"THE TREATMENT OF DIPHTHERIA BY REFINED DIPHTHERIA ANTITOXIN".

WITH PARTICULAR REFERENCE TO THE INCIDENCE OF SERUM REACTIONS.

THESIS FOR DEGREE OF M.D., PRESENTED BY:

ALEXANDER HUTCHISON, M.B., Ch.B., F.R.F.P.S., D.P.H.

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789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

C O N T E N T S.

CONTENTS (Contd.) PAGE.

CONTENTS. (Contd.)

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Since 1931, when Anderson et al. published their account of the three different types of c. diphtheriae, much work has been done on this subject in Britain and also in other countries in the world. It was soon realized that the percentage incidence of the various types varied in different parts of Britain in the same year, and that they also varied from year to year. Comparative figures for the same areas in different parts of the world were difficult to obtain.

Table showing Different Percentage Incidence in the Same year in , Different Areas throughout the World.

Area	Year	Percentage Gravis	Percentage Intermedius	Percentage Mitis	Percentage Aberrant
Glasgow (Carter 1936)	1935	5.8	65.1	24.1	4.96
Dundee (Murray)	1935	6.0	54.2	39.8	-
Liverpool (Private Communication)	1935	50.0	26.0	24.0	-
Manchester (Robinson and Marshall) ...	1935	50.9	19.9	27.8	1.4
Romford (Tannahill 1936)	1935	63.2	21.7	10.5	4.45
Cork (Cooper et al.1936)	1935	91.3	3.7	3.7	1.2
Berlin (do.)	1935	78.2	8.8	9.2	3.8
Maryland, U.S. (Whitley)	1935	6.3	1.8	91.9	-
Iowa, U.S. (Wright and Christison)	1935	9.1	-	81.8	9.1
Australia (Anderson et al.1936) ...	1935	52.2	-	47.8	-

Table showing Different Percentage Incidence in the Same Area in different years.

Area	Year	Percentage Gravis	Percentage Intermedius	Percentage Mitis	Percentage Aberrant
Glasgow (Carter 1933)	1933	3.1	59.2	36.5	1.17
Glasgow (Carter, 1936)	1935	5.8	65.1	24.1	4.96
Manchester (Robinson and Marshall)	1934	23.8	54.6	19.2	2.4
Manchester (Robinson and Marshall)	1935	50.9	19.9	27.8	1.4
Liverpool (Private Communication)	1935	50.0	26.0	24.0	-
Liverpool (Do.)	1937	34.2	24.7	41.1	-
Hull (Leete et al)	1933	59.4	29.0	8.7	2.3
Hull (M.O.H. Report)	1938	21.4	68.4	10.2	-

It was also recognised that the severity of the clinical case did not always coincide with the isolation of the gravis strain, as had been suggested by Anderson et al. (1931), though in some areas this was the case, while in others the intermediate type was associated with the more severe clinical type of diphtheria.

Severity of Clinical Type of Diphtheria assessed on Percentage Death Rate.

	Percentage Death Rate		
	Gravis	Intermedius	Mitis.
Glasgow (Carter,1933)	-	7.8	7.5
Dundee (Murray,1935)	-	11.1	3.4
Manchester (Robinson and Marshall 1934)	13.8	15.7	2.6
Hull (Leete et al., 1933)	19.4	5.7	3.8
Leeds (Cooper et al. 1936)	6.3	4.9	1.4
Berlin (do.)	13.2	9.4	-
Australia (Anderson et al., 1935)	26.0	-	7.9
Port Elizabeth, S.A. (Emmerson, 1937) ..	11.1	100.0	2.3

In Glasgow, from the reports by Carter (1933 and 1936) and the Medical Officer of Health's Report, 1935 (Knightswood Hospital),

it was observed that the percentage incidence of the gravis strain was increasing in the Glasgow area.

As the literature on the severity of the clinical case correlated with the strain of c. diphtheriae recovered from the case was not extensive, an investigation was commenced in Belvidere Fever Hospital, Glasgow, in 1937, to correlate the strain of c. diphtheriae recovered from each clinical case of diphtheria with the clinical severity of the case. At the same time as the investigation commenced, Messrs. Burroughs Wellcome placed at my disposal a highly concentrated diphtheria antitoxin, which, they claimed, greatly reduced the incidence of serum reactions when it was administered. This serum is called "Refined Diphtheria Antitoxin".

As this antitoxic serum was claimed to cause no reaction after its administration, each case was kept under observation to determine whether or not this was the case, and to observe the time and type of serum reaction (if any). For comparison, a series of cases of diphtheria treated with Burroughs Wellcome "concentrated" (Banzhaf and Gibson, 1907, and Banzhaf, 1912-13) diphtheria antitoxin was also investigated. This involved an examination of the case records of all cases of diphtheria which had received Burroughs Wellcome concentrated diphtheria antidiphtheritic serum between the years 1930-1936 inclusive. From the case record there was noted the age, sex, day of disease, the amount and route of administration of the serum, the time taken for the serum reaction, if any, to be manifest, and the clinical type of serum reaction.

1,000 cases of diphtheria were treated to a conclusion. 150 received the antitoxin by intravenous injection, 250 by combined

intramuscular and intravenous therapy, and 600 by intramuscular injection alone. Four-hourly observations were made upon the temperature, pulse and respirations, the rate of disappearance of toxaemia, membrane and/or discharges, and complications.

The 1,000 cases were swabbed by myself and then inoculated on Loeffler's medium, and on to Kerrin and Gaze (1936) media plates, where the different colonies were identified. From these colonies the biochemical and haemolytic properties of the different types of c. diphtheriæ were investigated.

All the laboratory work, including the preparation of the media, was done by me.

This thesis is presented in three sections:-

(1) A survey of the methods used to concentrate diphtheria antitoxin.

(2) Serum reactions: Their incidence and manifestations, together with a comparison of their occurrence and clinical findings following the injection of concentrated and refined diphtheria antitoxin.

(3) An analysis of the results of treatment of 1,000 cases of clinical diphtheria treated with refined diphtheria antitoxin.

SECTION 1.

A Survey of the methods used to concentrate diphtheria antitoxin.

Since the introduction of anti-Diphtheritic serum by Behring and Kitasato (1890) repeated efforts have been made to reduce to a minimum the content of non-specific substance. The early workers in in this field of medicine hoped to produce a pure antibody. In view of the clearer understanding reached on the nature of antibodies the work done has been to reduce the ratio of the antibody titre to the protein concentration of the resultant product.

The methods employed have been:-

(1) Non-Specific - fractional precipitation of the serum and adsorption.

(2) Specific methods depending upon the precipitation of the antibody by the specific antigen and extracting the compound.

(1) Non-Specific methods are divided into the following groups:-

(a) Variation of the salt concentration.

(b) Precipitation with alcohol.

(c) Adsorption.

(d) Cataphoresis.

(e) Enzymes.

(a) Brieger and Ehrlich (1893). They used a 27-30 per cent. solution of Ammonium Sulphate and their experiments were done upon milk from cows which had been immunized. From their results they concluded that Ammonium Sulphate in concentrations between 27-30 per cent. precipitated most of the antitoxin from the milk, and of the other salts they tried, Magnesium Sulphate came next in efficacy to Ammonium Sulphate in precipitating the antitoxin. The precipitate

obtained was dissolved in water, dialysed against flowing water, filtered and finally dried. The powder they obtained by this method had, weight for weight, 400 - 600 times the antitoxic strength of the original milk.

Brodie, 1897.

He was the first to prove that Diphtheria antitoxin was precipitated from an anti-Diphtheritic serum by any means that completely precipitated the Globulins in the serum. He made fractional precipitations of the serum, using as his precipitant Ammonium Sulphate. He found four different portions in the serum, all of which contained antitoxin in equal quantities.

Freund and Sternberg, 1899.

They found that by adding one-third in volume of a 5 per cent Potash alum solution to one volume of serum that all the antitoxin remained in the filtrate - the albumin was precipitated and the filtrate containing only the globulins contained all the antitoxin. The globulin was then precipitated by Ammonium Sulphate and so a concentrated antitoxin was produced.

Fuld and Spiro, 1900.

From experiments they determined that there were two globulin fractions - (1) one which was precipitated by a 28-33 per cent. solution of Ammonium Sulphate, and this they called "Euglobulin", and (2) one which was precipitated by a 34-46 per cent. solution of Ammonium Sulphate. They called this fraction "Pseudoglobulin".

Hiss and Atkinson, 1901.

They estimated the globulin content of the serum

of horses at various stages during their immunization against Diphtheria. The precipitant used was Ammonium Sulphate. They obtained the total protein content of the serum by heating and the albumin content was estimated by the difference. They found that the globulin content of the serum increased during immunization against diphtheria, and that the albumin fraction diminished. In 10 c.c's of a normal horse's serum they found the globulin content to be .3235 gramme. When this same horse had been immunized against diphtheria so that 10 c.c's of serum contained 120 anti-diphtheritic units, the globulin content had risen to .8987 gramme.

Pick, 1902:

From numerous experiments he concluded that the antitoxin was associated with the psuedoglobulins, but not with the euglobulins. This was the case when horses were immunized, but not for goats, where the conditions were the reverse.

Gibson, 1905-6:

He published a method whereby the protein content of a serum could be greatly reduced, and he claimed that he could concentrate a serum to at least double its original antitoxic strength. His method was:-

(1) Precipitation of the globulins with an equal volume of saturated ammonium sulphate solution.

(2) Filtration.

(3) Extraction of the residue with saturated sodium chloride solution, so re-dissolving the pseudoglobulin with the antitoxin.

(4) Filtration.

(5) Precipitation with half saturation of ammonium sulphate.

(6) Drying of the precipitate and dialysing in parchment.

(7) Sterilization of the resultant solution by double filtration through Berkefeld filters.

(8) Addition of .25 to .5 per cent. solium chloride and a preservative.

Ledingham. 1907:

He found that in a horse where the serum was low in antitoxic value the total protein content showed no tendency to increase during immunization. The slight increase found was due to a rise in the albumin content. During the immunization of a horse which produced a high grade antitoxic serum, the percentage globulin content of the total protein increased progressively. Finding that the euglobulin was more affected than the pseudoglobulin, he concluded that the pseudoglobulin contains most, if not all, the antitoxin, but that it seemed probable that this relationship only held good when the antitoxic content of the serum was steadily rising. Ledingham also found that in goats the antitoxic content of the euglobulin and pseudoglobulin fractions may vary at different periods in the course of immunization.

Gibson and Collins, 1907:

Experimenting with rabbits, goats and horses, and using various organisms such as B. diphtheria, Flexner manila dysentery, Shigas dysentery, etc., as antigens they found that there was great distribution of the antibodies in the different fractions of a serum and that the distribution of antibodies varied from time to time in

the same animal. They used ammonium sulphate to precipitate the various fractions and of ammonium sulphate they said that it was not a reliable means of differently classifying proteids, the precipitation characters of which are not widely separated.

Banzhaf and Gibson, 1907:

They produced a serum which was concentrated about 4-5 times its original antitoxic strength. The following method was used to separate the pseudoglobulin-antitoxin combination from the other serum proteins.

(1) The serum was diluted with one-third its volume by water, and then heated to a temperature of 57° C. for 15 hours or to a temperature of 58° C. for 7 hours. Ammonium sulphate was then added until its concentration was 30% of the whole volume of liquid. The precipitate formed (Fraction 1) was obtained by filtration.

(2) Fraction 1 was suspended in a saturated solution of brine. The solution was filtered and the filtrate containing the soluble pseudoglobulin plus antitoxin, which had been present in fraction 1, was made .25% acid with Glacial acetic acid. The ensuing precipitate was filtered, pressed and dialysed.

(3) To the filtrate from fraction 1 was added ammonium sulphate until it was in a concentration of 54% and the precipitate thus formed was filtered, pressed and dialysed.

The residue from the dialysis of the second fraction

precipitate was the more potent, but the residue from both dialyses from fractions 1 and 2 was mixed and the requisite amount of sodium chloride and preservative added. The finished product had a protein content of about 18-20%, and was concentrated to about 4-5 times that of the original serum.

Banzhaf, 1912-13:

Following the principle in the last method, he produced a "one fraction method". The concentrated serum he produced had 4-5 times the antitoxic strength, and a protein content about 20% of the original serum. The method was:-

(1) The serum was diluted by one-half of its volume by water, and the whole made up to 30% of saturation with ammonium sulphate.

(2) The mixture was heated to 60°C. and kept at that temperature for a few minutes.

(3) The hot serum was filtered.

(4) The precipitate of euglobulin was washed with 33% ammonium sulphate. The washings were filtered and were added to the main bulk of the filtrate, which was then made up to 50% saturation with ammonium sulphate.

(5) The precipitate was filtered, pressed and dialysed. The dialysed residues were treated as in the Banzhaf-Gibson method.

This method, Banzhaf claimed, was quicker than the

previous method, as the time taken to extract the first fraction with brine was not required. It was a cheaper method because less materials were used.

The drawback however was that the production of a readily filterable end product depended a great deal upon the condition of the serum mixtures after the preliminary heating. If the latter had not filtered readily the end product was found to be unsatisfactory.

Homer, 1916[1]

The disadvantage of the Banzhaf one fraction method was that the final product might be opalescent due to a suspension of euglobulin and which was difficult to get rid of by filtration. From her experiments Homer found that the successful preparation of an easily filterable end product depended upon the initial heating of the mixtures. The advantages of using sodium chloride in serum mixtures were:-

(a) During the heating of the mixtures, if the temperature was kept at 63-65°C., the addition of 1.5% sodium chloride did not change the amount of protein remaining in solution but if the temperature were raised to 67°C., then a change took place.

(b) If a solution of pseudoglobulin and euglobulin in saturated brine was heated to 61°C. and kept at that temperature for a few minutes, 60% of the soluble globulin was converted into insoluble globulin, and there was a loss of antitoxin.

(c) The heating of the serum with sodium chloride denaturated the protein and made it more easily precipitated with

sodium chloride or 30% of saturation of ammonium sulphate.

The sodium chloride had a favourable influence on filtration by re-adjusting the pH value of the serum mixtures.

Homer (1916[2]).

Following the general principles of the Banzhaf and Gibson and the Banzhaf methods, she produced a serum which she claimed was 8-9 times the strength and which contained 17-19% of the total protein of the original antitoxic serum. Her method was:-

(1) The serum was diluted with 1/5 to 1/3 its volume by water, and then made 1.5-2.0% with sodium chloride.

(2) The mixture was heated to 56-57°C. for 15 hours or 57-58°C. for 8 hours.

(3) The heated serum was then made 30% saturated with ammonium sulphate and the mixture heated to 61°C. and kept at that temperature for a few minutes.

(4) After it had cooled to 40-45°C. the mixture was filtered.

(5) The precipitate was then washed with a 33% solution of ammonium sulphate, and the washings were added to the main bulk of the filtrate which was then made up to 50% saturation of ammonium sulphate.

(6) The precipitate was filtered, pressed and dialysed.

(7) This was now treated with the necessary amount of sodium chloride and preservative.

This method was an improvement on the Gibson-Banzhaf method for the following reasons:-

(1) There was only one fraction, and the extraction with brine was unnecessary, so time, labour and expense were saved.

(2) The heating of the serum made up to 30% saturation with ammonium sulphate served to agglutinate the suspensoid particles of protein into conglomerates large enough to be retained in the filter paper. In the Gibson-Banzhaf process it was often necessary to increase the percentage of ammonium sulphate to 35 to ensure the retention of the particles.

(3) The potency of the serum was much greater and the protein content much less.

(4) The total loss of antitoxic units was not greater than 10%.

Over the Banzhaf process its advantages were:-

(1) As more protein was removed as a result of heat denaturation of serum proteins and their increased precipitability by 30% saturation of ammonium sulphate there was less chance of filtration difficulties.

(2) By the addition of 1.5-2.0% sodium chloride the reaction of the medium tended to be more suitable for complete precipitation of the proteins thrown out of the emulsoid solution by the 30% saturation of ammonium sulphate.

(3) The finished product was nearly double the antitoxic strength of the Banzhaf product.

Homer 1916[3].

Experimenting with electrolytes she found that, if a serum is heated with an electrolyte, much stronger concentrations per gramme of protein can be obtained, but that there is also a limit to which a serum can be concentrated. She used sodium chloride

and ammonium sulphate in concentrations of 10-20% and 30% respectively. The strongest serum she produced contained 22,500 units per gramme of protein.

Homer, 1917[1].

In a further investigation into the factors influencing the production of concentrated sera, Homer found that the pH of the serum was of great importance and that the best results were obtained when the pH of the serum - ammonium sulphate mixture - was adjusted to between 5.3 - 5.0. Further, the filtration of the mixtures could be improved by the addition of 2% sodium chloride to the mixtures, and the improvement was due to a specific action by the sodium chloride upon the globulins. If the serum was heated for some hours, up to 8, at a temperature of 57-58°C., there was a greater precipitation of proteins by the ammonium sulphate. She also found that by the addition of phenol .35 - .5% there was an alteration in the surface tension of the proteinsin colloidal solution and an increase in their precipitation.

Homer 1917[2].

In a further investigation she found that if 2% sodium chloride and .3 - .35% cresylic acid were added to the serum prior to the heating of the plasma - ammonium sulphate mixture - the tendency of the end products to be cloudy was diminished. There was no destruction of antitoxin provided that the 2% sodium chloride was added, and that the serum - ammonium sulphate mixture - was not heated to above 60°C. and was not allowed to remain at that temperature for more than 2 hours.

Homer, 1917[3].

She found that for a serum whose pH value is above 7 the serum must be heated to a temperature of 57.5°C. for not less than 4 and not more than 5 hours for the maximum precipitation to be obtained, if 30% saturated ammonium sulphate were used. Homer also found that, if she included in the second fraction the protein precipitated by varying concentrations between 30 - 44% of saturation with ammonium sulphate instead of 30 - 50%, the amount of albumin which appeared in the final fraction was much reduced. Finally, the serum produced was clearer in colour, contained less protein and was more concentrated than that produced when 30 - 50% of saturation of ammonium sulphate was used.

Homer 1918.

In 1918 she suggested a new modification of her previous method for concentration of anti-diphtheritic sera. The technique was:-

(1) Pooled batches of undiluted oxalated or citrated plasma were treated in the following manner -

(a) The pH was adjusted to 8.

(b) If this could not be done, between .25 - .3% cresylic acid or trikresol was added to the plasma.

(c) 2% sodium chloride was added.

The plasma thus treated was heated in two stages:

(i) The plasma was heated in jars at a temperature of 57.5°C. for 4 hours.

(ii) The plasma was then made 30% of saturation with ammonium sulphate and the temperature raised to

58°C. and then cooled to 45°C. The mixtures were constantly stirred.

(2) The mixture was filtered and Fraction 1 obtained. This first fraction precipitate, consisting of euglobulin, heat denaturated protein and pseudo globulin, was now washed with 30% saturation of ammonium sulphate - about one-half volume of ammonium sulphate to one volume of plasma. The washings were now added to the main bulk of the filtrates, and then to the main bulk was added a saturated solution of ammonium sulphate to bring the ammonium sulphate content up to 46% of saturation. The precipitate produced was now filtered, pressed and dialysed until it was free of sulphate. To the residue was added .85% sodium chloride and .35% cresylic acid. About 18% of antitoxin was lost. To avoid this 18% loss of antitoxin the following method was employed; after the first precipitates had been washed with 30% of saturation ammonium sulphate, they were put into canvas bags and these were placed in a saturated solution of brine. The first precipitates of many plasmas were added to the brine tub. This was closed at room temperature for some time and then the brined extract of all the precipitates was filtered. To the filtrate was added .3% glacial acetic acid and the precipitate produced was filtered, pressed and dialysed after 3% washing soda had been added to the contents of each dialysing bag. The dialysing was continued until the contents of the bags

were free from salt. To these residues were added .85% solid sodium chloride and .35% cresylic acid.

This method had the following advantages:-

(1) The adjustment of the undiluted plasma removed the difficulties experienced in the filtration of the hot plasma-ammonium sulphate mixture.

(2) The two stages could be completed in 7 hours.

(3) By precipitation with 40% and not 50% saturation ammonium sulphate the heat denaturated albumin in the final product was less and the serum was clearer.

(4) The loss of antitoxin was reduced to 2.5%.

Homer 1919[1].

From further investigations upon the concentration of antitoxins, she concluded that where a serum showed a 35% or less, heat denaturation, the second fraction must be precipitated by ammonium sulphate between 30 - 45% of saturation. If ammonium sulphate of less than 45% of saturation was not used, then some of the antitoxin was discarded with the albumin filtrates. Further, if the lower figure of 30% was raised, some of the antitoxin was again lost because it was precipitated with the first fraction and in such a form that it was insoluble in brine. Homer also concluded that where the heat denaturation was 35% or less the antitoxin was precipitated with saturations of ammonium sulphate varying between 36 - 45%. From her observations she thought that to isolate antitoxins completely, other means than fractional precipitation would have to be used.

Homer 1919[2].

Investigating further into the concentration of antitoxin, Homer showed that the increased precipitability of pseudoglobulin by saturations of ammonium sulphate varying between 20 - 47% depended upon the heating of the serum. She also demonstrated that there was no need to have a preliminary heating of the serum. When ammonium sulphate was used in saturations varying between 30 - 44% with heated serum, the same results could be obtained using ammonium sulphate in saturations of 36 - 50%, and unheated serum. The heating of the serum, however, reduced the toxicity of the cresylic acid - protein complex.

Homer 1919[3].

Comparing the uses of sodium sulphate and ammonium sulphate as precipitants of antitoxin, Homer found that there were no critical points which marked out the precipitation limits of the individual proteins, and that the concentrations of sodium sulphate and ammonium sulphate varied with the pH value of the sera. They also varied depending upon whether or not the serum had been treated with cresylic acid. She stated that when sera are being heated prior to precipitation, the use of cresylic acid is beneficial, but with unheated serum it must not be used. When sodium sulphate was used the agglutination of the particles of precipitated protein in the euglobulin and pseudoglobulin zone was better than if ammonium sulphate was used. The reason for this is that the sodium sulphate does not hydrolyse in solution,

but ammonium sulphate does, and so the sodium sulphate first fractions could be heated for 4 - 5 hours at 58°C. without losing any of the antitoxin. The end product was clearer than that produced by the two-stage ammonium sulphate method. Barr, Glenny and Pope, 1931.

For greater accuracy, they concluded solid ammonium sulphate should be used in precipitation, as the solubility of salts varies with the temperature. When a solution of ammonium sulphate was added to a serum, the protein filtrate became diluted with each addition of the ammonium sulphate solution. The quantities of ammonium sulphate should be expressed in grammes per litre of the serum, and not as a percentage of saturation as this varies with the temperature.

They found that if albumin were added to a serum a greater quantity of ammonium sulphate was required to precipitate the antitoxin, and that the amount required increased as they increased the amount of albumin.

Further, they found that if two antisera, which showed differences in antitoxin distribution as determined by salting-out methods, were mixed, some redistribution of antibodies took place.

(b) Precipitation by Alcohol.

Mellanby, 1908.

He found that, working at room temperatures, the greater proportion of the antitoxin was precipitated by a

concentration of alcohol between 35 - 45%. When working at a temperature of 2°C. he found that most of the antitoxin was precipitated by a concentration of alcohol between 16 - 28%. The advantage of the cold method was that the total protein precipitated was soluble and so the whole of the antitoxin could be recovered. At the higher temperature, some of the antitoxin was lost through coagulation of the protein.

Mellanby also found that if alcohol was added to an antitoxic serum, it did not destroy the antitoxins so long as none of the proteins were coagulated. When coagulation of the proteins took place, destruction of antibody also occurred. He concluded that, so far as the coagulating action of alcohol was concerned, there is no physical difference between diphtheria antitoxin and proteins from normal serum.

Hardy and Gardiner, 1910.

They precipitated the proteins in antisera by absolute alcohol or acetone at 0°C. The product they obtained was a white powder. This was washed with ether at 0°C. to remove the alcohol or acetone. The precipitate was then extracted with boiling ether, filtered and dried in vacuo with sulphuric acid. This precipitate lasted indefinitely, and when redissolved showed the antitoxin to be present undamaged.

Moloney and Taylor, 1928.

Using various concentrations of alcohol at various pH values, they found the optimum concentration factor to be about 30%. They treated a citrated plasma whose pH value was

5.3 with 14% alcohol at a temperature of 40°C. They also found that the same concentration factor could be obtained working at room temperature if they used citrated plasma, pH 5.3 but 27% alcohol.

Using the serum they obtained in intradermal tests upon known serum sensitive patients, they found that their alcohol produced serum caused less reaction than that produced by the use of ammonium sulphate.

Merrill and Fleisher, 1932.

They used as precipitants for the antitoxin methyl, ethyl and propyl alcohol in varying strengths, at various temperatures and at varying pH concentrations. They found that -

(1) Between 70 - 75% concentration there was complete precipitation of proteins, but that the precipitate was almost all insoluble in water.

(2) With higher concentrations of alcohol, protein precipitation was also complete, but that the precipitate was readily soluble in water.

(3) The loss of agglutinins was almost parallel with the loss of solubility.

(4) If the temperature of the serum was raised the solubility of the precipitate diminished even when alcoholic concentrations of 90% were used.

(5) At low temperatures - 5°C. - all precipitates, no matter what concentration of alcohol had been used, were

soluble in water.

(6) The pH value for maximum precipitation is 6, and precipitation is less, above and below this value.

(7) The greater the concentration of the serum, the greater the amount of protein precipitated by any given concentration of alcohol.

Their method for producing an immune serum in a dry state was:-

(1) They added one volume of serum to 10 of acetone.

(2) The precipitate was washed with acetone and then three times with anhydrous ether.

(3) The white powder was placed in an incubator for one hour at 37°C.

(4) The powder was redissolved in distilled water or .85% sodium chloride.

They found that there was no loss of antibody if this method was carried out at a temperature of 20 - 25°C. and if the incubation did not exceed one hour.

(c) Adsorption.

Much of the experimental work has been done on antibodies other than diphtheria antibodies.

