Thesis for M.D. degree

The Treatment of Diphtheria by the injection of the Erysipelas Albumose

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I have for some time been vainly trying to think of some treatment of Diphtheria that would give a medical man some hope of combating successfully that dreadful scourge. All methods so far have been more or less failures but I give the following short account of the more common forms of treatment based on Bristowe (1) before describing the treatment that I would suggest should in future be carried out. In Bristowe it is recommended as a general principle that as much nourishment as possible is to be given, combined with tonic medicines. Food in a fluid form is best and as medicine our chief standby is the tincture of the Perchloride of Iron. There is no reason however why other preparations of Iron should not be used, with or without Quinine. Chlorate of Potash and Hydrochloric Acid are also recommended. Bretonneau and afterwards Trousseau strongly advocated the use of strong Hydrochloric Acid to the mucous membrane. Others again prefer a strong solution of Nitrate of silver, or of Bi-carbonate of Soda, or pure tincture of the

(1) Theory and Practice of Medicine 6th Edition p.218.

Perchloride of Iron or Creasote. Some use the following to wash out or gargle the throat viz:- Chlorate of Potash, thymol Chlorinated Lime, Carbolic Acid, Permanganate of Potash, Salicylic Acid etc. Small lumps of ice have been in many cases of use. I do not here require to refer to the local treatment of the nares or larynx, my endeavour being solely to give an outline of the more common methods of treating the disease. Emetics are said to do good in cases where asphyxia is imminent, the most useful emetics for that purpose being Ipecacuanha or Sulphate of Copper. As a last resort we fall back on Tracheotomy. This becomes necessary as soon as the symptoms of asphyxiation become marked. The mortality where it has been had recourse to is however very great. In my own experience 80% of those operated on have died whereas of those who were not operated on only 35% died. That the disease is caused by bacilli and their products is now I think clearly admitted by all. The most important discoveries with regard to its etiology being made by Loeffler (1) and confirmed more recently by Klein.(2) Taking for granted that the cause of the disease is a bacillus the production of immunity

(1) Mittheit aus dem K.Gesundheitsamte Vol.II. 1884.

(2) Proc. Roy.Soc. May 22.90.p.71.

naturally comes to be the next and most important question. This has always been looked on as not only quite possible but quite probable and a large number of experiments have been made with this object in view. So far none of them have proved successful however. Leoffler(1) by his careful study of this disease has certainly helped us greatly in the investigation, but he like the rest never succeeded in producing, either in animal or man, immunity.

Roux and Yersin (1) from pure cultivation of the bacilli succeeded in isolating a soluble poison by which they were able to produce the symptoms of Diphtheria varying in intensity according to the quantity of the poison injected. By boiling the poison for about the space of ten minutes they destroyed its virulence and it therefore appeared to them to be more of the nature of a ferment than of a ptomaine.

Brieger and Frankel (2) have since tried their hands at producing immunity but they have likewise failed. They consider that the product they isolated is not a ferment but rather of

(1) Mittheit aus dem K. Gesundheitsamte Vol II. 1884
(1) Annales de l'Institute Pasteur Nos 11 & 12 1888
(2) Untersuchungen uber Bakteriengifte Berl. Klinische Woehenschrift 1890 p. 241 - 248.

the nature of a tox-albumin (albumose) but their method would also extract any ferment present. To show that it was not a ferment they treated it at 55°C for six hours in the presence of Hydrochloric Acid. I think however that that hardly proves their case.

From the foregoing it will be seen that numerous experimenters have tried without success to produce immunity and I think we may safely say that so far the right means for doing so has not yet been found.

The symptoms of Diphtheria, apart from the purely local symptoms all point to the presence of some soluble animal poison circulating in the blood. What this poison is has not yet been definitely determined but the experiments of Roux and Yersin, and of Brieger and Frankel all point to its probable nature. Death from Diphtheria may either be caused by Asphyxia, in which case the local symptoms are the more prominent or by Syncope or by Asthenia in either of which case the constitutional symptoms are the more marked. As would be expected where the poison is of the nature of an animal poison or product the nervous symptoms are common and severe. The temperature is seldom high but the heart's action is usually much interfered with, death often

