated seven remained ' cured ' for 12–15 months, twelve for 9–12 months, two for less than nine months, four were improved only. Goadby [2] in a later paper gives further details of the treatment of pyorrhœa with vaccines.

[1] *Proc. Roy. Soc. Med.* iii. December 1909 (Odontolog. Sect.).
[2] *Ibid.* February 1910 (Odontolog. Section).

The causative organisms were in 19 cases the *S. longus*, in 23 cases the *M. catarrhalis*, in 5 cases the pneumococcus, in 12 cases the *M. pyogenes aureus*, in 14 cases the *B. necrodentalis*, in 15 cases the *B. septus*. In 70 cases of early pyorrhœa treated, of 21 with no general symptoms 15 were cured, and of 49 with general symptoms 30 were cured. The doses varied from five to fifteen in number, given at intervals of 10–20 days and controlled by opsonic determinations, and the amount of vaccine was 50,000,000–250,000,000 organisms. Local treatment was also used.

Pyocyaneus Infections

A case of severe *B. pyocyaneus* pyæmia treated with vaccine is described by Groves.[1] The condition was secondary to operation on the hip-joint for caries of the bones. The vaccine was prepared from the patient's own bacillus (present in pure culture in the abscesses) by heating an emulsion to 60° C. for one hour. Six injections in all were given, 40,000,000 bacilli November 6, 60,000,000 November 14 (temperature became normal the next day), 100,000,000 November 24, December 8, January

[1] *Brit. Med. Journ.* 1909, i. p. 1169.

1 and 16. The lesions healed up and the patient recovered completely.

Epidemic Cerebro-Spinal Meningitis

An anti-serum, as already recorded, has been successfully used in this disease (p. 199).

Vaccine treatment has also been employed apparently with good effect. The dose is 500,000–1,000,000 cocci injected preferably intraspinously.

In the later stages of this disease the opsonic index may be very high, indexes of five and even ten having been recorded.

Vaccine Treatment in other Diseases

Streptothrix infections, *e.g.* actinomycosis, the various forms of conjunctivitis and keratitis and other infective diseases may all be treated by vaccine therapy.

Bruce [1] has treated cases of mania and neurasthenia with vaccines (*e.g.* injections of 7,000,000–10,000,000 streptococci) with apparent benefit.

Bruce believes that the vaccine injections alter the nitrogenous metabolism and improve

[1] *Brit. Med. Journ.* 1910, i. p. 430.

nutrition, and to this he attributes the beneficial action.

Diphtheria-Bacilli Carriers

The diphtheria bacillus may persist for a long period after an attack ; in one case followed by the writer it persisted for twenty-two weeks, and the organism was virulent to the last. He has seen other cases in which it was present for six or seven months, and still longer periods have been recorded. The bacilli may also be found in well persons, particularly in contacts.

The question what to do with such cases is a very important one. It is generally agreed that they should be segregated. Antiseptic douches, gargles and paints occasionally bring about the disappearance of bacilli, but more often fail.

Antitoxin has been tried but usually fails, and this treatment has the objection that supersensitation may supervene in cases previously treated with it. An anti-microbial diphtheria serum has been recommended, being applied locally as a douche or made into tablets to be sucked, but these, too, on the whole, are not very successful, though it occasionally seems to act.

Wassermann suggested the use of a polyvalent antibacterial diphtheria serum in such cases (*N.Y. Med. Journ.* and *Phila. Med. Journ.* October 15, 1904, p. 722). The serum is prepared by the injection of diphtheria bacilli (therefore antibacterial) of many strains (therefore polyvalent) into an animal, whereby a serum is obtained having a powerful agglutinating action for the diphtheria bacillus. The serum is evaporated to dryness *in vacuo*, mixed with milk-sugar, and pressed into tablets. A tablet is allowed to dissolve in the mouth every two hours, and then fifteen minutes later the child's naso-pharynx is rinsed out with some indifferent fluid in the form of spray or gargle. The isolated diphtheria bacilli scattered diffusely throughout the naso-pharynx are agglutinated into masses by the action of the serum, and are then removed by the subsequent rinsing. In this way the bacilli are so decreased in number that the natural power of the organism is able much more quickly to make away with those remaining.

Vaccine treatment has also been applied. Petruschky [1] of Dantzig treated six cases with sterilised cultures with favourable results.

Goodall has treated some cases of persistence

[1] *Arbeit. a. d. Geb. der patholog. Anatomie*, Bd. vi. Heft 2, 1908.

of bacilli after an attack of diphtheria by injections of diphtheria endotoxin prepared by the writer by trituration of the washed bacilli from serum cultures. The dose given was 0·2–1·0 mgrm., and it was apparently successful in four cases. This endotoxin vaccine deserves an extended trial.

Anti-rabic Inoculation (for Hydrophobia or Rabies)

Anti-rabic inoculation being essentially a vaccine treatment after infection may conveniently be considered here.

Hydrophobia or rabies, the ætiological micro-organism of which is not known with certainty, is caused by the bites of rabid animals. The infective agent, whatever it be, resides in the central nervous system, in the saliva, the lachrymal glands and suprarenal capsules, but the other tissues and fluids of the body are non-infective.

Hydrophobia attacking man is invariably contracted through the bite of an animal affected with the disease. It is most frequent in the dog, but the cat, wolf, jackal, and deer are also subject to it, and other animals can be infected by inoculation. In the lower animals the disease is

termed rabies, and takes two forms, either the raging or the paralytic. The latter is not met with in man, unless certain rare forms of acute ascending paralysis (*e.g.* Landry's) be manifestations of it. In the dog either may occur, but in rodents the paralytic form is almost always the one assumed. In man the incubation period is very variable ; it is never less than about twenty days, and may possibly be as long as two years, or even more ; the average seems to be about ten weeks. In the rabbit after inoculation from the dog, the incubation period is about two to three weeks.

Pasteur showed that the virus could be attenuated by desiccating the infective nerve matter, and in this way was able to prepare a vaccine which would protect animals from otherwise fatal doses of the virus. Advancing a step further, he used his vaccines to treat individuals who had been bitten by rabid animals, but in whom the symptoms had not yet developed, and so inaugurated the present system of anti-rabic inoculation as carried out at the Pasteur and other Institutes.

To prepare the anti-rabic vaccines a rabbit is inoculated subdurally with an emulsion prepared from the medulla of a rabid dog. When the animal dies a second rabbit is similarly inoculated

from the first, and the passage through rabbits is continued until a 'fixed virus' is obtained, with which the first symptoms appear on the seventh or eighth day, and which kills with certainty in about ten days. This having being attained, two or three rabbits are inoculated subdurally every day, so that there is a daily supply of animals dead of the disease. The spinal cord is removed with aseptic precautions, and cut into convenient segments, which are suspended in bell-jars containing a layer of stick caustic potash at the bottom; this serves to desiccate them. The jars are dated and preserved in glass cases in a dark room, which is kept at a constant temperature of about 23° C. In Paris the vaccine fluids are prepared by triturating portions of the dried cords in sterile broth, so as to form an emulsion—1 centimetre of cord in 5 c.c. of sterile broth, of which 1 c.c. (*i.e.* 2 mm. of cord) forms a single dose. At the commencement of treatment the cords which have been dried for fourteen days are used, at the end of treatment those which have been dried for only three days; the latter are much more virulent, and would communicate the disease but for the previous treatment. The rabbits employed should all be of the same weight (2½ kilograms at Paris); if the rabbits are small, a slightly

shorter period of desiccation of the cords would be necessary. The treatment varies in duration according to the severity of the case, which is gauged by the number and situation of the bites and by the species of animal. Bites on exposed parts are regarded as much more serious than those through clothing, and on the face, where efficient treatment is difficult, than on the hands, and wolf bites than dog bites.

The doses are injected subcutaneously in the flank, and do not produce much constitutional disturbance. At first there is a feeling of lassitude and considerable muscular tenderness at the seat of inoculation, which later on passes off. At Lille, where there are only a few cases under treatment at a time, the cords after drying for the requisite period are placed in pure sterile glycerine. In this they retain their virulence unimpaired for about a month. This method does away with the necessity for the daily inoculation of rabbits, a rabbit being inoculated occasionally as required. The system of dosage employed at the various anti-rabic stations differs somewhat ; the following is that employed at Lille, 2 mm. of cord being emulsified in 5 c.c. of sterile broth or physiological salt solution :

ORDINARY TREATMENT		ORDINARY TREATMENT	
Day of Treatment	Days of Desiccation of Cord	Day of Treatment	Days of Desiccation of Cord
1 (two injections)	14 and 13	13 . . . 3	
2 ,,	12 and 11	14 (two injections)	9 and 8
3 ,,	10 and 9	15 ,,	7 and 6
4 ,,	8 and 7	16 . . . 5	
5 . . . 6		17 . . . 4	
6 . . . 5		18 . . . 3	
7 . . . 4			
8 . . . 3		FOR SEVERE BITES, in Addition	
9 (two injections)	9 and 8		
10 ,,	7 and 6	19 (two injections)	7 and 6
11 . . . 5		20 ,,	5 and 4
12 . . . 4		21 . . . 3	

At Buda-Pest, a dilution method has been employed; instead of drying the spinal cords, an emulsion is made with the fresh cord, and this emulsion is considerably diluted for the earlier doses, dilutions of 1 in 10,000 to 1 in 6,000 corresponding to cords dried for from fourteen to eight days.

The Pasteur inoculations will undoubtedly protect animals from rabies, the duration of immunity after vaccination in the dog being at least three years. In man the efficacy of the treatment can only be judged by statistics. The mortality after bites by supposed rabid animals is variously stated, the most favourable being about 16 per cent. (Leblanc). Remlinger[1] has

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[1] *Bactériothérapie, Vaccination, et Sérothérapie,* p. 114 (Baillière et Fils, 1909).

collected statistics of 131,579 persons treated at various Institutes from 1886 to 1905. Of these 549 died from hydrophobia, a mortality of 0·41 per cent.

The failure of the treatment may be due to two causes : (1) delay in commencement, and (2) a short incubation period. The principle of the treatment probably depends upon the long incubation period of the disease, owing to which it is possible to forestall the disease and to immunise the body by the inoculations before its onset. If, unfortunately, the infective material should be very virulent, and the incubation period thereby reduced to the lower limit, it may be impossible to do this before the onset of the disease, and the same is the case if the commencement of the treatment be delayed. Pasteur's system of inoculation is useless when the disease has declared itself.

Coley's Fluid

This preparation consists of the organisms and toxins of the streptococcus of erysipelas and the *Bacillus prodigiosus*. It was devised by W. B. Coley, of New York, as a cure for inoperable malignant tumours. The treatment is based on the undoubted fact that malignant growths may

decrease or even disappear completely after an attack of erysipelas. The fluid was originally prepared by growing the streptococcus, obtained from a fatal case of erysipelas, and rendered highly virulent by a succession of passages through rabbits in bouillon for about ten days ; the *B. prodigiosus* is next added, and the two organisms are allowed to grow together for another week or ten days. The culture is then heated to from 58° to 60° C. for one hour and a piece of thymol added to preserve it.

The fluid is now prepared by growing the two organisms separately and then mixing with the sterilised streptococcus culture a certain definite quantity of the sterilised prodigiosus culture. In this way a more constant preparation is obtained, and one that can be standardised as to dose. Full directions for preparation are given in Coley's last paper (1909).

The primary dose recommended is $\frac{1}{4}$ minim injected into the buttock or pectoral muscle ; in this way the susceptibility of the patient to the fluid is ascertained. Subsequently, the doses are injected into, or into the neighbourhood of, the tumour, if this be accessible, otherwise into the buttock or elsewhere. The dose injected into the tumour should be one-fourth that injected elsewhere. Daily injections are

given, increasing by one-fourth of a minim, until the desired reaction, a temperature of 102°–104° F., is obtained. The dose should then be not increased until it fails to give a reaction, when it may again be increased by one-fourth of a minim. A good plan is to give the daily injections alternately into the tumour and into the buttock. The dose varies in different individuals; the largest reached in many successful cases has been 7–8 minims, though this has occasionally been exceeded (in one case 20, in another 30 minims). The treatment may have to be continued for several months. The fluid may be used not only for direct treatment, but also as a prophylactic to prevent recurrence after operation.

