ment is carried out. Modern operative technique only gives a low mortality, and in 467 cases which have come under my supervision during the twenty-year period 1915-1935, I have lost twenty-six, a mortality of 5.5 per cent. This corresponds almost exactly with some American statistics, for Fowler of Brooklyn has reported a mortality of 5.9 per cent. in 1,206 cases, while Erdmann of New York reports a record of 525 operations with a mortality of fifteen cases, equalling 2.85 per cent. Thus this operation, which had a high mortality thirty years ago, may be said to have an extremely low one at the present time.

Analysis of 505 Cases of Cholecystectomy, 1915-35

Total Mortality: 7.3 per cent.

Diagnosis	Number of Cases	Sex	Deaths	Per-centage	Total
Acute cholecystitis ...	26	Females 21 Males, 5	5 1	23.8 20.0	23 %
Chronic cholecystitis ...	467	Females, 386 Males, 81	21 5	5.4 6.2	5.5 %
Malignant cholecystitis	10	Females, 8 Males 2	4 1	50.0 50.0	50.0 %
Torsion of gall-bladder...	1	Female	0	Nil	Nil
Double gall-bladder ...	1	Female	0	Nil	Nil

Malignant Disease of the Gall-bladder

Carcinoma of the gall-bladder is in itself a rare disease, but it would be excessively rare if patients with chronic cholecystitis were more often submitted to operation, for fully 95 per cent. of cases of malignant disease of the gall-bladder are produced by calculi. Ten cases of malignant disease of the gall-bladder have come under my care, and in all these calculi were present. Most of these were operated upon under the mistaken diagnosis of chronic cholecystitis with gall-stones, and it is a curious fact that the operation is usually quite easy, and there is no suspicion of malignant disease until the gall-bladder is opened and submitted to histological examination. We shall only come to know the true incidence of carcinoma of the gall-bladder when every operation specimen is submitted to full histological examination.

FIG. 5.—Gall-stones which had ulcerated through the gall-bladder wall into the duodenum and become impacted in the lower ileum.

Diseases of the gall-bladder, which in the main are due to infection, are quite common, and can only be cured by surgical measures. Radical operation gives a low mortality rate and results in a cure of the condition in most cases; it prevents innumerable complications and sequelae in later life, not to mention malignant disease.

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**SOME IMMUNOLOGICAL ASPECTS OF
THE CURE AND PREVENTION
OF DIPHTHERIA ***

BY

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BACTERIOLOGIST TO THE COUNTY OF STAFFORD

From the clinical point of view this subject can be limited to the consideration of passive and active immunization and the immunological accidents attending these procedures. In passive immunization ready-made antitoxin is introduced into the patient's circulation. In active immunization the patient manufactures his own antitoxin as the result of natural or arranged antigenic stimuli. Passive immunization is of short duration, and is used for treatment. Active immunization stimulates a tissue habit which lasts for years, and is the ideal prophylactic.

The theme of this address centres round a patient who died of acute anaphylaxis nearly a quarter of a century ago. A boy, aged 9 years, suffering from scarlet fever, was admitted to an institution. As cross-infection was prevalent he was given on admission 500 units of diphtheria antitoxin intramuscularly. The acute course of the scarlet fever was uneventful. Three weeks later he was allowed up and was transferred to a convalescent ward. A week later he developed acute faucial diphtheria. He was then ordered 2,000 units of diphtheria antitoxin. Immediately after the injection he showed symptoms of acute respiratory distress and collapsed and died in fifteen minutes.

Active and Passive Immunization

The prophylactic use of antitoxin was more prevalent then than now. Many years previously attention had been drawn to the temporary nature of passive immunization. Globulin solution remains slightly longer in the blood than unconcentrated serum, but it is doubtful if any form of antitoxin is retained in sufficient quantity to prevent infection three weeks after administration. Its use for this purpose merely gives a false sense of security; sensitizes the patient and increases the risk of unpleasant reactions if he should afterwards need serum treatment; may lead to a higher carrier rate; and may delay the development of natural active immunity. Some people recommend during an epidemic an initial dose of 1,000 units of antitoxin followed by active immunization fourteen days later, but there are scientific objections to this procedure. As one antigen now available can produce a good response in fourteen days it is better to segregate and actively immunize the susceptibles at once if their swabs are negative. Those showing positive swabs may be virulent carriers—if so, they are already immune; or they may be incubating the disease—if so, clinical signs will soon appear and antitoxin can then be injected. The old objections to active immunization during an epidemic do not hold if toxoid preparations are used. A small number may develop the disease before protection is established, and parents should be warned of this possibility.