resulting from its failure. The nervous symptoms usually appear late on in the disease thus pointing all the more strongly to the cause. Dilatation of the ventricles of the heart might be explained on the theory of some poisonous material acting directly on the arteries and on the cardiac muscle. An interesting explanation might also be given of the irregularity of the pulse - its action being slow in some cases and rapid in others, other conditions being apparently the same. The first case being due to irration of the vagus and the second to paralysis- both conditions being caused by the poison circulating in the blood of the brain. In several cases of canerous stricture of the Oesophagus a very slow pulse has been noted owing to the direct irritation of the vagus in its course. And if thus easily in its course, how much more easily at its centre by a poison in the blood. The presence of the animal poison is easily accounted for as the bacilli are present in enormous numbers in the false membrane and outer layers of the mucous membrane. Their products will therefore be very readily absorbed by the congested blood vessels and lymphatic. The presence of the bacilli in the mucous membrane of the larynx may account for the common occurrance of paralysis of that organ as they will not only act locally but also centrally by their products. If

further proof be needed that the disease, at least its more dangerous symptoms, is caused by a soluble animal poison, the fact that the bacilli are never found in any part of the body beyond the local lesion will I think be sufficient. No bacilli, for example, have ever been found in the kidney even although that organ is most seriously affected and showing distinct pathological signs of disease. This holds true also in the case of all the animals hitherto experimented on except in the case of the cow. Klein (loc.cit.) found in the cow that the bacilli passed into the blood and thus into the milk.

It is a well known fact that bacteria alone will not cause the symptoms of a so-called parasitic disease, beyond the mere local symptoms, whereas the products will. Although necessary to the production of the disease they are not the most important element in it and the presence of any bacteria in a disease can only suggest an enquiry into the antecedent factors which have combined to bring about the disease or morbid condition favourable to the development of the bacteria. It is quite certain that molecular death must precede all evidence of existing bacteria. Although the matter on which they thrive is found wherever dead organic matter exists they are not able to settle in a

perfectly healthy body but are only able to develop when the physico-chemical constitution of the tissues is altered so as to correspond to their requirements. The old belief that bacteria act injuriously by blocking up the capillaries has now been abandoned and some new theory must be advanced to explain their action. From all that we so far know I think that the theory of the chemical products producing the chief symptoms is the correct one. The following examples may be taken as proofs of the above statement:

I. It has been concluded that a fluid which can retain its specific property after being filtered, boiled, evaporated to dryness and the residue digested in cold and then in boiling alcohol and then redissolved and again filtered cannot owe its toxic property to any living organism.(1)

II. The experiments of Onimus (1) who dialysed the blood, proves that the poison is not dialysable. In most cases he was able to produce symptoms of the disease before dialysis and not afterwards. The toxic element remaining in the non-dialysable part. (This is noticed also when albumoses form the poisonous product).

(1) Aitken - Science & Practice of Medicine 7th edit. p. 376.

III. Burt[1] found that compressed Oxygen destroyed all living organisms but that the blood from a case of Anthrax so treated was still virulent - proving that the cause was not a living organism.

IV. It has been shown by M.M.Jaillert and Laplat [1] that Anthrax is not a parasitic disease; that bacteridia are not a cause and the fewer bacteria present in the blood the more virulent is it. It has also been found that blood not containing any bacteria will propogate the disease.

(Three ideas have been advanced with regard to the causation of the symptoms in Anthrax viz:-

(a) That the bacilli block up the vessels and cause an embolism to form.

(b) That they produce a ferment capable of decomposing the tissues.

(c) And that they give rise to one or more definite poisons.

All these theories may be correct but the last seems to be the most probable.)

(1) Comptes Rendus LXXXIV p. 1130 May 1877 also Lewis Memorial Volume.

V. Hoffa from a pure cultivation of the Anthrax bacillus has isolated a ptomaine which when injected subcutaneously produced symptoms of Anthrax.

VI. Pasteur (1) showed that the Anthrax bacillus does not act by producing a ferment for by filtering the blood through porous cylinders he obtained an inert fluid. He also found that the blood from an infected animal when sterilized and injected into other animals appeared to produce immunity.

He further found that by subjecting the bacilli to abnormally high temperatures or to small doses of certain poisonous substances that they lose their pathogenic properties while their morphological and biological characters remain otherwise unaltered.