The temperature is the chief guide in estimating the dose, and the frequency of injections depends upon the general condition of the patient and upon the rapidity of recovery from the depression of the preceding dose. The injection must not be repeated until the temperature has completely fallen.

The treatment is especially indicated in sarcoma, particularly the spindle-celled variety; the round-celled and the melanotic varieties are not nearly so amenable. Carcinoma is only exceptionally benefited.

Coley himself advocates the treatment only in inoperable cases.

According to Coley's older statistics, he has treated in all 140 cases of sarcoma, of which 84 were of the round-celled type, 21 spindle-celled, 9 melanotic, 2 chondro-sarcomata. Of the round-celled, 40 were more or less improved but only 3 cured ; of the 21 spindle-celled, 10 had disappeared completely and the remainder were much improved. Of the cases cured, 16 had remained well for 3–8½ years ; of these two had recurred at three and eight years respectively and both died.

Coley's most recent statistics show 52 cases of inoperable sarcoma cured by the treatment.

Coca and Gilman have treated cancer cases with a vaccine prepared by triturating the patient's own tumour tissue in a vaccine grinder. Quantities of 10 to 15 grains of the cancer tissue were injected subcutaneously. Indications of a favourable result were obtained.

LITERATURE

Coley, *Amer. Journ. of Med. Sc.* cxii. 1896, p. 251 ; *Johns Hopkins Hosp. Bull.* vii. 1896, p. 157 ; *Ann. of Surgery,* xxv. 1897, p. 174 ; *ibid.* xxvi. 1897, p. 232 ; *Philad. Med. Journ.* 1901, May 25, p. 1013 ; *Proc. Roy. Soc. of Med.* iii. 1909–1910 (Surgical Sect.), p. 1 ; Coca and Gilman, *Philippine Journ. Science,* iv. 1909, p. 391.

Cancroin

Adamkiewicz has devised a mixture, termed by him ' cancroin,' which is stated by himself and others to be of service in the treatment of carcinoma. It was originally prepared by extracting carcinomatous tumours, but the active principle is stated to be identical with neurin, and an artificial substitute has been prepared, viz. :

	Parts
Neurin (25 per cent. solution) . .	10
Ac. citric to saturation . . .	1·82
Ac. carbolic to saturation . .	1·25
Distilled water	27

The solution is diluted with an equal quantity of water, and one gram is injected.

See *Lancet,* 1902, i. p. 288 and p. 322 ; *Berl. klin. Wochenschr.* 1902, No. 24, No. 28, and No. 36.

Pyocyanase

Emmerich and Loew [1] isolated from cultures of the *B. pyocyaneus* a ferment, ' pyocyanase,' which possesses powerful digestive properties on proteins, and which has been employed as an application for digesting and removing the membrane in diphtheria.

Mühsam [2] believes that pyocyanase is a useful adjunct in the treatment of diphtheria, as it assists in

[1] *Zeitschr. f. Hyg.* 1899 ; *Centr. f. Bakt.* (Orig.), xxxi. p. 1.
[2] *Arch. f. Kinderhlkde.* xliv. p. 95.

the removal of the membrane. It was applied as a spray, 2 c.c. of the solution warmed to 40° C. being used for an application. Zuker[1] found pyocyanase a very useful agent in the treatment of septic diphtheria. Simon used pyocyanase with benefit in follicular tonsillitis. Piasecki, on the other hand, obtained somewhat negative results.[2] Kulakowsky[3] also found pyocyanase to possess a powerful bacteriolytic action and has suggested its use in typhoid and cholera. Escherich[4] has likewise found pyocyanase of service for the disinfection of the nasal cavities.

Yeast

Ordinary brewer's yeast (*Saccharomyces cerevisiæ*) is a well-known therapeutic agent and is a popular remedy for boils. In the Pharmacopœia of 1885 it was employed for poultices (*Cataplasma fermenti*).

Sergent investigated the action of yeast on suppurative cutaneous processes. Confirmation was obtained of the fact already observed clinically, that yeast when administered internally has a favourable action on small suppurative foci, such as furuncles, though it is useless in extensive abscess formation. Idiosyncrasy also plays a part, and in some persons furunculosis is entirely uninfluenced by yeast. The experiments were carried out on white or black rabbits, the skin of which is susceptible to infection with the staphylococcus.

[1] *Deut. med. Woch.* Feb. 6, 1908.
[2] Refs. in *Centr. f. Bakt.* Abt. I. (Referate), xlv. 1909, p. 30.
[3] *Ibid.*   [4] *Wiener klin. Woch.* 1906, No. 25.

The fur over a small area was shaved or epilated, and a few drops of a broth culture of *Staphylococcus aureus* was rubbed in with a sterile lifter, until the epidermis was slightly excoriated. Two days later 40 to 100 pustules of the size of a pin's head appeared. On the third day the pustules were larger, and surrounded by a red areola. On the fourth day they began to dry up, and on the fifth or sixth day desquamation was complete. If 20 to 30 c.c. of a thick emulsion of yeast were injected down the throat of the rabbit as soon as the pustules appeared on the second day, and the dose was repeated on subsequent days, the suppurative process was shortened. The pustules began to dry up on the third day, and the scabs fell off on the fourth. If yeast were given as a prophylactic twenty-four hours before the inunction of staphylococci, the results were still more striking : only a few scattered vesicles, which dried up in a few hours, appeared. The action of yeast is evanescent. If it is given for a week or a fortnight, but the treatment is suspended for twenty-four hours before inoculation, an unmodified eruption results. Administration of yeast *per os* was the only practicable method, as intravenous inoculation was generally followed by death, peritoneal inoculations were not absorbed, and subcutaneous inoculations produced nodules.

For furunculosis drachm doses may be given two or three times a day. Landau and Albert have used vaginal injections of 10–22 c.c. in leucorrhœa. It has also been used in constipation, meteorism, and diabetes.

Since it contains large quantities of nuclein, a phos-

phorised protein, yeast has been recommended in phthisis.[1]

In de Backer's method,[2] pure cultures of yeast are stored under pressure in glass vessels, resembling soda-water siphons, from which the yeast can be injected hypodermically by means of a hollow needle attached to them. It is employed in the treatment of tuber-culosis and cancer. The exact mode of preparation of the cultures does not seem to have been published.

For therapeutic use, it would be preferable to employ *pure* cultures of yeast, if obtainable.

Anti-Ferment Treatment of Acute Suppuration

If the pus from an acute coccal abscess be dropped on a plate of coagulated blood-serum, pits form showing the presence of a proteolytic ferment derived from the polymorphonuclear leucocytes. If a little normal blood-serum be mixed with the pus this action is pre-vented, showing that an anti-ferment is present in the serum. On this observation a treatment of acute suppurative conditions has been based, consisting in the application of the anti-ferment after evacuation of the pus.

In order to obtain increased anti-ferment action the serum of an animal which has been subjected to a course of injection with pancreatic ferment is made use

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[1] On the therapeutic use of yeast, see Ullman, *American Medicine*, October 11, 1902, p. 582 ; Merck's *Annual Rep.* for 1902, p. 64 ; *Rev. méd. de la Suisse romande*, 1901, No. 8 ; Sergent, *Ann. de l'Inst. Pasteur*, xvii. 1903.

[2] See *Brit. Med. Journ.* 1897, ii. p. 802.

of. This anti-tryptic serum has been placed on the market by Messrs. Merck under the name of ' leuco-fermantin.' [1]

Klotz[2] has obtained good results in several cases suffering from acute suppurative processes, but in two cases in infants he met with disastrous results, and doubts if leucofermantin should be used in diseases of children.

(Sterile normal blood-serum has been used as an application to septic wounds. See p. 248.)

Extracts of Leucocytes in Acute Infective Diseases

Hiss suggested the use of extracts of leucocytes in acute infective conditions, and Lambert[3] records several cases treated by this method. The best results were obtained in six cases of recurrent boils and two cases of erysipelas. The leucocytes are obtained by injecting rabbits in the pleural cavity with aleuronat (vegetable protein), the exudate after centrifugalisation is washed free from serum with sterile salt solution, and then extracted with distilled water equal in quantity to the original amount of the exudate.

[1] See D. MacEwan, *Brit. Med. Journ.* 1910, i. p. 185.
[2] *Berl. klin. Woch.* October 18, 1909.
[3] *Amer. Journ. of Med. Science*, April 1909.

CHAPTER VIII

*METHODS OF PRODUCING ACTIVE IMMUNITY—
SENSITISED VACCINES — VACCINE LYMPH
—ANTI-CHOLERA VACCINE—ANTI-TYPHOID
VACCINE—ANTI-DYSENTERY VACCINE—ANTI-
PLAGUE VACCINE — ANTI-MEDITERRANEAN
FEVER VACCINE*

As detailed in the introductory portion of this book, an immunity to disease and disease-producing organisms is frequently acquired by an attack of the disease or may be artificially induced in various ways, *e.g.* by the use of a living but attenuated culture, by the injection of an antitoxic or anti-microbic serum, or by the injection of sterilised cultures or microbial products. Efforts are now being made artificially to induce immunity in many diseases.

The use of living cultivations the virulence of which has been artificially diminished in some way—*e.g.* by continuous culture *in vitro*, or passage through other animals, by culture at a high temperature, in the presence of traces of antiseptics, &c.—has been largely used for the

protection of animals against anthrax, black quarter, and rinderpest, and in man in vaccination with vaccine lymph (anti-variola vaccine).

Generally, however, for man dead (*i.e.* killed) cultures have been used for prophylaxis, as in anti-typhoid and anti-plague vaccinations.

An antitoxic or an anti-microbic serum will generally rapidly confer a high degree of immunity, and may be employed when it is desired to protect quickly and for a comparatively short time. As already pointed out, however, the protection conferred by a single dose of serum passes off in 2–3 weeks, and this method, therefore, cannot be employed for inducing a lasting degree of immunity except by repeated doses of the serum, a procedure which is inconvenient and also inadvisable, on account of the danger of the occurrence of the anaphylactic state (p. 96). Hence the general use of living or sterilised culture when a lasting protection is desired.

Although dead cultures, or preparations derived therefrom, have during the last few years been largely used for the production of artificial immunity, many investigators, in particular Kolle, have emphasised the fact that a greater immunity is conferred by the use of attenuated living cultures, and this principle has been applied in cholera, typhoid, and even in plague.

It has been objected that the use of cultures, &c., will immediately and for a short time after inoculation induce a negative phase (p. 270) and render the person more susceptible during this period. This objection, while valid experimentally, does not seem in practice to be one which need be taken into account.

'Sensitised' Vaccines

Besredka[1] has shown that, for prophylaxis, vaccines that have been 'sensitised' by means of an immune serum cause less disturbance and produce a more rapid and lasting immunisation than the ordinary bacterial vaccines. The method of preparation is as follows :

The culture grown on agar is emulsified in sterile physiological salt solution, the homologous anti-serum is added, and the mixture is allowed to remain in a cylinder for about twelve hours for the microbes to fix the immune body (amboceptor) of the anti-serum. The bacteria at the end of this time will have sunk to the bottom, the supernatant fluid is then decanted, and the bacteria are washed by centrifugalising several times with physiological salt solution to remove all excess of anti-serum. The

[1] *Bull. de l'Inst. Pasteur*, viii. 1910, p. 241.

bacterial mass is then made into a fine and homogeneous emulsion with physiological salt solution. Presumably, the emulsion is standardised to contain a definite bacterial content in a given volume : this could be done by the method employed for therapeutic vaccines (p. 280).

In the case of plague, the organisms are grown on agar in plate bottles (p. 202) for forty-eight hours, emulsified, and the emulsion is heated to 60° C. for one hour *previously* to being treated with the anti-plague serum. This sensitised vaccine, under the name of ' Vaccin Antipesteux Sensibilisé ' is official in the French Pharmacopœia of 1908.