Eisler and Spiegel - Adolf, 1929. have studied the adsorption of typhoid antibodies with aluminium hydroxide and elution with N/10 sodium hydroxide. They succeeded in concentrating the serum to a slight extent. When Spiegel - Adolf (1932) attempted to purify other antisera by the same method, he had no success.

Fränkel, 1932

Adopting the method described by Olitzki and Fränkel (1931) he prepared elutes from diphtheria sera which contained 12 - 24% of the original antibody, but this was later disproved by Pope and Marrack.

(d) Cataphoresis.

Mellanby, 1908.

He passed a constant current of 100 volts through zinc sulphate electrodes into an antitoxic serum for 3 hours. He obtained a deposit of insoluble protein on the anode. The antitoxic value of the remaining serum was found to have remained constant. The antitoxin had neither accumulated nor diminished in the remaining serum. He concluded that diphtheria antitoxin possessed the same properties as normal proteins when tested by an electric current passing through it.

When the insoluble mass of protein at the anode was redissolved in sodium hydroxide, it was found to have an antitoxic value proportional to the amount of protein dissolved - that is, the precipitate possessed the antitoxic value of the original protein.

Pauli, 1924[1 & 2].

He used a three chambered cell containing the serum in the middle compartment, which was separated from the other two chambers by two semi-permeable membranes. The two end compartments contained platinum electrodes placed in flowing distilled water. When a current was passed the ions of the

serum passed to the electrodes, the equilibrium between the electrolytes and the proteins was disturbed and the globulins were precipitated. The euglobulin separated first and after prolonged electrodialysis the pseudoglobulin was also precipitated, and so a globulin free solution of albumin was obtained.

Adolf, 1924.

Using the same method, she found that the precipitates produced last in electrodialysis contained little antitoxin, but that the amount of antitoxin increased as precipitation increased. She claimed by her method to be able to remove all the antitoxin.

Wernicke, 1925.

He used the same method as Adolf, but was unable to precipitate all the antitoxin.

(e) Enzymes.

Mellanby, 1908.

He found that if 0.2% hydrochloric acid were added to a serum it did not destroy the antitoxin, but if pepsin were also added, the antitoxin was rapidly destroyed. Trypsin also had this effect, but acted more slowly. He experimented with trypsin and found that the diminution of the amount of protein precipitated by ammonium sulphate after digestion with trypsin was constant for all concentrations of ammonium sulphate.

Pope 1938.

He obtained two pseudoglobulin solutions from one

original anti-serum by the following method:-

He obtained a precipitate, which consisted of fibrin, euglobulin and some pseudoglobulin, by precipitation of the diphtheria plasma by 30% saturation with ammonium sulphate. The antitoxic pseudoglobulin was extracted by solution and reprecipitation with ammonium sulphate.

When this had been diluted to approximately 2% protein and when 5% sodium chloride had been added, solutions of varying pH value were obtained and heated at various temperatures to denaturate the proteins. The temperatures varied between 58 and 65^{o}C., depending upon the pH value of the solution. The antitoxic content was measured by the flocculation method and the protein by the Zeiss dipping Refractometer.

It was found that where the pH value had been adjusted to around 4.0 and certain temperatures had been used, the destruction of antitoxin was much less than the denaturation and loss of solubility of protein. After filtration, the residual antitoxin and protein were found. The number of antitoxic units per gramme of protein was as much as five times as many, per gramme of protein of the original solution.

When a similar experiment was carried out on another pseudoglobulin solution obtained by fractionation by ammonium sulphate in varying concentrations between 30 and 50%, there was no increase in antitoxic units per gramme of

protein.

The difference between the two pseudoglobulin solutions was that in one case the pseudoglobulin had been acted upon by the enzyme fibrinolysin, whilst in the other it had not. The difference in the results obtained was in no way dependent upon the pseudoglobulin because when fibrolysin was added to the second solution, it behaved in the same manner as the first.

He visualized the intact antitoxic pseudoglobulin molecule as having two components, one non-antitoxic with a low heat denaturation temperature and the other bearing antitoxic properties and less easily denaturated. Under certain conditions critical denaturation, denaturates the first of these and renders the whole molecule insoluble, but by means of enzyme action the undenaturated protein may be split off as a soluble protein, still having its specific antitoxic activity.

If the proteolytic enzyme is allowed to act only on the undenaturated protein then, for a degree of hydrolysis which cannot be detected as a change in the total protein content, a change is produced which allows the easily dentaurable portion to become insoluble without interference with the other antitoxin bearing fraction.

Pope found all proteolytic enzymes to give the same results as fibrinolysin when employed under correct conditions. Pope, 1939 (1)

He used various proteolytic enzymes such as pepsin (BP $^{1}/_{2000}$ - $^{1}/_{10,000}$), Trypsin and activated papain to digest

the non-antitoxic protein in serum and so increase the purity of the antitoxin. He defined true digestion of protein as "Its conversion into a form no longer coagulable by heat at the isoelectric point of the protein (in the presence of a suitable electrolyte) ".

He found the factors to be observed when true digestion is being considered were:-

(1) Where the temperature was 20°C., the reaction of the solution may be pH 3, but where the temperature was 35 - 40°C., the pH must be higher than 4. If not, there was marked loss of antitoxin.

(2) Where the reaction of the solution was pH 4 the ratio of pepsin to protein must be kept high - 1/10 (Pepsin B.P. 1/10,000), if not, a precipitate was formed and this carried out the enzyme with it. At lower pH values this precipitate was not formed, and so lower pepsin-protein ratios could be used.

(3) During the digestion by enzymes, proteoses were formed, and so pure antitoxin could not be obtained by salting out methods, as these proteoses were also precipitated with antitoxin.

He found that not more than 90% of the original protein could be digested without much of the antitoxin being lost. He also found, from a study of the plasma from individual horses that protein digestion and antitoxin stability varied greatly, even although the conditions of the peptic digestion were

kept as nearly identical as possible. Some plasmas showed marked antitoxic loss for the digestion of the first portions of protein while others showed an antitoxin loss which was constant in terms of "units per gram protein loss, per gram of protein digested" until most of the protein had been digested. Serum from horses which had been immunized rapidly to a high titre gave the best results with a simple digestion method.

The antitoxin molecule, when altered by enzyme action, was more stable to phenol than that prepared by other methods.

Pope concluded that the purification of antibodies by enzyme action had little advantage over salting out methods unless it was followed by the use of adsorption procedures to remove the inert proteins.

Pope, 1939[2].

Experimenting further with enzymes he tried the effect of:

(1) Heating the plasma before treatment with the enzyme

(2) After slight enzyme action, heating the serum and separating the proteins by critical heat denaturation.

The second was the better method.

From his further experiments he substantiated his theory that the antitoxic globulin is a complex molecule which is composed of two protein components, one of which is non-antitoxic and is easily denaturated in acid solutions and the other which is antitoxic and is less easily denaturated. The antitoxic globulin can easily be disaggregated into its two

components by enzyme action.

The second portion is, however, denaturated by alcohol, acetone or urea. Pope also found that if the original antitoxic solution or the separated antitoxic portion was heated to excess, or heated in a solution whose pH value was 3 or less, the antitoxic property was permenently lost.

His experiments showed that the disaggregation of the antitoxic molecule into its two component parts could be accomplished by a very small amount of enzyme in a very short time.

His method for concentrating antitoxin on a large scale is -

The original plasma containing .4% tricresyl is diluted with volumes of water before the pH is adjusted, and before the pepsin is added. If toluol is added prior to heating, the lipoids in the serum come out with the coagulated protein when the mixture is heated to 55°C. for two hours. The enzyme is added, and it is arranged that a true digestion will not take place, as this would (1) produce proteoses which would be salted-out later in the process, and (2) make it impossible for differential heating to be employed to separate the proteins. A disaggregation is made to take place, and the conditions for this vary with the enzyme chosen. For pepsin it is a pH of 3.2 and a temperature of 20 - 23°C. for 30 minutes.

The enzyme action is then stopped by changing the

pH of the solution from 3.2 to 4.1, using sodium hydroxide and adding a high concentration of an electrolyte (saturated ammonium sulphate) to precipitate some protein and the enzyme. The mixture is now heated to the temperature which coagulates the non-antitoxic protein. The pH of the filtrate is adjusted to 7 and the antitoxin is precipitated by 30 - 32% ammonium sulphate. The precipitate is pressed and dialysed. The final product has a protein content of 10% and an increase of antitoxic units per millilitre of 8 - 12 times. The antitoxin is about 60% of the original total. This is called "Refined serum".

(2) Specific Methods.

The active immunization of human beings against diphtheria by the use of slightlyn toxic mixtures of toxin and antitoxin was for many years attended by unpleasant reactions in the patient. These reactions were in the main due to the presence of non-specific poisonous substances arising mainly from the culture medium used for the production of the toxin, the products of bacterial growth other than toxin, bacterial protein and horse serum. These undesirable constituents of diphtheria prophylactics have been difficult to remove. It was Ramon (1922) who first suggested that floccules which form when neutral or nearly neutral mixtures of toxin and antitoxin are left at room temperature for some time might be used to concentrate anti-diphtheritic sera. From this experiment much work along these lines has evolved.

Ramon, G. 1923.

To 500 c.c's of fresh diphtheria toxin he added 40 c.c's of antitoxin containing 200 units per c.c. He separated the precipitate of toxin-antitoxin floccules by centrifuging, and then the floccules were washed, first with physiological salt solution, and then with distilled water. The preparation was next dissolved in 75 c.c's of $^{N}/_{100}$ acetic acid, and the mixture heated for one hour at a temperature of 58 - 60°C. It was estimated to contain a concentration of 60,000 units per gram of dried floccule and to represent a recovery of 75% of the original titre.

Hartley, 1925.

He prepared floccules in which the antitoxin was in excess of the toxin. These floccules were insoluble in saline, and after repeated washings contained less than 1% of the nitrogen compounds of the original mixture. These floccules caused no reaction when injected into guinea pigs, even in large doses.

When guinea pigs were injected with a quantity of the emulsion of the floccules which contained less than a hundredth of a milligramme of floccule, they became immune to diphtheria toxin.

Hartley found that the value of the floccules varied with the original toxin-antitoxin mixture. If the antitoxin had been much in excess of the toxin, the floccules did not induce a high degree of immunity in the guinea pigs. If the

antitoxin were only slightly in excess, then the floccules produced good immunity. If the toxin was very slightly in excess of the antitoxin, the floccules produced the highest degree of immunity of these three types of floccules.

Hartley 1926.

He substantiated his findings on the antigenic properties of the three different types of floccule and he observed that the floccules formed in excess of toxin took longest to precipitate. He also found that the solution which remained when neutral floccules had been obtained, was also able to produce a slight immunity.

The floccules contained less than 1.0% of the total nitrogen in the mixtures from which they separated. The most active preparation in his series contained only 0.02% of the total nitrogen occurring in the mixture from which it was derived.

He had some evidence that the large amount of extraneous nitrogenous compounds which toxin-antitoxin mixtures contain, interferes with the production of antitoxin. If the floccules were repeatedly washed with normal saline solutions, there was little alteration in their nitrogen content and their antigenic activity.

Antitoxin production followed the injection of small amounts of floccules which separate in neutral or toxic mixtures. Thus the injection of amounts of floccules from a neutral mixture containing $^{1}/_{4,000}$ of a mmgr. of nitrogen produced 0.02 - 0.1 units of antitoxin per c.c. of serum in six guinea pigs.

Doses a hundred times as large produced 0.1 - 0.6 units. The floccules from a toxic mixture were even more active. Doses containing less than $^{1}/_{10,000}$ of a mmgr. of nitrogen produced from 0.02 - 0.1 units of antitoxin per c.c. of serum in inoculated animals and doses ten times as large, produced from 0.2 - 1.3 units of antitoxin per c.c.

Marrack and Smith, 1930.

They thought that the floccules obtained from an antitoxin-toxin mixture resembled very closely pseudoglobulin, as the ultra-violet absorption curves of the floccules and of pseudoglobulin are identical. They found also that floccules contained a very small amount of lipoid material. They finally concluded that floccules consisted mainly of antitoxin, and that antitoxin was not precipitated with the pseudoglobulin fraction of a serum, but that antitoxin is actually pseudoglobulin.

S U M M A R Y.

The non-specific methods of concentrating serum have been more fully investigated than the specific methods.

The early investigators used fractionation with a reversible precipitant such as ammonium sulphate or sodium sulphate which removed from the serum, fibrinogen, part of the euglobulin and the albumin. It was soon realized however, that this method did not produce a pure antitoxin but a product which as well as containing antibodies, also contained lipoids, euglobulin and albumin. Further, this preparation deteriorated with age.

Further investigations led to repeated fractionation with ammonium sulphate or dialysis to a salt free condition which was followed by dilution and adjustment of the pH of the serum to the iso-electric point of the water insoluble protein-lipoid complex.

None of the methods used produced a very concentrated serum when considered in terms of units of antitoxin per gramme of protein. The concentrated sera produced by these methods contained about 15% of pseudoglobulin but much of this was non-specific.

The early investigations of the use of enzymes to purify antitoxins did not give promising results. The attempts were handicapped by two main reasons (a) lack of control factors such as the reaction (pH) of the digestion mixtures; and (b)

lack of a simple method for estimating the antitoxic content during digestion and isolation of fractions. The introduction of an in vitro method by Ramon has led to an increase in the amount of experimental work carried out on diphtheria toxin and antitoxin.

Pope described a method for concentrating antisera whereby he slightly altered the antitoxic pseudoglobulin of the antiserum by the action of enzymes. This was followed by critical heat denaturation of the non-antitoxic proteins.

His conception of the antitoxic globulin was that it is composed of a complex molecule, one portion of which is non-antitoxic with a low heat denaturation temperature and the other antitoxic and less easily denaturated.

By the use of certain proteolytic enzymes, under certain specified conditions, he attempted to digest the non-antitoxic protein and so increase the purity of the antitoxin.

In this process however proteoses were formed which reduced the increase of purity of the antitoxin. To remove these proteoses adsorption methods were necessary.

He found that there was a limit to which digestion could be allowed to progress, after which destruction of antitoxin took place.

The serum he obtained had a protein content of 16% and an increased value in units per ml. of 8 - 12 times the original serum.

Specific Methods: The use of floccules which

precipitate when diphtheria toxin and antitoxin are mixed, to induce immunity, have produced good results. Very small amounts of these floccules were able to induce a high degree of immunity in animals immunized by this method. The three different types of floccules have been investigated as to their efficacy in producing antibodies and the "toxic" floccules have been found to give the best results. Floccules closely resemble pseudoglobulin, which is, in the opinion of Marrack and Smith (1930), actually antitoxin.

SECTION 11.

**Serum Reactions:
Their incidence and manifestations, together with a comparison of their occurrence and clinical findings following the injection of concentrated and refined diphtheria antitoxin.**

SERUM REACTIONS.

Definition - Serum reaction is the reaction of the human body to the parenteral injection of foreign proteins contained in animal serum. The degree of this reaction varies in different people. In some there may only be a few urticarial wheals, whilst in others who are serum sensitive, there may be a severe reaction, accompanied with cyanosis, oedema, pains in the joints and an urticaria covering most of the body. The incubation period varies. In the serum sensitive individual it may come on immediately, but the average for the non-sensitive patient is 8 - 10 days. The sensitive patient may have been sensitised by a previous injection of serum, by contact with animal protein or he may be naturally allergic to serum proteins. It is usually in the serum sensitive people that the fatal cases are recorded.

The sudden and immediate reactions are very often termed anaphylactic reactions, as they are comparable to the anaphylaxis of sensitised animals.

1. Historical.

The first observations on serum reactions were made by Dallera in 1874, who observed urticarial rashes in patients following transfusion with lambs' blood. These rashes appeared 7 - 10 days after the transfusion.

Shortly after the introduction of anti-diphtheritic serum by Behring and Kitasato in 1890, and more so after

its therapeutic value had been reported upon by Emile Roux at the International Congress of Hygiene at Budapest (1894), serum reactions were observed in patients following its parenteral administration. These reactions were not new complications of the disease, but complications directly due to the serum.

Lublinsky (1894) first observed the symptom complex of fever, joint pains and rashes which occurred some days after injection of the serum. Heubner and Bokay (1895) said that the symptom complex was due to the non-antitoxic proteins present in the serum, and their view was confirmed by Johannessen and Heubner (1895), who demonstrated that serum reactions took place after the injection of normal horse serum and so the reactions could not be attributed to the antibodies present in the serum.

Hartnung (1896[1]) published the first statistics on serum reactions. The percentage varied from 8 - 11, and of serum rashes there were four types (a) Urticarial, (b) Morbilliform, (c) Polymorphus or circinate, and (d) Scarlatiniform. He also recorded two cases of abnormal reactions after a second injection of serum (1896[2]). Similar reactions were reported by Denys and Leclef (1896) and Goodall (1898). The first fatality after serum administration was recorded in 1896. The child was an allergic individual who had had no previous injection of serum, but was given a prophylactic dose of diphtheria antitoxin. The case was

reported by Professor Langerhans.

The term serum sickness or serum disease was first used by Von Pirquet and Schick in 1903. In 1905 these two investigators published their book "Die Serumkrankheit", which contained a full account of the etiology, types and symptoms of serum reactions in man.

2. Mechanism of Serum Reactions.

Attempts to demonstrate that the course of serum reactions is due to an antigen-antibody reaction have resulted in many experiments being carried out. The first important work in this field was done by Von Pirquet and Schick (1903 - 1911). Most of the work done since then has been towards verification of their findings, which were mainly the relationship between the appearance in the blood-stream of precipitins and the onset of serum reaction. These precipitins appear in the blood 6 - 9 days after serum injection and never before the symptoms of serum disease. Von Pirquet (1911) concluded that normal serum reactions in man occurring 8 - 12 days after injection were analagous to anaphylaxis in animals, because rabbits and guinea pigs became sensitised and antibodies appeared in the blood-stream after a similar interval. He also stated that a serum injection repeated while specific antibodies are still present in the body causes an immediate reaction. After a longer interval antibodies may not still be present, but the body has been sensitised to produce antibodies more

rapidly, causing an accelerated reaction after 4 - 7 days.

Animal Experiments.

As our knowledge of the mechanism of serum reactions has been greatly enhanced by the injection of animals with serum, the results recorded by three observers will be quoted to illustrate the value of animal experiments.

(a) Fleischer and Jones (1931-1934) working with albino rabbits, found that serum reaction characterized by erythema and oedema was apparent in the ears of 68.9% of rabbits, 3 - 9 days subsequent to the subscapular injection of horse serum. They could not discover any evidence that the occurrence of serum reaction was directly associated with the production or appearance of precipitins. There was no difference in the precipitin curves of injected rabbits who did or did not develop serum reaction. The variation in incidence of serum reaction using serum from different horses was 24 - 93 per cent. and they concluded that the active factor is present in all sera, the important factor in production of serum reactions being the reactivity of the individual rabbits.

In reactions after a second injection the time between the first and second injection is the important factor, up to 12 days a normal reaction occurred, between 2 - 4 weeks reactions were of the accelerated type, from 5 - 7 weeks there were both immediate and accelerated

reactions, and from 3 - 5 months only immediate reactions occurred. After 6 months reactions tended to revert to the normal type.

"These reactions in rabbits are essentially analagous to reactions appearing in man subsequent to second injections of serum."

Fractional precipitation of serum with ammonium sulphate proved that the pseudoglobulin was primarily concerned with the cause of serum reaction, euglobulin only when it was injected in large quantities and albumin not at all. Abnormal reactions after second injection were equally frequent with pseudoglobulin and euglobulin, rare with albumin.

(b) Kahn (1933[1 & 2]) also experimenting with albino rabbits found that following the injection of white of egg or horse serum, skin sensitivity reactions and serum precipitins were parallel for some weeks, while a month or more later skin sensitivity reactions were still present in the absence of serum precipitins.

Mechanism of Serum Reactions in Man.

To substantiate the assumption that serum reactions in man are due to an antibody-antigen reaction, it must be proved that (a) following the parenteral administration of antisera, a specific antigen appears in the blood plasma (b) where serum reactions follow specific antibodies appear in the blood-stream (c) where no serum reactions follow,

no antibodies are elaborated or if they are, their presence cannot be detected in the blood-stream.

(a) Presence of a Specific Antigen in the Blood-Stream.

Longcope and Rackemann, 1918, concluded that during the incubation period of serum disease the amount of antigen present in the blood-stream remains constant and is dependent upon the amount of serum administered .

Mackenzie and Leake, 1921, using serum from rabbits which had been immunized against horse serum, precipitated out of human serum the horse serum present in it, after the patient had received anti-serum derived from horses. In eleven of the patients who developed severe serum reactions, the horse serum (precipitinogen) disappeared from the human serum when the symptoms of the serum reaction were passing off. Where the patients developed no reaction, the precipitinogen remained in the blood for as long as the observations were carried out (two months).

Mackenzie and Hangar, 1930, proved that horse serum can be found in the blood-stream for 2 - 3 weeks following administration, but if a serum reaction occurred, it rapidly disappeared.

(b) Presence of Antibodies.

Wells, 1915, demonstrated the presence of precipitins in the blood plasma 4 - 5 days after the administration of antiserum. He thought that the concentration of the precipitins increased with the amount of antiserum given and

that the symptoms of serum reactions disappeared when the precipitins were liberated.

Longcope and Rackemann, 1918, were of the opinion that serum reactions were not due to the appearance of antibodies in the blood plasma, but that the antibodies neutralized the antigen and so the patient recovered from his serum reaction.

Mackenzie and Leake, 1921, tested for precipitins by adding an equal volume of normal saline to a quantity of patients' serum and precipitated the precipitins by various dilutions of horse serum. In eleven patients who had severe serum reactions they found a high concentration of precipitins and this concentration rose near the time when the symptoms were passing off. In four patients who had mild serum reactions the concentration of the precipitins was very low or absent.

Wyard, 1922. From 35 cases he found by precipitation with horse serum, specific antibodies in 24 cases. He was of the opinion that although precipitins may be found in the patient's serum, serum reactions have no relationship with them.

Tuft and Ramsdell, 1929, conducted many experiments for the presence of antibodies by precipitation and passive transfer. They obtained blood from patients at different intervals following injection or antitoxic and immune horse serum. Their results showed that, where reactions occurred, antibodies were demonstrable in many of the patients' sera by

means of the Prausnitz - Kustner reaction, and also that passive sensitisation of guinea pigs' ear skin could be produced.

Mackenzie and Hangar, 1930, demonstrated the presence of precipitins in human sera from non-sensitive patients 9 - 21 days after the injection of the anti-serum. They thought that shortly after the injection of serum the cells of the body began to produce precipitins which remained intracellular. At the end of the incubation period, the concentration of the precipitins was such that it reacted with the horse serum in the blood-stream. Where serum reaction took place, the precipitins were in excess in the patient's serum.

(c) Absence of Antibodies where no Serum Reaction occurs.

Longcope and Rackemann, 1918, considered that it was impossible for serum reaction to occur if no antibodies could be found in the patient's serum.

Mackenzie and Leake, 1921, in four cases where no serum reaction occurred, were unable to demonstrate precipitins in the blood-stream.

From the observations made above it will be seen that there is considerable experimental proof that serum reactions are dependent upon an antibody - antigen reaction. Coca, 1931, has, however, been able to demonstrate the presence of precipitins in the blood before and after the occurrence of symptoms and also in the absence of reactions. He also has

shown that symptoms could occur without precipitins being present.

It might be that the elaboration of antibodies depends more on the individual than on the serum injected, but there is no doubt that antibodies do appear in the patient's serum. The presence or absence of demonstrable antibodies must depend a great deal upon the patient. In one patient, who shows serum reaction, the antibodies might only be in slight excess and so their presence might not be detectable; in another with no serum reaction, the antibodies might be in great excess and easily demonstrable.

Conclusions.

Following upon the parenteral administration of foreign serum in man, antigens can be demonstrated inthe blood. During the incubation period there is a gradual formation of specific antibodies in the special cells of the body (reticulo-endothelial system, skin, etc.). The process may end there, but in certain cases, for no apparent reason, a reaction takes place between the antigen and the specific antibody, histamine is liberated in the tissues and the onset of the serum reaction occurs. The antibodies extruded in excess from the cells into the blood neutralize the antigen and so the symptoms of the reaction diminish. Some antibody remains in the cells for a prolonged period. This can be demonstrated by skin sensitivity tests. When a second injection of serum is given, the incubation period is reduced

because there is some antibody already present in the tissue cells.

3. Serum Reactions in Man.

A. Types of Reaction (Von Pirquet and Schick, 1905)

(i) Normal: After primary injection and a latent period of 7 - 15 days.

(ii) Accelerated: In which the latent period is reduced to less than 7 days. This type usually follows the reinjection of serum after a period of several months (7 months to 7½ years in Von Pirquet's series of cases).

(iii) Immediate: Occurs within 24 hours from the time of administration and occurs usually where a previous injection of serum has been received from 2 weeks to 6 months previously. (12 - 50 days in Von Pirquet's series).

The latter two groups may be termed abnormal reactions.

Coca, 1931, differentiates the immediate type of reaction which occurs in sensitive patients after a primary injection of small quantities of serum into two classes:-

(i) Where the reaction occurs within a few hours and where the symptoms are similar to those developing in the normal reinjected patient.

(ii) The severe and occasionally fatal immediate reaction which occurs within a few minutes, characterized by dyspnoea, cyanosis, vomiting etc.

B. Factors which influence the Incidence of Serum Reactions.

(i) Age: There is difference of opinion on this subject. Coca (1922) states that the incidence of serum reactions varies little at different ages. Goodall (1928) is of the opinion that the rate is highest in young people. Hunt (1932) found the incidence highest in the 10 - 20 age group. Hecksher (1926) recorded that with large doses of serum the incidence and severity of serum reactions increases with age.

(ii) Sex: Goodall (1928) in cases treated with unconcentrated diphtheria antitoxin found a higher proportion of reactions in females than males. With refined serum, the reverse was observed. The general concensus of opinion is, however, that sex has no influence on the incidence.

(iii) Race: Mackenzie and Hangar (1930) found a low incidence of serum reactions in negroes and North American Indians. Coca, Deibert and Menger (1922) observed in a series of cases that 46% of American Indians and 75% Whites developed serum reactions. The Indians had a milder reaction than the Whites.