VII. Dr.Sydney Martin(1) has succeeded in getting a proto - and a deutero-albumose from the bacilli of Anthrax and he also at the same time isolated a ptomaine. . With all these he has been able to produce the symptoms of the disease.

(1) De l'Attenuation des virus et de leur retour a la virulence Competes Rendus

(1) Proc.Roy.Soc. May 22. 90 Vol XLVIII No 292.

VIII. The latest and by far the most important discovery has however been made by Dr.Hankin[1] who has isolated an albumose in the same manner as Dr.Martin. He has however gone further and has succeeded in producing immunity by excessively small doses of the albumose. This discovery opens the way I hope to the production of immunity in many other diseases.

IX. Brieger[1] has isolated four separate ptomaines from among the products of Tetanus namely-

(a) Tetanine - This substance produces tetanus in mice when injected in small doses subcutaneously.

(b) Another, not named, also causes tetanus along with a free flow of saliva and tears.

(c) Tetanatoxine - This substance first produces tremor, then paralysis and lastly violent convulsions.

(d) Spasmotoxine - This causes severe convulsions both tonic and clonic.

It is probable that the poison formed by the bacilli in Tetanus is chiefly ~~treated~~ located either in the cord or brain and that it does not enter into the general circulation. The flesh of animals killed by Tetanus has been proved not to be

(1) British Medical Journal p.66 July 12. 1890
(1) Weitere Untersuchungen uber Ptomaine.

poisonous.

X. As mentioned before Brieger and Frankel have isolated what they call a tox-albumin from Diphtheria, Tetanus, Cholera and Typhoid fever cultivations.

XI. Roux and Yersin as was also mentioned before have isolated a soluble poison from Diphtheritic cultures.

XII. The virus of rabies is still unknown although the virus has been artificially attenuated by Pasteur(1) and he has thus succeeded in producing immunity. He thinks the virus is a soluble chemical substance formed by an unknown microbe.

XIII Prof.A.Babes(2) has informed Dr Hankin that he has just isolated an albumose from the central nervous system of animals dead of rabies.

XIV. Since the above was written Dr.E.A.V.Sehmenitz(3) has given some preliminary notes of a study of the products of Hog Cholera. He has isolated a salt which he says is Cadaverine and also a primary amine which he has not yet identified. He has also obtained an albumose which gives rise to well marked symptoms.

(1) Comptes Rendus Tome XCVIII p. 457 & p.1229
(2) British Medical Journal p. 67. July 12. 1890
(3) Medical News Sept 6th 1890.

He has been able to produce immunity by means of certain chemical compounds which however he does not reveal.

XV. Selander[1] also gives his results with regard to Hog Cholera. Death he says is due to poisoning as when virulent filtered blood is injected into the veins of a healthy animal, the animal dies rapidly with symptoms of paralysis which occur in a definite and regular order. The poison, when the animal recovers is excreted by the kidneys. The poison is not destroyed at 58°C but it loses its power at 100°C and retains only part of it at 60°C. He states that it is not an alkaloid but a toxic albumin which has a cumulative action. He was fairly successful in some experiments he made to produce immunity but not entirely so.

From the above facts it is argued that the cause of death is poisoning by these products and that the bacteria are not merely the means of spreading infection but are also actually the makers of it. On such principles rests the protective inoculation by chemical substances. By introducing, little by little, these chemical substances, produced by pathogenic bacteria, into the bodies of animals in such a way as to avoid

(1) Annales de l'Institute Pasteur p.545 No 9 T.4 Sept 25. 90

speedy poisoning but so as gradually to accustom the animal to its presence, it becomes refractory not only to toxic doses which would at first have caused death but also to the micro-organisms themselves. And now the immunity which hitherto we could only produce by introducing a living virus into the body is effected by introducing a chemical substance and these inoculable substances being those that we have observed caused the poisoning. The question now comes to be has the animal become refractory in consequence of the presence of these substances in its tissues thus preventing the growth of the bacteria? Upon this point we know that as the products of growth accumulate in certain tube cultivations of bacteria so do its powers of growth lessen until finally they cease entirely. We must however be careful to avoid as yet forming an opinion as to what happens in the human body. If we take a little blood from a sheep rendered immune to Anthrax and inoculate it with Anthrax bacilli they will grow - thus showing that the blood contains no substance capable of destroying the life of the bacilli.