In the case of typhoid and cholera, the *living* organisms are treated with the anti-serum, and *after* washing are heated to 56° C. for one hour.

Immunity is established by these vaccines within 24–48 hours, and the injection causes no reaction, either local or general.

Vaccine Lymph [1]

At the present time, arm-to-arm vaccination, undoubtedly the most efficient form, has almost

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[1] In addition to references given on the preparation of vaccine lymph, see Sacquépée in *Bactériothérapie, Vaccination, et Sérothérapie*, p. 44 (Baillière et Fils, 1909).

entirely been given up, owing to the risk of conveying such diseases as syphilis, and vaccination with calf lymph has been substituted. In order to destroy pyogenic or other organisms, which are not essential, and may cause unpleasant or dangerous effects, the lymph is treated with glycerine or some other agent, which destroys these micro-organisms, but leaves uninjured the true vaccinating agent. The mode of preparation of glycerinated lymph, as carried out in the laboratories of the Local Government Board, is as follows : [1]

' Calves of suitable age (three to six months), breed, and condition, are kept in quarantine for a week to see that they are healthy. If so, when required for vaccinating purposes, the calf is strapped to a large tilting table, and the lower part of the abdomen, extending as far forward as the umbilicus and backwards into the flanks, is carefully shaved. This shaved area is first washed with a 5 per cent. solution of carbolic acid or lysol, then well syringed with tap-water, and finally cleansed with sterilised water. The moisture from such washing is removed from this shaved area, and from the adjacent skin, by means of sterilised gauze sponges.

[1] Report by Dr. Blaxall, *Twenty-eighth Ann. Rep. Loc. Gov. Board, Supp. containing Rep. of Med. Officer for* 1898–99, p. 35.

' The calf is then vaccinated with glycerinated calf lymph, introduced into the skin in numerous parallel linear incisions by a sharp scalpel, previously sterilised, which is dipped from time to time in the vaccinating fluid. The incisions are designed to penetrate the epidermis and to open up the rete Malpighii, if possible without drawing blood ; and as they are made, additional glycerinated lymph is run in along the whole length by the aid of a sterilised blunt instrument, such as an ivory or bone spatula. The inoculation of the incisions is effected immediately they are made, otherwise the lips of the wound are apt to swell and close the opening. After vaccination, the calf is removed from the table, and is then so stalled in a stable as to prevent any injury to the vaccinated surface. The temperature of this stable is not allowed to fall below 60° Fahr.

' *Collection of the Vaccine Material.*—After five days (120 hours) the calf is again placed on the table, and the vaccinated surface is thoroughly washed with soap and warm water, gently rubbed over it by the clean hands of the operator. It is again washed with tap-water and finally cleansed with sterilised water. Next, any crusts that may have formed upon the vesicular lines and any epidermal débris are removed by

the careful use of a sterilised india-rubber pad. Superfluous moisture is absorbed by sterilised gauze sponges. At this stage the site of each incision should present a line of continuous vesiculation.

' The skin having been put firmly on the stretch, the vesicles and their contents are collected with a sterilised Volkmann's spoon, each line being treated in turn and scraped once only, care being taken that the edge of the spoon does not touch the neighbouring lines of vesicles. In this way the vesicular pulp is removed without admixture of blood. The pulp obtained by the above procedure is received into a previously sterilised stoppered bottle of known weight.

' The abraded surface of the calf is gently washed with warm water, and dusted over with starch powder or boracic acid powder. The calves after use should be slaughtered and the carcases carefully examined. If any carcase show disease, the lymph from that animal should be discarded.

' *Glycerination of the Vaccine Material.*— The bottle containing the lymph pulp from each calf is then again weighed, so that the exact weight of the material is ascertained. The pulp is next transferred to a triturating machine ;

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that employed being either one invented by
Dr. Chalybäus, of Dresden, or a modified form
of it. All the parts of the machine which come
in contact with the lymph pulp are previously
sterilised by prolonged steaming. The vaccine
material, just as it is derived from the calf, is
then passed through the machine, which is
worked by an electric motor. When the pulp
has been triturated in this way, the amount
of subdivision it has undergone can be ascer-
tained by suspending a loopful of the ground-up
material in a watch-glass containing distilled
water. If the trituration has been effectual,
such suspension should show only the minutest
particles of pulp : causing the water to appear
merely cloudy. The pulp is then passed through
the machine a second time, together with six
times its weight of a sterilised mixture of 50 per
cent. pure glycerine in distilled water. The
resulting mixture is then once more passed
through the machine ; thus producing a fine
and intimate emulsion. At this stage a loopful
of the emulsion is withdrawn with a sterilised
platinum needle, and agar-agar plates are estab-
lished, in order to estimate both the number and
the quality of the organisms present in the lymph.

'Storage of Emulsion.—The emulsion is next
received into conical glass receptacles, previously

sterilised. By means of a stopcock at the point of the cone, the glycerinated lymph is run into small sterilised test-tubes capable of holding 4 to 10 c.c. Each tube is filled as completely as possible, so that very little air remains in contact with the emulsion. It is plugged with a sterilised cork, is sealed with melted paraffin wax, which has been rendered aseptic with carbolic acid, and is then placed in a dark cool cupboard or ice-chest. Week by week, agar-agar plates are established from the emulsion, with the result that the number of colonies is shown to diminish successively in the several plate cultures. At the end of a month the plates rarely show colonies of any sort. When the stage is reached at which agar plates show no growth after inoculation with the emulsion, samples of the lymph are drawn up into capillary tubes, and may be tested on children. The results of these vaccinations are recorded a week later, and from the number and size of the vesicles obtained, an estimate is made as to the potency of the lymph. If satisfactory, the lymph is then filled into the fine-bore vaccine tubes, which are sealed in a flame and are then ready for distribution.' The tubes are usually filled by a machine which drives the lymph into them by means of compressed air.

The use of unripe lymph—*i.e.* lymph with glycerine but not kept sufficiently long for the glycerine to destroy the contaminating microbes —is followed by excessive fever, inflammation, pus production, and other symptoms of septic infection. The use of stale lymph—that is, lymph that has been kept so long that the glycerine is beginning to destroy the vitality of the vaccine organism—is a less serious evil. Copeman has stated that the glycerinated lymph may be kept for considerable periods of time without deterioration—*e.g.* for eight months or even longer (' Milroy Lectures,' 1897)—but the Chicago Health Department does not agree with this view. Their experience is that in the glycerinated lymph, stored under proper conditions, the extraneous pathogenic organisms are destroyed in from fifty to sixty days, and that at the end of this period the lymph continues active for another period of forty or fifty days, after which it deteriorates. If stored in the cold ($18°$ F. or $-5°$ C.) the lymph will retain its activity for a much longer time.

The amount of glycerine added to the lymph varies at different establishments.[1] At Berlin the proportions are :

[1] *Rep. to the Loc. Gov. Board on the Preparation and Storage of Glycerinated Calf Vaccine Lymph*, 1897.

Vesicle pulp . 　 . 　 . 　 1 part.
Glycerine 　 . 　 . 　 . 　 7 parts.
Boiled water . 　 . 　 . 　 7 　,,

At Cologne :

Vesicle pulp . 　 . 　 . 　 1 part.
Glycerine 　 . 　 . 　 . 　 10 parts.
Water . 　 . 　 . 　 . 　 5 　,,

At Geneva :

Vesicle pulp . 　 . 　 . 　 1 part.
Glycerine 　 . 　 . 　 . 　 2 parts.
Water . 　 . 　 . 　 . 　 1 part.

Green[1] has found that the vesicle pulp, finely ground, and mixed with five or six times its volume of saturated chloroform water, soon becomes freed from extraneous organisms. His most recent method is to pass air charged with chloroform vapour through the emulsified vesicle pulp. This kills the extraneous micro-organisms in a few hours.

No unbiased person can doubt the protective power of vaccination against variola. In many instances, however, vaccination is performed in a perfunctory manner, even by

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[1] *Thirtieth Ann. Rep. Loc. Gov. Board, Rep. Med. Off.* for 1900–1901, p. 639, and Royal Society, London, April 30, 1903.

medical men who should know better. At least three, preferably four, places should be inoculated, and vaccination should be repeated at intervals of a few years (7–10) until late adult life, especially if an epidemic of variola is in progress.

It can now hardly be doubted that vaccinia is modified variola, and this would explain the rationale of the process. In a few instances variola has been inoculated upon the calf, and after three or four passages becomes indistinguishable from true vaccinia. It is very difficult, however, to inoculate the calf successfully from a case of variola ; but Copeman has shown that *inoculated* small-pox can be much more readily inoculated upon the calf, and he suggests, therefore, that spontaneous vaccinia has developed from *inoculated* small-pox, since small-pox inoculation was practised at the time Jenner made his discovery.

Anti-cholera Vaccine

The vaccines employed in the anti-cholera vaccination are two in number, a first or weak, and a second or strong. The following is Haffkine's method : [1]

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[1] *Brit. Med. Journ.* 1893, i. p. 227 (Wright and Bruce).

The first vaccine is prepared from attenuated cultures of the cholera spirillum. The ordinary laboratory cultures are usually considerably attenuated, but to be sure that they are sufficiently so they are grown for several generations on surface agar at 38° C. in tubes through which a current of moist air is continuously passed. Such a culture causes only a local œdema instead of necrosis when injected into the subcutaneous tissue of a guinea-pig.

The second, or strong vaccine, is prepared from cholera cultures, the virulence of which has been artificially increased by passing through the peritoneal cavity of guinea-pigs. This is done by first of all preparing standard cultures from any ordinary culture of the cholera vibrio. Test-tubes measuring 15 cm. in length are employed ; of the 15 cm., 10 cm. are occupied by the sloping surface of ordinary nutrient agar. The whole surface of the nutrient medium is inoculated and the inoculated tubes are incubated at 35° C. for twenty-four hours. The whole growth from the surface of the agar is then scraped off with a sterilised platinum needle of stout wire and made into an emulsion with about 3 c.c. of sterile broth. A guinea-pig (300–400 grams) is etherised, a small patch of hair on the abdomen cut short, and a spot

cauterised with a hot iron to sterilise it. The
emulsion of cholera bacilli is then drawn up into
a sterile syringe or glass pipette and injected
into the abdominal cavity through the cauterised
area. Two guinea-pigs should be injected at
the same time, using for each one a standard
cholera culture. The guinea-pigs so treated
will die within twenty-four hours.

The animals are then pinned out and the
peritoneal cavity is opened with strict aseptic
precautions ; with a sterile forceps the intestines
are thrown upwards and to the right, and with a
sterile glass pipette the peritoneal fluid is sucked
up from the iliac fossæ. The whole of the peri-
toneal fluid from one guinea-pig is introduced
into a sterile test-tube, which is well plugged
with cotton-wool, and is placed in the oblique
position (for aëration) in the incubator at
35° C. for about ten hours in order to induce
proliferation of the cholera bacilli. After this
treatment the fluid is similarly injected into a
second guinea-pig, the size of which, however,
has to be taken into account. If the peritoneal
fluid in the first guinea-pig be abundant it will
contain comparatively few cholera bacilli, and
a smaller animal should be chosen, but if it be
scanty the comma bacilli will be numerous, and
a larger animal may be used. After twenty to

thirty passages through guinea-pigs the virus will have attained its maximum virulence, which is known by the fact that further passages do not shorten the period that elapses between inoculation and death.

Throughout all the manipulations the greatest care must be taken to prevent contamination, and the cultures, &c., should be controlled by subculturing and microscopical examination. The 'exalted' cholera cultures do not retain their maximum virulence for longer than ten days, and have again to be passed through guinea-pigs (three or four).

In order to prepare the vaccines a 'standard' agar tube is inoculated over its whole surface and incubated at 35° C. for twenty-four hours. Three or four cubic centimetres of sterile broth are introduced into the tube and an emulsion is made with the whole of the growth. The emulsion is measured by drawing it up into a sterile syringe, the contents of which are then introduced into another sterile glass and made up to a volume of 8 c.c. by the addition of more sterile broth. One cubic centimetre of this emulsion constitutes the dose for vaccination. Carbolised vaccines may be prepared by using a $\frac{1}{2}$ per cent. solution of carbolic acid (sterilised by boiling) for making the emulsions and diluting

them to 6 c.c., and not 8 c.c., as in the un-carbolised. The carbolised vaccines may be preserved for some time in sealed tubes.