(iv) Amount of Serum injected: Broadly speaking, the incidence of serum reactions is directly proportional to the amount of serum injected. This fact is substantiated by the results of

Sturtevant (1916), Goodall (1928) and Hunt (1932). Hunt's figures for diphtheria serum were - under 10 c.c's of serum injected, the incidence was 14.3%; where over 100 c.c's were injected, the incidence rose to 61.7%.

(v) Type of Serum:

(a) Whole and concentrated.

As has been demonstrated above, antitoxin is mainly associated with the pseudoglobulin fraction of serum protein. The various methods used to isolate this have been described. With concentrated serum the bulk of serum injected is diminished as more units of antitoxin are present in each cubic centimetre of antitoxic serum.

Toomey and August (1930) reported on a large series of cases treated between 1924 - 1929. With unconcentrated serum the percentage was 10.6 and with concentrated serum it was 16.6. The higher dosage employed in recent years may account for this.

A full investigation into the incidence of serum reactions following the administration of unconcentrated and concentrated serum was published in the M.A.B. report of 1928 - 1929. During 1925 - 1926, 711 cases received unconcentrated serum (400 units of antitoxin per c.c)

The percentage of serum reactions was 44.3 During 1926 - 27, 770 cases received 75% unconcentrated serum and 25% concentrated serum, and the percentage reaction was reduced to 31.4%. From 1927 onwards only concentrated serum was used (800 - 1000 units per c.c), and the incidence of serum reactions fell to 15.7%.

Variation in incidence of serum reactions with concentrated and unconcentrated sera.

Author	Type of Serum	Percentage of Serum Reaction	
		Unconcentrated	Concentrated
Park and Throne (1906)	Diphtheria A.T.	70.0	46.0
Ronaldson (1923)	Diphtheria A.T.	76.5	
Von Pirquet and Schick (1926)	Scarlet Fever	85.0	
M.A.B. Report (1928-29)	Diphtheria A.T.	44.3	15.7
Goodall (1928)	Do.	40.1	
Toomey and August (1930)	Do.	10.6	16.6
Cecil and Plummer (1930)	(Intravenous Pneumococcal)	53-92	20.6
Hunt, (1932)	Diphtheria A.T.		28.1
Davis (1938)	Do.		17.3
Belvidere Fever Hospital (1930-36)	Do.		9.9
Present Investigation	Do. (refined)		2.4

(b) Sera derived from Animals other than the Horse:

Diphtheria and tetanus antitoxins have been derived from immunizing cattle. Kraus et al (1921[1 & 2]) found only 4.5% of reactions in a series of 198 cases treated with diphtheria antitoxin derived from cattle. Of the 198 cases, 93 had had a previous injection of horse serum, and of this number the percentage reaction rate was 5.3.

(c) Homologous Sera:

These are rare but do occur. Dooley (1932) reported a case of a boy who was given, intramuscularly, 45 c.c's of pooled poliomyelitis convalescent serum. The patient developed a generalized urticaria, oedema of the face and a mild arthritis three days later. One month later he showed skin sensitivity to the same batch of serum but to no other. Tedstrom (1934) reported a case where a blood donor, who, at the time of transfusion, was suffering from urticaria due to strawberries, transmitted the urticaria to the recipient. The urticaria developed in the patient in thirty minutes. A transfusion was done some time later when the donor's urticaria had passed off, and this time the

recipient did not develop urticaria.

(d) Serum from certain horses would appear to cause a greater incidence of serum reactions than others (Goodall, 1928; Coca, 1931).
The practice nowadays is to pool all sera and so eliminate this factor.

(e) Some types of sera produce a higher rate of reactions - pneumococcal and anti-scarlatinal sera. The figure from the scarlet fever case reports in Belvidere Hospital during the years 1930 - 36 was 10.7% against 9.9% for diphtheria. The reason for the difference may depend upon the concentration, the amount and route of administration.

(vi) Presence of Preservative: This does not influence the number of reactions occurring. It has no action on the sensitising property of horse serum (Rosenau and Anderson, 1907), nor does it cause reactions (Park, 1913).

(vii) Hypersensitivity of the Individual:

(a) Previous Serum Injections.

Both Currie (1907) and Sturtevant (1916) found no proof of an increased reaction rate if the serum were administered in two or more injections, provided that the interval between the first and last dose

did not exceed 8 - 10 days. The latent period is not reduced and the duration of the reaction is not increased.

Where a second dose of serum is given after a period of more than 10 days the percentage of serum reactions is definitely increased.

Author	No. of Cases	No Previous Serum % of Reactions	Previous Serum Type	Previous Serum % of Reactions
Stewart, 1926	6		T.A.T.	100
Spicer, 1928	164	13.4		
	45		Diph.A.T.	17.8
	28		T.A.T.	14.3
Goodall, 1928	8,523	39.5		
	203		Diph.A.T.	63.5
Gordon and Creswell 1929	1,750	16.0		
	151		Diph.A.T.	43.0
	556		T.A.T.	74.1
Toomey and August 1930	53		Diph.A.T.	26.4
	42		T.A.T.	45.0
Belvidere Hospital Case Reports 1930-36	6,046	8.6		
	554		Diph.A.T.	18.4
	120		Sc.Fev.A.T.	36.6
Present Investigation	884	2.4		
	96			2.1
	20			5.0

Note: Diph. A.T. = Diphtheria Antitoxin; T.A.T. = Tetanus Antitoxin; and Sc. Fev. A.T. = Scarlet Fever Antitoxin.

(B) Previous injection of T.A.T., T.A.M., etc:

Gordon and Creswell, 1929. As noted above, they reported a 74.1% of serum reactions in children who had previously been immunized with T.A.T. The high incidence was thought to be due to the children's having received several small doses of horse serum.

Park 1928. In his opinion, previous immunization with material containing small doses of horse serum had little effect upon the incidence of serum reactions.

Fleming and Petrie, 1934. Noting the fact that most immunizing solutions consisted mainly of formalized toxoid solution with little antitoxin present, they were of the opinion that this factor (previous immunization) would shortly be eradicated as a possibility in increasing the percentage of serum reactions.

(C) Natural Allergy to Horse Proteins -

See section on serum accidents (Page 64)

(viii) Methods of Administration: Opinion varies as to whether serum reactions are more frequent after intravenous injection or after intra-muscular injection.

Coca, Deibert and Menger (1922), stated

that reactions are much more frequent after intravenous injection than after intramuscular. In one series of 367 cases the incidence was 92.4% after intravenous administration of the serum (Boston City Hospital).

Heckscher (1926), dealing with a series of severe cases of diphtheria considered that reactions were less frequent with intravenous than with intramuscular therapy.

Mackenzie and Hangar (1930) could find no definite difference between the two methods.

Hunt (1932) found that reactions were more frequent after intravenous therapy and that the reaction was usually of the immediate type.

London County Council Report (1936) stated that reactions may follow the administration by any route, but were more frequent when the serum was given intravenously.

When considering the "pros" and "Cons" in connection with intravenous therapy we must bear in mind two points -

(a) Intravenous injection is usually only resorted to when the patient is very ill and requires a large dose of serum.

(b) The immediate reactions may be of the thermal, non-antigenic type, and so should not be

classed as serum reactions.

C. Signs and Symptoms of Serum Reaction:

Since the introduction of concentrated sera the incidence of serum reactions has definitely diminished. The various manifestations of serum reaction can be classified under the following headings:-

(i) Prodomal Period. The prodomal signs may come on a few days before the rash appears. These signs are, usually, sweeting, drowsiness, oliguria (Ronaldson, 1923) and elevation of temperature - up to 106°F (Coca, 1931).

(ii) Local Rash. This usually appears between the second to the sixth day and before the general rash (Coca, 1931; Bray 1937).

(iii) Generalized Rash.

(a) Day or onset. Hunt (1932) found that 85.8% appeared before the eleventh day, the average being between the 5th. and the 15th. day, and the day on which most rashes appeared was the 9th. day after the administration of the serum.

Coca, Deibert and Menger (1922) in a series of 223 cases found an 80% incidence of rashes. 70% of these appeared between the 1st. and the 30th. day.

Goodall (1928) observed rashes from the 1st. to the 29th. day.

Ronaldson (1923) reported a circinate rash which appeared on the 33rd. day

Goodall (1932) observed an erythematous rash on the 40th. day.

Belvidere Hospital Case Records (1930-36):

The days on which most rashes appeared were the 8th. and 9th., rashes still developed up to the 18th. day, and 98% of rashes occurred before the 13th. day.

(b) Types of rash. From two series reported by Davidson (1919)[1] and Goodall (1928) the percentages are:-

Morbilliform, 16 - 30% of all rashes.

Urticarial, 57 - 77.6% ,, ,,

Circinate, 7 - 12% ,, ,,

Urticarial appear first, average, 9th. day.

Morbilliform ,, second, ,, 12th. ,,

Circinate ,, last, ,, 14th. ,,

Stanley (1932) reported 52% erythematous rashes and 26% urticarial.

Figures from the Belvidere Hospital:

Diphtheria Case Reports (1930 - 36) are:-

Urticarial, 86.5% of all rashes;

Erythematous, 4.7% ,, ,,

Morbilliform, 4.2% ,, ,,

Circinate, 2.3% ,, ,,

Polymorphus, 2.3% ,, ,,

and for the time of appear ance:-

Urticarial appear	first,	average	8th. day
Erythematous ,,	second,	,,	9th. ,,
Morbilliform ,,	third,	,,	10th. ,,
Polymorphus ,,	fourth,	,,	11th. ,,
Circinate ,,	last,	,,	12th. ,,

(c) Recurrent Rashes. These were more common after the administration of whole serum.

Davidson (1919)[1] found in his series an incidence of 3.6% but in the Belvidere Hospital Diphtheria Case Records (1930 - 36) the incidence is 1%.

(iv) Arthritis. Following the administration of whole serum an incidence of 1 - 3% has been reported and in 14 - 20% of all serum reactions.

Coca, Deibert and Menger (1922), reporting upon a series of 223 cases, found arthritis in 36.7% of the cases.

Bray (1937) observed that the commonest joints to be affected were the interphalangeal joints. The patient complains of pain and tenderness in the joints, but swelling is slight or absent.

Boots and Swift (1923). Six out of ten cases

which had received Type 1 pneumococcal anti-serum intravenously developed arthritis. Four of the patients had pain and swelling of the knees. The knee joints were aspirated, and in two of the cases horse serum was found in the aspirated fluid.

(v) Oedema. This occurs usually in the loose tissues of the face and hands.

Sturtevant (1916), Davidson (1919[1]), and Mackenzie and Hangar (1930) found this in 4.8%, 7 - 8% and 33% respectively of reacting cases. Oedema in other regions is rare, but it does occur in the mucous membrane of the bronchi, the bowel and the conjunctivae (Davidson, 1919[2]; Coca, 1931).

(vi) Adenitis. Von Pirquet and Schick (1905) noted the presence of an inguinal adenitis on the side nearest the seat of injection of the serum. This usually occurred during the first 7 days following the injection and prior to the appearance of the rash; as the symptoms of the serum reaction subsided, so also did the glands.

Rolleston (1908), investigating a large series of diphtheria cases where the adenitis due to the diphtheria had subsided, found adenitis due to

the serum to occur in 9% of cases. in 111 out of 133 cases reported, the fauces were normal. Most of the cases occurred within the second week. He reported that serum adenitis is more frequent after severe attacks of diphtheria than after mild, and may occur at any age, whereas the late adenitis of diphtheria bears no relation to the severity of the attack and is confined to young children.

Coca, Deibert and Menger (1922) observed a 19.5% incidence of adenitis in a series of 223 cases treated at the Hospital of the Rockefeller Institute.

Davidson (1919[1]) gave as his findings 5% incidence of adenitis following the administration of antiserum.

(vii) Albuminuria. Sturtevant (1916) found a percentage of 7 in cases showing serum reaction.

Davidson (1919[1]) concluded that albuminuria had no relationship with serum disease and occurred in only .4% of reactions and after the first injection of serum.

Mackenzie and Hangar (1930) are of the opinion that albuminuria associated with serum

reaction is due to temporary impairment of renal function, during which chlorides are retained and the volume of urine passed is diminished.

(viii) Fever. This occurs in about 30% of reactions in cases treated with whole serum. The average temperature is 100 - 102°F. and the fever is of the intermittent type. The highest incidence of fever reaction is associated with the morbilliform and circinate types of rashes. (Davidson 1919[1]; Mackenzie and Hangar 1930; Bray 1937)

(ix) Blood Changes. These are not characteristic and depend a great deal upon the nature of the illness.

Von Pirquet and Schick (1905) found, in children, a leucocytosis during the incubation period and a poly-morphonuclear leucopenia with the onset of the symptoms.

Mackenzie and Hangar (1930) observed in some cases a leucocytosis and an eosinophilia towards the end of the symptoms.

(x) Orchitis and Abdominal Pain.

Rolleston (1924) quotes three cases from French observations of orchitis, but the

condition is not a common one.

Goodall (1925) reports a case of a boy of 10 years, suffering from severe diphtheria, who developed urticaria on the 13th. day after serum injection, abdominal pain and arthritis on the 18th. and 19th. days, and acute bilateral orchitis on the 20th. day. A recurrent erythematous rash appeared on the 22nd. and 32nd. days.

(xi) Neurological Complications. These were first reported upon by Roux, Martin and Chaillons at the Budapest Medical Congress of 1894.

Allen (1931). Reviewing the literature on this subject, he states that over a period of 40 years only about 50 cases have been reported and these were mainly in French literature. One case is reported in England in 1918 by Dyke. Allen divided the cases into four groups:-

(a) Radicular Type - Erb-Duchenne paralysis of acute onset.

(b) Neurotic - Single nerve trunks affected.

(c) Polyneuritic - Resembles toxic polyneuritis.

(d) Intracranial - Probably due to Cerebral Oedema.

The neuritis is due to oedema of the peri-neural tissues and there is a compression paralysis. Recovery is slow, but a good prognosis can be given. (Foster Kennedy, 1929; Gordon 1932).

Young (1932), reporting upon 7 cases, found an aseptic meningitis involving the central nervous system.

Wilson and Hadden (1932) describe six cases of multiple neuritis following the administration of tetanus and diphtheria serum. They found that the upper cord of the brachial plexus was most often involved.

Doyle (1933), analysing 49 cases, found that 34 followed tetanus antitoxin, 7 diphtheria, 5 scarlet fever, and 3 pneumonia, anti-serum. 44 of the cases were males and 5 were females. The average age was 27. Onset of symptoms occurred most often on the 9th. day after injection and about 2 days after the reaction had been present.

Cathala, Garcia, Gabriel and Laplane (1934), describe a case of a woman of 25 years who had had a previous injection of anti-

diphtheritic serum, and who, on being given 60 c.c's of anti-scarlatinal serum developed recurrent urticaria and multiple neuritis on the 14th., 57th. and 73rd. day.

Roger and Paillas (1936) define 3 cerebral forms which may occur (a) Isolated paralysis of the cranial nerves, including optic neuritis, (b) Cerebral symptoms in the course of general paralysis and (c) Isolated encephalopathy in the form of hemiphlegia, choreiform movements and various psychical symptoms.

(xii) Arthus Phenonema.

Gatewood and Baldridge (1927) report 6 cases who had repeated injections of serum and developed pain, redness and swelling, which progressed to actual necrosis. This is more liable to occur if the reinjection is given during a serum reaction from a previous dose of serum.

Dudgeon (1927) reported a similar case in a horse sensitive adult who was given subcutaneous doses for porposes of desensitisation.

(xiii) Serum Accidents. Severe and fatal reactions following the administration of serum are rare. They come on within 2 - 3 minutes of the giving of the serum, and if death does follow it is usually within 5 - 10 minutes. According to Lamson (1924) these severe reactions are most common in patients who have not been sensitised by a previous injection of serum, but who, if they had been tested by skin tests, would have shown reaction to horse serum. From a series of 41 cases he found that 34% had a history of asthma or hay fever and that 19.5% had had a previous injection of serum.

Park (1928) has estimated the number of fatalities occurring after the administration of serum, and being directly due to the serum, to be 1 in 50,000.

Ratner (1930), sensitised guinea pigs by inhalations of horse dander and induced serum shock in the same animals by injecting them with horse

serum. He thought that a similar sequence might occur in man.

Bray (1937), thought that the severe reactions to a first injection of serum might be caused by the patient's having been sensitised by lying on horse-hair beds or chairs.

Walker (1917), proved that although a person may suffer from asthma it did not necessarily mean that he would exhibit signs of serum reaction when he was injected with serum. His findings were: 20% of asthmatics were sensitive to horse protein, but only 4% reacted to horse serum.

In man the train of symptoms in all cases of serum reaction which prove fatal, is remarkably constant. These are dyspnoea, cyanosis, restlessness, coughing and extreme anxiety. These are later followed, in cases where death comes slowly, by headache, urticaria and oedema. The actual cause of death is usually respiratory failure, the heart continuing to beat even after respiration has ceased. The post mortem findings are, there is

pulmonary emphysema with enormous distension of the lungs, dilatation of the right heart, general venous stasis and visceral congestion (Boughton 1919; Bullows and Jacobi, 1930).

The majority of fatal cases have followed the intravenous injection of serum, but this is not always so.

Boughton (1919) recorded a case where the patient died after having received only one minim of horse serum intravenously as a desensitising dose for horse asthma.

Park and Bolduan (1908) describe a case of a young man of 16 years who received his serum subcutaneously. There was no immediate reaction and the doctor left the room. A few minutes later he returned to find the patient dead, apparently due to the serum.

Freedman (1935[1]) described a fatal case in an asthmatic child following the administration of 0.05 c.c's of horse serum intracutaneously for desensitisation purposes.

(xiv) Severe Serum Shock with Recovery:

Numerous cases have been recorded of patients

who were, and others who were not, sensitised by previous injection of serum; similarly, where the patient was, or was not, allergic to horse serum.

Park and Bolduan (1908). A young man with a negative history, suffering from diphtheria, was given 5 c.c's of serum subcutaneously. In about two minutes he felt tingling sensations, faintness and a sinking feeling. He quickly became cyanosed, had a rapid pulse and then passed into coma. He was given strychnine and artificial respiration and he recovered.

Fawcett and Ryle (1923). The patient had had anti-diphtheritic serum 19 years previously. On being given 50 c.c's of antistreptococcal serum, he developed symptoms on the 8th. day. These were dyspnoea, urticaria and oedema of the face. Adrenalin was administered, and the symptoms passed off in 20 minutes.

Dudgeon (1927). A known asthmatic who was sensitive to horse serum was given 0.25 c.c's of normal horse serum subcutaneously. Five minutes later he developed oedema of

the face, dyspnoea, cyanosis and then fell into coma. An hour later a diffuse mottled rash appeared which persisted for 12 hours. The patient made a complete recovery.

D. Desensitisation;

As it is known that the administration of small doses of serum desensitises animals and prevents anaphylaxis, it was thought that the same principle could be applied to man.

Gordon and Creswell (1929) concluded that desensitising of horse serum sensitive patients prevented immediate reactions, but had little influence on the development of late serum disease.

Battley (1931) reported a case of severe serum shock after an injection of serum in a patient who had been desensitised 17 days previously. He concluded that a longer period is necessary for desensitisation than is usually allowed.

Method of Desensitising:

Desensitisation is based on the partition of the dose and slow administration of the serum.

The routine to be adopted is: primary injection of ½ c.c. of adrenalin (1 in 1,000) and atropine sulphate gr. $^{1}/_{100}$ subcutaneously. This is followed by 0.2 c.c's of a 1 in 10

dilution of the therapeutic serum given subcutaneously. If, after half-an-hour, no reaction is observed, 0.5 c.c's of undiluted serum may be given intramuscularly, and if, after another half-an-hour has elapsed, no reaction has occurred, the full dose of serum may be given intramuscularly. If reaction should occur at any of the stages, the dose should be reduced and the process continued over a longer period (Bray, 1937; Fleming and Petrie, 1934; L.C.C. Report, 1936).

If the preliminary injection is given into the forearm and a general reaction occurs, this can be minimized by the application of a tourniquet to the arm. This prevents the antigens from entering the general circulation. This method has been used following an acute reaction in an allergic infant who was being Schick Tested (Freedman, 1935[2]).

E Prevention and Treatment of Serum Reactions:

(A). Prevention. The serum to be used must be considered.

Early observations revealed that serum from certain horses was more apt to cause serum reactions than others, and also that freshly drawn serum caused reactions more frequently

than that which had been left for longer periods.

Serum stored in excessive cold or heat had also the same fault. Nowadays serum is pooled from several horses, is kept for some time before use and the storage temperature is carefully regulated.

Concentrated serum is now generally used, and this, as has been shown, in consequence of its lower content of non-specific substance, has lessened the incidence and severity of serum reactions.

Inquiry should always be made from the patient (or relatives) for a history of allergy or previous injection of serum. Desensitisation should be carried out if a positive answer is obtained, as this lessens the possibility of the rare acute shock in the horse-serum sensitive individual.

If a patient is known to be sensitive to horse serum, serum derived from another animal should be used - bovine or goat. Certain drugs have a preventive action:-

Anderson and Schultz (1910). They found that by using atropine sulphate, adrenalin and choral hydrate, and inhalation of oxygen

in anaphylactic animals who were given serum, they could reduce the incidence of reactions to 28%, 41% and 43% respectively. Park (1913) and Bray (1937) both advised the injection of atropin sulphate and adrenalin hydrochloride (1 in 1000) in known or suspected sensitive patients.

Arvray (1933), advocated the oral administration of ephedrène chloride tablets (1 to 3 cg. according to age) one hour before the serum was given, and every 16 hours for 14 days thereafter.

Mercier (1934). He reduced the incidence of reactions in a series of cases to 6%. To each patient he gave an intramuscular injection of camphosulphate and ephedrene.

When serum is being given intravenously, it should be heated to blood heat and injected very slowly. It may be diluted with saline or glucose, and 2-5 minims of adrenalin hydrochloride added to it.

No matter how serum is given, adrenalin hydrochloride (1 in 1000) should always be at hand on the serum tray.

B. Treatment:

(i) Serum Shock. Treatment must be immediate

to relieve the respiratory distress. Artificial respiration, inhalation of oxygen and carbon dioxide, and injection of adrenalin to reduce the bronchial spasm, have all been tried with success even where the patient was in coma (Mavor 1934; Grant and Scott, 1934).

Hurst (1934). He recommended an initial dose of 2-3 minims of adrenalin (1 in 1000) followed by one minim every 30-60 seconds, according to the reaction of the patient, until recovery ensued.

(ii) Serum Reaction.

Acute urticaria and oedema of the tissues usually respond well to injection of ½ to 1 c.c. of adrenalin. Cooling applications, such as calamine lotion are welcomed by the patient. (Curphey and Solomon, 1936). They recommended large doses of calcium in an assimilable form (10 to 20 c.c's of 20% calcium gluconate intravenously and 10 c.c's of 10% solution intramuscularly, as soon as the rash appeared - the intramuscular injection to be repeated every 12 hours if necessary).

Most serum reactions do not require more than local treatment, but salicylates do help in cases of arthritis.

SUMMARY AND CONCLUSIONS.

The types of serum reactions occurring in man are:

(1) Normal - after primary injection and a latent period of from 7 - 15 days.

(2) Abnormal - in a sensitised individual, which may be immediate, occurring within 24 hours, or accelerated, when the latent period is 2 - 6 days.

(3) Serum accidents or serum shock - the severe but rarely fatal reactions which usually occur immediately after injection in a horse serum sensitive or allergic person.

The main factors influencing the incidence of serum reactions are:

(1) The quantity of serum given.

(2) The type of serum - unconcentrated or concentrated.

(3) Presence or absence of sensitivity in the patient due either to previous serum injection or natural horse serum sensitivity.

(4) The method of administration, intravenous or intramuscular.

An account has been given of the manifestations of serum reaction in man and

their incidence. The question of desensitisation in man and the prevention and treatment of serum shock and serum reactions has been discussed.

The results when "Refined" diphtheria antitoxin was used are reported in the next section of this work.

Analysis and Comparison of the Incidence and Manifestations of Serum Reaction occurring in a Series of 6,720 Consecutive Cases of Diphtheria treated with concentrated Diphtheria Antitoxin, and 1,000 Consecutive Cases of Diphtheria treated with refined Diphtheria Antitoxin.

All the cases were treated at the Belvidere (City of Glasgow) Fever Hospital. The 6,720 represent all the cases of diphtheria in the Hospital during the years 1930 to 1936 inclusive. The 1,000 cases treated with refined antitoxin were in the Hospital during the period August 1937 to April 1939.

Definition of Reactions.

For the purpose of this analysis, serum reaction is interpreted as including any reaction which may be attributed to the injected serum, apart from the immediate thermal reaction after intravenous therapy.

Serum reactions are therefore divided into:-

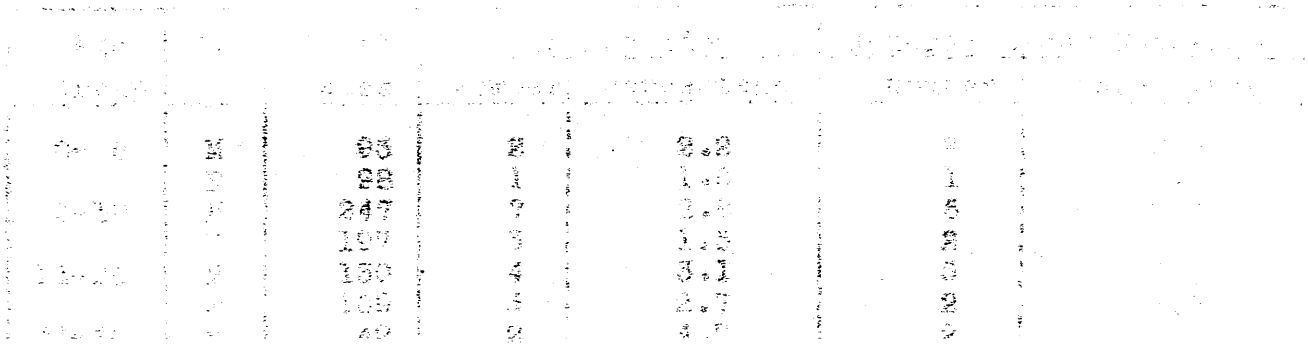
(1) Local reaction where the rash is limited to the site of serum injection.