Bouchard[1] on this point says that after eliminating one by one the different possibilities as to how the chemical action

(1) Action des produits secretes par les microbes Pathogenes Paris 1890.

of a few cubic centimeters of injection acts he finally considers that the products of a microbe act by diminishing the power of the cells to act as phagocytes. A microbe is then apparently pathogenic if it can produce a poison capable of paralysing the leucocytes. If the leucocytes have acquired tolerance against this paralysing agent, the animal has acquired immunity against the microbe.

The two products that I have here mentioned as being produced by bacteria are alkaloids or ptomaines and poisonous proteids or albumoses.

The present state of our knowledge with regard to these substances is still very elementary but the following few facts may be of interest.

The Ptomaines or Animal Alkaloids were first discovered by Panum in dead animal tissues produced by putrefactive decomposition. Since then a large number of discoveries have been made with regard to them by a number of workers but more especially by Brieger. He has studied them very closely and has done more than anyone else to forward our knowledge of them. They have been found to resemble very closely the known vegetable alkaloids.

Dr.Lauder Brunton[1] writes of them "as products of albuminous decomposition whether their albuminous precursor be contained in the cells of plants and altered during the process of growth, or whether the albuminous substances undergo decomposition outside or inside the animal body or by processes of digestion as by unorganised ferments."

They are crystalisable bodies, consisting of nitrogenous bases. They have been discovered in nearly all bacterial cultivations but so far no one has successfully induced immunity by their means. When injected into the bodies of animals they give rise to the well-known symptoms of alkaloid poisoning.

Albumoses or poisonous proteids. Little is known about this class of product, but the little that is known shows that they are of a very powerful class in their action. Hankin[1] mentiones "that the only cases of tolerance which resemble the tolerance implied in disease immunity are those of tolerance against albumoses" Immunity against a disease produced by a micro-organism suggests the fact that immunity against the disease is also immunity against an albumose." That immunity can be produced by this class of product has already been proved by

(1) Pharmacology & Therapeutics 3rd Edit. p.100
(1) British Medical Journal p.65 July 12. 90

Hankin and taking everything into consideration I think we must look to this class for our means of treating and of producing immunity against the so-called parasitic diseases.

The poisonous proteids give all the reactions of the proteids formed during digestion.

I will now turn more particularly to the treatment and producing of immunity against Diphtheria. Considering the failures of so many in the treatment of this disease I was much pleased to see a report in the "Lancet"(1) of some cases successfully treated by Dr.Babchinski of Kieff by the inoculation of the Erysipelas virus. Dr.Babchinski(1) had his attention first drawn to this mode of treatment by his son who was suffering from gangrenous diphtheria that had extended to the nares. The irritation caused by this made the lad scratch himself until he produced ulceration. This was followed by Erysipelas and the boy at once appeared to improve and was shortly out of danger and well. Following this up he began inoculating patients suffering from Diphtheria with the Erysipelas virus and he was rewarded with most satisfactory results. He injected the virus into the patients submaxillary region.

(1) "Lancet" p. 922 Vol I 1890
(1) Internationale Klinische Rundschau May 1890

After thinking the above over I came to the conclusion that I had at last got a clue to the treatment of Diphtheria. As holding out still more hope that I might prove to be right was the fact that Erysipelas has been used for ages as a curative agent in various affections but more especially of malignant disease of the external surfaces. It seems first to have been employed in the 17th century and amongst other early observers was Hebra. He expressed great faith in its use for tumours. Record and Despres[1] appear to have used it largely in the treatment of phagedenic chancres.

Busch[1] also employed it in malignant new formations of the lymphatic glands. Fehleisen[2] who was the first to isolate successfully the micro-coccus records seven cases in which he treated, with more or less success for the time being, malignant disease. He also mentions the following as examples of what it was supposed to do good to viz:- mental diseases, neuralgia, typhus, acute rheumatism, chronic joint affections, syphilis, keloid, epithalioma, carcinoma and enlargement of the lymphatics.