The dose of vaccine (1 c.c.) is injected hypodermically into the flank, the second or strong vaccine being injected three to five days after the first or weak one.

The statistics of the value of anti-cholera inoculation are very favourable. In a certain district in Calcutta, Simpson had under observation for a period of two years 8,000 individuals who had been inoculated; these lived with their uninoculated relatives and neighbours in the same huts. During 738 days, cholera occurred among the uninoculated on 78 days, among the inoculated on only 10 days. From the 4th to the 216th day after inoculation, *one* case only of cholera occurred among the inoculated, and from the 5th to the 420th day after inoculation the number of deaths among the inoculated was 22·62 times smaller than among the uninoculated. The case mortality, however, of those vaccinated who may be attacked does not seem to be influenced by the inoculations.[1]

Strong [2] suggests the use of vaccine prepared

[1] Haffkine, *Brit. Med. Journ.* 1899, ii. p. 12.
[2] *Bull*, No. 16, 1904, Bureau of Gov. Laboratories, Manila.

by autolysis. The surface of agar in a plate bottle (fig. 22, p. 202) is inoculated by spraying with a 24-hour broth culture of virulent cholera, and incubated at 37° C. for twenty hours. The growth is then scraped off, suspended in sterile water, the suspension is kept at 60° C. for several hours, and afterwards at 37° C. for from two to five days, and is finally filtered through a porcelain filter. About 3 c.c. of the filtrate constitutes a dose.

Besredka has prepared a ' sensitised ' vaccine (p. 333).

Anti-typhoid Vaccine

In Wright's method, a virulent typhoid bacillus (the virulence being kept up by intraperitoneal passage through guinea-pigs) is grown in peptone beef broth in flasks at 37° C. for from fourteen to twenty-one days. The flasks are then so heated that their contents attain, and remain at for a few minutes, a temperature of 60° C. To obtain uniform toxicity the contents of several flasks should be mixed, and to safeguard the vaccine from contamination one-tenth of its volume of 5 per cent. lysol or carbolic acid is added. Various ingenious devices have been adopted by Wright and Leishman (*loc. cit.*) to prevent contamination and for standardisation.

Further details of standardisation are given by Lamb and Forster and by Leishman.

The use of suspensions of agar cultures in physiological salt solution and killed by heat has also been recommended and has been adopted in Germany.

Maceration of agar cultures suspended in salt solution by keeping the suspension at 37° C. (after killing by heating to 60° C.) for two or three days and subsequent filtration through a porcelain filter is another method that has been used.

Even living attenuated cultures have been suggested, but may entail some risk and the possibility of spreading infection if spilled.

The most recent method for the preparation of typhoid vaccine is that of Russell. He selects a culture, not necessarily virulent, but having good ' binding ' powers and producing large quantities of anti-bodies on injection. This is grown on agar slants for 18–20 hours, the tubes being of uniform size and having the same surface of agar in each. The cultures are sown with a uniform quantity of a broth suspension of a 20-hour agar culture ; in this way ' standard ' cultures are obtained, and 200-300 tubes are prepared at one time. After incubation, 2 c.c. of physiological salt solution

are introduced into each tube and the growth is thoroughly broken up and emulsified. A sample is taken from the bulk and used for a bacterial count (see p. 280) and also for tests of purity. The suspension is filled into large (50 c.c.) tubes which are sealed in the blow-pipe flame. The tubes are then completely immersed in a water-bath and heated to 60° C. for 75 minutes. After the cultures are thus killed, the suspension is diluted up to about 15–20 c.c. for each agar slant. The quantity is varied according to the results of the bacterial count, the aim being to obtain about 1,000,000,000 bacilli per cubic centimetre, which is the dose. One-quarter per cent. of trikresol is added and the suspension is filled into ampullæ. Before use, a mouse and a guinea-pig are inoculated, and aërobic and anaërobic tests for sterility are made with each batch. Up to the present 1,400 men have been inoculated, of whom about 1 per cent. had severe, 7·7 per cent. moderate, 30 per cent. mild, and about 60 per cent. practically no reactions.

Besredka has prepared a sensitised vaccine (p. 333).

The Dosage and Strength of the Anti-typhoid Vaccines.—The strength of a typhoid vaccine depends upon the number of bacilli it contains

and their virulence. It may be standardised by ascertaining the degree of opacity, by counting the number of bacilli, and by determining the toxicity.

The dose of Wright's vaccine for an adult varies from 0·5 c.c. to 2 or 3 c.c. according to the strength of the preparation. A second increased dose given ten to fourteen days after the first one increases the immunity and should always be adopted if possible. The injection should be made subcutaneously in the flank.

Clinical Symptoms which supervene upon the Inoculation of the Anti-typhoid Vaccines.—The symptoms are comparatively slight when small doses are used, *e.g.* tenderness at the seat of inoculation, a chilly feeling two or three hours after inoculation, slight rise of temperature, and restlessness at night, but these symptoms pass away in about twenty-four hours. With larger doses all the symptoms are severe, and are described as commencing two or three hours after injection, with tenderness, which gradually increases in severity and extends upwards into the armpits and downwards into the groin ; a patch of congestion two or three inches in diameter develops round the site of inoculation, and red lines of inflamed lymphatics

can be traced extending into the armpits. These symptoms gradually subside in about forty-eight hours.

The constitutional symptoms are marked by some degree of faintness and collapse, in some cases accompanied by nausea and vomiting, which commence about three or four hours after injection, entire loss of appetite, disturbed sleep, and high temperature, all of which pass off in a few hours. The blood and serum of individuals vaccinated in this way give the agglutination reaction in a marked manner.

To obtain more complete protection a second inoculation of one-and-a-half to twice the original dose should be given after an interval of ten to fourteen days. There is practically no risk, and the immunity conferred will probably last for two or three years.

As regards the sphere of application of the anti-typhoid vaccination, it would be expedient in the case of young soldiers and other individuals going abroad to infected districts, for those living in a district visited by an epidemic, or for those in attendance upon typhoid patients.

The value of anti-typhoid inoculation is still *sub judice* and can only be gauged by statistics. From an analysis of statistics dealing with some 15,000 inoculated individuals, mostly soldiers,

in Great Britain, India, Egypt, Cyprus, and South Africa, Wright deduced the following conclusions : (1) As regards the *incidence* of typhoid fever, there is a reduction of at least twofold, but it may be as great as 28-fold ; (2) As regards *case mortality* there is a reduction of one-half ; (3) as regards *death-rate* there is at least a twofold reduction, generally a fourfold one, among the inoculated.

Some of the most recent results of anti-typhoid vaccination are given by Leishman. Of 12,083 men, 5,473 were inoculated and 6,610 were uninoculated. Among the former, 21 cases of typhoid occurred with two deaths ; among the latter there were 187 cases and 26 deaths.

The writer has found that typhoid endotoxin prepared by the Macfadyen method (p. 50) possesses powerful immunising properties, and it is possible that this substance may be utilised as a vaccine.

See Wright and Semple, *Brit. Med. Journ.* 1897, i. p. 256 ; Wright and Leishman, *ib.* 1900, i. p. 122 ; Wright, *Lancet,* 1902, ii. September 6 ; Lamb and Forster, *Sc. Mem. Gov. of India,* No. 21, 1906 ; Russell, *Johns Hopkins Hosp. Bull.* xxi. No. 228, 1910, p. 83 (a good summary) ; Leishman and others, *Journ. Roy. Army Med. Corps,* x. 1908, p. 583 ; Leishman, *ib.* xii. 1909, p. 166, and *Journ. Roy. Inst. Public Health,* xviii. 1910, p. 385 *et seq.*

Anti-plague Vaccine

An anti-plague vaccine was first prepared by Haffkine, is frequently termed the Haffkine prophylactic, and is the one most generally employed. The method of preparation is comparatively simple. A virulent plague bacillus is cultivated in flasks in a special broth ; this consists of ordinary peptone beef broth to which a trace of butter is added. In Hindu countries, in which the cow is sacred, goat's flesh or wheat flour may be substituted for beef for making the broth. The butter melts at the temperature of incubation ($35°$ C.) and forms little islets upon the surface of the medium which serve as nuclei from which growth starts. The flasks must be kept absolutely still, in which case copious flocculent pyramidal growths depend downwards into the medium forming the so-called stalactite growth of Haffkine. This is shaken down, allowed to re-form, again shaken down, and the process is repeated several times. Haffkine gives the following details of the mode of preparation of his prophylactic. Mutton is finely minced and infused in dilute hydrochloric acid. The exact proportions of the materials are as follows : $1\frac{1}{2}$ kilos of mutton are infused in 3

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litres of water plus 225 cubic centimetres of hydrochloric acid. As a rule, the material is kept in this infusion for two or three days in the cold. Afterwards it is subjected to a high temperature, 130° to 140° C., which corresponds to a pressure of about $2\frac{1}{2}$ atmospheres. It is kept at that temperature for six hours. The fluid is then siphoned off, filtered, and the filtrate is diluted with a sufficient quantity of water to bring the amount to $4\frac{1}{2}$ litres. This solution is neutralised with 60 grams of caustic soda and again heated to a temperature equal to the previous one, for only half an hour, then filtered again, and whatever solid residue is produced by the neutralisation and second heating is again rejected, and only the liquid part employed. This liquid is called Warden's bouillon. For the cultivations for the plague prophylactic it is mixed with a small quantity of ghee or of cocoanut oil, distributed into large flasks, sterilised, and inseminated with a minute quantity of the most virulent plague microbes which can be obtained, and the inoculated liquid is incubated. During the first two or three days scarcely any signs of change are observed, but then minute flakes appear underneath the floating droplets of oil or ghee, which in the course of from

twelve to twenty-four hours grow down in the form of shaggy stalactites. The liquid remains clear, except for a small quantity of powder-like residue, which very early in the process falls to the bottom of the flask. The stalactites in the course of two or three days fill the upper half, or sometimes even the whole volume, of the liquid. The least oscillation of the vessel is sufficient to detach the suspended masses from the drops of ghee or oil, and the whole growth in the course of a day or so falls to the bottom of the flask, while the liquid appears again perfectly limpid. After the first growth of stalactites has been brought down by shaking, a new crop of flakes appears underneath the droplets of oil or ghee. The same course of growth as described above is repeated, but may be a little slower in development. After another two or three days the flask is again shaken and the second crop brought down. This process is repeated ten or twelve times, the development taking from five to six weeks before it is perfectly accomplished, and the growth becoming slower and slower, until it stops entirely. The cultures are then sterilised by heating to 65° C. for one hour, and carbolic acid in the proportion of 0·5 per cent. is added. The dose employed is 2·5 c.c., which is injected

into the flank. A second injection given a week after the first increases the immunity.

In the Bombay Laboratory minced goat's flesh or wheat flour is suspended in water and hydrochloric acid is added until the mixture is decidedly acid. The whole is kept at a temperature of 70° C. for three days, caustic soda solution is then added until neutral, and the medium is distributed in flasks and sterilised. After inoculation the cultures are allowed to grow for six weeks. They are then sterilised at 55° C. for fifteen minutes, 0·5 per cent. of carbolic acid is added, and the vaccine is distributed into bottles with aseptic precautions. The dose for an adult is 4 c.c. The greatest care must be exercised both in preparation and in inoculation to avoid contamination, for on one occasion, owing to the carelessness of a native assistant, a bottle became contaminated with the tetanus bacillus and nineteen deaths from tetanus ensued (at Mulkowal in 1903).

Lustig and Galeotti also prepared an anti-plague vaccine by growing the plague bacillus upon agar for three days, scraping off the growth and treating with 1 per cent. caustic soda. The fluid is then filtered through paper and precipitated with dilute (0·1 per cent.) acetic or hydrochloric acid, or by saturation

with ammonium sulphate. The precipitate is dissolved in a 0·5 per cent. solution of sodium carbonate, and filtered through a Chamberland filter. This is the vaccine fluid and the dose corresponds to 1 milligram of solid matter.