* Read before the North Staffordshire Medical Society, June 6th, 1935.

Dosage of Antitoxin

You will notice that the patient quoted received 2,000 units of antitoxin when he developed diphtheria. Unfortunately these small doses are still often given for economic reasons. The size of the dose to some extent depends on the day of disease and the severity of the case. Toxin is rapidly fixed by the tissues and cannot then be easily dissociated. It is important to administer antitoxin at once in every case of suspected diphtheria without waiting for removal to hospital or for bacteriological diagnosis. A small proportion of diphtheria patients may have an initial negative swab, and in some instances a report may not be available for forty-eight hours. If the infection turns out to be due to yeasts, streptococci, pneumococci, or Vincent's organisms, the protein associated with the antitoxin often produces beneficial effects. Mildness of symptoms does not justify waiting or the administration of a very small dose of antitoxin, for a patient with slight initial symptoms may become gravely ill later.

The minimum dose for a child should be 8,000 units, and 100,000 units or more should be administered in severe cases. These large doses can now be concentrated in very small bulk. Concentration is based on the fact that in the horse antitoxin is associated with the pseudoglobulin fraction of the serum, which can be isolated from the troublesome euglobulin by salting out or by electrodialysis. These methods have reduced the volume of 10,000 units from 50 c.cm. to 4 c.cm. or less. As well as the fatalities which result from under-treatment, an increase in the incidence of paralysis is a notable feature, and it is a truism of antitoxin treatment that it is easier to give too little than it is to give enough.

Some contend that antitoxin is useless after the fifth day of disease, but patients seen for the first time after this day ought to have it if toxin is still being elaborated. In mild and moderate cases it is injected intramuscularly. In severe cases the intravenous route is used, but the patient should be desensitized if he has previously received antitoxin manufactured by the same species of animal. The serum should be warmed before intravenous administration. Some authorities recommend intraspinal injections in special cases, but although increased protein and a leucocytosis have sometimes been found in the cerebrospinal fluid of patients suffering from diphtheritic paralysis, the administration of antitoxin in this way is not necessary if it is given early by the usual routes. It has been stated that antitoxin has little or no effect in "gravis" infections, but this seems to be merely a local strain peculiarity, as some "gravis" epidemics respond well to the ordinary antitoxin at present on the market.

It is useless taken by the mouth or per rectum, and absorption is very slow by the subcutaneous route. By intraperitoneal inoculation antitoxin does not reach its maximum in the blood until thirty hours after administration. In this country it is the custom to give the total antitoxin in one dose. Stocks should be stored in a dark, cool place, but should not be frozen, as this leads to rapid deterioration. Sound antitoxin treatment implies three maxims: (1) give it early; (2) give sufficient; (3) give the total amount in one dose by a route through which it will be easily absorbed. Simple injunctions, far removed from counsels of perfection, but often ignored by some of us.

Anaphylaxis and Serum Sickness

The use of concentrated antitoxin has reduced the ill-effects which follow serum injections, but they are still sufficiently common to require mention. These can be discussed under the headings of anaphylaxis and serum sickness.

Anaphylaxis may be here confined to the unpleasant symptoms which follow repeated injections of protein substances in subtoxic or non-toxic amounts, a suitable interval elapsing between the first or sensitizing dose and the second or assaulting dose. The minimum incubation period is usually from seven to ten days before the patient becomes sensitized from the first injection. The period of maximum sensitization, although usually from three to six weeks, varies with the size of the dose and other factors. The larger doses prolong the incubation period.

All sensitizing or assaulting substances are antigens—that is, they must be capable of producing antibodies or they must be the portions of antigens known as haptenes, which are capable of combining with antibodies. The anaphylactic reaction appears to be the result of antigen-antibody combination, which has selective effects on the cells of unstriated muscle and on the capillary endothelium. The effects depend to some extent on the anatomical distribution of plain muscle, as is well shown by the diversity of the findings in different species of animals. In the guinea-pig the predominant symptom is bronchospasm; in the dog, liver derangement owing to spasm of the muscular coat of the hepatic veins; and in the rabbit, respiratory arrest due to spasm of the pulmonary vessels. Human anaphylaxis resembles that in the guinea-pig, and patients often complain of a peculiar sensation of constriction in the chest after administration of the second dose of serum. This symptom also occurs as an allergic phenomenon. In anaphylactic shock the blood changes common to all species are: diminished coagulability; increased concentration of protein and red cells; pronounced leucopenia; and reduction of complement.