(1) Fehleisen -Die Oetiologie des Erysipels Berlin 1882

Deutsche Zeitschr.f.chirugie Band.16.1882
Uberden Erysip.Wurburger phys.med.Ges Aug 1882

(1) W.Busch.Berlin Klin.Wochenschrift '66
(2) loc.cit. (p.38)

In the cases treated by Fehleisen he injected pure cultivations of the micro-coccus and in every case he succeeded in producing Erysipelas. The period of incubation varied from 16-60 hours. In no case did any evil result to the patient from the induction of the disease. He also found that after the patients had once suffered from the disease that they were immune from a further attack for a short period. This immunity does not appear to be lasting like that of many other infectious diseases - in fact it would appear as if repeated attacks of erysipelas actually predisposed patients after a certain time to other attacks. This is supported by observation made in a large general hospital where the larger proportion of idiopathic attacks were either second or even third or fourth attacks. Although the inoculation with the Erysipelas virus may be effective in treating diphtheria it is open to many objections and it has occurred to me that it would be much better if possible, to do so by the products of the Erysipelas micro-coccus. In that case you would only inject a known quantity and could thus regulate its strength and result. You would not only run less danger than must always be present in injecting living organisms into the human system but would also avoid

in all probability any chance of setting up local mischief. I may here mention the fact mentioned by Bourehard[1] that many observers have succeeded in protecting an animal against a microbe by inoculating it with another. More than half a dozen different species protect against Anthrax and if against Anthrax why not against Diphtheria?

I shall now give some details of my experiments carried out with the production of immunity or treatment of Diphtheria being the end always kept in view.

In the following experiments the Klebs-Loeffler No 2. bacillus[1] was entirely used. It was obtained from a small piece of Diphtheritic membrane embedded in blood serum. From among the numerous bacterial growths resulting, the No 2. Bacillus was isolated and proved to be the Diphtheritic Bacillus by control inoculations of guinea-pigs. The colonies growing on the nutrient jelly were round or oval in shape, dark brown or greyish yellow in colour, according to their situation. On closer examination they were seen to be coarsely granular and had irregular outlines. When injected into guinea-pigs a

(1) Action des produits secretes per les microbes Pathogenes
(1) Mitth.aus dem K.Gesundheitsante Band 2.

a whitish or hemorrhagic exudation occurred at the spot of inoculation, rapidly followed by subcutaneous oedema and death. After death large quantities of bacilli were found in the local lesion and were readily isolated and cultivated in nutrient jelly. In no case were any bacilli found in the internal organs although these were greatly congested. The principal organs affected were the lungs, intestines and kidneys.

The Bacilli when examined under the microscope were found to be immobile. Their length corresponded closely to that of a tubercle bacillus but they were thicker. They may vary very considerably in length. The best stain to use is methylene blue.

The Erysipelas micro-cocci were obtained by embedding a small piece of skin taken from a case of Erysipelas in man. It was placed in a gelatine medium while in a fluid state. In about 24 to 36 hours when the tube was kept at 20°C, small colonies appeared near the piece of skin. By subsequent inoculation of other tubes a pure cultivation was got. The organisms were found to grow in chains and were easily stained by Gram's method. On coagulated blood serum it grows very well, at the temperature of the body, as a white layer easily removed from the surface. When inoculated on rabbits very characteristic

results were got. The site of the inoculation as recommended by Fehleisen[1] was the ear. After 36 to 48 hours a sharply defined reddening appeared and spread from the seat of inoculation, especially along the blood vessels, to the root of the ear and neck. The disease after the space of a day or two waned and finally disappeared. I also obtained similar results in the case of guinea-pigs except that perhaps the course of the disease was not quite so well seen.

In the first series of experiments I inoculated the micro-organisms of both diseases simultaneously and in only one case did death ensue. In all ten guinea-pigs were so treated.

Series No I. Animal- Guinea-pig. (Both micro-organisms injected together)

Animal No I. At the point of inoculation after about 36 hours the subcutaneous tissues were seen to have become oedematous and a small tumour occurred with the formation of a false membrane. Shortly after, the surrounding skin was seen to be much reddened and this appearance gradually extended in all directions. At the same time severe constitutional disturbences were noticed.

(1) Fehleisen loc.cit.

The temperature rose 1 to 2°C.- the animal was restless, it constantly moved about and did not sleep. It refused all food but drank readily; the respiration was hurried. In about 36 hours more the severity of the disease had passed and the animal gradually recovered its normal appearance with only slight sloughing of the tissues at the seat of inoculation.

No.2.3.4 & 5. all followed a more or less similar course and all ultimately came round.