(2) General reaction ; results of reaction due to circulating serum, including rashes occurring apart from the site of injection and symptoms such as fever, oedema and arthritis, which may occur in the absence of a rash.

Analysis of Serum Reactions.

	Concentrated Serum	Refined Serum
Total Number of Cases.....	6,720	1,000
Total Number with no Serum Reaction.....	6,050	976
Total Number with Serum Reaction.....	670	24
Percentage of Serum Reactions..	9.9	2.4
General Serum Reaction.....	594 = 8.8%	19 = 1.9%
Local Reaction only.....	76 = 1.1%	5 = 0.5%

The main fact derived from the above table is the definite reduction in the incidence of serum reaction from 9.9% to 2.4%.



Detailed Analysis of the Incidence of Serum Reactions in 6,720 Cases of Diphtheria Treated with Concentrated Serum, and of 1,000 Cases of Diphtheria treated with Refined Antitoxin.

Concentrated Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage
0- 5	M	953	69	7.2	61	6.4
	F	982	72	7.3	62	6.3
6-10	M	780	189	24.2	176	22.5
	F	1068	158	14.8	146	13.7
11-20	M	785	47	6.0	40	5.1
	F	1112	83	7.5	74	6.7
21-30	M	149	17	11.4	10	6.6
	F.	449	22	4.9	16	3.5
31-40	M	138	6	4.3	4	2.9
	F	281	7	2.4	5	1.7
41-50	M	12	-	-	-	-
	F	10	-	-	-	-
51-60	M	1	-	-	-	-
	F	-	-	-	-	-

Refined Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage
0- 5	M	93	2	2.2	2	2.2
	F	98	1	1.0	1	1.0
6-10	M	247	7	2.8	5	2.0
	F	197	3	1.5	2	1.0
11-20	M	130	4	3.1	3	2.3
	F	109	3	2.7	2	1.8
21-30	M	42	2	4.7	2	4.7
	F	29	1	3.4	1	3.4
31-40	M	15	1	6.7	1	6.7
	F	12	-	-	-	-
41-50	M	20	-	-	-	-
	F	3	-	-	-	-
51-60	M	5	-	-	-	-
	F	-	-	-	-	-

With concentrated serum the incidence of reaction is highest in the 6-10 age group, and thereafter diminishes with age, except for the 21-30 group (male) where there is a sharp rise. In the refined series there is a gradual increase in incidence with age, but after 40 no reactions were observed. With both sera, males would appear to have a higher incidence of reactions than females.

A comparison is drawn between the incidence of serum reactions correlated with the dosage of serum administered.

A. Up TO 8,000 UNITS

Concentrated Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage
0- 5	M	357	15	4.2	12	3.3
	F	376	17	4.5	11	2.9
6-10	M	271	31	11.4	27	9.9
	F	397	26	6.5	23	5.8
11-20	M	246	7	2.8	5	2.3
	F	406	11	2.7	8	1.9
21-30	M	39	4	10.2	3	7.7
	F	107	3	2.8	2	1.8
31-40	M	47	1	2.1	1	2.1
	F	99	1	1.0	1	1.0
41-50	M	6	-	-	-	-
	F	5	-	-	-	-
51-60	M	1	-	-	-	-
	F	-	-	-	-	-

UP TO 8,000 UNITS

Refined Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage
0- 5	M	21	-	-	-	-
	F	25	-	-	-	-
6-10	M	94	-	-	-	-
	F	61	-	-	-	-
11-20	M	27	-	-	-	-
	F	24	-	-	-	-
21-30	M	11	-	-	-	-
	F	9	-	-	-	-
31-40	M	6	-	-	-	-
	F	3	-	-	-	-
41-50	M	4	-	-	-	-
	F	-	-	-	-	-
51-60	M	5	-	-	-	-
	F	-	-	-	-	-

The fact that no reactions were observed in any patients who received 8,000 units or less of refined serum, while reactions were fairly common after injection of concentrated serum, is the most notable feature of the tables, and is probably due to the patients who were given refined antitoxin receiving only very minute quantities of non-specific protein. From these tables it is seen that the reaction rate in males is slightly higher than in females.

B.

UP TO 40,000 UNITS

Concentrated Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage
0- 5	M	501	39	7.8	37	7.4
	F	451	38	8.4	37	8.2
6-10	M	349	106	30.4	102	29.2
	F	481	89	18.3	84	17.4
11-20	M	401	30	7.5	27	6.7
	F	472	52	11.0	49	10.4
21-30	M	89	9	10.1	5	5.6
	F	232	12	5.2	9	3.6
31-40	M	52	3	5.7	2	3.8
	F	94	4	4.2	3	3.2
41-50	M	3	-	-	-	-
	F	3	-	-	-	-
51-60	M	-	-	-	-	-
	F	-	-	-	-	-

UP TO 40,000 UNITS

Refined Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage.
0- 5	M	42	1	2.4	1	2.4
	F	44	-	-	-	-
6-10	M	89	3	3.4	1	1.2
	F	72	1	1.4	-	-
11-20	M	65	2	3.1	1	1.5
	F	43	1	2.4	-	-
21-30	M	17	1	5.8	1	5.8
	F	11	-	-	-	-
31-40	M	6	-	-	-	-
	F	5	-	-	-	-
41-50	M	12	-	-	-	-
	F	3	-	-	-	-
51-60	M	-	-	-	-	-
	F	-	-	-	-	-

The percentage of serum reactions following the administration of refined serum is very much lower than that

following the injection of concentrated serum, and the percentage with doses of 8000 units or less of concentrated serum is higher than that when the largest dose of refined antitoxin given, was 40,000 units. With both sera males have the slightly higher rate, and the rate is highest in the 6-10 years group with concentrated, and in the 21-30 years (male) group with refined serum. The incidence of reactions with concentrated serum has increased with the increase in the dose of serum given.

C. 40,000 UNITS OR OVER

Concentrated Serum.

Age Group	Sex	Total Cases	Serum Reaction		General Serum Reaction	
			Number	Percentage	Number	Percentage
0- 5	M	95	15	15.8	12	12.6
	F	155	17	10.9	14	9.0
6-10	M	160	52	32.5	47	29.3
	F	190	43	22.6	39	20.8
11-20	M	138	10	7.2	8	5.7
	F	234	20	8.8	17	7.6
21-30	M	21	4	19.0	2	9.5
	F	110	7	6.4	5	4.5
31-40	M	39	2	5.1	1	2.6
	F	88	2	2.3	1	1.2
41-50	M	3	-	-	-	-
	F	2	-	-	-	-
51-60	M	-	-	-	-	-
	F	-	-	-	-	-

40,000 UNITS OR OVER

Refined Serum.

Age Group	Sex	Total Cases	Serum Reactions		General Serum Reactions	
			Number	Percentage	Number	Percentage
0- 5	M	30	1	3.3	1	3.3
	F	29	1	3.4	1	3.4
6-10	M	64	4	6.3	4	6.3
	F	64	2	3.1	2	3.1
11-20	M	38	2	5.2	2	5.2
	F	42	2	4.7	2	4.7
21-30	M	14	1	7.1	1	7.1
	F	9	1	11.1	1	11.1
31-40	M	3	1	33.3	1	33.3
	F	4	-	-	-	-
41-50	M	4	-	-	-	-
	F	-	-	-	-	-
51-60	M	-	-	-	-	-
	F	-	-	-	-	-

Again the incidence rate is on the upgrade with both sera. Males again have the higher incidence. Using concentrated serum the age group 6-10 has the highest percentage of reactions, but with refined serum it is the 31-40 (male) group which is highest.

FACTORS INFLUENCING THE INCIDENCE OF SERUM REACTIONS.

(1) Sex.

Concentrated Serum.

Sex	Total Number	Serum Reaction		General Serum Reaction	
		Number	Percentage	Number	Percentage
M	2,818	328	11.6	291	10.3
F	3,902	342	8.6	303	7.8

Refined Serum.

Sex	Total Number	Serum Reaction		General Serum Reaction	
		Number	Percentage	Number	Percentage
M	552	16	2.9	13	2.3
F	448	8	1.8	6	1.3

With both sera males have much the higher rate, the

difference being more marked with refined serum than with concentrated - 66.6% and 48.9% respectively.

(2) Age

Concentrated Serum.

Age Group	Number of Cases	Serum Reactions	
		Total %	General %
0- 5	1,935	7.3	6.3
6-10	1,848	18.7	17.3
11-20	1,897	6.8	6.0
21-30	598	6.5	4.3
31-40	419	3.1	2.1
41-50	22	-	-
51-60	1	-	-

Refined Serum

Age Group	Number of Cases	Serum Reactions	
		Total %	General %
0- 5	191	1.6	1.6
6-10	444	2.3	1.6
11-20	239	2.9	2.1
21-30	71	4.2	4.2
31-40	27	3.7	3.7
41-50	23	-	-
51-60	5	-	-

Using concentrated serum the percentage incidence of reactions fluctuates from age group to age group, being highest in the 6-10 group and falling suddenly over the age of 30. With refined serum the percentage incidence is steadily progressive up to the 31-40 group, when it falls slightly, and over 40 no reactions were recorded.

(3) Dosage of Serum.

Concentrated Serum.

Dosage (Units)	Serum Reactions	Male %	Female %	Total %
8,000	Total	5.9	4.1	5.0
	General	4.9	3.2	3.9
8-40,000	Total	13.4	11.2	12.2
	General	12.7	10.4	11.4
Over 40,000	Total	18.2	11.2	14.0
	General	14.3	9.7	11.9

Refined Serum.

Dosage (Units)	Serum Reactions	Male %	Female %	Total %
8,000	Total	-	-	-
	General	-	-	-
8-40,000	Total	3.0	1.1	2.05
	General	1.7	-	0.85
Over 40,000	Total	5.9	4.0	4.95
	General	5.9	4.0	4.95

These tables show that as we increase the dose of serum the percentage incidence of serum reaction increases, and that the rate is higher for males than for females. This conclusion holds for both refined and concentrated antisera.

(4) Intravenous Injection of the Serum:

Concentrated Serum.

Number of Cases.	Serum Reaction		General Serum Reaction	
	Number	Percentage	Number	Percentage
476	91	19.1	72	15.1
759	81	10.7	74	9.7

Refined Serum.

Number of Cases	Serum Reaction		General Serum Reaction	
	Number	Percentage	Number	Percentage
150 intravenous	5	3.3	5	3.3
250 (intravenous and intramuscular	16	8.0	14	5.6
600 intramuscular	3	0.5	-	-

With concentrated serum none of the cases received the serum by intravenous injection alone. 476 cases received a combined intravenous and intramuscular injection, and all of these cases had over 40,000 units of serum. In the tables above, with concentrated serum the comparison is made with the cases who received the serum by combined intramuscular and intravenous injection and the cases who received over 40,000 units of serum intramuscularly. With refined serum the table is self-explanatory.

It is seen that with concentrated serum the percentage of reactions is higher where the part of the serum was given intravenously, but this figure is much higher than for the same group in the refined serum table. In the latter table it is seen that the incidence of reactions is lowest where the serum was given intramuscularly, highest when given by

the combined method, and that intravenous therapy occupies the intermediate place. The patients in the intramuscular-intravenous group were the most seriously ill, and so received most serum, and this probably accounts for the higher serum reaction rate.

(5) Yearly Variations:

Year	No. of Cases	Serum Reaction		General Serum Reaction		No. with Previous Serum	Percentage Over 20,000 Units.
		No.	%	No.	%		
1930	1,177	118	10.0	107	9.1	121 (10%)	521(44.1)
1931	929	92	9.9	79	8.5	93 (10%)	417(44.8)
1932	952	97	10.2	88	9.2	98(10.3%)	435(45.7)
1933	837	85	10.1	72	8.6	85(10.1%)	391(45.5)
1934	1,054	101	9.5	89	8.5	101(9.5%)	416(39.3)
1935	962	99	10.3	90	9.3	99(10.3%)	419(44.6)
1936	809	78	9.7	69	8.5	77(9.5%)	365(45.1)
Total.	6,720	670	9.9	594	8.8	674 (10%)	2,964(44.3)
Cases treated with refined antitoxin, Aug.1937-April 1939.	1,000	24	2.4	19	1.9	116(11.6%)	650(65.0)

From the table it is seen that the percentage rate varies little from year to year. The slight increases are either associated with an increased percentage of previous cases who had been sensitised to serum or with a high incidence of patients who had received large doses of serum.

(6) Previous Injection of Serum:

(a) Concentrated Serum:

Of 6,720 cases treated, 674 had received previous serum = 10.0%
Of 6,050 cases with no serum reaction, 528 had received previous serum..... = 8.7%
Of 670 cases with serum reaction, 146 had received previous serum..... = 21.6%
Of 594 cases with general serum reaction, 132 had received previous serum..... = 22.2%

(b) Refined Serum:

Of 1,000 cases treated, 116 had received previous serum = 11.6%
Of 976 cases treated with no serum reaction, 113 had received previous serum..... = 11.5%
Of 24 cases with serum reaction, 3 had received previous serum..... = 12.5%
Of 19 cases with general serum reaction, 3 had received previous serum..... = 15.8%

Concentrated Serum

Type of Case	Total Treated	Serum Reaction		General Serum Reaction	
		Number	Percentage	Number	Percentage
No previous serum.	6,046	524	8.6	462	7.6
Previous serum.	674	146	21.6	132	19.5
Combined Group	6,720	670	9.9	594	8.8

Refined Serum.

Type of Case	Total Treated	Serum Reaction		General Serum Reaction	
		Number	Percentage	Number	Percentage
No previous serum.	884	21	2.4	16	1.8
Previous serum.	116	3	2.6	3	2.6
Combined Group	1,000	24	2.4	19	1.9

CONCENTRATED

General Serum Reaction divided into Dosage Groups.

Dosage in Units	No Previous Serum					Previous Serum.				
	No Serum Reaction	General Serum Reaction	Local	Total	% General Serum Reaction	No Serum Reaction	General Serum Reaction	Local	Total	% General Serum Reaction
-8,000	2,077	62	17	2,156	2.9	164	31	6	201	15.4
8,000-40,000	2,487	284	23	2,794	10.1	259	71	4	334	21.2
40,000+	958	116	22	1,096	10.6	105	30	4	139	21.6
	5,522	462	62	6,046	7.6	528	132	14	674	19.5

REFINED

General Serum Reaction divided into Dosage Groups.

Dosage in Units	No Previous Serum					Previous Serum.				
	No Serum Reaction	General Serum Reaction	Local	Total	% General Serum Reaction	No Serum Reaction	General Serum Reaction	Local	Total	% General Serum Reaction
-8,000	270	-	-	270	-	20	-	-	20	-
8,000-40,000	361	4	5	370	1.1	39	-	-	39	-
40,000+	232	12	-	244	4.9	54	3	-	57	5.2
	863	16	5	884	1.8	113	3	-	116	2.6

Conclusions:

The effect of previous injection of serum is illustrated in the foregoing tables. Patients who have had a previous injection of serum, are, with concentrated serum two-and-a-half times as liable to develop serum reaction as those receiving a first injection. Using refined serum, this liability is reduced to 1.4 times. With both sera the high dosage of serum, correlated with previous injection of serum, gives the highest reaction rate, but the difference between the incidence after the injection of a second dose of concentrated and of refined serum is marked, being 21.6% and 5.2% respectively. With no previous injection of serum, as the dosage increases, so does the percentage incidence of serum reactions; with concentrated serum it rises from 2.9% to 10.6%, and with refined serum from 0 to 4.9%.

SERUM REACTIONS.

An account is given of the incubation period, duration and manifestations of the serum reactions which occur after concentrated and refined sera have been injected.

(a) Incubation Period.

Day of Occurrence	Concentrated Serum Length of Incubation Period Number of Cases.			Refined Serum Length of Incubation Period Number of Cases.		
	No Previous Serum	Previous Serum	Total	No Previous Serum	Previous Serum	Total
1	8	14	22	3	1	4
2	8	10	18	1	-	1
3	9	18	27	3	-	3
4	21	19	40	1	1	2
5	40	29	69	2	1	3
6	51	40	91	5	-	5
7	61	11	72	3	-	3
8	91	4	95	3	-	3
9	94	1	95	-	-	-
10	92	-	92	-	-	-
11	29	-	29	-	-	-
12	11	-	11	-	-	-
13	2	-	2	-	-	-
14	1	-	1	-	-	-
15	2	-	2	-	-	-
16	2	-	2	-	-	-
17	1	-	1	-	-	-
18	1	-	1	-	-	-
Total.	524	146	670	21	3	24

Summary of Occurrence of General Serum Reaction.

Concentrated Serum.

Type of Case	No. of Cases	Highest % of Reactions	3-Day Group of Highest % of Reactions	Length of Incubation Period.			
				Less than 6-Days	6-12 Days incl.	13-18 Days incl.	19 Days and Over.
No Previous Serum	524	9th. Day 17.9%	8-10th. Day 52.8%	86= 16.4%	429= 81.8%	9= 1.8%	-
Previous Serum	146	6th. Day 27.4%	4-6th. Day 58.8%	90= 61.7%	56= 38.3%	-	-
Total	670			176= 26.3%	485= 72.4%	9= 1.3%	-

Refined Serum.

Type of Case	No. of Cases	Highest % of Reactions	3-Day Group of Highest % of Reactions	Length of Incubation Period.			
				Less than 6-Days	6-12 Days incl.	13-18 Days incl.	19 Days and Over.
No Previous Serum	21	6th. Day 26.3%	6-8th. Day 57.8%	10= 47.6%	11= 52.4%	-	-
Previous Serum.	3	1,4 & 5th. Day 33.3%	4th. & 5th. Day 66.6%	3= 100%	-	-	-
Total	24			13= 54.2%	11= 45.8%	-	-

From the foregoing tables it is seen that with concentrated serum the 8th. and 9th. day are the days on which most serum reactions appear, but for refined serum it is the 6th. day. Using concentrated and refined sera the majority of reactions appear in non-sensitised people between the 5th. - 11th. day and the 3rd. - 8th. day respectively, and with both sera between the day of injection and the 6th. day in sensitised people. A previous injection of serum appears to hasten the onset of the serum reaction.

Immediate and Accelerated Reactions.

An immediate reaction is one occurring within 24 hours of injection with the serum, and an accelerated reaction is one occurring between the 2nd. and 7th. days (Von Pirquet and Schick, 1905).

The following tables show the number of these types of reaction occurring after the administration of concentrated serum, compared with those where refined serum was used.

Concentrated Serum.

Type of Reaction.	No.Previous Serum		Previous Serum		Total.	
	No.	%	No.	%	No.	%
Immediate: within 24 hours	8	1.5	14	9.5	22	3.3
Accelerated: 2nd.-7th. Day.	190	36.2	127	86.9	317	47.3

Refined Serum.

Type of Reaction.	No Previous Serum		Previous Serum		Total.	
	No.	%	No.	%	No.	%
Immediate within 24 hours.	3	15.8	1	20	4	16.6
Accelerated: 2nd.-7th. Day	13	68.4	4	80	17	70.8

After the patient has been sensitised by a previous injection of serum the percentage of both immediate and accelerated reactions increases. This is true for both sera. It is also seen that with refined serum in both non-sensitised and sensitised patients the majority of reactions are of the accelerated type. With concentrated serum, in non-sensitised patients the percentage of accelerated reactions is 36.2, whereas in sensitised patients it rises to 86.9.

Duration of Serum Reactions.

In the tables below a comparison is drawn between the duration of the reactions which followed the administration of the two types of serum.

CONCENTRATED SERUM

Duration in Days.	No Previous Serum.		Previous Serum		Combined Total.	
	No.	%	No.	%	No.	%
1	156	29.7	17	11.8	173	25.8
2	107	20.4	29	19.8	136	20.3
3	121	23.1	18	12.3	139	20.8
4	41	7.8	19	13.0	60	8.9
5	43	8.2	17	11.8	60	8.9
6	29	5.5	16	10.9	45	6.8
7	17	3.3	15	10.2	32	4.7
8	8	1.6	10	6.8	18	2.7
9	2	.4	5	3.4	7	1.1
Total	524	100.0	146	100.0	670	100.0

REFINED SERUM.

Duration in Days.	No Previous Serum.		Previous Serum		Combined Total.	
	No.	%	No.	%	No.	%
1	2	9.5	1	33.3	3	12.5
2	4	19.1	-	-	4	16.7
3	9	42.9	1	33.3	10	41.7
4	2	9.5	1	33.3	3	12.5
5	2	9.5	-	-	2	8.3
6	2	9.5	-	-	2	8.3
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
Total	21	100.0	3	100.0	24	100.0

Part of the serum in some of the cases, was administered by intravenous injection.

Duration in Days.	Concentrated		Refined.	
	No.	%	No.	%
1	7	7.7	2	9.5
2	15	16.5	4	19.1
3	18	19.7	9	42.9
4	22	24.2	2	9.5
5	26	28.6	2	9.5
6	2	2.2	2	9.5
7	1	1.1	-	-
8	-	-	-	-
Total	91	100	21	100

Conclusions.

From the data given it is seen that the effects of serum reaction in non-sensitive patients has in 73% of cases (concentrated serum) passed off in 3 days, and that the effect of all serum reactions has passed off within 9 days. In sensitised patients the effect lasted longer in the majority of cases, but they too had all recovered within 9 days. Using refined serum, it is seen that in non-sensitised patients the effects of serum reaction have passed off in 3 days in 71% of cases, and all were better by the end of the 6th. day. In the sensitised patients in this group all had fully recovered by the 4th. day.

Where the serum was given intravenously, the duration of the reaction tended to be prolonged, but this was

probably due (especially with the concentrated serum cases) to large doses of serum having been given.

TYPES OF RASHES.

The three main types which occur in serum reactions are urticarial, erythematous and morbilliform, but others have been described, such as macular, circinate, papular and polymorphus. In the 670 reactions following the administration of concentrated serum, 668 exhibited rashes, and in the refined series, 24.

The following table shows the percentage of each type and the day on which the different types of rash appeared.

Type	Concentrated Serum					Refined Serum.				
	Rashes		Day of Appearance			Rashes		Day of Appearance		
	No.	%	0-5	6-12	13-	No.	%	0-5	6-12	13-
Urticarial	578	86.5	148	426	4	10	41.6	6	4	-
Erythematous	32	4.7	10	20	2	8	33.3	5	3	-
Morbilliform	28	4.2	9	18	1	4	16.7	2	2	-
Circinate	15	2.3	5	10	-	1	4.2	-	1	-
Polymorphus	15	2.3	4	9	2	1	4.2	-	1	-
Total.	668	100.0	176	483	9	24	100.0	13	11	-

The urticarial rash predominates and the incidence is higher with concentrated than with refined serum where the percentage difference between the urticarial and the erythematous is not so marked (41.6 - 33.3, as against 86.5 - 4.7). There was a higher percentage incidence of erythematous, morbilliform, circinate and polymorphus rashes when refined serum was used than when concentrated serum was

administered. Circinate and polymorphus rashes occurred in the same number with each serum.

Recurrent Rashes.

These are of rare occurrence with concentrated sera, and did not occur with refined serum. With concentrated serum only 7 were observed. They were not confined to any definite batch of serum, and the serum was given by the intramuscular route. Three of the cases were males and four were females.

Age Groups: 0-5 years 2 cases.
6-10 ,, 3 ,,
11-20 ,, 2 ,,

Dosage of Serum Given: 8,000 units or less..... 1
8,000-40,000 units..... 4
40,000 units or more.... 2

In all of them two rashes appeared. In two the time between the rashes was three days, in four it was five days, and in one it was six days. The rashes were all of the generalized urticarial type. Four of the cases had had a previous injection of serum, and three had not.

Signs of General Serum Reaction other than Cutaneous.

Below, in tabular form, is an analysis of the main signs of general serum reaction other than serum rashes.

	Concentrated			Refined.		
	Oedema	Arthritis	Fever 99°F. Upward	Oedema	Arthritis	Fever 99°F. Upward
No. of Cases....	19	17	418	-	1	4
Percentage of Cases treated...	.28%	.25%	6.2%	-	.1%	.4%
Percentage of Serum reactions.	2.8%	2.5%	62.4%	-	4.1%	16.6%
Sex: Male.....	10	12	256	-	1	2
Female....	9	5	162	-	-	2
Average Age. Years.....	8.7	17.2	11.1	-	14	16
Dosage: Up to 8,000 units..	2	3	56	-	-	-
8,000-40,000.	6	5	256	-	-	-
40,000 units & over.....	11	9	106	-	1	4
No Previous Serum; Number.....	9	6	187	-	-	3
No Previous Serum: Day of Occurrence	2-12	4-11	8-9,	-	-	1
Previous Serum: Number.....	10	11	231	-	1	1
Previous Serum: Day of Occurrence.	1-4	2-7	5-6	-	5	1
Number who had Intravenous Serum.	1	4	87	-	1	4
Concomittent Signs: General Rash.....	16	12	418	-	1	4
Local Rash.....	2	4	-	-	-	-
Rash Absent.....	1	1	-	-	-	-
Albuminuria.....	8	7	106	-	-	2
Oedema.....	-	-	12	-	-	-
Arthritis.....	-	-	12	-	-	1
Adenitis.....	-	-	-	-	-	-
Fever.....	8	12	-	-	1	-

Conclusions.

The all-round reduction in the complications due to serum by the use of refined serum is striking. The higher incidence of complication in males is shown, except in fever caused by refined serum, where the incidence was equal. As the dose of serum increases so does the liability to serum complications. A previous injection of serum increases this liability, and also, in the use of concentrated serum, reduces the incubation period of the reaction. Fever is common, more so with concentrated serum, where it occurred in 6.2% of all cases, as compared with .4% with refined antitoxin. Albuminuria was a frequent complication with concentrated serum, but only occurred in two cases treated with refined antitoxin.

General Serum Reaction with No Rash.