Value of Anti-plague Inoculation.—The value of the Haffkine anti-plague inoculation can only be estimated from statistics.

Major Wilkinson, I.M.S., formerly Chief Plague Medical Officer in the Punjab, gives the following statistics relating to villages where 10 per cent. or more of the inhabitants had been inoculated, and where plague was present or subsequently appeared not later than four months afterwards :

—	Population	Attacks	Deaths	Case Incidence per cent.	Case Mortality per cent.
Inoculated	186,797	3,399	814	1·8	23·9
Non-inoculated	639,630	49,433	29,723	7·7	60·1

From this it is evident that both the incidence of plague, and the mortality among the inoculated who happen to contract the disease, are very much lessened by the inoculation. In these villages inoculation saved some eight thousand lives.

Calmette asserted, as the outcome of his experiments upon animals, that a person

inoculated with the prophylactic during the incubation period of the disease would have the disease in so aggravated a form that it would almost certainly prove fatal. Bannerman, however, finds that this is not the case ; among persons inoculated with the prophylactic and who developed plague within ten days, the case mortality was never more than 62·5 per cent., and averaged only 47·0 per cent., while among the uninoculated the case mortality was 73·7 per cent. among the same population during the same period. Bannerman also believes that protection is secured within twenty-four hours of the inoculation, and that it lasts as long as eighteen months.

Calmette suggested that in order to procure a *rapid* immunity 10 c.c. of anti-plague serum may be injected, followed by the Haffkine prophylactic to obtain the prolonged immunity. Such a combined procedure might be expedient for those suddenly called upon to tend the sick. No statistics seem to have been published of the use of Lustig and Galeotti's vaccine.

Strong found in a series of investigations that living but attenuated plague cultures produce experimentally far greater immunity than dead cultures, *e.g.* the Haffkine prophylactic, and has applied this method in the vaccination of man against plague. The greatest

care has to be taken that the culture employed for this purpose has lost its virulence almost completely, and Strong believes that if a whole agar slant culture no longer kills a 250-gram guinea-pig, it is probably quite safe to inject into human beings in small amount, nor is there any evidence that such a culture will under ordinary laboratory condition regain any of its lost virulence.

The culture used by Strong for the preparation of his vaccine was obtained from Professor Kolle, who received it originally from Dr. Maassen. The dose employed is one 24-hour agar slant culture suspended in 1 c.c. of salt solution for an adult, for a child from one-third to one-half this quantity. The reaction following injection is not severe ; a few hours after inoculation there is some fever and by the evening of the first day the temperature may reach 101·5°–102° F., occasionally even 104° F., then it declines and becomes normal by the third or fourth day. The injections were made deeply into the deltoid muscle, and there may be some induration, redness, and soreness locally, which soon pass off. Klein has prepared a plague prophylactic, which experimentally seems to be effective, by drying *in vacuo* the necrotic organs (bubo, liver, spleen, and lungs) of guinea-pigs inoculated with plague bacilli so as to induce a sub-acute infection. The dried material is pulverised, extracted with water, and the watery extract heated to 70° C. ; this forms the prophylactic fluid.

Besredka has prepared a 'sensitised' vaccine, which is official in the French Pharmacopœia of 1908 (p. 334).

See Haffkine, *Rep. of the first Indian Plague Commission ;* Lustig and Galeotti, *Brit. Med. Journ.* 1897, i. p. 1057 and *ib.* 1900, i. p. 311 ; Bannerman, *Centr. f. Bakt.* Abt. I. Bd. xxix. p. 873 and *Lecture on the Plague Prophylactic,* Bombay, 1905 ; *The Preparation and Use of Anti-Plague Vaccine* (The Bombay Laboratory), Bombay, 1907 ; Wilkinson, *ib.* p. 8 ; Strong, *Philippine Journ. of Science,* ii. 1907, p. 155 ; Klein, *Rep. Med. Officer, Loc. Gov. Board,* 1905–1906 and 1906–1907.

Anti-Dysentery Vaccine

Shiga in 1898 made experiments on prophylactic vaccination against dysentery, first by the use of killed cultures, but finding that this vaccine gave rise to local trouble, he subsequently used killed cultures together with anti-dysentery serum.

Dopter [1] using killed cultures has found that a prolonged negative phase with hypersensibility, lasting four to six weeks, is produced (in the light of Wright's work it seems likely that a much smaller dose of culture would have proved efficacious as a protective, and would not have induced this profound negative phase). Finally, Dopter adopted the method of sensitising the organisms, devised by Besredka and previously referred to (p. 333). The dysentery

[1] *Ann. de l'Inst. Pasteur,* xxiii. 1909, p. 677.

bacilli are killed by heating to 60° C. for one hour, and dried *in vacuo*. The dry mass is emulsified in physiological salt solution, to which is then added a fresh unheated anti-dysentery serum, and the mixture is then allowed to remain at room temperature for an hour. At the end of this time the organisms will have agglutinated and settled to the bottom. The fluid is decanted and the sediment washed twice with salt solution by centrifugalisation. The deposit is then suspended in salt solution and forms the vaccine. With it an immunity of some months' duration was obtained experimentally, and the negative phase was practically negligible. It provokes no reaction, either general or local.

Mediterranean or Malta Fever

Eyre has employed a killed culture of the *M. melitensis* for prophylaxis. The initial dose was usually 400,000,000 cocci, and a second dose was sometimes given after a suitable interval.

No data are available as to the preventive efficiency of the vaccine.

See Eyre, *Rep. Mediterranean Fever Commission*, pt. **vi.** 1907, p. 115.

CHAPTER IX

The Tuberculins

Tuberculins are fluids containing toxic products, bacterial cell extractives, or bacterial cell constituents of the tubercle bacillus. As two types of the tubercle bacillus are now recognised, the human and the bovine, *tuberculin may be prepared either from a human or from a bovine type of bacillus*, and thus for every tuberculin two preparations may be obtained. According to German nomenclature, the letter P (*Perlsucht*) is appended to a preparation derived from bacilli of bovine origin.

The *old tuberculin*, tuberculin A (alt), introduced by Koch so long ago as 1890, consists essentially of a boiled and filtered broth culture of the tubercle bacillus. The mode of preparation is briefly as follows. The tubercle bacillus is grown in a glycerin veal broth for

six to twelve weeks in a shallow layer in flat flasks (fig. 32), so that the supply of oxygen is free and an abundant growth with copious film formation results. The latter seems to be essential, but it does not appear to matter whether the bacilli are virulent or non-virulent. The cultures, bacilli, and all are concentrated over
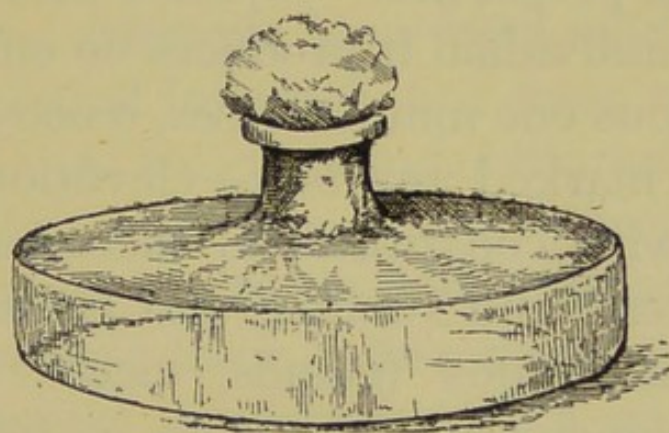
FIG. 32.—FLASK FOR GROWING TUBERCULIN.

a water-bath to about one-tenth of their volume, and then filtered through porous porcelain ; the resulting fluid is quite clear, thick, owing to the concentration of the glycerin by the evaporation, of a dark amber colour, and possesses a curious characteristic smell. The large proportion of glycerin preserves the fluid, which keeps indefinitely in a cool, dark place.

It is perhaps preferable first to concentrate to one-half over the water-bath, then to filter through a Chamberland filter, and, after filtration,

to concentrate further over the water-bath until the fluid is reduced to one-tenth of the original volume. If first concentrated to one-tenth, filtration is slow and there is considerable loss owing to the thick nature of the fluid.

So prepared, the old tuberculin possesses remarkable properties. Injected into a healthy animal or individual it produces no effect, but in a tuberculous one minute doses, 0·0003 c.c., give rise to a marked reaction—elevation of temperature with constitutional disturbance more or less severe, and swelling and tumefaction of tuberculous lesions (glands, ulcers, &c.). By cautiously increasing the dose a tolerance is gradually induced, so that large doses cause little or no disturbance. Under certain conditions the injections of tuberculin produce marked changes in the tuberculous parts, leading to necrosis and exfoliation, with subsequent healthy reaction and repair ; this is especially seen in cases of lupus. By continued injections a marvellous improvement results, so much so that a cure is apparently effected ; but, unfortunately, when the tuberculin treatment is discontinued the scar usually breaks down and the disease returns. Nevertheless, some cases remain permanently healed.

Healthy guinea-pigs bear considerable injections of tuberculin without harm ; but if they be tuberculous, doses of 0·01 gram produce death if the disease is advanced (eight or ten weeks after inoculation) ; if less advanced (four to five weeks after inoculation), a larger dose, 0·2 to 0·3 gram, is required ; but 0·5 gram always proves fatal. There is no method of accurately standardising tuberculin : attempts have been made to do so by intracerebral injection, but have not proved successful. A rough standardisation may be effected by injecting guinea-pigs ; healthy animals should be able to withstand an injection of 1 c.c. without harm, tuberculous ones, inoculated six weeks previously with a pure culture, should be killed by a dose of 0·1 c.c.

The *new tuberculin*, introduced by Koch in 1897, differs from the old by being prepared with *virulent* cultures and by being *unboiled*. It consists essentially of a solution or emulsion of the tubercle bacilli themselves, without their toxic products.

Young cultures of a virulent tubercle bacillus are used. The bacillus may be grown on glycerin serum and the resulting growth scraped off, desiccated *in vacuo*, and then triturated. This is a dangerous process and can only be done with safety by machinery. The dry masses of

bacilli are introduced into steel vessels containing unglazed porcelain balls, these are closed and kept in movement for five or six days ; the continual movement of the porcelain balls grinds up the tubercle bacilli. The triturated bacilli are then emulsified in distilled water and the emulsion is centrifugalised for 30–45 minutes at a speed of 4,000 revolutions per minute. The upper slightly opalescent layer, termed tuberculin O (O = *obere* = upper), is pipetted off, and the residue of powdered bacilli again dried, triturated, emulsified and centrifugalised. The upper layer is the new tuberculin R (R = residual). The residue is again similarly treated and a second fraction of tuberculin R obtained, and further fractions are obtained by the same process until the residue is entirely used up. To get rid of the bacilli, the fluids are mixed and filtered through a Chamberland filter, and, in order to preserve the mixture, glycerin to the amount of 2 per cent. is added. The fluid is standardised to contain 2 milligrams of solid matter per cubic centimetre.

The tuberculin R is the one employed therapeutically. It is stated to possess powerful immunising properties, produces little reaction in small doses in a tuberculous individual, and causes no local irritation at the seat of inocula-

tion. The tuberculin O, the fluid from the first centrifugalisation, much resembles the old tuberculin.

Another tuberculin preparation, *new* tuberculin A (A = alkaline), obtained by emulsifying tubercle bacilli in a 10 per cent. solution of caustic soda, was also prepared, but since it invariably caused local abscesses, its use was given up, and the tuberculin R substituted.

Tuberculin, bacillary emulsion, Tuberculin B E, is of still more recent introduction. It is prepared by triturating unwashed tubercle bacilli and making an emulsion in 50 per cent. glycerin. It therefore consists of the total cellular products, with adherent toxins, of the tubercle bacillus. The emulsion is standardised to contain 5 milligrams of solid material per cubic centimetre.