No.6. For some reason which I could not explain this case ended fatally. From the appearance at the seat of inoculation it seemed as if the Erysipelas virus had not taken effect and on making cultivations from the tissues only the Diphtheritic Bacillus was got.

No.7.8.& 9 Ran a course similar to No.1.

No.10. In this case the amount of Erysipelas virus was in much greater proportion than the Diphtheritic and the disease ran a much milder course than in any of the other cases.

No.11. Control for Diphtheritic virus.

No.12. For Erysipelas.

From this series it will be seen that Dr.Babchinski's view is upheld and it becomes clear that the virus of Erysipelas can

prevent a fatal termination in Diphtheria. Whether immunity can be produced by these means has yet to be worked out. I now began my search for the chemical products by means of which I hoped to effect as much as the virus itself did without many of the latter's evil effects. I first then tried to isolate a ptomaine from pure cultivations of the Erysipelas micro-cocci.

The method employed was as follows - The cultivation was first turned out of the tube into a porcelain capsule which was gently heated until it was evaporated to dryness and remained as a brownish deposit at the bottom of the capsule. I thus extracted with successive small quantities of alcohol to remove various insoluble salts and I then removed the sulphates, phosphates.etc.,by precipitating with an alcoholic solution of the neutral acetate of lead. After filtration the remaining alcoholic solution was then precipitated with a warm alcoholic solution of mercuric chloride. By these means most of the organic bases present were thrown down in the form of insoluble mercuric compounds. Some however as a rule always remained in solution.Both precipitate and filtrate were kept and treated separately. They were freed from the Mercury by heating at the same time driving off the alcohol. The remaining solution

could now be tested with Liquor Potass. to show the absence of any Mercury. It is now rendered strongly alkaline by the addition of 10% solution of caustic soda and then treated with either Benzoyl Chloride, Gold Chloride or Platinum Chloride. By these means I succeeded in isolating a compound from the pure cultivations, that answered in all respects to the characters of a ptomaine. To obtain the alkaloid pure I treated the double Benzoyl compound with alcohol in which it was freely soluble. It was insoluble in water and crystalized out of alcohol in long fine needles arranged in feathery bunches. Its melting point was found to be between 170° and 173°C. It gave reactions with Potassio-mercuric-Iodide, Phospho-molybdic Acid and Phospho-tungstic Acid.

When injected into rabbits it gave rise to general uneasiness, a rise in temperature, refusal of food, vomiting, purging, salivation, dyspnoea, paralysis and death according to the quantity injected.

I was not in any case able to produce immunity by it from Erysipelas and it appeared also to be useless in the treatment of Diphtheria.

I now turned my attention to the poisonous proteids and my

search for an albumose was conducted as follows - The cultures of the Erysipelas micro-coccus were made in a 0'1% solution of Liebegs extract of meat to which some fibrin was added. The Liebegs extract was sterilized by heating for several consecutive days in a steam sterilizer for two or three hours daily. The fibrin was added after this and the whole resterilized by repeatedly heating to the boiling point for a short time on each occasion. After inoculating with the micro-cocci from a pure cultivation the tube is kept at the ordinary temperature for two days or so. The liquid is then filtered and the albumose extracted. At first I employed naCl as the reagent for extracting the albumose but I finally got better results with $(NH_4)_2 SO_4$. After filtering, the cultivation fluid is rendered acid with Acetic Acid and saturated with $(NH_4)_2SO_4$. A bulky white precipitate formed which was filtered off and the salt separated by dialysis. The best means to prevent putrefaction at this stage is to carry on the process over running water at a temperature of 48° Cent. After dialysing for 24 to 48 hours the albumose will be found in solution with a considerable quantity of water, which has passed through the parchment.