This occurred in two cases treated with concentrated serum. Both cases were males. Summary of each case:-

(1) A boy of 9 years received 20,000 units intramuscularly. He developed arthritis, albuminuria and a temperature of 100.6° on the 7th. day. He was given adrenalin m.5.t.i.d. for two days, together with aspirin gr. 5. q.i.d. In four days the albuminuria and the arthritis had passed off. His temperature settled on the third day.

(2) A young man of 19 was given 16,000 units intramuscularly. 6 days later he developed a temperature (101.4°) and arthritis of his interphalangeal joints of both hands. He was given aspirin gr. 10. q.i.d., and after two days his temperature settled, and in three days more the last trace of his arthritis had passed off.

Conclusion.

Arthritis, albuminuria and pyrexia can develop in the absence of cutaneous signs.

LOCAL SERUM RASH.

Definition- Where the rash is confined to site of injection.

This occurred in 76 of the concentrated serum series, and in 5 of the refined series. In the concentrated group 6 cases were associated with signs of general reaction, 2 having oedema and four having arthritis, plus the rash at the site of injection. These 6 cases had all been sensitised to horse serum by a previous injection of antiserum. In the remaining 70 cases, analysis of the rashes was: 64 urticarial, 4 erythematous, and 2 morbilliform. Of these cases 62 had had no previous serum, while 8 had had serum previously. The day of occurrence of the rash in the 76 cases was: 0-6 days, 22. 6-12, 48, and 12 days upwards 6. With refined serum, 5 local rashes were observed. These 5 cases had had no previous injection of serum and were not accompanied by any other manifestation of serum disease. The 5 rashes were of the

urticarial type, and occurred 3 on the 5th. day and 2 on the 6th. day after injection.

Conclusion.

Local rashes can occur even although the patient has been sensitised by a previous injection, but a previous injection of serum makes the patient liable to a general reaction to the serum. The rashes are mainly of the urticarial type. The day of occurrence of these local rashes approximates to the day of onset of general serum reaction.

RECURRENT LOCAL RASHES.

None were observed in this series.

ALBUMINURIA.

The difficulty of determining whether albuminuria is caused by the toxins circulating in the blood stream or whether it is due to the serum per se is obvious. In this analysis it was regarded as a complication due to serum when it occurred during the period when serum reactions do occur, and in the absence of any other cause for albuminuria.

Incidence of Serum Albuminuria.

	Concentrated Serum.	Refined Serum.
Number of Cases.....	122	2
Albuminuria and General Reaction.	121	2
Albuminuria and Local Reaction...	1	-

Day of Appearance of Albuminuria.

	Concentrated Serum.	Refined Serum.
Under 6 days.....	48	1
6 - 12 days.....	73	1
12 days.....	1	-
	122	2

Relation to Appearance of Rash.

Time of Appearance.	Concentrated Serum.	Refined Serum.
Before the rash appeared.....	22	-
After " " "	100	2
Total.....	122	2

Duration of Albuminuria.

Time.	Concentrated Serum.	Refined Serum.
1 - 5 days.....	76	2
5 - 10 days.....	44	-
10 days.....	2	-

Conclusions.

Albuminuria occurs as a serum phenomenon in 1.8% of all cases treated with concentrated serum, and in 18.4% of cases of serum reactions caused by the same serum. With refined serum the figures are .2% of all cases and 8.3% of serum reactions. With concentrated serum it makes its appearance most often between the 6th. and the 12th. day, mostly after the rash has appeared, and persists for 1 - 5 days. With refined serum the incidence

is equally divided between the 1 - 6, and 6 - 12 day periods, always after the rash has appeared, and lasts for 1 - 5 days.

THERMAL REACTIONS.

These occur most frequently after the intravenous administration of serum. With concentrated serum 476 cases received serum intravenously, and 87 (18.2%) had a febrile reaction. With refined serum 400 had serum intravenously, and 4 (1%) had a thermal reaction. A thermal reaction was defined as a rise of temperature above 99.4° and persisting for more than two hours. The total number of patients who received concentrated serum and who showed a thermal reaction was 126, which is 1.8% of all cases receiving serum, and 18.8% of all reactions. Using refined serum, 4 cases, representing .4% of all cases and 16.6% of all reactions, had a thermal reaction.

Conclusion.

Refined serum definitely reduces the incidence of thermal reactions.

PREVIOUS HISTORY OF ALLERGY.

In the concentrated serum series, 18 gave a history of asthma or previous attacks of urticaria. In this group 14 developed general serum reactions and 4 did not. The reactions were accompanied by urticarial rashes. Of the 1,000 cases treated with refined serum 12 gave an allergic history (asthma or urticaria) but none developes serum reaction.

Conclusion.

Where a history of allergy is given it is unsafe to use concentrated serum unless the patient has been desensitised. Refined serum should be used.

SERUM ACCIDENTS.

This term is used to denote the occasional severe and sometimes fatal reaction occuring after serum has been given. In neither of the series under consideration was there a fatal accident, but severe reactions were observed.

(1) Concentrated Serum. After the use of intravenous serum 22 such reactions were observed. This represents 0.3% of all cases receiving serum, 4.6% of all cases receiving the serum intravenously, and 3.3% of all reactions. 14 had received a previous injection of serum. There were no serum accidents where the serum was given intramuscularly. The train of signs and symptoms in all the cases were the same. They were - tightness across the chest, palpitation, cyanosis, breathlessness, cold perspiration, extreme

anxiety and vomiting. The patients were given .5 c.c's of 1/1000 adrenalin hydrochloride, oxygen and kept in Fowler's position - adrenalin was readministered where necessary. All cases recovered.

(2) Refined Serum. Two similar cases occurred with intravenous injection. This represents .2% of all cases, .5% of cases who were given serum intravenously, and 8.3% of all reactions.

Conclusions.

Serum accidents are rare and their incidence is increased by intravenous therapy. Refined serum should be the serum of choice. Adrenalin should always be kept at hand on the serum tray.

GENERAL SUMMARY.

An analysis has been made and a comparison drawn of the manifestations of serum reactions following upon the injection of concentrated and refined anti-diphtheritis sera.

The total incidence was -

	Concentrated.	Refined.
Total Serum Reaction.....	670(9.9%)	24(2.4%)
General Serum Reaction.....	594(8.9%)	19(1.9%)
Local Reaction.....	76(1.1%)	5(0.5%)

The factors which influence serum reactions are -

(a) Sex and Age. Males have a higher rate than females, and this rate varies in the different age groups. The incidence is lower where refined serum is used.

(b) Dosage. As the dose of serum increases, so also does the incidence of reactions. The incidence is more marked in males than in females. The serum reaction rate is lower where refined serum was given.

(c) Intravenous Injection. Where this method of therapy is employed the incidence of serum reactions rises. Intravenous therapy is usually combined with intramuscular injection, and the total dose given is usually large. This is a factor in causing an increased incidence. Using concentrated serum, the incidence was 19.1%. In the refined series, where intravenous serum was given alone, the reaction rate was 3.3%, while with combined intravenous-intramuscular injection, it was 8.0%.

Here again refined serum had the lower reaction rate.

(d) Different Batches of Serum. This would appear to have little influence on the incidence of serum reactions. Over a period of six years, using concentrated sera, the rate varied from 9.5% - 10.3%.

(e) Reinjection of Serum. This has a definite bearing on the incidence of serum reactions. With concentrated serum the incidence rose from 7.6% to 19.5%, and with refined serum from 1.8% to 2.6%. These are significant increases. The much lower percentage of reactions after refined serum is noteworthy.

Observations on Serum Reactions.

(a) Incubation Period. The 9th day after injection of concentrated serum is the day on which most reactions occur. This is reduced to 6 days in the case of refined serum, and, in both, a previous injection of serum brings about a reduction in the incubation period.

(b) Duration. The effects in most cases have passed off within 3 days, but a previous injection of serum would appear to prolong the symptoms.

(c) Types of Rash encountered. Five different rashes have been described. For both sera, the urticarial type of rash is most frequent, but the percentage incidence of this type is much higher for concentrated than for refined. Urticarial rashes are the rule in sensitised patients.

(d) Recurrent Rashes. These are rare and are not confined

to any age group, but no cases were recorded in this series in patients over 20.

(e) Oedema. This usually developes in localized sites such as the face or the hands. It is commoner in males than in females, where large doses of serum were given, and previous injection of serum had little influence on its incidence. It tends to occur in young people. No cases of oedema were recorded where refined serum was used.

(f) Arthritis. This occurred in 2.5% and 4.1% of serum reactions following the administration of concentrated and refined sera respectively. It was more frequent in males than in females, and was more common in sensitised patients.

(g) Fever. Some fever is present in most general reactions after concentrated serum - 62.4% of reactions. This figure fell to 16.6% when refined serum was used. In the concentrated series previous injection of serum and high dosage of serum were contributory factors to this high incidence. In the other series, high dosage was a factor, but previous injection of serum was not.

Local Serum Rashes.

These usually have no accompanying general reaction where rash is limited to a small area around the site of injection, but in 6 of the recorded cases where concentrated serum was used, generalized symptoms were also present.

Albuminuria.

This occurs in almost 1/5th of all cases as a sign of general serum reaction. It makes its appearance mostly

after the rash has appeared, and between the 6th and the 12th day after injection. It is less common after the administration of refined serum than after concentrated serum.

Serum Accidents.

These are rare and occur after intravenous injection. They are more likely to happen in sensitised patients. With refined serum their incidence is much reduced. No fatal case was recorded.

An analysis of the results of treatment of 1,000 cases of clinical diphtheria treated with refined diphtheria antitoxin.

SECTION 111.

An analysis of the results of treatment of 1,000 cases of clinical diphtheria treated with refined diphtheria antitoxin.

TREATMENT OF DIPHTHERIA BY REFINED ANTITOXIN.

A series of 1,000 consecutive cases of clinical diphtheria was investigated in Belvidere Fever Hospital, Glasgow, from August 1937, to April 1939. All were given refined antitoxin. This was supplied in ampoules containing (with slight variations) -

4,000 units in 0.8 c.c's of serum.
6,000 units in 1.3 c.c's of serum.
8,000 units in 1.75 c.c's of serum.
20,000 units in 3.0 c.c's of serum.

In each case the following particulars were noted:-

(a) Age of patient.

(b) Sex of patient.

(c) Clinical type of diphtheria.

(d) Strain of c. diphtheriæ recovered from the case.

(e) Time of occurrence of serum reactions or rashes.

(f) Time and type of complication.

(g) History of previous injection of serum, asthma or urticaria.

Four-hourly observations were made upon:-

(a) Temperature and pulse rate.

(b) Rate of disappearance of membrane / or discharges / or adenitis.

(c) Degree of toxaemia.

The 1,000 cases were divided into three groups:-

(a) 600 who were given the serum by intramuscular injection alone.

(b) 250 who received a combined intravenous and intramuscular injection; and

(c) 150 who received the antitoxin by intravenous injection alone.

Outline of General Treatment and Nursing of the Patients.

Each case was reviewed separately and treated according to its special requirements. Generally speaking, however, the routine procedure in the wards was:-

Immediately after the patient had been admitted to the ward and had been bathed, the temperature, pulse and respiration rates were taken, and a specimen of urine obtained (if possible). The patient was then examined and the extent of the membrane / or discharges / or adenitis was noted. The degree of toxicity was observed and recorded. Prior to the serum being given, a swab was taken of the membrane / or discharges. This was inoculated on to a Loeffler slope and on to a plate of the media of Kerrin and Gaze (1936). These were then placed in the incubator at 37° C.

From the patient, or from the nurse's case record, obtained at the patient's home (if the patient were too ill or too young to assist) it was ascertained whether the patient had suffered from asthma or urticaria or whether he had had a previous injection of serum. Previous illnesses were also recorded. The patient was now examined as to his soundness of heart and lung, and for any evidence of other disease.

The serum was then given. Although a previous history of asthma, urticaria or previous injection of serum was given, the patient received the refined antitoxin without desensitisation. On the serum tray, a syringe charged with .5 c.c's of adrenalin hydrochloride solution 1/1000, was kept at hand in case it was

required.

The serum having been given, the patient was closely observed for signs of serum reaction. The temperature, pulse and respiration rates were taken 5, 10 and 30 minutes afterwards, thereafter, 4 hourly observations were made. The urine was examined daily for abnormal constituents, such as albumin, blood, sugar and acetone.

All patients were kept lying down for three weeks, and cases which received more than 40,000 units of antitoxin were kept flat for a minimum period of four weeks. Those receiving very large doses - 200,000 units - were not allowed to sit up in bed for prolonged periods, extending in one case to 14 weeks. If a complication was observed, the patient was kept lying down until the complication was cured.

Following the lying-down period, patients were allowed to sit up in bed, for a minimum period of one week. Patients who had had large doses of serum or those who had developed complications were brought gradually to the sitting position by means of pillows. After the sitting-up period, the patients were allowed up to walk about the ward and the grounds of the hospital for another minimal period of one week.

Additional Non-Specific Treatment of the Cases.

Schwentker and Noel, 1930. They found, in cases which had a fatal termination, a hypoglycaemia. If death did not take place, the blood sugar content rose until finally there was a definite hyperglycaemia. They found, also, a reduction

of the hepatic and muscular glycogen during the toxaemia, and a definite reduction in the ability to assimilate dextrose. They advocated the use of insulin and intravenous dextrose. Begg and Harries (1935) also recommended the use of intravenous dextrose but found no benefits from the use of insulin. As the content of dextrose in the blood rose, so did the liability to cardiac paresis.

McLean, 1937, thought that the hypoglycaemia found in the acute stages of diphtheria was due to the patient's fasting. He found that the glucose tolerance curves in the acute stages of diphtheria took the form commonly called the "lag" curve. In convalescent patients the curves were of the normal type. He thought that the "lag" curve was due not to diminished absorption of glucose from the alimentary tract but to a diminution of the ability of the peripheral tissues to assimilate it from the blood.

Muir 1924, noted that the suprarenal glands showed degeneration and haemorrhages into their substance following death from diphtheria.

Rolleston, 1925, agreed with the findings of Muir but only in the case of guinea pigs which had died from diphtheria. In humans there were few "naked eye" changes, and the microscopical changes were not constant.

Ker, 1929, substantiated the observations of Muir.

Harrop et al, 1933, found that, in dogs where the suprarenal glands had been removed, the dogs lived longer if

they were given injections of sodium chloride. They concluded that the suprarenal cortex had a regulating influence over the excretion of water and sodium chloride.

Thaddea, 1935, showed that, following upon the injection of guinea pigs with diphtheria toxin, there is necrosis and haemorrhage of the suprarenal cortex and a disappearance of lipoid material also from the cortex. These changes could be avoided or diminished if the guinea pigs were given extracts of suprarenal cortex combined with ascorbic acid (Vitamin C) which is normally found in the cortex.

McLean, 1936, in a controlled series obtained good results by administering one teaspoonful of salt per mouth, three times a day for the first four weeks that the patient was in hospital. In patients who were too ill to take salt by mouth he employed continuous rectal salines. In the patients who did not receive the additional sodium chloride there was a higher complication rate, and their stay in hospital was longer than those who did receive the extra sodium chloride.

McLean 1937. In cases where death took place, he found that there was a fall in the serum sodium and in the serum chlorine. By treating the patients with sodium chloride this fall was mitigated.

From the data given above it is seen that in diphtheria there is:-

(a) A hypoglycaemia in prelethal cases and in cases which recover, a hyperglycaemia.

(b) There is some upset in metabolism whereby the peripheral tissues lose their power to assimilate the dextrose in the blood.

(c) During diphtheria there is damage to the suprarenal glands.

(d) In diphtheria there is a deficiency in the blood of sodium and chlorine.

(e) The effects of the damage to the suprarenal glands can be lessened by the administration of sodium chloride and ascorbic acid.

(f) Dextrose is a useful adjunct in the treatment of diphtheria.

All patients on admission to hospital were treated with fluids, glucose (to which one teaspoonful of salt per pint of glucose solution had been added) and liberal supplies of fruit juices, the fruit juices supplying the ascorbic acid (Vitamin C). Three days following the disappearance of the membrane / or discharges, some solid food was gradually introduced into the diet, and slowly the patient was brought back to normal diet, the time taken for this being 10-16 days. As many of the patients liked ice-cream and as it contains sugar, milk and cornflour, this was given each day, commencing on the 3rd. day following the disappearance of the membrane or discharges.

Where, in conjunction with the diphtheria, there

appeared a streptococcal or staphylococcal infection of the throat, the patients were given a sulphanilamide preparation - the preparation used being Streptocide (Evans), the dose varying from ½ to 1 gramme 4 hourly.

The patients were given mouth washes of glycothymolene every four hours, and where the patient was too ill or too young the mouth was cleaned by nurse.

Where cervical adenitis was present, when the patient had sufficiently recovered from the initial toxaemia, he was given chewing gum. This appeared to reduce the adenitis by causing massage of the cervical glands by the action of the jaw in chewing.

When the patient had returned to normal diet, an iron tonic was given. The prescription was:-

Syr. Ferri Phos. Co.
Syr. Ferri. Iod. ā ā ʒ iv.
Syr. Simplex ʒ i.
Aq. ad. ʒ vi.
Sig. ʒ ii t.i.d.m ex aq. p.c.

Treatment of Complications due to the Toxin of Diphtheria.

(a) Cardiac Paresis. When during the patient's stay in hospital, an elevation of the pulse rate of more than 10 beats per minute was observed, the patient was kept lying flat in bed. If he had been on normal diet, he was put on fluids until the pulse rate had fallen. The patients were examined frequently in order to determine the tone of the

heart muscle, and to detect any arrhythmia which might be present. If no marked evidence of cardiac disease was found, the patient was kept lying down for one week after his pulse rate had returned to normal. Thereafter he was brought to a sitting posture in bed by pillows and so on until he could get up from bed.

When a definite arrhythmia developed, the patient, in addition to the treatment detailed above, was given ½ c.c. adrenalin hydrochloride solution 1/1000 4-hourly until the normal rhythm was restored. The foot of the bed was elevated on blocks.

In acute cardiac failures, the patient was immediately given .5 c.c's of 1/1000 solution adrenalin hydrochloride, the foot of the bed was elevated, and oxygen was given continuously. No food was given by mouth, but the patient was given spicules of ice to cool his mouth. This treatment was persisted in for at least 24 hours. If the patient had improved, some fluids were allowed by mouth, but if not, rectal glucose was given. This treatment was then persisted in until the patient had improved sufficiently to be allowed some fluids by mouth. The reason for the stopping of oral feeding is that one of the cardinal symptoms of cardiac failure in diphtheria is vomiting. If no food is put into the stomach, the risk of vomiting is lessened.

(b) Palatal Paresis. As soon as this complication was recorded, the patient was kept in the recumbent position until

one week after his speech had returned to normal. While suffering from this paresis, he was given a fluid diet which was administered to him by nurse, who took care that none of the food passed into the respiratory tract. The patient was gradually brought to the sitting position, and finally allowed to get up from bed.

(c) Albuminuria. This was treated by the patient's being given a diet consisting mainly of fluids, carbo-hydrate and milk. Return to normal diet was gradual and was on the lines followed in the nursing of acute nephritis.

(d) Ocular Paresis. This occurred in six patients, all of whom were in the same ward at the same time. The paresis took the form of a third nerve palsy, with external strabismus, diplopia, dilated pupils, loss of power of accommodation and no reaction to light. The six patients were kept together in the convalescent part of the ward, which was darkened. They were kept lying flat, fed on fluids, and were not allowed to read. They all recovered. No pathological condition of the retina or optic nerve was seen on ophthalmoscopic examination.

Treatment of the complications due to serum.

(a) Serum Rash. The patient was given ½ to 1 c.c. of 1/1000 adrenalin hydrochloride solution, and cooling applications of calamine lotion or methylated spirit were applied. At the same time, calcium gluconate (10 grs. t.i.d.) was also given.

(b) Arthritis. This was treated by sodium salicylate and sodium bicarbonate ā ā gr. 5 - 10 q.i.d. In this case the pain in the ankle joints was very severe, a cage being used to take the weight of the blankets from the feet. The ankles were rubbed with methyl salicylate and wrapped in cotton wool. Recovery was complete.

(c) Albuminuria. The two cases which developed this complication also had an accompanying serum rash. .5 c.c's of a 1/1000 solution of adrenalin hydrochloride was given subcutaneously every four hours until the albuminuria had cleared up.

(d) Adenitis. This involved the cervical glands. The patient was given adrenalin .5 c.c's 4-hourly. The condition was cured in three days.

(e) Serum Accident or Shock. This occurred in two cases and was treated by elevation of the foot of the bed, intramuscular injection of .5 c.c. 1/1000 adrenalin hydrochloride solution, repeated in 30 minutes and in one hour's time, and administration of oxygen.

Strains of C. Diphtheriæ recovered from the 1,000 Cases.

The literature on the various strains found is abundant. Anderson et al, (1931) found in Leeds that the Gravis strain predominated. Carter (1933 and 1936) found the intermediate type to be the most common in the Glasgow area. In 1936, Carter reported for the first time in the Glasgow area the presence of the two types, named iv and vi

by Wright and Christison (1935). These observers used McLeod's Tellurite Medium (1931). In Belvidere Hospital the medium used was that of Kerrin and Gaze (1936). All cultures were tested for their biochemical and haemolytic reactions and for their growth in broth. To differentiate between types iv and vi, animal virulence tests were necessary.

Taking of the Swab.

Many patients are swabbed and a negative result is returned when the clinician is sure that the case is diphtheria. The reasons for this may be:-

(a) The case is not diphtheria.

(b) There is frequently present a good deal of exudate which is staphylococcal or streptococcal in origin.

(c) The nurse fails to swab the membrane because of the resistance of the patient.

Swabbing a patient is an operation in which the doctor taking the swab requires the help of a nurse. The doctor stands at the side of the bed on the patient's left with a tongue depressor in his left hand, the swab in the right. The nurse is at the opposite side of the bed, a pencil torch in her left hand, her right hand steadying the patient's head. The patient's mouth is opened, the light shone into the back of the mouth, the amount of membrane noted and then swabbed.

Immediately this had been done it was inoculated on to

a Loeffler's egg medium slope (1884) and, at the same time, on to a plate of Kerrin and Gaze medium. These were then incubated at 37°C. for 24 hours before being examined. If, after this period, the cultures on these media were negative, they were reincubated for a further 24 hours, when they were re-examined. In the meanwhile, a second swab had been taken after the first negative had been returned. The second cultures were subjected to the same treatment. If the result were negative from all the cultures, a third swab was taken and all six xultures were incubated. Each culture was examined at the end of 24 hours until it had been in the incubator for 72 hours. If at the end of this period all had been reported negative, the swab was reported as negative. If a positive culture was obtained, it was tested for its growth in broth, its biochemical and haemolytic reactions. If the result were still doubtful when these results were correlated with the colony appearance on the tellurite medium, the culture was sent for a virulence test.

Preparation of the Medium of Kerrin and Gaze.

A. One-and-a-half pounds of minced ox heart muscle is added to 1,000 c.c's of tap water and the mixture heated to 48°C. and kept at that temperature for one hour. The juice is then squeezed out through muslin and filtered through chardin filter paper in the ice chest. The volume is not made up with water. To every 1,000 c.c's of this filtrate, 20 grammes of Witte's peptone and 5 grammes of

Sodium Chloride A.R. are added. The mixture is now heated to 45° C. and kept at that temperature until all the peptone and salt are dissolved. The pH of the mixture is adjusted as follows:-

50 c.c's of the mixture are heated to 80-90°C. for 5-10 minutes. The mixture is filtered through paper and allowed to cool. The amount of N/1 NaOH required to bring a quantity of the mixture to a pH of 7.6 is calculated and added. The mixture is now filtered through chardin paper and then through a seitz E.K. filter disc into a sterile flask. The filtrate is stored in 100 c.c flasks and tested for sterility by incubating for 24 hours at 37° C.

B. A 5 per cent. solution of agar is made in water. The reaction of this does not require to be adjusted. Quantities of 100 c.c's are placed in 250 c.c's flasks and autoclaved at 115° C. for 20 minutes.

C. A 1 per cent. solution of potassium tellurite (B.D.H.) is made in distilled water. This must be freshly prepared.

D. A quantity of ox serum is sterilized by filtration.

To 100 c.c's of mixture (A), 8 c.c's of the potassium tellurite solution, 15 c.c's of sterile ox serum, 2 grammes of saccharose (B.D.H. and A.R.) and 2 c.c's of Andrade's indicator are added , The mixture is heated to 75-80°C. and kept at that temperature for 10 minutes. During this heating the mixture should be well shaken to avoid clumping. This mixture is now added to a flask containing

5 per cent. agar (B) at 75° C. and this mixture is kept at that temperature for 10 minutes. The medium can now be placed in sterile tubes for slopes or cooled to 45° C. for plates. The finished product is a semi-opaque buff-coloured substance.

The appearance of the diphtheria colonies when grown on this medium are:-

(a) Gravis. After 24 hours, a well-separated colony which is approximately two mms. in diameter, has a raised centre and a slightly irregular edge. The surface is usually dullish and finely granular. The central part of the colony is pale grey with a lighter periphery which is translucent at the edge. This periphery shows some tendency to radial striation. After 48 hours' incubation the diameter of the colony is increased to 3-5 mms. The centre is dark, the periphery grey, and the striations are well marked, giving a "daisy head" appearance.

(b) Intermedius. After 24 hours' incubation they are small umbonate colonies about .5 mm. in diameter. The outline is regular, the surface smooth, the centre dark brown and the periphery translucent. In 48 hours the size of the colony has increased to 1.5 mms. in diameter, the brown colour is more intense and the periphery is reduced in size. The edge may be slightly irregular, and some fine concentric rings are often seen on the surface of the colony, which may also be finely granular.

(c) Mitis. After 24 hours the colony is about 1 mm. in

diameter, low and convex, has a regular edge and a smooth surface. The central part is black, the periphery pale grey. In 48 hours the diameter has increased to 2-3 mms. and the colony is much blacker. The surface is shiny and only the extreme periphery remains a pale colour.

Biochemical Reactions of the Various Strains.