Tuberculo-toxoidin.—Ishigami [1] has prepared this substance by treating tubercle culture with sulphuric acid. He claims that it is an endo-toxin, produces no irritation on injection, and possesses powerful immunising properties. He has treated several cases of tuberculosis with it with benefit. By injecting tuberculo-toxoidin into animals an active immune serum is obtained, and may be used in conjunction with

[1] *Philippine Journ. Science,* iii. 1908, p. 379.

the tuberculo-toxoidin. The substances were best administered in pill form.

Denys' Tuberculin

Denys [1] details a method he has employed for the treatment of pulmonary tuberculosis somewhat similar to the old tuberculin treatment. Apparently cultures of the tubercle bacillus (? virulent) in glycerin broth are filtered through a porcelain filter and the filtrate is employed for injection. The culture is not heated. In cases without fever, treatment is commenced with small doses, 0·000,001 milligram. This produces a febrile reaction, and the same dose is administered until tolerance is established, and if this does not occur after two or three doses, the dose is diminished to one-half or one-quarter (for dosage, see p. 292). Should febrile attacks occur independently of the injections, it is well to reduce the dose.

In febrile cases, after rest in bed, &c. for a fortnight, a dose of 0·000,000,01–0·000,000,1 milligram is given, and injections are administered every second day, slowly and gradually increasing the dose.

[1] *Bull. de l'Acad. Roy. de Méd. de Belg.* t. xvi. No. 3, 1902, p. 153. See also *Brit. Med. Journ.* Epit. 1906, i. No. 309.

By this method, tested on 442 patients with pulmonary tuberculosis in the cavitation stage, 193 or 43·6 per cent. are stated to have been cured. Denys claims that there is considerable improvement in the physical signs, diminution and generally cessation of the cough and expectoration, and disappearance of the bacilli, increase of the appetite and gain in weight, together with cessation of fever and night sweats when present (see also p. 292).

THERAPEUTIC USE OF TUBERCULINS

Preparation of Dilutions

1. *The Old Tuberculin*

The following directions for the preparation of dilutions are issued by Messrs. Meister, Lucius, and Brüning :

Tuberculin must be diluted before use. As diluent $\frac{1}{2}$ per cent. carbolic acid solution is selected. The dilution is best carried out by taking 1 c.c. tuberculin from the original bottle by means of a pipette and adding to this 9 c.c. $\frac{1}{2}$ per cent. carbolic acid solution. By this means a 10 per cent. tuberculin solution = Tub. Dil. 1 is obtained. One c.c. of this Tub. Dil. 1 is again diluted with 9 c.c. $\frac{1}{2}$ per cent. carbolic acid solution and thus

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a 1 per cent. tuberculin solution = Tub. Dil. 2 is obtained.

In the same way by diluting 1 c.c. of the Tub. Dil. 2 with 9 c.c. carbolic acid solution a 0·1 per cent. tuberculin solution = Tub. Dil. 3 is obtained, and so forth.

The different dilutions contain in 1 c.c. :

Tub. Dil. 4 . .	0·0001 c.c. Tuberculin
Tub. Dil. 3 . .	0·001 c c. Tuberculin
Tub. Dil. 2 . .	0·01 c.c. Tuberculin
Tub. Dil. 1 . .	0·1 c.c. Tuberculin

The undiluted tuberculin will keep for any length of time.

Likewise Tub. Dil. 1 keeps well, whilst the higher dilutions will only keep for from four to five days. It is therefore advisable only to prepare such quantities of the higher dilutions as are needed for a few days' use.

Turbid solutions are on no account to be used.

The dilute solution is administered by subcutaneous injections by means of a 1 c.c. or 2 c.c. syringe, similar to an antitoxin syringe ; each should be graduated into tenths. The syringe should be sterilised and the skin may be disinfected with absolute alcohol. The best seat for the injections is in the back, between the scapulæ. The writer has given some thousands of injections without any local trouble at the seat of inoculation.

2. *The New Tuberculins : Tuberculin R*

The *new tuberculin*, of which only tuberculin R (T R) is employed, is used solely for treatment and not for diagnostic purposes. The fluid is supplied by Meister, Lucius, and Brüning of Höchst-on-Maine, and the following are the directions for use issued with it :

The New Tuberculin R is supplied in liquid condition. It is an opalescent liquid similar in appearance to a mixture of 5 or 6 drops of milk in half an ounce of water. It must be kept in a cool, dark, and dry store.

The solution contains 2 milligrams of solid substance in each cubic centimetre.

For dilution of the liquid 20 per cent. glycerine should be employed. This may be prepared by mixing 80 c.c. of distilled water and 20 c.c. of glycerine, and boiling the mixture for twenty minutes and using cold. The dilutions are preferably made in the following manner :

1. With a 1 c.c. pipette, calibrated to $\frac{1}{10}$, 0·3 c.c. is withdrawn from the bottle, and mixed with 2·7 c.c. of the 20 per cent. glycerine solution, making in all 3 c.c. This 10 per cent. dilution contains 0·2 milligram of solid substance.

2. From this 10 per cent. dilution 0·1 c.c. is taken and made up to 10 c.c. with glycerine solution. Thus a 1 per mille dilution of the original fluid is obtained.

Of this dilution 0·2 c.c. contains 0·0002 c.c. of the original fluid or 0·0004 milligram of solid substance. Further dilutions may be prepared in a similar manner.

Instruments and pipettes must, before use, be sterilised with absolute alcohol and ether and then rinsed out with sterilised glycerine solution, in order to remove every trace of alcohol and ether.

Dilutions which present a turbid appearance, or show a deposit which does not dissolve upon shaking, must not be employed. Generally the dilutions keep for a fortnight in a cool and dark place.

3. *Tuberculin, Bacillary Emulsion (B E)*

The following directions are issued by Messrs. Meister, Lucius, and Brüning for the preparation of dilutions of this tuberculin :

Dilutions are prepared with sterile 0·8 per cent. sodium chloride solution, to which 0·5 per cent. carbolic acid may be added if the dilutions are to be kept for several days.

Dilutions are prepared by the use of sterile graduated pipettes and vessels. Instead of pipettes, the graduated syringe may be employed.

The first dilution is prepared by diluting 0·1 c.c. of the original fluid with 9·9 c.c. of the diluting fluid.

The second dilution is prepared by taking 1 c.c. of the first dilution and adding 9 c.c. of the diluting fluid, the third dilution by taking 1 c.c. of the second and adding 9 c.c. of the diluting fluid, the fourth dilution by taking 1 c.c. of the third and adding 9 c.c. of the diluting

fluid, the fifth dilution by taking 1 c.c. of the fourth and adding 9 c.c. of the diluting fluid.

The dilutions will then contain :

1. First dilution, 0·01 c.c. of the original solution, or 0·05 mgrm. of solid substance.

2. Second dilution, 0·001 c.c. of the original solution, or 0·005 mgrm. of solid substance.

3. Third dilution, 0·0001 c.c. of the original solution, or 0·0005 mgrm. of solid substance.

4. Fourth dilution, 0·00001 c.c. of the original solution, or 0·00005 mgrm. of solid substance.

5. Fifth dilution, 0·000001 c.c. of the original solution, or 0·000005 mgrm. of solid solution.

In order, therefore, to give the smallest dose usually employed, viz. $\frac{1}{100,000}$ mgrm., the fifth dilution would have to be prepared and of this 0·2 c.c. would contain this amount.

The dose is given subcutaneously. For injection, such parts of the body should be selected at which large folds of skin may be raised. The local reaction that not unfrequently appears in the locality of the injection generally disappears within twenty-four hours, and must be taken into account in increasing the dose.

Diagnosis of Tuberculosis in Man by Means of Tuberculin

1. *By Injection.*—For this purpose the *old* tuberculin is used. Since the tuberculin, even in comparatively large doses, produces no effect in a non-tuberculous individual, while in a

tuberculous one minute doses cause a rise of temperature, this reaction may be made use of for diagnostic purposes in suspected cases of obscure tuberculosis. The concentrated fluid is diluted with 0·5 per cent. aqueous carbolic, and injected in precisely the same manner as for treatment (see p. 370).

The Following Doses refer to the Concentrated (i.e. *Undiluted*) *Fluid.*—Some persons being very sensitive, it is wisest to give a preliminary dose of 0·0001 c.c. If this causes no reaction, as is usually the case, it may be followed by a dose of 0·001 c.c. ; if this produces no result, 0·002 c.c. may be given after an interval of two days ; if this again produces no reaction, 0·005 c.c. may be given after a further interval of two days ; if no reaction is obtained with this dose, the condition may be considered to be non-tuberculous. In certain instances, if these three injections produce no reaction, a fourth of 0·01 c.c. may be given. The reaction consists of a rise of temperature above the normal of 2° F. or more, with general malaise, and swelling, redness, desquamation, and irritability of local tuberculous lesions, such as glands and ulcers. The temperature should be taken every two hours. A rise of temperature of no more than 1° F. if it does not occur before or after,

and coincides with, the injection, may be considered sufficient, especially if it happens again on giving a slightly larger dose.

Injections of tuberculin for diagnostic purposes should not be made if the temperature is 1° F. above normal.

2. *The Cutaneous Reaction.*—Von Pirquet[1] observed that if the skin be scarified and a dilute solution of the old tuberculin applied, an area of redness and swelling with papulation, somewhat resembling that of vaccinia, appears in forty-eight hours in tuberculous individuals but not in non-tuberculous ones. The solution employed consists of one part of the old tuberculin, one part of a 5 per cent. solution of carbolic acid in glycerine, and two parts of physiological salt solution. Two small superficial scarifications of the skin of the arm are made through two drops of the tuberculin solution placed a little distance apart, and between the two a control scarification is made in a similar manner through a drop of the same solution *minus* the tuberculin.

The method causes practically no general disturbance, and hence can be used in cases in which the injection of tuberculin is contra-indicated. The reaction is of particular value

[1] *Wien. med. Woch.* July 6, 1907.

in young children, in whom it is practically diagnostic whether positive or negative ; but after the age of eight years an increasing number of persons give the reaction, although there may be no sign or suspicion of tuberculosis, the reason being probably that old and healed tubercles still react.

McNeil [1] records the local tuberculin reactions obtained in 153 consecutive cases of tuberculosis and other diseases. All were tested by the cutaneous method of v. Pirquet, and 78 of them also by the conjunctival test (ophthalmic reaction of Wolff-Eisner or of Calmette). The cutaneous reaction was done by chafing off the epidermis with the point of a straight surgical needle, thus exposing a small circular patch of the pink cutis vera, bleeding or oozing being avoided. On this vascular surface the tuberculin solution was placed and rubbed in by rotation of the head of the needle. A control surface was similarly prepared and inoculated with 50 per cent. glycerine in water. A positive reaction consisted in the development of a papule of a deep livid red hue persisting for a week : the mere development of a red patch or ring is to be disregarded.

The general results were that the cutaneous

[1] *Brit. Med. Journ.* 1909, ii. p. 1335.

reaction thus performed gives very reliable indications, *provided the disease is not extensive and the general vigour of the patient maintained.* In *advanced* cases of tuberculosis the reaction was often negative. Of 41 non-tuberculous cases, the reaction was negative in 40. Of the 78 cases also tested by the conjunctival method, all were negative in those which were negative by the cutaneous method.

Moro[1] has modified the cutaneous method by applying the tuberculin in the form of an ointment consisting of equal parts of lanolin and old tuberculin.

3. *The Ophthalmo-Reaction.*—Calmette and Wolff-Eisner independently observed that the instillation into the eye of a dilute solution of tuberculin gives in tuberculous cases a distinct reaction consisting of lachrymation, redness and swelling of the conjunctiva, with in severe cases a semi-purulent discharge. The reaction appears in the majority of cases after about 8 hours, sometimes only after 20–24 hours, and occasionally it may be delayed 48 hours.

The solution is prepared by precipitating the old tuberculin with 95 per cent. alcohol, collecting, washing and drying the precipitate, and dissolving in sterile water so as to form a

[1] *Münch. med. Woch.* 1908, lv.–lxi. p. 216,

1 per cent. solution. For children the solution may be half this strength.