Opinions differ as to how anaphylaxis is produced. The humoral theory is obsolete. The reaction is too explosive to be explained by ferment action. Dale¹ (1912) has suggested that the antigen-antibody union upsets the colloidal state of the cells, leading to intracellular precipitation. Doerr² (1922) thought that the union and precipitation occurred on the surface of the cells. Lewis³ (1927) has shown the importance of histamine in producing the capillary reaction of skin irritation. He believes that histamine, or a complex of similar activity, is present in normal body cells, and it is not difficult to imagine its liberation as the result of injury caused by antigen-antibody union. This conception of anaphylaxis brings it into line with shock resulting from grave destruction of tissue, such as that taking place in extensive burns, in which, if the theory is correct, there must be an extensive liberation of histamine. There is a remarkable similarity between histamine shock and the anaphylactic reaction, and this likeness extends to the peculiarities dependent on species. Animals resistant to anaphylaxis are also resistant to histamine shock. It is difficult to sensitize the white rat, which is 1,000 times more resistant to histamine than is the guinea-pig.

Acute anaphylactic shock ensues within a few minutes of the injection, and death may occur in from eight to fifteen minutes, but sometimes the symptoms may be more prolonged. Fatal anaphylaxis is rare in man, and most of the serious accidents follow the administration of antitoxin intravenously. Richet estimates the incidence at 1 in 300,000. During the war sixty cases of shock were reported in the British Army, but since eleven of these followed a first prophylactic injection of serum they cannot be classed as true anaphylaxis. Forty-nine cases of shock with twelve deaths were reported as resulting from antitoxin treatment of tetanus, but intravenous injection accounted for the majority of these accidents (Rouse, 1921). In England alone at least two million antitoxin injections were given during the war.

Prevention of Anaphylaxis

The fear of anaphylaxis must therefore not curtail treatment. With intramuscular administration the risk is infinitesimal. With intravenous injection the risk is slightly greater, and the patient should be desensitized. The following methods are available:

1. The intradermal or subcutaneous injection of minute doses, or the continuous or intermittent administration of very dilute antigen. It is best to begin with 0.01 c.cm. of serum, increasing the dose every half-hour so that a 1 c.cm. dose is given at the end of four hours. The full amount required for treatment can then be given three hours later.
2. Horse serum diluted 1 in 10 with distilled water and heated to 80° C. desensitizes without risk of shock, the initial dose being 1 c.cm.
3. The various narcotics. They are, however, dangerous in diphtheria.
4. Atropine 1/200 to 1/100 grain, and adrenaline 5 to 15 minims of 1 in 1,000 solution subcutaneously, and the intravenous injection of sodium chloride, may also be employed.
5. Non-specific desensitization. Sensitive guinea-pigs can be protected against shock by the prior injection of normal guinea-pig serum, so in man the previous injection of normal human serum may have similar effects.

Serum sickness may be revealed by:

1. Acute fatal shock, which is rare. This is characterized by fainting, dyspnoea, collapse, and sometimes by vomiting, diarrhoea, and cough. Oedema of the face, throat, and lungs may also be present. It follows serum administration immediately.
2. The more common and less serious form manifested by (a) scarlatiniform, morbilliform, and urticarial skin eruptions; (b) associated fever; (c) swelling of the joints; (d) enlargement of glands; (e) albuminuria; (f) sometimes hypocholesterolaemia. This milder form comes on six to fourteen days after the administration of serum, and may recur after intervals of two to five days, sometimes showing two or three recurrences. Recovery is the rule.

Occasionally the period of onset of the two forms is reversed. The acute attack occurs in about 1 in 100,000 cases. The exanthematous variety affects 20 to 90 per cent. of those who have had a single injection of serum. The incidence of serum sickness is commoner in patients who have received serum previously, and then the incubation period of the exanthematous form is reduced to four days or less. The immediate shock is probably due to idiosyncrasy, but the delayed symptoms may be regarded as a variety of anaphylaxis. The severity of serum sickness has been reduced by the use of concentrated serum, but it is still fairly common. The administration of soluble calcium salts also lessens the tendency to it.

Natural Immunity

A proportion of human beings acquire active immunity as the result of natural infection. Such people give a negative Schick reaction when the antitoxin in the blood is in the region of 1/30 of a unit per cubic centimetre. The Schick toxin dose used in the test is usually 1/50 M.L.D. Some authorities, however, suggest that smaller amounts of antitoxin in the blood will lead to a negative Schick reaction, and it is difficult to be dogmatic on the point owing to the many variables involved, not the least of which is the variation in combining power of different toxins.