	Starch.	Glycogen
Gravis.....	+	+
Intermedius.....	-	-
Mitis.....	-	-
Type iv.....	-	-
Type vi	-	-

Growth in Broth.

Gravis..... - Pellicle and Granular Deposit.

Intermedius - Granular.

Mitis..... - Uniform Turbidity.

Type iv..... - Pellicle and Granular Deposit.

Type vi ..... - Pellicle and Granular Deposit.

Test for Haemolysis (Hammerschmidt, 1924 and 1925)

Gravis..... - Non-Haemolytic.

Intermedius.. - Non-Haemolytic.

Mitis..... - Haemolytic.

Type iv..... - Non-Haemolytic.

Type vi..... - Non-Haemolytic.

Animal Pathogenicity.

Gravis..... +

Intermedius.. +

Mitis..... +

Type iv..... +

Type vi..... -

No mention has been made of the microscopical appearances of the different strains of c. diphtheriae as, in my opinion, it is not a reliable test.

Analysis of the 1,000 cases treated with refined serum is shown (exclusive of negative cases).

	Gravis	Intermedius	Mitis	Type iv.	Type vi
Appearance on Tellurite Medium	295 + 17	263	374	8	9
Granular + Pellicle in Broth.....	295 + 17	-	-	8	9
Granular in Broth.....	-	263	-	-	-
Uniform Turbidity in Broth.....	-	-	374	-	-
Fermentation of Starch and Glycogen.....	295	-	-	-	-
Haemolytic.....	-	-	374	-	-
Pathogenicity for Animals.....	295 not tested	Not tested	Not tested	8	-

Conclusions.

The combination of the colony appearances on the

medium of Kerrin and Gaze, together with the appearance in broth, the biochemical and haemolytic reactions, affords a conclusive method, in most cases, of differentiating between the different strains of c. diphtheriæ. In a few cases an animal pathogenicity test is necessary to differentiate between certain rarer types (iv and vi).

The incidence of the Gravis strain is definitely higher in the Glasgow area when compared with the reports by Carter (1933 and 1936) and the Glasgow Medical Officer of Health's Report, 1935 (Knightswood Hospital).

Detailed Analysis of the 1,000 Cases treated with Refined Serum.

1. Age and Sex.

Age Groups	0-5	6-10	11-20	21-30	31-40	41-50	51-60
Males.....	93	247	130	42	15	20	5
Females....	98	197	109	29	12	3	0
Total.....	191	444	239	71	27	23	5

Of the total, 552 (55.2%) were males and 448 (44.8%) were females. 635 (63.5%) of the patients were aged 10 or under.

2. Age of Patients and Type of C. Diphtheriae recovered from Each Case (expressed first as a Percentage of Age Groups and then as a Percentage of Type of C. Diphtheriæ.

Age Groups	Gravis	Intermedius	Mitis	Aberrant	Negative.
0-5	61(31.9)	56(29.3)	68(35.6)	3(1.55)	3(1.55)
6-10	133(29.9)	122(27.5)	161(36.3)	7(1.6)	21(4.7)
11-20	84(35.1)	49(20.5)	85(35.6)	5(2.1)	16(6.7)
21-30	14(19.7)	17(23.9)	38(53.5)	2(2.9)	-
31-40	3(11.1)	14(51.9)	6(22.2)	-	4(14.8)
41-50	-	5(21.7)	11(47.8)	-	7(30.5)
51-60	-	-	5(100)	-	-
Total	295	263	374	17	51

Age Groups	Gravis	Intermedius	Mitis	Aberrant	Negative.
0-5	61(20.7)	56(21.5)	68(18.2)	3(17.6)	3(5.9)
6-10	133(45.1)	122(46.3)	161(43.1)	7(41.3)	21(41.2)
11-20	84(28.5)	49(18.6)	85(22.7)	5(29.4)	16(31.4)
21-30	14(4.7)	17(6.5)	38(10.2)	2(11.7)	-
31-40	3(1.0)	14(5.3)	6(1.6)	-	4(7.8)
41-50	-	5(1.8)	11(2.9)	-	7(13.7)
51-60	-	-	5(1.3)	-	-
Total.	295	263	374	17	51

Of the 0-10 age group, the ages in which the death rate in diphtheria is highest (Graham Forbes, 1932), 30.9% of the cases had Gravis infections and 28.3% had Intermedius. These two types are usually associated with the severest clinical types of diphtheria. (Anderson et al 1931). From the age of 40 upwards, most infections were caused by the Mitis strain.

The Gravis, Intermedius and Mitis strains had their highest incidence in the 6-10 age group, the Intermedius having the highest percentage - 46.3. Gravis came next with 45.1, and Mitis last with 43.1. Of the Gravis infections, 65.8% occurred in children of 10 and under, and 67.8% of the

Intermedius occurred in this same age group. With Mitis, Aberrant and Negative cases, the highest proportion of these was found in the 6-20 age groups.

3. Dosage of Serum given in the Various Age Groups (expressed first as a Percentage of the Age Groups and then as a Percentage of the Number receiving the various doses).

Age Group.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
0-5	19 (9.9)	121 (63.3)	51 (26.8)
6-10	41 (9.2)	263 (59.2)	140 (31.6)
11-20	29 (12.1)	118 (49.4)	92 (38.5)
21-30	9 (12.7)	32 (45.1)	30 (42.2)
31-40	5 (18.5)	14 (51.8)	8 (29.7)
41-50	6 (26.1)	10 (43.5)	7 (30.4)
51-60	2 (40)	3 (60)	-
Total.	111	561	328
0-5	19 (17.1)	121 (21.5)	51 (15.6)
6-10	41 (36.9)	263 (46.9)	140 (42.7)
11-20	29 (26.1)	118 (21.0)	92 (28.0)
21-30	9 (8.1)	32 (5.7)	30 (9.1)
31-40	5 (4.5)	14 (2.5)	8 (2.4)
41-50	6 (5.4)	10 (1.8)	7 (2.2)
51-60	2 (1.9)	3 (0.6)	-
Total	111	561	328

The majority of patients (56.1%) received a dose of serum between 8,000 and 40,000 units. Doses of 40,000 units and over came next in frequency and small doses of less than 8,000 units were used least of all. The percentage of patients who received doses of less than 8,000 units increased with age. The percentage of patients receiving 8,000 to

40,000 units varied from age group to age group, being 63.3% in the 0-5 age group and 45.1% in the 21-30 group. Of the patients who received 8,000 units or over, 575 (57.5%) were between the ages of 0-10.

4. Day of Disease correlated with Type of C. Diphtheriæ recovered from the case (expressed first as a percentage of Cases per Day of Disease and then as a Percentage of the Various Types found).

Day of Disease	Gravis	Intermedius	Mitis	Aberrant	Negative.
1	4(11.8)	8(23.4)	19(55.9)	-	3(8.9)
2	66(23.9)	92(33.3)	92(33.3)	3(1.1)	23(8.4)
3	117(32.7)	73(20.4)	148(41.5)	7(1.9)	12(3.5)
4	59(32.9)	42(23.4)	62(34.6)	3(1.8)	13(7.3)
5	20(28.9)	33(47.8)	13(18.9)	3(4.4)	-
6	17(24.6)	13(18.9)	38(55.1)	1(1.4)	-
7	-	2(50.0)	2(50.0)	-	-
8	12(100)	-	-	-	-
Total.	295	263	374	17	51
1	4(1.3)	8(3.0)	19(5.1)	-	3(5.9)
2	66(22.4)	92(34.9)	92(24.6)	3(17.6)	23(45.1)
3	117(39.6)	73(27.4)	148(39.6)	7(41.2)	12(23.5)
4	59(20.0)	42(15.9)	62(16.5)	3(17.6)	13(25.5)
5	20(6.8)	33(12.5)	13(3.5)	3(17.6)	-
6	17(5.8)	13(4.9)	38(10.1)	1(6.0)	-
7	-	2(0.6)	2(0.6)	-	-
8	12(4.1)	-	-	-	-
Total.	295	263	374	17	51

Of the 1,000 cases, 846 (84.6%) had received serum treatment within four days from the onset of their illness. Of the cases which had a Gravis infection, only 1.3% received serum on the first day of their illness. By the end of the fourth day however, 83.3% had received serum treatment. Of the Intermedius, Mitis, Aberrant and Negative cases, 81.2%,

85.8%, 76.4% and 100% respectively had been given antitoxin by the end of the fourth day of illness.

5. Day of Disease correlated with the Age of the Patient (expressed first as a Percentage of the Number of Cases occurring Each Day and secondly as a Percentage of the Number of Cases in Each Age Group.

Day of Disease	0-5	6-10	11-20	21-30	31-40	41-50	51-60
1	4(11.7)	7(20.6)	11(32.4)	6(17.7)	4(11.7)	2(5.9)	-
2	54(19.5)	128(46.4)	78(28.3)	10(3.6)	4(1.5)	2(0.7)	-
3	58(16.2)	161(45.1)	74(20.8)	37(10.3)	13(3.6)	10(2.8)	4(1.2)
4	46(25.6)	79(44.1)	29(16.1)	15(8.4)	3(1.7)	6(3.4)	1(0.7)
5	14(20.3)	35(50.7)	18(26.2)	1(1.4)	1(1.4)	-	-
6	9(13.1)	24(34.8)	29(42.0)	2(2.9)	2(2.9)	3(4.3)	-
7	-	4(100)	-	-	-	-	-
8	6(50)	6(50)	-	-	-	-	-
Total.	191	444	239	71	27	23	5
1	4(2.1)	7(1.5)	11(4.6)	6(8.4)	4(14.8)	2(8.7)	-
2	54(28.3)	128(28.9)	78(32.6)	10(14.1)	4(14.8)	2(8.7)	-
3	58(30.3)	161(36.3)	74(30.9)	37(52.1)	13(48.2)	10(43.5)	4(80)
4	46(24.2)	79(17.9)	29(12.2)	15(21.1)	3(11.1)	6(26.1)	1(20)
5	14(7.3)	35(7.9)	18(7.5)	1(1.4)	1(3.7)	-	-
6	9(4.7)	24(5.4)	29(12.2)	2(2.9)	2(7.4)	3(13.0)	-
7	-	4(0.9)	-	-	-	-	-
8	6(3.1)	6(1.2)	-	-	-	-	-
Total.	191	444	239	71	27	23	5

In this series, in the age groups where the mortality is highest (0-10 years), 537 out of 635 cases had received serum before the end of the fourth day. The earlier the serum is given, the better the prognosis and this holds especially in those age periods mentioned above. Most of the cases that received serum very late were in the 0-10 age group.

For all age groups, with the exception of the 11-20 group, the percentage of cases which came under treatment rose

to a maximum on the third day of illness, and thereafter became less. The rate of diminution varied in the different groups.

6. Day of Disease correlated with Dose of Serum given (expressed first as a Percentage of Cases per Day and secondly as a Percentage of Cases receiving the defined Doses).

Day of Disease.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
1	26 (76.5)	4 (11.75)	4 (11.75)
2	46 (16.7)	168 (60.9)	62 (22.4)
3	36 (10.1)	226 (63.3)	95 (26.6)
4	2 (1.2)	127 (70.9)	50 (27.9)
5	1 (1.5)	21 (30.4)	47 (68.1)
6	-	11 (15.9)	58 (84.1)
7	-	3 (75.0)	1 (25.0)
8	-	1 (8.3)	11 (91.7)
Total.	111	561	328
1	26 (23.4)	4 (0.7)	4 (1.2)
2	46 (41.4)	168 (29.9)	62 (18.9)
3	36 (32.4)	226 (40.3)	95 (28.9)
4	2 (1.9)	127 (22.6)	50 (15.2)
5	1 (0.9)	21 (3.8)	47 (14.3)
6	-	11 (1.9)	58 (17.8)
7	-	3 (0.6)	1 (0.3)
8	-	1 (0.2)	11 (3.4)
Total.	111	561	328

As the duration of illness lengthened before the administration of antitoxin, the dose required, increased.

In the under 8,000 units series, the highest number of patients receiving this dose did so on the second day of illness. With the 8,000 to 40,000, and 40,000 units and over, the highest number receiving this dose did so on the third day.

Thereafter the percentage number fell with the increase of time but the fall was slower with the over 40,000 units series.

7. The Type of C. Diphtheriae recovered from the Individual Cases is correlated with the dose of Serum given (expressed as a Percentage of Type of C. Diphtheriae).

Type of C. Diphtheria.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
Gravis.	17 (5.8)	121 (41.0)	157 (53.2)
Intermedius.	14 (5.3)	121 (46.0)	128 (48.7)
Mitis.	55 (14.7)	281 (75.1)	38 (10.2)
Aberrant.	3 (17.6)	10 (58.9)	4 (23.5)
Negative.	22 (43.1)	28 (55.0)	1 (1.9)
Total.	111	561	328

In the Gravis infections, the majority of the patients (53.2%) required a dose of serum of over 40,000 units, 41.0% required doses varying between 8,000 and 40,000 units and only a small percentage (5.8) were given less than 8,000 units. With the Intermedius infections similar figures were obtained, these being 48.7%, 46% and 5.3%. In the Mitis infections most of the cases (75.1%) were given doses varying between 8,000 and 40,000 units, while 14.7% and 10.2% received less than 8,000 units and over 40,000 units respectively. In the aberrant cases much the same results were obtained, the figures being 58.9%, 17.6% and 23.5%. In the negative cases 43.1% were given less than 8,000 units, 54.9% between 8,000 and 40,000 units and 2% over 40,000 units.

The Gravis infections required the highest

doses of serum. Next in order came (1) intermedius, (2) mitis, (3) aberrant, and (4) negative cases, which required least of all.

Faucial diphtheria is divided into four different clinical types according to the amount of membrane present.

(a) Mild: Where there was membrane amounting to the size of a threepenny piece on one or divided between both tonsils, these cases required a dose of serum up to 8,000 units for their cure.

(b) Moderate: Where the total membrane on one or divided between both tonsils amounted to the size of a florin, they required a dose of serum varying between 8,000 and 32,000 units.

(c) Severe: Where the membrane was present on both tonsils and had spread on to the uvula, these cases required a dose of serum varying from 32,000 units to 80,000 units.

(d) Haemorrhagic: Where both tonsils, uvula and the hard palate were covered with membrane and there was bleeding from the edges of the membrane, these cases required from 80,000 units upwards for their cure.

8. **Type of C. Diphtheriæ recovered from the Different Clinical Varieties of Diphtheria, expressed as a Percentage**

of Clinical Cases. Cervical Adenitis is expressed as a Percentage of Cases.

Clinical Type.	Gravis	Intermedius	Mitis	Aberrant	Negative.
Faucial:					
(a) Mild	24(14.1)	20(11.7)	94(54.9)	5(2.9)	28(16.4)
(b) Moderate	112(28.9)	103(26.4)	152(39.4)	8(2.1)	12(3.2)
(c) Severe	68(41.7)	51(31.3)	38(23.3)	2(1.2)	4(2.5)
(d) Haemorrhagic	23(47.0)	14(28.5)	12(24.5)	-	-
Nasal	4(21.0)	2(10.5)	13(68.5)	-	-
Laryngeal	-	-	13(65.0)	-	7(35.0)
Faucial and Nasal	64(33.3)	73(38.3)	52(27.4)	2(1.0)	-
Cervical Adenitis	220(74.6)	160(60.8)	140(31.6)	6(35.3)	10(19.6)
Total.	295	263	374	17	51

As the clinical severity of the case increased, the percentage of Gravis and Intermedius infections increased. As these types increased, the percentage of mitis, aberrant and negative cases decreased.

Cervical adenitis was common in diphtheria, occurring most frequently in Gravis infections and decreasing in frequency in the intermedius, aberrant, mitis and negative cases, in that order.

Death and Complication Rates.

These factors vary greatly in different series. Anderson et al (1931) found the Gravis strain to be associated with a high death and complication rate, while Carter (1936) found the intermedius strain to be the type having the highest death and complication rate.

Serum reactions have been dealt with in previous chapter.

(a) The following table shows the Type of C. Diphtheriae

recovered correlated with the various complications observed and with the death rate (expressed as Percentages of the number of the Types found).

Complication.	Gravis.	Intermedius.	Mitis	Aberrant	Negative.
Albuminuria	16(5.4)	9(3.4)	8(2.1)	1(5.9)	2(3.9)
Cardiac Paresis.	22(7.4)	12(4.5)	2(0.5)	-	-
Palatal ,,	18(6.1)	16(6.1)	6(1.5)	1(5.9)	1(2.0)
Ocular ,,	-	6(2.3)	-	-	-
Death Rate	16(5.4)	8(3.0)	3(0.8)	1(5.9)	1(2.0)

The Gravis infections, with the exception of the aberrant strain (see below) had the highest complication rates, with the intermedius cases coming next in frequency. The mitis cases had the fewest complications. The complications shown under the aberrant column all occurred in the same case - a case of Type iv. The negative cases with complications were two very ill patients from whom a persistent negative swab was obtained, although the cases were typically diphtheria. Three of the deaths were not due directly to diphtheria. One was due to a nursing accident and two were due to septic meningitis following upon mastoiditis. The two cases developed mastoiditis some weeks after admission to hospital. They first had a septic tonsilitis then developed otitis media, which progressed into mastoiditis. Both cases were operated on for mastoiditis, but developed septic meningitis and died.

(b) Death and Complication Rates correlated with the Age of the Patient (expressed as Percentage of the Age Group).

Age Group.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
0-5	8(4.2)	7(3.7)	10(5.2)	3(1.6)	11(5.8)
6-10	17(3.8)	16(3.6)	12(2.7)	3(0.7)	8(1.8)
11-20	4(1.6)	12(5.0)	10(4.2)	-	9(3.8)
21-30	6(8.5)	4(5.6)	3(4.2)	-	1(1.4)
31-40	1(3.7)	3(11.1)	1(3.7)	-	-
41-50	-	-	-	-	-
51-60	-	-	-	-	-
Total.	36	42	36	6	29

Generally, the percentage of complications was highest in the 0-5 age group.

Albuminuria occurred in 4.2% of children under five. Thereafter the percentage fell until the 21-30 group was reached, where there was a sharp rise, even above the 0-5 figure, and then it fell again.

Palatal paresis was, apart from a slight fall in the 6-10 age group, a progressively more frequent complication with age, up to the age of 40.

Cardiac paresis, typified by rise in pulse rate, with or without irregularity of the rhythm, loss of tone of the heart, muscles and softness of the pulse, was most frequently found in the 0-5 age group. The incidence rose in the 11-20 and the 31-40 age groups, but was never so high as in the first age group.

Ocular paresis occurred most frequently in the 0-5 age group.

Death rate was highest in the very young. It fell

in the 6-10 group but rose again in the 11-20 group, but not so high as in the 0-5 group.

(c) Death and Complication Rates correlated with Day of Disease (expressed as Percentages of Day of Disease).

Day of Disease.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
1	-	-	1(2.9)	-	-
2	3(1.2)	7(2.7)	4(1.6)	-	1(0.4)
3	7(1.7)	10(2.8)	8(2.2)	4(1.1)	7(1.7)
4	14(7.8)	12(6.7)	10(5.6)	2(1.1)	6(3.4)
5	10(14.6)	8(11.6)	4(5.8)	-	6(8.7)
6	2(2.9)	5(7.3)	6(8.7)	-	6(8.7)
7	-	-	1(25.0)	-	1(25.0)
8	-	-	2(16.7)	-	2(16.7)
Total.	36	42	36	6	29

As the time before treatment was commenced increased, so did the incidence of complications increase. In all complications (with the exception of the cardiac paresis) the incidence increased up to the fifth day. Thereafter the incidence of complications became irregular. This was probably due to some of the cases being very mild and having been swabbed by the practitioner because of some small patch becoming apparent.

(d) Death and Complication Rates correlated with the Clinical Types of Diphtheria (expressed as Percentages of Clinical Types).

Clinical Type.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
Faucial:					
(a) Mild	2(1.2)	3(1.8)	-	-	-
(b) Moderate	3(0.8)	4(1.0)	-	-	-
(c) Severe	5(3.1)	7(4.3)	1(0.6)	6(3.6)	1(0.6)
(d) Haemorrhagic	13(26.5)	14(28.7)	14(28.7)	-	16(32.7)
Nasal	-	-	-	-	-
Laryngeal	-	-	4(20.0)	-	2(10.0)
Faucial and Nasal	13(6.8)	14(7.3)	17(8.9)	-	10(5.2)
Total.	68	42	36	6	29

With the exception of the moderate faucial cases, as the clinical severity of the case increased, so did the incidence of complications. The faucial-nasal cases occupied an intermediate position between severe and haemorrhagic faucial cases when considered in terms of complications.

Cardiac complications occurred in 20% of the cases of laryngeal diphtheria.

Dose of Serum.

The tendency is to increased dosage. This has been made more easy through the serum being available in concentrated form. Park (1921) advocated 50,000 units as the largest dose of serum that should be given. Bie(1922) gave up to 340,000 units with good results. In London (privately communicated) large doses up to 400,000 units have been given. In the series under consideration the largest dose given was 312,000 units (with recovery).

Park (1921) was of the opinion that one large dose should be sufficient for a case, but Bie (1922) and Friedmann (1922) thought that additional doses should be given if necessary. In the 1,000 cases dealt with, second, third and fourth doses were given. This should be done, because frequently with Gravis and Intermedius cases which have received a large initial dose the membrane spreads rapidly instead of diminishing. If after 24 hours from the time of the administration of the serum the membrane is still

spreading, more serum should be given. Of the 1,000 cases treated, 205 had two injections of serum, 44 had three and 5 had four separate injections.

An Analysis of the Groups of Cases treated according to the Different Routes of Administration of the Serum.

1. Intravenous.

Method of Administering the Serum intravenously.

A 10 c.c. side nozzled (record) syringe was used. The ampoule containing the serum was heated in water to blood-heat. The patient's arm was cleansed with methylated spirit, dried and held flat on the bed. Pressure on the upper arm was exerted by the hand of the sister in charge of the ward. This was applied for 30-60 seconds and the patient told to open and shut his hand. The serum was then slowly injected into the cephalic or basilic vein, about 2 c.c's of serum per minute. The patient was kept under close observation for any sign of serum reaction.

Analysis of the 150 cases treated.

(1) Age and Sex

Age Groups	0-5	6-10	11-20	21-30	31-40	41-50	51-60
Males	13	41	17	7	2	3	-
Females	16	27	15	6	2	1	-
Total.	29	68	32	13	4	4	-

Of the total, 83 (55.3%) were males and 67 (44.7%) were females, 97 (64.7%) of the patients were in the 0-10 age group.

(2) Age of Patient and Tupe of C. Diphtheriae recovered from each Case (expressed first as a percentage of age groups and then as a percentage of Type of C. Diphtheriae).

Age Groups	Gravis	Intermedius	Mitis	Aberrant	Negative.
0-5	8(27.6)	6(20.7)	14(48.3)	-	1(3.4)
6-10	19(27.9)	18(26.5)	28(41.2)	-	3(4.4)
11-20	9(28.1)	7(21.9)	15(46.9)	-	1(3.1)
21-30	7(53.8)	4(30.8)	2(15.4)	-	-
31-40	2(50.0)	1(25.0)	1(25.0)	-	-
41-50	-	2(50.0)	2(50.0)	-	-
51-60	-	-	-	-	-
Total.	45	38	62	-	5
0-5	8(17.8)	6(15.8)	14(22.6)	-	1(20.0)
6-10	19(42.2)	18(47.4)	28(45.2)	-	3(60.0)
11-20	9(20.0)	7(18.4)	15(24.2)	-	1(20.0)
21-30	7(15.6)	4(10.5)	2(3.2)	-	-
31-40	2(4.4)	1(2.6)	1(1.6)	-	-
41-50	-	2(5.3)	2(3.2)	-	-
51-60	-	-	-	-	-
Total.	45	38	62	-	5

Of the 0-10 age groups, 27.8% had gravis infections and 23.6% intermedius. There was a high incidence of the gravis strain in the 21-30 age group, where it was 53.8%. Of four cases in the 41-50 age group, two were of the intermedius and two were of mitis strain.

The gravis, intermedius and mitis strains had their highest incidence in the 6-10 age group and thereafter fell progressively in the succeeding age groups. There was a slight rise in the intermedius and mitis percentages in the 41-50 age group. No aberrant types were found and five

clinical cases of diphtheria had negative swabs.

(3) Dosage of Serum given in the Various Age Groups (expressed first as a Percentage of the Age Groups and then as a Percentage of the Number receiving the various Doses).

Age Group.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
0-5	8(27.6)	12(41.4)	9(31.0)
6-10	12(17.7)	31(45.6)	25(36.7)
11-20	9(28.1)	16(50.0)	7(21.9)
21-30	2(15.4)	4(30.8)	7(53.8)
31-40	2(50.0)	-	2(50.0)
41-50	2(50.0)	-	2(50.0)
51-60	-	-	-
Total.	35	63	52

Age Group.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
0-5	8(22.9)	12(19.1)	9(17.3)
6-10	12(34.3)	31(49.2)	25(48.1)
11-20	9(25.7)	16(25.4)	7(13.5)
21-30	2(5.7)	4(6.3)	7(13.5)
31-40	2(5.7)	-	2(3.8)
41-50	2(5.7)	-	2(3.8)
51-60	-	-	-
Total.	35	63	52

Of the 150 cases treated, 63 (42.0%) received a dose of serum varying from 8,000 to 40,000 units. Doses of over 40,000 units came next in frequency, and the smallest dose was employed least of all. In the 21-30 age group 53.8% of the cases received over 40,000 units.

All three doses had their highest incidence of administration in the 6-10 age group and thereafter the incidence fell in the older age groups.

(4) Day of Disease correlated with the type of C. Diphtheriæ recovered from the cases (expressed firstly as a percentage of cases per day, and secondly as a percentage of the various types found).