The reaction generally disappears 18–24 hours after it has developed, occasionally it may be severe and persist for days. Any affection of the eye already present contra-indicates its application.

Experience indicates that the reaction, positive or negative, is very reliable in diagnosing the presence of, or excluding, active tuberculosis, both in children and in adults. On the other hand some cases have been reported in which the reaction was so severe as to cause considerable anxiety as regards the ultimate recovery of the eye.

Wolff-Eisner[1] surveys the various tuberculin reactions as follows: With the subcutaneous application of Koch's tuberculin a positive reaction indicates the presence of tuberculosis, but by no means indicates that the disease is active. The cutaneous reaction is a safe and handy method of applying tuberculin for diagnostic purposes, but it also indicates latent as well as active tuberculosis. The conjunctival reaction, on the other hand, discovers an active form of the disease. Negative reactions in obvious cases occur and indicate a bad

[1] *Münch. med. Woch.* November 10, 1908.

prognosis. Positive reactions are obtained in 5–8 per cent. of apparently healthy persons, but these may ultimately prove to be tuberculous. Negative results do not exclude active tuberculosis. The method is a safe one provided concentrated preparations be not used, and contra-indications (*e.g.* tuberculosis of the eye) are noted.

Row[1] has attempted to apply the precipitin reaction in cases of tuberculosis by making use of an alcoholic extract of the caseated spleen of tuberculous guinea-pigs. The results, however, were very erratic.

Cutaneous Reaction in Leprosy.—Teague[2] has applied the cutaneous reaction to leprosy. Glycerin extracts (5 per cent. glycerin) were made from nodules excised from living lepers, and from the nodulous skin and from the spleen of a dead leper. The extracts were evaporated to about one-tenth of the original volume on a water-bath. Fifty lepers were vaccinated with these extracts, a control vaccination with an extract similarly prepared from the skin of a cholera corpse being made in each instance. In two or three cases there was a doubtful reaction, but otherwise the results of the vaccinations were in all respects like the controls.

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[1] *Brit. Med. Journ.* 1909, ii. p. 1333.
[2] *Philippine Journ. of Science*, iv. 1909, No. 5, p. 323.

Diagnosis of Tuberculosis in Cattle by Means of Tuberculin

For this purpose the *old* tuberculin is employed. The dose of the Meister, Lucius, and Brüning preparation recommended is 0·2 c.c. for calves up to 6 months old, 0·3 c.c. for young cattle up to 2 years old, and 0·4 c.c. for full-grown cattle, corresponding to 2 c.c., 3 c.c., and 4 c.c., respectively, of a 10 per cent. solution. The reaction consists in a rise of temperature above the normal, and the mode of applying the test is indicated in the following directions, issued by the Royal Veterinary College, London.

DIRECTIONS FOR USING TUBERCULIN

1. While under the tuberculin test cattle ought to be kept in the house, fed on their usual food, and protected from draughts. They ought not to be allowed to drink large quantities of cold water between the sixth and fifteenth hours after injection. It is well to take their temperature at least once on the day preceding the test.

2. The dose of tuberculin (*i.e.* the Veterinary College preparation) for a medium-sized cow is 3 cubic centimetres, or 50 minims, and it may be varied above or below that, according to the size of the animal. Large bulls ought to receive 4 c.c.

3. It ought to be injected under the skin with a clean hypodermic syringe. The most convenient points are in front of the shoulder, or on the chest wall behind the point of the elbow. The best form of syringe is

one with an asbestos piston, as the whole instrument may be sterilised by boiling it in water for five minutes before use.

4. The tuberculin must be injected into the sub-cutaneous connective tissue, and care must be taken that the whole dose is introduced.

5. The temperature must be taken at the time of injection, and at the 9th, 12th, and 15th hours after-wards.

6. Animals in which the temperature during the fifteen hours following the injection rises *gradually* to 104° or more may be classed as *tuberculous*, and those in which it remains under 103° *as not tuberculous*. When the maximum temperature attained is under 104° but over 103°, the case must be considered doubt-ful, and the animal may be re-tested after a month.

7. The test is not reliable in the case of animals in the last stage of the disease, or in those in which the temperature is over 103° before injection.

8. The tuberculin should be kept in a cool place, and protected from light. Should it become turbid or cloudy it must not be used.

9. The tuberculin test does not render the milk in any way injurious.

The proper dose, as indicated in the directions issued with each brand, should of course be given.

Employed in this manner, and with the limi-tations indicated above, tuberculin is acknow-ledged by all veterinary authorities to be the

most certain test for tuberculosis in cattle, especially when incipient and showing no manifest clinical symptoms. It is quite harmless, and does not affect the milk.

By repeated injections (three or four) of tuberculin, an immunity is induced, so that, if so treated, a tuberculous beast may not react when subsequently injected, and this method has been employed to evade the enactments against the admission of tuberculous cattle into certain countries.

Campbell[1] has also employed the ophthalmo-reaction for cattle testing with very promising results, but the ordinary preparation of tuberculin was found unsatisfactory. That used was 0·25 c.c. of the ordinary tuberculin supplied by the U.S. Agricultural Department.

Ophthalmo- and Cutaneous Reactions in Typhoid Fever

Chantemesse has prepared a toxin for carrying out an ophthalmo-reaction in typhoid fever, analogous to that for tuberculosis. Chantemesse prepares his toxin as follows : A virulent typhoid bacillus is grown on the surface of agar in plate bottles (fig. 22, p. 202) for 18–24 hours.

[1] *Journ. Exper. Med.* March 1, 1908.

At the end of this time 4 or 5 c.c. of sterile water are introduced into each bottle, and the growth is scraped off and emulsified. The suspension is decanted into stout glass test-tubes which are heated to 60° C. in a water-bath. The suspension is then centrifugalised, and the dead bacterial paste is collected and dried *in vacuo*. The dried bacterial mass is then ground up in an agate mortar, 3 grams with 1 gram of salt, for two or three hours, sterile water being added drop by drop until 5–6 c.c. have been added ; finally the ground-up mass is made up to 100 c.c. with water. The suspension is then heated in tubes in a water-bath to 60° C. for half an hour. The supernatant liquid is next decanted from the sediment and is poured slowly into ten times its volume of absolute alcohol, and the mixture is allowed to stand for three hours. The fluid is decanted, and the precipitate collected and dried rapidly *in vacuo*. The dry material is for use dissolved in water, 8–10 milligrams to each cubic centimetre, and forms the fluid for instillation into the eye, one drop being used. A reaction consists in the development of a conjunctivitis, commencing with redness two or three hours after instillation, and becoming slightly fibrinous six to ten hours after the instillation.

Chantemesse obtained the reaction in seventy cases of typhoid fever, but in one case only out of forty-nine cases of disease not typhoid. The one exception had had two years previously a continued fever of some duration and might therefore have had typhoid.

Meroni investigated the ophthalmo-typhoid reaction of Chantemesse in twenty-six cases. He found that a positive reaction might occur six hours after instillation in diseases other than typhoid, but that if the reaction is delayed for twenty-four hours it occurs only in typhoid. He considers the reaction to be of practical value though not absolutely reliable.

Hewlett and Goodall have tried an ophthalmo-reaction in typhoid fever, carried out with typhoid endotoxin prepared by the Macfadyen method (p. 50). The endotoxin was carefully prepared, so as to elimate so far as possible extraneous substances, and dried *in vacuo*. One per cent. solutions were used, and three different batches were employed. Of ten cases of enteric on which it was tried, five gave a marked reaction and five a slight reaction. Of ten cases not enteric, in one case of scarlet fever the reaction was very marked (probably accidental as it developed several days after application), in three cases of scarlet fever it

was slight, and in six cases (four of scarlet fever, one of indefinite pyrexia, and one normal person) there was no reaction.

Link has employed the cutaneous method, applying it to fourteen cases of typhoid and paratyphoid fevers. Killed cultures of *B. coli*, *B. typhosus*, and *B. paratyphosus* were prepared, and all three suspensions were used on all the patients. In general the reactions agreed with the agglutination tests, but on the whole were not altogether satisfactory.

Deeham has used a vaccine prepared from an agar culture and containing three billion bacilli per cubic centimetre of salt solution for carrying out a cutaneous reaction. In twelve cases of typhoid the reaction was obtained one or more days before the Widal. In eight control cases not typhoid the reaction was negative.

See Chantemesse, *Bericht ü. d. xiv. Internat. Kongress f. Hyg. u. Demog.* 1907, Bd. i. p. 205 ; Meroni, *Münch. med. Woch.* 1908, lv. p. 1379 ; Link, *ib.* p. 730 ; Hewlett and Goodall, *Proc. Roy. Soc. Med.* ii. 1908–1909, Med. Sect. pp. 253 and 257 ; Deeham, *Univ. of Pennsylvania Med. Bull.* August 1909

Mallein

Mallein is a fluid employed for the diagnosis of glanders and is a preparation analogous to the old

tuberculin (see p. 362), only substituting the glanders bacillus (*B. mallei*) for the tubercle bacillus. The shallow flasks of veal broth are inoculated with a *virulent* culture of the glanders bacillus, and grown for three to four weeks; the culture is then autoclaved, concentrated to one-tenth of its volume and filtered. The mode of preparation is practically precisely the same as for the old tuberculin.

Mallein is rarely if at all employed on human patients, and then only for diagnostic purposes (see also p. 388). It possesses little, if any, curative properties. Its chief use is for the diagnosis of glanders in the horse or other equines, and for this purpose it is admittedly the most certain test, especially in the early stages before the clinical signs are obvious.

The reaction consists in a rise in temperature above the normal, and in the formation of a local swelling at the seat of inoculation, as indicated in the following directions issued by the Royal Veterinary College, London.

DIRECTIONS FOR USING MALLEIN

1. While under the mallein test, horses ought to be left at rest in the stable and protected from draughts. The rectal temperature ought to be taken once or twice on the day before the test is applied.

2. The dose of mallein for a horse is 1 cubic centimetre, or 18 minims. It ought to be injected about the middle of the side of the neck, with a clean hypodermic syringe. The best form of syringe is one with

an asbestos piston, as the whole instrument may then be sterilised by boiling it in water for five minutes before use.

3. The mallein must be injected into the sub-cutaneous connective tissue, and care must be taken that the whole dose is actually introduced.

4. The temperature must be taken at the time of injection, and at the 9th, 12th, and 15th hours afterwards.

5. Provided the temperature were normal (under 101° F.) before the injection, it will rise 2° or more (to 103°–105°) during the next fifteen hours if the horse is glandered, but it will remain practically unaffected (under 102°) if the horse is not glandered.

6. Attention must also be paid to the swelling that forms at the seat of injection. When the horse is glandered this goes on increasing in size during the second twenty-four hours after the injection, and it seldom declines before the third or fourth day. The maximum diameter of this swelling in glandered horses varies from 5 to 10 inches.

7. In horses that are not glandered the local swelling attains its maximum size during the first fifteen hours, and by the twenty-fourth hour it has almost entirely disappeared. Its maximum diameter is usually about 3 or 4 inches.

8. When the temperature gradually rises from the normal to 104° during the first fifteen hours, and a large slowly disappearing swelling forms at the seat of injection, the horse may confidently be declared glandered.

9. If, with a normal temperature at the time of injection, a horse displays only the temperature reaction, or only the local reaction, the case must be considered doubtful, and the test repeated after the lapse of a week.

10. When the temperature is 102° or more at the time of injection the temperature reaction is unreliable, but in such a case the diagnosis may be based on the characters of the local swelling.

11. The mallein should be kept in a cool place and protected from light. Should it lose its transparency or become cloudy, it must not be used.

The proper dose, as indicated in the directions issued with each brand, should of course be given.

Ophthalmo-reaction.—An opthalmo-reaction, using diluted mallein, is stated to have given good results in the diagnosis of glanders in horses and has the advantage that there is no need to watch the temperature. Martel[1] has reported four cases in man which gave a positive reaction with mallein diluted to one-tenth of its ordinary strength.

[1] *Berl. klin. Woch.* 1908, Bd. xlv. p. 451.