Gröer and Kassowitz⁴ worked out the percentage of humans of different ages who showed antitoxin in the blood. At birth it was present in detectable amounts in 84 per cent. of infants. There was a rapid fall to 32 per cent. at 9 to 18 months, and the lowest figure (28 per cent.)

was reached at 2½ to 3½ years. Thereafter there was a gradual rise, which reached the maximum at 17 to 18 years. The antitoxin present in the blood at birth is due to passive immunization, being transmitted through the placental circulation. Later the child receives it through the mother's milk, and breast-fed infants have a higher blood antitoxin than bottle-fed children. This fact does not contradict the statement that antitoxin by mouth is of no use for older children, as the physiological conditions and other circumstances are different. The Schick test is no criterion to immunity in newly born infants, as the skin does not react allergically until later, and this explains the apparent inconsistency of Schick-positive mothers giving birth to Schick-negative children. The danger period for infection lies between the first and fifth years. At or after the fifth year active immunization due to subliminal infection begins to make itself felt, and the ratio of immunes to susceptibles increases, and this is why it is customary to immunize all children under 5 years of age without a preliminary Schick test.

The degree of natural immunity depends on the extent of contact with *C. diphtheriae*, and this in turn varies with age, density of population, contact with known cases, and increase in the immunity rate. Increase in the immunity rate may mean increase of virulent carriers, who may then mix freely with the susceptibles without being detected, and may either immunize or give them the disease. Enlarged tonsils and adenoids impede natural immunization.

Principles of Artificial Immunization

Larval diphtheria plays a part in the spread of infection and of immunization. It is a mild infection without toxic symptoms, and may pass unnoticed. Nasal infection often assumes this form.

Early workers tried to induce artificial immunity with ordinary toxin, but it proved too potent to be injected into human beings in sufficient amount to be of value. When Theobald Smith⁵ in 1907 demonstrated that the addition of antitoxin did not inhibit the antigenic powers of toxin the way became clearer. Von Behring⁶ (1913), and Park⁷ in the same year, introduced toxin-antitoxin mixtures. In 1923 Schroder and Park⁸ introduced a new mixture containing a reduced amount of toxin. It has since been found that modified toxin or toxoid has antigenic powers. Toxoid is toxin minus the toxic properties. Such toxoid appears spontaneously in toxin kept under suitable conditions. It can be prepared by the addition of formaldehyde to toxin. Ramon⁹ (1923) applied the term "anatoxine" to formalized toxin, but as this substance is no different from toxoid this term is not in favour in this country. Sodium ricinoleate has been employed for detoxication, but it merely masks the toxic effects, which may become evident on dilution.

When toxin or toxoid and antitoxin are mixed in equivalent proportions a precipitate is formed which is comparatively free from deleterious proteins. These floccules contain only 1/50 of the nitrogenous material present in the original mixture, and are very useful for immunization. Ramon¹⁰ (1926) has demonstrated that irritating substances, such as tapioca, increase the antigenic activity of anatoxine. Glenny, Pope, Waddington, and Wallace¹¹ (1926), and Glenny and Waddington¹² (1928), have proved that the addition of potassium alum to toxoid has similar effects. Toxin-antitoxin mixture is not now used in England, as it is rather risky, owing to the occurrence of dissociation of the toxin. Toxin-antitoxin floccules are not in favour, as this mixture is not neutral to animals at the point where specific flocculation first takes place. The discarding of these toxin preparations has eliminated those fatal and serious accidents which still arise on the Continent.

Methods of Immunization

Artificial active immunization can best be induced in the following ways:

1. Toxoid-antitoxin: in some preparations goat's serum is the vehicle for the antitoxin, so that patients are not unnecessarily sensitized against future injections of horse serum, although there is no definite evidence that sensitization can occur with such small doses. Toxoid-antitoxin mixture is especially recommended for younger children when it is not convenient to do preliminary testing and when reactions must be avoided at all costs.

2. Formol-toxoid is recommended for routine use in children where the Schick and Moloney tests can be carried out, for it may cause severe reactions in Moloney-positive children, especially those over 8 years old. This liability increases with age, so that it is not a suitable preparation for adults unless they give a definite negative Moloney reaction. Formol-toxoid should not contain less than 20 to 35 Lf.