Day of Disease	Gravis	Intermedius	Mitis	Aberrant	Negative.
1	1(11.1)	2(22.2)	5(55.6)	-	1(11.1)
2	12(24.5)	17(34.6)	19(38.8)	-	1(2.1)
3	15(28.3)	12(22.6)	24(45.3)	-	2(3.8)
4	9(50.0)	3(16.7)	5(27.8)	-	1(5.5)
5	3(37.5)	1(12.5)	4(50.0)	-	-
6	2(28.6)	1(14.3)	4(57.1)	-	-
7	-	2(66.7)	1(33.3)	-	-
8	3(100.0)	-	-	-	-
Total	45	38	62	-	5
1	1(2.2)	2(5.3)	5(8.1)	-	1(20.0)
2	12(26.7)	17(44.7)	19(30.7)	-	1(20.0)
3	15(33.3)	12(31.6)	24(38.7)	-	2(40.0)
4	9(20.0)	3(7.9)	5(8.1)	-	1(20.0)
5	3(6.7)	1(2.6)	4(6.4)	-	-
6	2(4.4)	1(2.6)	4(6.4)	-	-
7	-	2(5.3)	1(1.6)	-	-
8	3(6.7)	-	-	-	-
Total.	45	38	62	-	5

Of the 150 cases treated, 129 (86.0%) had been given serum before the end of the fourth day of their illness. Of the Gravis infections only 2.2% had received antitoxin by the end of the first day. By the end of the fourth day 82.2% had been given serum treatment. For intermedius, mitis and negative cases the figures were 5.3%, 8.1%, 20% and 89.5%, 85.6%, 100% respectively. No aberrant types were found in this group of cases.

(5) Day of Disease correlated with the Age of the Patient (expressed first as a percentage of cases per day and secondly as a percentage of the number of cases in each age group).

Day of Disease.	0-5	6-10	11-20	21-30	31-40	41-50	51-60
1	2(22.2)	2(22.2)	3(33.4)	1(11.1)	-	1(11.1)	-
2	9(18.6)	22(44.9)	10(20.1)	6(12.2)	1(2.1)	1(2.1)	-
3	10(18.8)	25(47.2)	10(18.8)	4(7.6)	3(5.7)	1(1.9)	-
4	5(27.8)	6(33.3)	4(22.2)	2(11.1)	-	1(5.6)	-
5	2(25.0)	3(37.5)	3(37.5)	-	-	-	-
6	1(14.3)	4(57.1)	2(28.6)	-	-	-	-
7	-	3(100)	-	-	-	-	-
8	-	3(100)	-	-	-	-	-
Total	29	68	32	13	4	4	
1	2(6.9)	2(2.9)	3(9.4)	1(7.7)	-	1(25.0)	-
2	9(31.1)	22(32.4)	10(31.2)	6(46.1)	1(25.0)	1(25.0)	-
3	10(34.5)	25(36.8)	10(31.2)	4(30.8)	3(75.0)	1(25.0)	-
4	5(17.2)	6(8.8)	4(12.5)	2(15.4)	-	1(25.0)	-
5	2(6.9)	3(4.4)	3(9.4)	-	-	-	-
6	1(3.4)	4(5.9)	2(6.3)	-	-	-	-
7	-	3(4.4)	-	-	-	-	-
8	-	3(4.4)	-	-	-	-	-
Total	29	68	32	13	4	4	

Of the 150 cases, 97 were under the age of eleven, and of this 97, 81 had received serum treatment by the end of the fourth day of their illness. Three cases in the 6-10 group did not receive serum treatment until the 8th. day of their illness.

In all ages, with the exception of the 21-30 and 41-50 age groups, the percentage number of cases sent to hospital rose to a maximum on the third day of illness and thereafter fell. In the 21-30 group the maximum was reached by the second day, and in the 41-50 group the percentage was the same on the first, second, third and fourth days.

Day of Disease.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
1	9(100.0)	-	-
2	15(30.6)	24(49.0)	10(20.4)
3	11(20.7)	24(45.3)	18(34.0)
4	-	9(50.0)	9(50.0)
5	-	3(37.5)	5(62.5)
6	-	-	7(100.0)
7	-	3(100.0)	-
8	-	-	3(100.0)
Total	35	63	52
1	9(25.7)	-	-
2	15(42.9)	24(38.1)	10(19.3)
3	11(31.4)	24(38.1)	18(34.6)
4	-	9(14.3)	9(17.2)
5	-	3(4.75)	5(9.7)
6	-	-	7(13.4)
7	-	3(4.75)	-
8	-	-	3(5.8)
Total	35	63	52

Delay in diagnosing the disease led to larger doses of serum having to be given.

The majority of patients who received less than 8,000 units of serum did so on the second day of disease. Of the patients who received 8,000 to 40,000 units or more than 40,000 units, the majority in the first series, were given serum on the second day, and in the latter group, on the third day. Thereafter the numbers decreased.

(7) Type of C. Diphtheriæ recovered from the individual cases correlated with the dose of serum given (expressed as a percentage of Type of C. Diphtheriæ).

Type of C. Diphtheriæ.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
Gravis	4(8.9)	17(37.8)	24(53.3)
Intermedius	5(13.2)	15(39.6)	18(47.2)
Mitis	25(40.3)	28(45.2)	9(14.5)
Aberrant	-	-	-
Negative	1(20.0)	3(60.0)	1(20.0)
Total.	35	63	52

In the gravis infections most of the patients (53.3% required over 40,000 units of serum. 37.8% and 8.9% required 8,000 - 40,000 and less than 8,000 units respectively. With the intermedius infections similar figures were obtained. In the mitis and negative cases the highest percentage of these required between 8,000 and 40,000 units of serum.

(8) Type of C. Diphtheriæ recovered from the different Clinical varieties of diphtheria (expressed as a percentage of clinical types. Cervical adenitis is expressed as a percentage of cases with each strain of C. Diphtheria).

Clinical Type.	Gravis	Intermedius	Mitis	Aberrant	Negative
Faucial:					
(a) Mild	8(23.5)	6(17.6)	17(50.0)	-	3(8.9)
(b) Moderate	9(20.5)	7(15.9)	27(61.4)	-	1(2.2)
(c) Severe	10(40.0)	4(16.0)	10(40.0)	-	1(4.0)
(d) Haemorrhagic	8(42.1)	7(36.8)	4(21.1)	-	-
Nasal	2(100.0)	-	-	-	-
Laryngeal	-	-	-	-	-
Faucial and Nasal	8(30.8)	14(53.8)	4(15.4)	-	-
Cervical Adenitis	32(71.1)	20(52.6)	25(40.3)	-	-
Total.	45	38	62		5

C. diphtheriae gravis and intermedius were associated with the more severe clinical types of diphtheria. The intermedius strain was found most frequently in faucial-nasal cases. The percentage incidence of cervical adenitis was highest with gravis infections, lowest with mitis and the intermedius occupied the intermediate position.

Death and Complication Rates.

(a) Type of C. Diphtheriae recovered correlated with the various complications observed and the death rate (expressed as a percentage of the number of the various types found).

Complication.	Gravis	Intermedius	Mitis	Aberrant	Negative.
Albuminuria	6(13.3)	4(10.5)	3(4.8)	-	2(40.0)
Cardiac Paresis	10(22.2)	6(15.8)	1(1.6)	-	-
Palatal ,,	9(20.0)	7(18.4)	2(3.2)	-	1(20.0)
Ocular ,,	-	-	-	-	-
Death Rate	6(13.3)	4(10.5)	1(1.6)	-	1(20.0)

Two negative cases had albuminuria. One had palatal paresis and one died. These two negative cases have been mentioned before. The case which died, died through an accident in nursing - a probationer giving the child a sweet which passed

into the respiratory passages, and death was due to asphyxia. Gravis had the next highest incidence of complications, intermedius following gravis, and mitis coming last.

(b) Death and Complication Rates correlated with the age of the patient (expressed as percentages of the age group).

Age Group.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
0-5	3 (10.4)	2(6.9)	4(14.5)	-	4(14.5)
6-10	6 (8.8)	9(13.2)	5(7.3)	-	3(4.4)
11-20	2 (6.3)	4(12.6)	5(15.6)	-	4(12.6)
21-30	4 (30.8)	2(15.4)	2(15.4)	-	1(7.7)
31-40	-	2(50.0)	1(25.0)	-	-
41-50	-	-	-	-	-
51-60	-	-	-	-	-
Total.	15	19	17		12

Albuminuria, palatal paresis and cardiac paresis all tended to become more frequent with age. Albuminuria and cardiac paresis had a high incidence in the 0-5 age group, fell in the 6-10 group and then rose again. The death rate was highest in the 0-5 and 11-20 age groups.

(c) Death and Complication Rates correlated with Day of Disease (expressed as percentages of Day of Disease).

Day of Disease.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
1	-	-	-	-	-
2	2 (4.1)	4(8.2)	3(6.1)	-	1(2.0)
3	4 (7.4)	5(9.4)	3(5.7)	-	3(5.7)
4	5 (27.8)	6(33.3)	4(22.2)	-	3(16.9)
5	4 (50.0)	3(37.5)	3(37.5)	-	2(25.0)
6	-	1(14.3)	2(28.6)	-	1(14.3)
7	-	-	1(33.3)	-	1(33.3)
8	-	-	1(33.3)	-	1(33.3)
Total.	15	19	17		12

No complications occurred when the serum was given on the first

day of illness. The incidence of all complications increased up to the end of the fifth day. Palatal paresis then fell in the sixth day and no further cases were recorded in the 7th. and 8th. days. Cardiac paresis and the death rate fell in the sixth day and rose again in the 7th. and 8th. day.

Death and Complication Rates correlated with the clinical Type of Diphtheria (expressed as percentages of clinical types).

Clinical Type.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
Faucial:					
(a) Mild.	1(2.9)	2(5.9)	-	-	-
(b) Moderate	2(4.5)	2(4.5)	-	-	-
(c) Severe	2(8.0)	2(8.0)	1(4.0)	-	1(4.0)
(d) Haemorrhagic.	5(26.3)	8(42.1)	9(47.4)	-	7(36.8)
Nasal	-	-	-	-	-
Laryngeal	-	-	-	-	-
Faucial and Nasal.	5(19.2)	5(19.2)	7(26.9)	-	4(15.4)
Total.	15	19	17		12

As the clinical severity of the case increased so did the incidence of complications. Faucial-nasal cases occupied the intermediate place between severe and haemorrhagic faucial diphtheria when considered in terms of complication and death rates.

Dosage of Serum given. The number of cases which received a dose of serum over 32,000 units was 74, almost 50% of the cases. 24 patients had two doses of serum, four had three doses, but none had a fourth injection of serum. The maximum dose given was 168,000 units, the minimum 4,000,

and the average, 42,500 units.

Rate of Disappearance of Membrane/ or Discharge/ or Cervical adenitis.

The maximum time taken for the membrane/or discharges/ of glands to clear up was 156 hours. This case was a boy of 14 years who was given serum on the second day of illness. The membrane was on both tonsils, soft and hard palate. The organism recovered was of the gravis strain. The minimum time was eight hours; the average, forty-seven hours.

Rate of Disappearance of Toxaemia.

Toxaemia was divided into five stages: (1) No toxaemia (2) Mild, (3) Moderate, (4) Severe, and (5) Comatose.

Mild Toxaemia - pulse normal in rhythm but slightly quicker than normal - up to 90 beats per minute. Respiration rate normal. Temperature slightly elevated (99° F.), but patient able to answer verbal questions.

Moderate Toxaemia - pulse - soft and regular in rhythm, but fast (120 beats per minute). Respiration rate quickened up to 26 per minute. Patient slightly mentally confused. Temperature elevated (100-101°F.) Patient may or may not have been sick.

Severe Toxaemia - pulse fast or slow, soft, irregular in rhythm. Respiration rate over 26 per minute and patient slightly cyanosed. Skin cold, temperature normal or slightly subnormal. Frequent sickness, tongue dry and furred. Patient mentally confused.

Comatose Toxaemia - patient very drowsy or unconscious. Pulse feeble, irregular, fast or slow. Respirations fast and gasping. Skin cold and bathed in perspiration. Temperature subnormal, eyes sunken and dry. Frequent sickness. Tongue furred on dorsal and ventral surfaces. Twenty-seven patients had severe toxaemia, sixty moderate, fifty-one mild and twelve showed no toxic effects. The maximum time was taken for the toxaemia to pass off was 160 hours. This case was a boy aged 8 years, who, although the membrane and discharges had cleared up in 48 hours, still remained toxic. The organism recovered was of the gravis strain. The minimum time was 12 hours, the average - 40 hours.

2 Intramuscular and Intravenous Injection.

250 cases were treated. The intravenous serum was given as before, the intramuscular serum was injected into the vastus externus muscle of the thigh. The same observations were made upon each case.

(1) Age and Sex.

Age Groups.	0-5	6-10	11-20	21-30	31-40	41-50	51-60
Males	23	72	26	8	4	6	-
Females	24	56	23	5	2	1	-
Total.	47	128	49	13	6	7	

The majority of the 250 cases (224) were under the age of 20. Of the 250 cases, 139 were males and 111 were females.

(2) Age of Patients correlated with the type of C. Diphtheriae recovered from each case (expressed first as a percentage of age groups and then as a percentage of the type of C. Diphtheriae)

Age Group	Gravis	Intermedius	Mitis	Aberrant	Negative.
0-5	17(36.2)	15(31.9)	14(29.8)	-	1(2.1)
6-10	36(28.1)	30(23.4)	60(46.8)	-	2(1.7)
11-20	12(24.5)	10(20.4)	16(32.6)	-	11(22.5)
21-30	4(30.8)	4(30.8)	5(38.4)	-	-
31-40	1(16.6)	4(66.6)	1(16.66)	-	-
41-50	-	-	7(100.0)	-	-
51-60	-	-	-	-	-
Total.	70	63	103	-	14
0-5	17(24.3)	15(23.8)	14(13.6)	-	1(7.1)
6-10	36(51.4)	30(47.6)	60(58.3)	-	2(14.3)
11-20	12(17.2)	10(15.8)	16(15.5)	-	11(78.6)
21-30	4(5.7)	4(6.4)	5(4.8)	-	-
31-40	1(1.4)	4(6.4)	1(0.9)	-	-
41-50	-	-	7(6.9)	-	-
51-60	-	-	-	-	-
Total	70	63	103		14

Of the 0-10 age group, 32.2% suffered from gravis and 27.6% from intermedius infections. In the 31-40 age group there was a high incidence - 66.7% of intermedius cases.

Gravis infections had the highest incidence in the 0-5 group, intermedius came next, followed by mitis and negative cultures, which occurred in the lowest percentage. From the age of 10 onwards there was a sharp fall in the incidence of gravis strains until after the age of 40 none were recorded. With intermedius and mitis the numbers also fell after the age of 10, but did not fall so rapidly as the gravis strains. In the negative series, no further cases were recorded after the

age of twenty. The highest incidence of gravis, intermedius and mitis cases occurred in the 6-10 age group, but of the negative cases the highest incidence occurred in the 11-20 age group.

(3) Dosage of Serum given correlated with age groups (expressed first as a percentage of the age groups then as percentages of the number receiving the defined doses).

Age Group.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
0-5	6(12.7)	19(40.4)	22(46.9)
6-10	20(15.6)	57(44.6)	51(39.8)
11-20	10(20.4)	26(53.1)	13(26.5)
21-30	4(30.8)	6(46.1)	3(23.1)
31-40	1(16.66)	4(66.66)	1(16.66)
41-50	1(14.3)	6(85.7)	-
51-60	-	-	-
Total.	42	118	90
0-5	6(14.3)	19(16.1)	22(24.4)
6-10	20(47.6)	57(48.3)	51(56.7)
11-20	10(23.8)	26(22.0)	13(14.4)
21-30	4(9.5)	6(5.1)	3(3.3)
31-40	1(2.4)	4(3.4)	1(1.2)
41-50	1(2.4)	6(5.1)	-
51-60	-	-	-
Total.	42	118	90

Of the 250 cases treated, 118 (47.2%) received an injection of between 8,000 and 40,000 units of serum. Doses of 40,000 units and over were used more than twice as often as doses of under 8,000 units (36% to 16.8%). With the 8,000 - 40,000 unit dose, the percentage frequency that this amount was given, rose progressively with the age groups, with the exception of the 21-30 age group.

The percentage of cases in the 0-5 age group which

received doses of 40,000 units and over was high (46.9). This number represented 24.4% of all doses of serum of 40,000 units and over given in this group of cases. Of the groups of doses, all had their highest incidence in the 6-10 age group and fell thereafter.

(4) Day of Disease correlated with the Type of C. Diphtheriæ recovered from the case (expressed first as a percentage of the cases occurring per day and secondly as a percentage of the various types of C. Diphtheriæ).

Day of Disease.	Gravis	Intermedius	Mitis	Aberrant	Negative.
1	1(8.3)	3(25.0)	7(58.4)	-	1(8.3)
2	19(24.1)	24(30.4)	28(35.4)	-	8(10.1)
3	21(29.2)	12(16.7)	39(54.1)	-	-
4	16(34.8)	11(23.9)	14(30.5)	-	5(10.8)
5	6(31.6)	9(47.4)	4(21.0)	-	-
6	4(22.2)	4(22.2)	10(55.6)	-	-
7	-	-	1(100.0)	-	-
8	3(100.0)	-	-	-	-
Total.	70	63	103		14
1	1(1.4)	3(4.8)	7(6.8)	-	1(7.1)
2	19(27.1)	24(38.0)	28(27.2)	-	8(57.1)
3	21(30.0)	12(19.0)	39(37.8)	-	-
4	16(22.9)	11(17.5)	14(13.6)	-	5(35.8)
5	6(8.6)	9(14.3)	4(3.9)	-	-
6	4(5.7)	4(6.4)	10(9.7)	-	-
7	-	-	1(1.0)	-	-
8	3(4.3)	-	-	-	-
Total.	70	63	103	-	14

Of the 250 cases, 209 (83.6%) had received serum treatment within four days from the onset of their illness. The incidence of the gravis strain was low on the first day and rose from then onwards until the fifth day, when it began to fall. The three cases which occurred on the 8th. day

were all gravis, giving a 100% incidence. The intermedius cases had a high incidence on the 1st. and 2nd. days; it then fell on the third day, rose on the 4th. and 5th. and fell again on the 6th. day. No further intermedius cases were observed after the 6th. day. Mitis cases had a high incidence on the 1st. day which fell on the 2nd. rose on the 3rd., fell on the 4th. and 5th., and rose again on the 6th. and 7th. No cases were observed in the 8th. day. With the negative cases the incidence was 8.3% on the 1st. day. This rose to 10.1% on the 2nd., fell to zero on the 3rd., rose to 10.8% on the 4th. day, and thereafter no cases were observed.

Of the various types of C. Diphtheriæ, 1.4% of gravis, 4.8% of intermedius, 6.8% of mitis and 7.1% of negative cases had received serum within the 1st. day of illness. By the end of the 4th. day the figures were 81.4% gravis, 79.3% intermedius, 85.4% mitis and 100% negative cases.

(5) Day of Disease correlated with the age group (expressed as a percentage of cases per day and secondly as a percentage of the cases in each age group).

Day of Disease.	0-5	6-10	11-20	21-30	31-40	41-50	51-60
1	1(8.3)	2(16.7)	4(33.3)	3(25.0)	2(16.7)	-	-
2	12(15.2)	48(60.7)	16(20.3)	2(2.5)	1(1.3)	-	-
3	16(22.2)	27(37.5)	20(27.8)	3(4.2)	2(2.8)	4(5.5)	-
4	12(26.1)	26(56.5)	3(6.5)	3(6.5)	1(2.2)	1(2.2)	-
5	4(21.0)	11(57.9)	3(15.8)	1(5.3)	-	-	-
6	2(11.0)	10(55.7)	3(16.7)	1(5.6)	-	2(11.0)	-
7	-	1(100.0)	-	-	-	-	-
8	-	3(100.0)	-	-	-	-	-
Total.	47	128	49	13	6	7	
1	1(2.1)	2(1.5)	4(8.2)	3(23.1)	2(33.3)	-	-
2	12(25.5)	48(37.6)	16(32.7)	2(15.3)	1(16.7)	-	-
3	16(34.1)	27(21.1)	20(40.8)	3(23.1)	2(33.3)	4(57.1)	-
4	12(25.5)	26(20.3)	3(6.1)	3(23.1)	1(16.7)	1(14.3)	-
5	4(8.5)	11(8.6)	3(6.1)	1(7.7)	-	-	-
6	2(4.3)	10(7.8)	3(6.1)	1(7.7)	-	2(28.6)	-
7	-	1(0.8)	-	-	-	-	-
8	-	3(2.3)	-	-	-	-	-
Total.	47	128	49	13	6	7	-

Of the 250 cases, 175 (70%) were aged 10 or under and of this number 144 (82.3%) had received serum before the end of the 4th. day of illness. Four patients who did not come under treatment until the 7th. and 8th. day of illness were in the 6-10 age group.

Of the age groups most cases came under treatment on the 3rd. day of illness with the exception of the 6-10 age group, when it was on the 2nd. day, and for the 21-30 age group when it was the 3rd. and 4th. days.

(6) Day of Disease correlated with the dose of serum given (expressed first as a percentage of cases per day and secondly as a percentage of cases receiving the defined doses).

Day of Disease.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
1	8(66.66)	2(16.66)	2(16.66)
2	16(20.3)	46(58.2)	17(21.5)
3	17(23.6)	30(41.7)	25(34.7)
4	1(2.2)	24(52.2)	21(45.6)
5	-	8(42.1)	11(57.9)
6	-	8(44.4)	10(55.6)
7	-	-	1(100.0)
8	-	-	3(100.0)
Total.	42	118	90
1	8(19.0)	2(1.7)	2(2.2)
2	16(38.1)	46(38.9)	17(18.9)
3	17(40.5)	30(25.4)	25(27.8)
4	1(2.4)	24(20.4)	21(23.3)
5	-	8(6.8)	11(12.3)
6	-	8(6.8)	10(11.1)
7	-	-	1(1.1)
8	-	-	3(3.3)
Total.	42	118	90

The longer the patient was ill before being treated, the larger was the amount of serum which was required.

The highest number of patients who received less than 8,000 units of serum did so on the 3rd. day of illness. In the 8,000-40,000 units group, the highest number of patients were given serum on the 2nd. day of illness and in the 40,000 units or over, it was on the 3rd. day.

(7) Type of C. Diphtheriæ recovered from the individual cases correlated with the dose of serum given (expressed as a percentage of Type of C. Diphtheriæ).

Type of C. Diphtheriæ.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
Gravis	6(8.6)	26(37.1)	38(54.3)
Intermedius	5(7.9)	31(49.2)	27(42.9)
Mitis	27(26.2)	51(49.5)	25(24.3)
Aberrant	-	-	-
Negative	4(28.6)	10(71.1)	-
Total.	42	118	90

In the gravis infections most cases (54.3%) required over 40,000 units of serum and 37.1% and 8.6% received doses of 8,000-40,000 units and less than 8,000 units respectively. With intermedius, mitis and negative infections the highest number of patients redeived between 8,000 and 40,000 units of serum.

(8) Type of C. Diphtheriæ recovered from the different clinical types of diphtheria (expressed as a percentage of the clinical types. Cervical adenitis is expressed as a percentage of the number of cases with each strain of c. diphtheriæ).

Clinical Type.	Gravis.	Intermedius	Mitis	Aberrant	Negative.
Faucial:					
(a) Mild	7(14.9)	5(10.6)	26(55.3)	-	9(19.2)
(b) Moderate	23(22.5)	28(27.4)	50(49.1)	-	1(1.0)
(c) Severe	17(31.5)	18(33.3)	16(29.6)	-	3(5.6)
(d) Haemorrhagic	7(53.8)	4(30.8)	2(15.4)	-	-
Nasal	1(16.66)	1(16.66)	4(66.66)	-	-
Laryngeal	-	-	-	-	1(100.0)
Faucial and Nasal	15(55.6)	7(25.9)	5(18.5)	-	-
Cervical Adenitis	62(88.6)	40(63.5)	28(27.2)	-	6(42.9)
Total.	70	63	103	-	14

C. Diphtheriae gravis and intermedius were associated with the more severe clinical cases of diphtheria. Mitis and negative cultures were found mostly in the milder clinical cases. Cervical adenitis occurred with all strains of diphtheria, but most often in the cases from which a gravis strain was isolated.

Death and Complication Rates.

(a) Type of C. Diphtheriae recovered from the cases correlated with the various complications observed (expressed as a percentage of the number of the various types found).

Complication.	Gravis	Intermedius	Mitis	Aberrant	Negative.
Albuminuria	2(2.9)	1(1.6)	1(1.0)	-	-
Cardiac Paresis	2(2.9)	2(3.2)	-	-	-
Palatal ,,	2(2.9)	3(3.2)	1(1.0)	-	-
Ocular ,,	-	-	-	-	-
Death Rate	2(2.9)	1(1.6)	-	-	-

With the exception of ocular paresis, all the complications mentioned above occurred in equal frequency with the gravis strain. With the intermedius cases, the cardiac paresis and palatal paresis rate were slightly higher than that for gravis infections, but the incidence of albuminuria and the

death rate was lower. Mitis infections were very mild, and in the negative cases no complications were observed. One of the deaths, with gravis infections, was due to the patient's developing an acute mastoiditis which was operated on but the patient developed septic meningitis and died.

(b) Death and Complication Rates correlated with the age of the patient (expressed as percentages of the age groups).

Age Group.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
0-5	1(2.1)	1(2.1)	1(2.1)	-	2(4.2)
6-10	2(1.5)	2(1.5)	2(1.5)	-	1(0.7)
11-20	1(2.0)	2(4.1)	1(2.0)	-	-
21-30	-	-	-	-	-
31-40	-	-	-	-	-
41-50	-	-	-	-	-
51-60	-	-	-	-	-
Total.	4	5	4	-	3

Complications were more frequent in the 0-5 and the 11-20 age groups.

The death rate was highest in the 0-5 age group.

(c) Death and Complication Rates correlated with day of disease (expressed as percentages of day of disease).

Day of Disease	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
1	-	-	-	-	-
2	1(1.3)	-	-	-	-
3	-	1(1.4)	1(1.4)	-	1(1.4)
4	1(2.2)	2(4.4)	2(4.4)	-	-
5	2(10.5)	2(10.5)	1(5.3)	-	2(10.5)
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
Total.	4	5	4	-	3

No complications were observed when the patient was given

serum on the first day of illness, and only one case which was given serum on the second day of illness developed a complication (albuminuria). From the third day of illness complications were more frequent and reached their highest incidence on the 5th. day, after this day no complications were recorded.