CHAPTER X

SOUR MILK

CAUSE OF SENILITY—MICRO-ORGANISMS OF THE DIGESTIVE TRACT—INHIBITION OF THE GROWTH OF PUTREFACTIVE FORMS BY LACTIC ACID—BULGARIAN AND OTHER SOUR MILKS— THE BULGARIAN BACILLUS OR BACILLUS OF MASSOL—PREPARATION OF SOURED MILK— THERAPEUTIC USES OF SOURED MILK—LACTIC CHEESE, TABLETS, &c.

Metchnikoff in a study of the nature of senility formulated the hypothesis that it is caused, in part at least, by an auto-intoxication with toxic substances produced by the action of micro-organisms in the digestive tract. These substances are partly derived directly from the *putrefactive* decomposition of proteins, others are formed by the secondary action of other organisms, *e.g. Bacillus coli*, on normal digestive products, or on the derivates of the putrefactive decomposition of proteins. The products which are formed in the putrefaction of proteins (in addition to proteoses, peptones, and amino-acids) are bodies belonging to (*a*) the phenol

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group, to which tyrosin, the aromatic oxyacids, phenol, and cresol belong ; (b) the phenyl group, including phenyl-acetic and phenyl-propionic acids ; and (c) the indol group, which includes indole, skatole, and skatolcarboxylic acid. In addition, many volatile fatty acids and sulphur compounds are formed, and probably also alkaloidal substances or ptomines.[1] In the putrefactive decomposition of proteins 'pressor' substances (i.e. substances which raise the blood pressure) are also formed (Taylor and Dixon, Rosenheim, Barger and Walpole[2]), e.g. iso-amylamine, penta-hydroxy-phenylethylamine and 'urohypertensine' (Abelous). Now it is common after middle life to find increased arterial tension and injections of adrenalin (also a 'pressor' substance) induce arterio-sclerosis experimentally. That derivatives of intestinal putrefaction may cause an auto-intoxication and arterial degeneration appears likely, and since a person is 'as old as his arteries' the condition of senility may well be due to excess of putrefactive products formed in the intestine.[3]

[1] See *Text-Book of Physiological Chemistry*, Hammarsten (Trans. by Mandel, 4th ed. 1904), pp. 21 and 503.
[2] See *Journ. of Physiol.* 1909 and 1910.
[3] Many cases of arterial degeneration and senility will, of course, be due to other causes ; the syphilitic toxin for example.

The putrefactive decomposition of proteins is largely brought about by *anaërobic* micro-organisms, and a large part of the intestine presents practically a strictly anaërobic condition. If an animal be fed with methylene blue and then killed, the contents of the intestine will be colourless from the middle of the small intestine onwards, indicating the absence of free oxygen.

The bacterial flora of the child's intestine essentially differs from that of the adult. In the child's dejecta Gram-staining microbes are relatively scanty and are mostly *B. bifidus* and *B. acidophilus*, both lactic-acid producing bacilli. In and after middle life Gram-staining forms usually become more and more numerous, the Gram-positive species now being principally *B. putrificus* and *B. welchii*, both of which provoke a putrefactive decomposition of proteins.[1] *B. coli* also becomes more numerous in the adult and elderly, and forms indole and bodies belonging to the phenol series. In seeking for some agent which would inhibit the growth of these undesirable bacteria, Metchnikoff conceived that lactic acid would probably effect the end desired since the growth of these organisms is inhibited by a moderate percentage of

[1] See Herter, *Bacterial Infections of the Digestive Tract*, 1907.

this acid. Simply to administer lactic acid, however, will not altogether effect this end, for it will be absorbed before reaching the large intestine. Metchnikoff therefore sought for some means by which the lactic acid might be formed *in situ*, and naturally resorted to the use of lactic acid producing organisms, which, if they can be established in the intestine, may produce sufficient lactic acid to inhibit the growth of the putrefactive and other undesirable forms. The problem, however, is not easy of solution, for it involves the use of organisms which will flourish well at blood heat, and the ordinary lactic ferments of milk do not, their optimum growth temperature being about 70°–80° F. Metchnikoff noted that in parts of Bulgaria certain peculiar soured milks, Yoghurt and Maya, form a staple article of diet, and that the peasants consuming them frequently live to an advanced old age. Investigation showed the presence of a peculiar lactic bacillus, which has been termed the *Bacillus bulgaricus* or bacillus of Massol, also the ' granule ' bacillus, capable of growing at blood heat ; indeed, it flourishes best at a temperature of 105°–110° F.

The use of soured milks is wide-spread in various parts of the world, in Sardinia

('Gioddu,' *B. sardous*), in Turkey and the near East, in Egypt ('Leben,' *Streptobacillus lebenis*), in Armenia ('Mazun,' *B. mazun*), in India ('Dadhi'),[1] and in South Africa. The various bacilli present in these sour milks all belong probably to one species, though they show some variation and form different races.[2]

The *B. bulgaricus* is a large, non-motile, non-sporing, Gram-staining organism, growing best in milk and culture-media prepared with milk or whey, and producing a relatively large amount of lactic acid. It is invariably associated in the sour milks with a lactic streptococcus. On these facts, the use of artificially prepared soured milks has been based. For the preparation of soured milk, the milk should be well sterilised by actual boiling for at least half an hour, preferably for an hour. It is then cooled to 40° C. (105° F.), inoculated with the lactic culture, and incubated at this temperature for twelve to twenty hours. Milk properly prepared should be well curdled without much separation of the whey, and possess a pleasant acid odour and an acid flavour.

Complete sterilisation of the milk to be treated

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[1] See Hewlett, *Nature*, April 7, 1910, p. 159.
[2] See Hewlett, *Brit. Med. Journ.* 1910, ii. (Bibliog.).

should be ensured, otherwise undesirable organisms may multiply and an improper, undesirable, or even unwholesome, product may result. Various apparatus [1] have been devised for the home production of soured milk, and the large dairy companies also supply the article in various forms.

As regards the culture employed for ' starting ' the souring, this should be a *fluid* one in milk or whey. It may contain one or more races of the *B. bulgaricus* and a lactic streptococcus which will grow well at blood heat, *e.g.* the *S. lebenis*. The addition of the streptococcus yields a better product, and it also aids the vigorous multiplication of the *B. bulgaricus* by producing an acid environment. Dry and tablet preparations are generally inefficient, as the *B. bulgaricus* rapidly dies out in the dry state, and are difficult to prepare without contamination. Although some are still sceptical as to the value of soured milk it can hardly be doubted that it is beneficial in many complaints. Even in comparatively well persons, the writer's experience is that it promotes a general feeling of ' well-being.'

[1] *E.g.* by Messrs. Allen & Hanbury. The writer's milk pasteuriser (made by Allen & Hanbury) can also be adapted for the purpose.

Herchell [1] defines the cases which are likely to be benefited as follows :

(1) Cases depending upon local irritation by the products of abnormal putrefaction of proteins in the intestinal tract, *e.g.* acute enteritis, entero-colitis, chronic colitis, mucous and muco-membranous colitis, and some forms of diarrhœa, especially in children.

(2) Auto-intoxication with the products of putrefaction, *e.g.* gradual failure of health, particularly in elderly people, some cases of skin disease, many cases of neurasthenia, malnutrition in children, some cases of anæmia, and some cases of arthritis, neuritis, and other obscure affections of nerve and muscle.

(3) Some forms of constipation.

(4) Before operations upon the intestine.

Minor ailments, such as lassitude, headache, dyspepsia, constipation and diarrhœa, rheumatic pains and the like, are frequently benefited. It must also be recognised that even if the soured milk as such does little good, it often enables an addition of valuable and easily assimilable food stuff to be made to the diet by its use.

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[1] *Proc. Roy. Soc. of Med.* iii. 1909–10 (Therapeutical Sect.), p. 51. Also 'Discussion on Sour Milk,' *Brit. Med. Journ.* 1910, ii. (Grünbaum, Hewlett, Bryce, Harley, Sahli, and others).

On the other hand, soured milk sometimes disagrees, it often tends *at first* to constipate, and has been stated occasionally to give rise to rheumatic pains, lumbago, &c., though many of the last-named cases and cases of rheumatoid arthritis improve under it.

Sour milk is contra-indicated in acid dyspepsia and in acid fermentations in the intestine. This condition may be recognised by the fact that the stools are *acid*, putrefactive stools being *alkaline*. Strasburger's fermentation apparatus may be usefully employed.[1] In healthy stools Gram-negative organisms preponderate, in abnormal putrefactive conditions Gram-positive organisms will be in excess. There can be no question that soured milk is by far the most efficient form by which to administer the lactic organisms. One pint may be taken daily, divided into two or three portions.

If soured milk disagrees, soured whey can often be substituted with advantage, and is an efficient preparation if the fattening qualities of soured milk are undesired.

Tablets, &c., are comparatively inefficient : whereas 1 c.c. of soured milk will probably contain at least 1,000,000,000 *B. bulgaricus*,

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[1] Herchell (*loc. cit.*), p. 56.

a tablet may contain only 1000 or 100 organisms,[1] and tablets generally contain other organisms and are therefore not suitable as 'starters' for the preparation of soured milk. The same applies to lactic chocolates. Lactic cheeses may be of some use, they form a pleasant variation of the diet, but must be consumed quite fresh.

[1] See Quant. *Brit. Med. Journ.* 1909, ii. p. 1738, and Hewlett, *ib.* (*loc. cit.*).

APPENDIX

1.—*Weights and Measures.*

I cubic centimetre (I c.c.) = 16 minims nearly.
10 cubic centimetres . = 2½ fluid drachms nearly.
I litre . . . . = 35 fluid ounces nearly.
I pint . . . . = 4/7 litre or 568 c.c.
I gram . . . = 15½ grains nearly.
I gram of dry serum . = 10 c.c. of fluid serum.

2.—*Physiological Salt Solution.*

This is a 0·7–0·8 per cent. solution of sodium chloride in distilled water (sometimes called ' normal saline solution ').

3.—' *Normal* ' *Solutions.*

By a ' normal ' solution is meant the equivalent weight, in grams, of a substance dissolved in (*i.e.* made up to) a litre of distilled water; a ' deci-normal ' solution $\left(\frac{N}{10}\right)$ contains one-tenth of, a 'centi-normal' $\left(\frac{N}{100}\right)$ one-hundredth of, a ' deka-normal ' (10N) ten times, this amount. Thus, a normal solution of caustic soda contains 40 grams of pure NaOH (NaOH = 40), caustic soda being monobasic ; a normal solution of sulphuric acid contains 49 grams of pure H_2SO_4 $\left(\frac{H_2SO_4}{2} = 49\right)$ per litre, sulphuric acid being a dibasic acid.

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4.—*Deterioration of Anti-sera.*

All anti-sera undergo a progressive diminution in strength, which is probably much more rapid in the case of anti-microbic sera than of anti-toxic sera. This deterioration is hastened by a high temperature and by the action of light; all sera should therefore be stored in a cool, dark place. Dried sera keep better than the fluid sera. Diphtheria and tetanus antitoxins do not undergo any serious deterioration in a less period than six or nine months (see also p. 67).

5.—*Firms supplying Antitoxins, Lactic Preparations, &c.*

Messrs. Allen & Hanbury, Vere Street, Cavendish Sq., W. (Agents for the preparations of the Lister Institute.)

* Messrs. Aplin & Barrett, Ltd., Yeovil.

Messrs. Burroughs Wellcome, & Co., Snow Hill Buildings, Holborn Viaduct, E.C.

* Messrs. Clay, Paget & Co., 71 Ebury Street, S.W.

Messrs. Meister, Lucius, & Brüning, 51 St. Mary Axe, E.C.

Messrs. Merck, 16 Jewry Street, E.C.

* Messrs. Oppenheimer, Son & Co., 179 Queen Victoria Street, E.C.

* Messrs. Parke, Davis, & Co., Beak Street, Regent Street, W.

* Dr. Renner, 75 Upper Gloucester Place, N.W.

* Messrs. Welford & Sons, Elgin Avenue, W.

Messrs. Willows, Francis, Butler, & Thompson, 40 Aldersgate Street, E.C.

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* See advertisements at end of the book.

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