3. Toxoid-antitoxin floccules are the method of choice for adults such as doctors and nurses, etc.

(These three preparations are given in three doses of 1 c.cm., usually at weekly intervals, but much better results are obtained by extending the interval to two or three weeks, especially that between the first and second doses. When reactions are likely with formol-toxoid some authorities recommend the initial injection of 0.1 c.cm. forty-eight hours before the first immunizing dose. In contrast to passive immunization, the subcutaneous route is used, as slow absorption and excretion are desirable.)

4. Alum-precipitated toxoid is the latest addition to the list. Its high immunizing value was discovered in the Wellcome Research Laboratories in 1926. One injection of 1/2 to 1 c.cm. of alum-precipitated toxoid immunizes well, in contrast to the three doses which are needed of the other preparations. It may cause severe local reactions in the form of induration or abscesses, and these are most apt to appear in children over 2 years of age and in adults. Attempts to eliminate these reactions reduce its immunizing efficiency, but at the present time investigations are being carried out to see how far the unpleasant manifestations can be reduced without seriously impairing the efficiency of the product. A reduction of the dose has been suggested as a way out, for it has been found that two small injections of 0.1 c.cm. each give a higher and more rapid immunity than one dose of 1 c.cm. This preparation can sometimes actively immunize in fourteen days, and may prove useful when it is desired to raise the threshold of protection quickly, as in epidemics. It must, however, be emphasized that one-shot immunization with alum-precipitated toxoid must not be used for older children or adults when they are Moloney-positive. In these cases, as already indicated, T.A.F. is the preparation of choice. Some authorities suggest that pronounced Moloney-positives may be left alone, as even though Schick-positive they have some immunity against diphtheria.

In carrying out active immunization care must be taken to exclude the introduction of pathogenic micro-organisms, and the needles and syringes should be thoroughly sterilized. Serious local and general reactions may result from neglect of this precaution, and a valuable preventive measure may thus be undeservedly discredited. On an average 95 per cent. of artificially immunized children are still negative when Schick-tested three to five years later. The figures vary with the preparation used. The order of antigenic efficiency is: (1) alum-precipitated toxoid; (2) formol-toxoid; (3) toxoid-antitoxin floccules; (4) toxoid-antitoxin mixture.

Variations arise with the same preparation in different districts both in the incidence of infection after immuniza-

tion and in the percentage of resulting immunes as confirmed by the Schick test. Allowing for differences in technique and errors in diagnosis there are times when other explanations must be sought for these discrepancies. Differences in local strains may be responsible. Some think that "gravis" and "intermediate" infections are more likely to break through the immune barrier than "mitis," but in Staffordshire I have recently examined swabs from five children who developed diphtheria after previous immunization, and in all these instances typical "mitis" was recovered. No doubt differences in immunological reactions of different individuals play a part. It is known that some adults are entirely refractory to immunization, and it is a peculiar fact that although some of these remain Schick-positive all their lives they never contract the disease. Their protection seems to be independent of antitoxin, and it has been suggested that it is hereditary.

The immunology of diphtheria is a vast subject, and it is only possible to touch the fringe of it. The modern progress made in the treatment and prevention of this disease stands to the credit of those great laboratory workers whose researches from 1880 onwards have extricated our conceptions from the realms of empiricism. They deserve at least the passing tribute of a thought.

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G. Loewy (*Bull. et Mém. Soc. Nat. de Chir.*, June 8th, 1935, p. 739) reports a case of cancer of the oesophagus, in which, after gastrostomy, normal feeding was resumed by mouth following dilatation of the oesophagus. The patient, a man of 57 years, had suffered from progressive dysphagia for some time, and was losing weight rapidly. Radiography and oesophagoscopy showed that the oesophagus was obstructed by a tumour, which biopsy proved to be a squamous epithelioma. A gastrostomy was carried out, and ten months later the patient was re-admitted to hospital for radium treatment and dilatation. The latter was a difficult procedure, but after the first dilatation the patient was able to swallow fluids, and after the second the passage was 9 mm. in diameter and allowed the swallowing of purées. Immediately after the normal feeding by mouth had become possible the patient gained rapidly in weight. After fourteen dilatations he was able to eat any form of food, and his mental outlook had completely changed. It would appear that mastication and deglutition are necessary for the functioning of the digestive tract and for the requisite nutrition. Another point of interest in this case was the slow development of the lesion. The danger of dilatation by fissure or rupture does not appear to be great, as in 137 dilatations there have only been two fatalities, and in another series of cases from the Mayo Clinic there were only nine deaths out of 794 patients treated by dilatation for cancer of the oesophagus.