(d) Death and Complication Rates correlated with the clinical types of diphtheria (expressed as percentages of clinical types).

Clinical Type.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
Faucial:					
(a) Mild	-	-	-	-	-
(b) Moderate	-	-	-	-	-
(c) Severe	1(1.8)	1(1.8)	-	-	-
(d) Haemorrhagic	2(15.4)	2(15.4)	2(15.4)	-	2(15.4)
Nasal	-	-	-	-	-
Laryngeal	-	-	-	-	-
Faucial and Nasal	1(3.7)	2(7.4)	2(7.4)	-	1(3.7)
	4	5	4	-	3

The more severe the clinical type, the higher was the incidence of complications. Faucial-nasal cases occupied an intermediate position between severe and haemorrhagic faucial diphtheria when considered in terms of complications.

Doses of Serum given. The maximum dose given was 312,000 units, the minimum 8,000 units, and the average 62,000 units. 148 cases had one dose of serum (actually one intramuscularly and one intravenously but these two doses were considered as one initial dose). 76 had 2, 21 had 3 doses, and 5 had 4 separate doses.

Rate of Disappearance of Membrane/ or Discharges/ or Cervical Adenitis.

The maximum time taken for the membrane/ or discharges/ or glands to clear up was 120 hours. The patient, a woman of 25 years, was admitted on the 5th. day of illness with a "bull neck" membrane on both tonsils, the hard and soft palate and a profuse nasal discharge. She was given 144,000 units (72,000 intravenously and 72,000 intramuscularly). The organism recovered was of the intermedius strain. The minimum time was 12 hours and the average, 68 hours.

Rate of Disappearance of Toxaemia.

20 patients were cometose on admission, 110 had severe toxaemia, 65 moderate, 45 mild and 10 showed no signs or symptoms of toxaemia. The maximum time taken for the toxaemia to pass off was 160 hours and was in the case mentioned above. The minimum time was 24 hours, the average, 62 hours.

INTRAMUSCULAR.

3 The serum was injected into the vastus externus muscle of the thigh.

Analysis of the 600 Cases treated.

(1) Age and Sex.

Age Groups.	0-5	6-10	11-20	21-30	31-40	41-50	51-60
Males	57	134	87	27	9	11	5
Females	58	114	71	18	8	1	-
Total.	115	248	158	45	17	12	5

Most of the cases (421 or 70%) were under the age of 20. Of the 600 cases, 330 (55%) were males, and 270 (45%) were

females.

(2) Age of Patient correlated with the Type of C. Diphtheriae recovered from each case (expressed first as a percentage of age groups and then as a percentage of the Type of C. Diphtheriae).

Age Group.	Gravis	Intermedius	Mitis	Aberrant	Negative
0-5	36(31.3)	35(30.4)	40(34.8)	3(2.6)	1(0.9)
6-10	78(31.5)	74(29.8)	73(29.4)	7(2.8)	16(6.5)
11-20	63(39.8)	32(20.2)	54(34.2)	5(3.2)	4(2.6)
21-30	3(6.6)	9(20.0)	31(68.9)	2(4.5)	-
31-40	-	9(52.9)	4(23.55)	-	4(23.55)
41-50	-	3(25.0)	2(16.7)	-	7(58.3)
51-60	-	-	5(100.0)	-	-
Total.	180	162	209	17	32
0-5	36(20.0)	35(21.6)	40(19.1)	3(17.7)	1(3.1)
6-10	78(43.3)	74(45.7)	73(34.9)	7(41.2)	16(50.0)
11-20	63(35.0)	32(19.9)	54(25.8)	5(29.4)	4(12.5)
21-30	3(1.7)	9(5.5)	31(14.8)	2(11.7)	-
31-40	-	9(5.5)	4(1.9)	-	4(12.5)
41-50	-	3(1.8)	2(1.0)	-	7(21.9)
51-60	-	-	5(2.5)	-	-
Total.	180	162	209	17	32

Of the 0-10 age group, 114 (19%) suffered from gravis infections and 109 (18%) from intermedius. In the 0-5 and 6-10 age groups the incidence of gravis, intermedius and mitis cultures occurred with fairly equal frequency.

All four types and the negative cases had their highest incidence in the 6-10 age group. No gravis cases were found in patients over 30. Intermedius and negative cultures were the most frequently found in the 31-40 and 41-50 age groups, but mitis was found most frequently in the 51-60 age group.

(3) Dosage of Serum given correlated with the age groups (expressed first as a percentage of the age groups and secondly as a percentage of the number of cases receiving the defined doses).

Age Group.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
0-5	5(4.4)	90(78.2)	20(17.4)
6-10	9(3.6)	175(70.5)	64(25.9)
11-20	10(6.3)	76 (48.1)	72(45.6)
21-30	3(6.7)	22(48.9)	20(44.4)
31-40	2(11.8)	10(58.9)	5(29.3)
41-50	3(25.0)	4(33.3)	5(41.7)
51-60	2(40.0)	3(60.0)	-
Total.	34	380	186
0-5	5(14.7)	90(23.7)	20(10.7)
6-10	9(26.5)	175(46.1)	64(34.4)
11-20	10(29,4)	76(20.0)	72(38.8)
21-30	3(8.8)	22(5.8)	20(10.7)
31-40	2(5.9)	10(2.6)	5(2.7)
41-50	3(8.8)	4(1.1)	5(2.7)
51-60	2(5.9)	3(0.7)	-
Total.	34	380	186

Of the 600 cases treated, 380 (63.3%) received a dose of serum between 8,000 and 40,000 units. Doses of over 40,000 units were used almost six times as often as doses of under 8,000 units.

Doses of serum between 8,000 and 40,000 units were employed most often in children between the ages of 6-10, but doses of under 8,000 units and over 40,000 units were used most often in the 11-20 age group.

(4) Day of Disease correlated with the type of C. Diphtheriae recovered from the case (expressed as a percentage of the cases occurring per day and secondly as a percentage of the various

types of C. Diphtheriæ).

Day of Disease.	Gravis	Intermedius	Mitis	Aberrant	Negative.
1	2(15.4)	3(23.1)	7(53.8)	-	1(7.7)
2	35(23.6)	51(34.4)	41(27.7)	3(2.0)	18(12.3)
3	81(34.9)	49(21.1)	89(38.4)	7(3.0)	6(2.6)
4	34(29.6)	28(24.4)	43(37.4)	3(2.6)	7(6.0)
5	11(26.2)	23(54.8)	5(11.9)	3(7.1)	-
6	11(25.0)	8(18.2)	24(54.6)	1(2.2)	-
7	-	-	-	-	-
8	6(100.0)	-	-	-	-
Total.	180	162	209	17	32
1	2(1.1)	3(1.9)	7(3.3)	-	1(3.1)
2	35(19.5)	51(31.4)	41(19.6)	3(17.6)	18(56.2)
3	81(45.0)	49(30.3)	89(42.5)	7(41.3)	6(18.8)
4	34(18.9)	28(17.3)	43(20.5)	3(17.6)	7(21.9)
5	11(6.1)	23(14.2)	5(2.7)	3(17.6)	-
6	11(6.1)	8(4.9)	24(11.4)	1(5.9)	-
7	-	-	-	-	-
8	6(3.3)	-	-	-	-
Total	180	162	209	17	32

Of the 600 cases, 508 (84.7%) had received serum by the end of the 4th. day of their illness. Gravis infections reached their highest incidence in patients who were admitted on the 3rd. day of illness, intermedius on the fifth day, mitis on the sixth day, aberrant on the fifth and negative cases on the second day.

The highest percentage of gravis, mitis and aberrant cases were admitted on the 3rd. day of illness. With intermedius and negative cases, the highest percentage was on the 2nd. day of illness. A small number of cases (6), admitted on the 8th. day of illness were found to be gravis infections.

(5) Day of Disease correlated with the age group (expressed first as a percentage of cases per day and secondly as a percentage of the cases in each age group).

Day of Disease.	0-5	6-10	11-20	21-30	31-40	41-50	51-60
1	1(7.7)	3(23.0)	4(30.8)	2(15.4)	2(15.4)	1(7.7)	-
2	33(22.3)	58(39.2)	52(35.1)	2(1.4)	2(1.4)	1(0.6)	-
3	32(13.8)	109(46.9)	44(18.9)	30(12.9)	8(3.5)	5(2.2)	4(1.8)
4	29(25.3)	47(40.9)	22(19.1)	10(8.7)	2(1.7)	4(3.4)	1(0.9)
5	8(19.0)	21(50.0)	12(28.6)	-	1(2.4)	-	-
6	6(13.4)	10(22.7)	24(54.6)	1(2.3)	2(4.7)	1(2.3)	-
7	-	-	-	-	-	-	-
8	6(100.0)	-	-	-	-	-	-
Total.	115	248	158	45	17	12	5
1	1(0.9)	3(1.2)	4(2.5)	2(4.4)	2(11.8)	1(8.3)	-
2	33(28.7)	58(23.4)	52(32.9)	2(4.4)	2(11.8)	1(8.3)	-
3	32(27.8)	109(43.9)	44(27.9)	30(66.7)	8(47.0)	5(41.8)	4(80.0)
4	29(25.3)	47(18.9)	22(13.9)	10(22.2)	2(11.8)	4(33.3)	1(20.0)
5	8(6.9)	21(8.5)	12(7.6)	-	1(5.8)	-	-
6	6(5.2)	10(4.1)	24(15.2)	1(2.3)	2(11.8)	1(8.3)	-
7	-	-	-	-	-	-	-
8	6(5.2)	-	-	-	-	-	-
Total.	115	248	158	45	17	12	5

Of the 600 cases, 363 (65%) were under the age of 11 years, and of this number 312 (52.0%) had received serum treatment before the end of the 4th. day of illness. Six patients who did not receive serum treatment until the 8th. day of their illness were in the 0-5 age group.

For the various age groups, there were different days on which most patients received treatment:- 0-5 and 11-20 were on the 2nd. day, while all the others were on the 3rd. day.

(6) Day of Disease correlated with the dose of serum given (expressed first as a percentage of cases per day and then as a percentage of the cases receiving the defined doses).

Day of Disease.	Under 8,000 Units.	8,000-40,000 Units	40,000 Units and Over
1	9(69.2)	2(15.4)	2(15.4)
2	15(10.1)	98(66.2)	35(23.7)
3	8(3.5)	172(74.1)	52(22.4)
4	1(0.9)	94(81.7)	20(17.4)
5	1(2.4)	10(23.8)	31(73.8)
6	-	3(6.8)	41(93.2)
7	-	-	-
8	-	1(16.7)	5(83.3)
Total	34	380	186
1	9(26.5)	2(0.5)	2(1.1)
2	15(44.1)	98(25.8)	35(18.9)
3	8(23.5)	172(45.4)	52(27.9)
4	1(2.95)	94(24.7)	20(10.7)
5	1(2.95)	10(2.6)	31(16.7)
6	-	3(0.7)	41(22.0)
7	-	-	-
8	-	1(0.3)	5(2.7)
Total	34	380	186

Delay in diagnosing the disease led to larger doses of serum having to be administered. Of the cases coming under treatment on the 8th. day, 16.7% required between 8,000 and 40,000 units, while 83.3% required doses of over 40,000 units.

The highest number of patients who received less than 8,000 units did so on the 2nd. day of their illness, those who were given between 8,000 and 40,000 units, on the 3rd. day and those who received over 40,000 units did so also on the 3rd. day.

(7) Type of C. Diphtheriæ recovered from the cases correlated with the dose of serum given (expressed as a percentage of Type of C. Diphtheriæ).

Type of C. Diphtheriae.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
Gravis	7(3.9)	78(43.3)	95(52.8)
Intermedius	4(2.5)	75(46.3)	83(51.2)
Mitis	3(1.4)	202(96.7)	4(1.9)
Aberrant	3(17.6)	10(58.9)	4(23.5)
Negative	17(53.1)	15(46.9)	-
Total.	34	380	186

The highest number of patients who had gravis and intermedius infections received over 40,000 units of serum, while most of the mitis and aberrant infections were given between 8,000 and 40,000 units. Of the negative cases, 53.1% received less than 8,000 units and 46.9% between 8,000 and 40,000 units.

(8) Type of C. Diphtheriae recovered from the various clinical types of diphtheria (expressed as a percentage of the clinical types. Cervical adenitis is expressed as a percentage of the number of cases with each strain of C. Diphtheriae).

Clinical Type.	Gravis	Intermedius	Mitis	Aberrant	Negative.
Faucial:					
(a) Mild	9(10.0)	9(10.0)	51(56.7)	5(5.5)	16(17.8)
(b) Moderate	80(33.2)	68(28.2)	75(31.1)	8(3.3)	10(4.2)
(c) Severe	41(48.8)	29(34.5)	12(14.3)	2(2.4)	-
(d) Haemorrhagic	8(47.1)	3(17.6)	6(35.3)	-	-
Nasal	1(9.1)	1(9.1)	9(81.8)	-	-
Laryngeal	-	-	13(68.4)	-	6(31.6)
Faucial and Nasal.	41(29.5)	52(37.7)	43(31.3)	2(1.5)	-
Cervical Adenitis.	126(70.0)	100(61.7)	87(41.6)	6(35.3)	4(12.5)
Total.	180	162	209	17	32

The gravis strain was associated with the most clinically severe cases and had the highest percentage of cervical

adenitis. The high incidence of mitis strains associated with haemorrhagic cases is a feature of the table. The aberrant strains were associated, in the majority of cases, with a moderately severe of mild illness. Of the nasal diphtherias a high percentage (81.8) were mitis types. Cultures from laryngeal diphtheria were either mitis or negative.

Death and Complication Rates.

(a) Type of C. Diphtheriæ recovered from the individual cases correlated with the various complications observed and the death rate (expressed as a percentage of the number of the Type of C. Diphtheriæ).

Complication.	Gravis	Intermedius	Mitis	Aberrant	Negative
Albuminuria	8(4.4)	4(2.5)	4(1.9)	1(5.9)	-
Cardiac Paresis	10(5.6)	4(2.5)	1(0.5)	-	-
Palatal ,,	7(3.9)	7(4.3)	3(1.5)	1(5.9)	-
Ocular ,,	-	6(3.7)	-	-	-
Death Rate	8(4.4)	3(1.9)	2(1.0)	1(5.9)	-

From the table the aberrant types would appear to have the highest albuminuria, palatal paresis and death rates, but, as previously explained, all these complications occurred in one case from which a Type iv organism was recovered. The patient died from septic meningitis following upon an operation for acute mastoiditis.

The gravis strain was associated with the highest complication rate except for palatal paresis. This occurred most often in intermedius cases.

(b) Death and Complication Rates correlated with the age of the patient (expressed as percentages of the age groups).

Age Group.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
0-5	4(3.5)	4(3.5)	5(4.4)	3(2.6)	5(4.4)
6-10	9(3.6)	5(2.0)	5(2.0)	3(1.2)	4(1.6)
11-20	1(0.6)	6(3.8)	4(2.5)	-	5(3.0)
21-30	2(4.4)	2(4.4)	1(2.2)	-	-
31-40	1(5.9)	1(5.9)	-	-	-
41-50	-	-	-	-	-
51-60	-	-	-	-	-
Total.	17	18	15	6	14

Albuminuria occurred with almost the same frequency in the 0-5 and 6-10 age groups. The incidence fell in the 11-20 group, rose in the 21-30 and the 31-40 age groups, but was not observed in any ages above 40. Palatal paresis, with the exception of a slight fall in the 6-10 age group, tended to become more frequent in the higher age groups. Cardiac paresis was highest in the 0-5 age group, fell in the 6-10 age group and rose again slightly in the two remaining age groups in which it occurred - 11-20 and 21-30.

Six cases of ocular paresis occurred, 3 in the 0-5 age group and 3 in the 6-10. The death rate was highest in the 0-5 age group, fell in the 6-10 and rose again in the 11-20 age group. No deaths were recorded in any of the other age groups.

(c) Death and Complication Rates correlated with day of disease (expressed as percentages of day of disease).

Day of Disease.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
1	-	-	1(7.7)	-	-
2	-	3(2.0)	1(0.6)	-	-
3	3(1.3)	4(1.7)	4(1.7)	4(1.7)	3(1.3)
4	8(6.9)	4(3.5)	4(3.5)	2(1.8)	3(2.6)
5	4(9.5)	3(7.1)	-	-	2(4.8)
6	2(4.5)	4(9.1)	4(9.1)	-	5(11.4)
7	-	-	-	-	-
8	-	-	1(16.7)	-	1(16.7)
Total	17	18	15	6	14

With the exception of cardiac paresis, where one patient, who was given serum on the first day of his illness, developed this complication, the incidence of all complications and the death rate increased, as the time before serum was given, was increased.

(d) Death and Complication Rates correlated with the clinical varieties of diphtheria (expressed as a percentage of the clinical types).

Clinical Type	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
Faucial:					
(a) Mild	1(1.1)	1(1.1)	-	-	-
(b) Moderate	1(0.4)	2(0.8)	-	-	-
(c) Severe	2(2.4)	4(4.8)	-	6(7.2)	-
(d) Haemorrhagic	6(35.3)	4(23.5)	3(17.7)	-	7(41.2)
Nasal	-	-	-	-	-
Laryngeal	-	-	4(21.1)	-	2(10.5)
Faucial and Nasal	7(5.1)	7(5.1)	8(5.8)	-	5(3.7)
Total	17	18	15	6	14

With the exception of the moderate cases, the more severe

the clinical case, the higher was the incidence of complications. Faucial-nasal cases occupied an intermediate place between severe and haemorrhagic diphtheria. The cardiac paresis in the laryngeal cases was high, but this was due to extra strain on the heart caused by the difficulty in respiration.

Dosage of Serum given. The maximum dose given was 256,000 units, the minimum, 4,000 units and the average 54,000 units. 476 patients had only one injection of serum, 105 had two, 19 had three but none had a fourth injection.

Rate of Disappearance of Membrane/ or Discharges/ or Cervical Adenitis.

The maximum time was 108 hours. The case was a girl of 2 years, with membrane on both tonsils, soft and hard palate, and with a bilateral cervical adenitis. She was in her fifth day of illness and was given 72,000 units of serum. The organism recovered was of the gravis strain. The minimum time was 16 hours, and the average, 44 hours.

Rate of Disappearance of Toxaemia.

On admission 30 patients were comatose, 156 had severe toxaemia, 288 moderate, 108 mild and 18 showed no signs or symptoms of toxaemia. The maximum time taken for the toxaemia to pass off was 120 hours. This was in the case mentioned above. The minimum time was 12 hours, the average, 39 hours.

Comparing the results of treatment by the three different methods of administering the serum.

Results of treatment are best judged by the complications and death rates in each method, after taking account of the various factors which might influence these results.

(a) Age (Percentages).

Age Groups.	0-5	6-10	11-20	21-30	31-40	41-50	51-60
Intravenous	19.3	45.3	21.3	8.7	2.7	2.7	-
Intramuscular) and Intravenous)	18.8	51.2	19.6	5.2	2.4	2.8	-
Intramuscular)	19.2	41.3	26.3	7.5	2.8	2.0	0.9

The percentage of children in the 0-5 age groups approximated in all three methods. Of the 6-10 group the intramuscular series had the lowest percentage of this age group, and the intramuscular-intravenous series had the highest. In the 11-20 age group the intramuscular series had the highest percentage of these. The ages mentioned (0-20) are the ages in which complications and the death rate are highest. (Forbes 1932). Thus all three have approximately the same percentage of cases in this group.

(b) Percentage of Types of C. Diphtheriae found in the various groups.

	Gravis	Intermedius	Mitis	Aberrant	Negative.
Intravenous	30.0	25.33	41.33	-	3.33
Intramuscular) Intravenous) Intramuscular	28.0	25.2	41.2	-	5.6
	30.0	27.0	34.8	2.8	5.4

In all three series the percentage of the various types was

fairly evenly distributed. The intramuscular series had a low incidence of mitis strains.

(c) Severity of Cases judged by amount of serum given (expressed as percentages of number in each group).

Dose of Serum.	Under 8,000 Units.	8,000-40,000 Units.	40,000 Units and Over.
Intravenous	23.3	42.0	34.7
Intramuscular & Intravenous	16.8	47.2	36.0
Intramuscular	5.7	63.3	31.0

Judged by dosage of serum given, the intramuscular - intravenous cases were most severe, as they had the highest percentage of cases who were given over 40,000 units of serum. Next came the intravenous, and lastly the intramuscular cases. However, the intramuscular had the highest percentage of cases in the 8,000 - 40,000 units group. If we consider the severity of the cases by the average dose given, the order of decreasing severity is - intramuscular-intravenous (62,000 units), intramuscular (54,000 units) and intravenous (42,500 units).

(d) Day of Disease - (expressed as a percentage of cases in each series).

Day of Disease.	Intravenous.	Intravenous and Intramuscular	Intramuscular.
1	9(6.0)	12(4.8)	13(2.2)
2	49(32.7)	79(31.6)	148(24.7)
3	53(35.3)	72(28.8)	232(38.7)
4	18(12.0)	46(18.4)	115(19.2)
5	8(5.3)	19(7.6)	42(7.0)
6	7(4.7)	18(7.2)	44(7.2)
7	3(2.0)	1(0.4)	-
8	3(2.0)	3(1.2)	6(1.0)

The third day was the day on which the highest percentage of cases received serum in the intravenous and in the

intramuscular series. In the intravenous-intramuscular series the highest percentage occurred on the second day. In the three series 14.0% of the intravenous, 16.4% of the intravenous-intramuscular and 15.2% of the intramuscular cases received serum after the fourth day of illness. The intramuscular series in the third and fourth days had the highest percentage of cases receiving treatment on these days. The other two had almost the same percentage.

S U M M A R Y.

In the three series there was little difference in the percentage of patients per age group and of types of c. diphtheriæ found. Judged by the amount of serum given, the intravenous-intramuscular series was clinically the most severe, the intramuscular being next, and the intravenous cases the mildest. The intravenous-intramuscular series had the highest percentage of cases receiving treatment after the fourth day, the intramuscular series being second and the intravenous last.

Thus the order of clinical severity is:- (1) intravenous-intramuscular cases, (2) intramuscular cases, and (3) intravenous.

Comparison of Complication Rates in the three series (expressed as a percentage of cases in each series).

Route of Injection.	Albuminuria	Palatal Paresis	Cardiac Paresis	Ocular Paresis	Death Rate.
Intravenous	10.0	12.7	11.3	-	8.0
Intravenous & Intramuscular	1.8	2.0	1.8	-	1.2
Intramuscular	2.7	3.0	2.6	1.0	2.3

The intravenous-intramuscular series of cases had the lowest percentage of complications. Next in order came the cases treated by intramuscular injection and lastly those which had received the serum by intravenous injection.

Conclusions.

A combined intravenous and intramuscular therapy is the best treatment for diphtheria. Where this cannot be used, the serum should be given by intramuscular injection. Intravenous therapy alone is not to be recommended. Comparison of Complications and death rates, correlated with type of C. Diphtheriae, when concentrated and refined sera were used.

	Author.	Complication	Gravis	Intermedius	Mitis	Aberrant	Negative.
x	Anderson et al 1931.	Albuminuria	44.4%	66.66%	17.1%	-	-
		Paralysis	22.2%	50.0%	0	-	-
		Death Rate	17.5%	0	0	-	-
x	J.F. Murray 1935.	Albuminuria	0	7.8%	3.4%		
		Paralysis	0	10.0%	6.8%		
		Death Rate	0	11.1%	3.4%		
x	Tannahill 1936.	Albuminuria	38.4%	40.4%	18.1%		
		Paralysis	20.8%	21.4%	11.3%		
		Death Rate	9.5%	13.8%	4.8%		
ø	Present series.	Albuminuria	5.4%	3.4%	2.1%	5.9%	3.9%
		Paralysis	13.5%	12.9%	2.0%	5.9%	2.0%
		Death Rate	5.4%	3.0%	0.8%	5.9%	2.0%

x Concentrated Serum.
ø Refined Serum.

McLeod, Murray and Tannahill all found the incidence of albuminuria and paralysis to be the highest in intermedius cases. In the cases treated in Belvidere Hospital, albuminuria had its highest incidence in aberrant cases and paralysis its highest incidence in gravis cases. The death rate in McLeod's series was highest in gravis strains. In

Murray and Tannahill's series it was with the intermedius strains and in the Belvidere cases it was with the aberrant strains.

Comparison of Results of Treatment.

	Albuminuria	Paralysis	Death Rate.
Rolleston,1925 (1)	53.3%	20.7%	10.1%
Goodall, 1928 (1)	-	15.0%	13.0%
Anderson et al 1931 (2)	36.5%	16.3%	10.6%
Murray, 1935 (2)	5.4%	7.8%	7.2%
Present Series (3)	3.6%	7.8%	2.9%

(1) Whole Serum. (2) Concentrated Serum. (3) Refined Serum.

Classified in order of efficacy from the above table, refined serum is the best, concentrated serum comes next, and whole serum gave the poorest results.

CONCLUSIONS.

(1) The death rate was highest in the 0-5 age group.

(2) The severest clinical cases were associated with the gravis strain.

(3) The cases where a gravis strain was isolated had the highest percentage of complications. As the number of Type iv strains recovered was very small, there is insufficient data for a reasonable comparison to be made.

(4) As the duration of the patient's illness increased before serum was given, so also did the percentage of complications.

(5) Haemorrhagic diphtheria was the most severe type of diphtheria.

(6) No hard and fast rule can be laid down on dosage of serum to be given. Each case should be reviewed separately. It is better to give excess serum than too little. Serum should continue to be given so long as the membrane is spreading. Combined intramuscular and intravenous therapy should be employed.

(7) Refined diphtheria antitoxin can be given intravenously with little fear of serum reaction. Given intramuscularly it causes slight discomfort to the patient owing to the large number of units of antitoxin per c.c.

(8) The incidence of serum reactions has been reduced.

(9) The incidence of c. diphtheriæ gravis is on the upgrade in the Glasgow area.

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