The next thing to do is to concentrate the solution by evaporating it "in vacuo" over Sulphuric Acid. When evaporated to a sufficiently small amount it is poured into absolute alcohol, washed in the same reagent and dried. I now had a substance that gave all the reactions of the albumoses formed in peptic digestion. When injected into animals it was found to produce local subcutaneous oedema with some sluggishness leading to prolonged stupor, coma and death according to the dose . At the same time there was especially at first great increase in the temperature, the pulse became full and bounding and the respiration much hurried. This was followed by a slowing of the pulse and loss of body heat as the comatose condition supervened. A fatal dose in guinea-pigs was found to be about 0·014 gram per 1 gram body weight of the animal. To free the solution of albumose from any ferments that might be present the solution was mixed with a quantity of lime water and a solution of Phosphoric Acid added. By this means a gelatinous precipitate formed of calcium phosphate.. The precipitate being filtered off a clear solution of the albumose remained - any ferment present being carried off with the precipitate . (The above method is almost identical with and was founded on that of

Hankin.)(1)

Now having obtained an albumose I carried out some experiments to see if it were possible to produce immunity against Erysipelas. I was only able to do so on five rabbits however and my results cannot therefore be in any way conclusive.

Series No II. Animal - rabbit.(dose of albumose varied from a $\frac{1}{10,000}$ to a $\frac{1}{5000}$

No 1. In this case the albumose was injected along with the micro-cocci.

No 2.Here it was injected 24 hours before.

No 3. Here 48 hours before and in

No 4. 72 hours before.

No 5. Was a control animal for the micro-cocci

In No 1 to 4 perfect immunity was obtained with the above doses given once. In No 5 a typical case of Erysipelas developed.

Series No III. Animal-Guinea -pig

Diphtheritic virus injected 24 hours after the Erysipelas albumose.

No I.Control animal for the Diphtheritic virus - died.

(1) Hankin - British Med. Journal p. 66 July 12. 90

No.2 & No.3 Dose of albumose a $\frac{1}{1000}$ of the body weight of the animal. In both these cases there were slight local signs of an irritating substance having been injected but no signs nor symptoms to show that it was caused by the virus of Diphtheria.

No.4 & 5 were quite immune.

Series No.IV. Animal - Guinea-pig. (albumose injected 7 days before virus.

In this series the dose at first was a $\frac{1}{5000}$ repeated when all signs of reaction had ceased. This was usually on the second day.

No.1. Control - died

No 2. Immune.

No 3. Only one dose given in this case. Animal contracted slight amount of diphtheria but recovered well.

No 4. Dose repeated twice in this case. Animal quite immune.

No 5. Slight local signs. Two doses of albumose given.

Series V. Animal - Guinea-pig. (Albumose injected 24 hours after virus)

No.I Twenty four hours after the injection of the Diphtheritic virus considerable local swelling was noticed as well as

slight general symptoms. In this case a $\frac{1}{5000}$ of albumose was given. This was followed almost at once by severe reaction. There was great increase in temperature and local swelling. These in about twenty four hours had subsided somewhat so another dose of a $\frac{1}{5000}$ was given and by this time the animal had apparently got over the worst of the disease. It finally recovered but not for nearly a week.

No 3. The dose in the first instance was a $\frac{1}{2000}$ and the reaction was very severe but it stopped all diphtheritic action and although another dose of a $\frac{1}{5000}$ was given it was not required.

No 4. Dose increased to a $\frac{1}{1000}$. In this case a large slough formed at the point of inoculation.

No 5. Dose a $\frac{1}{750}$. In both these two cases the reaction was most severe and the animals appeared as if they would succumb but they both rallied and certainly all traces of the Diphtheria disappeared rapidly.

Series VI. Animal - Guinea-pig. (Albumose injected 48 hours after virus)

No.I Control - died

No 2 & No 3. First dose a $\frac{1}{5000}$, repeated next morning when animals were found to be much better. On third morning a $\frac{1}{1000}$, was

given but no reaction took place.

No 4. Shortly after the first dose of a $\frac{1}{5000}$ was given the animal died apparently from Diphtheria.

No 5. Animal was very ill before the injection so that this was increased to a $\frac{1}{1000}$. Next morning after a very severe reaction animal was better and got another dose of a $\frac{1}{750}$. It steadily improved and when on the third morning a $\frac{1}{1000}$ was injected there was no reaction. In this series of cases the animals were all very ill before the albumose was injected and in all the reactions were severe and prolonged.

The conclusions I drew from the above experiments were.

Firstly That even although the Diphtheria had a good hold of the animal's system yet by injecting the Erysipelas albumose it could be arrested. This was true either where the symptoms were only beginning to show themselves as well as where they were well marked and severe. The albumose acted apparently by killing the bacilli, at least it rendered the surrounding ground unsuitable to their development and thus prevented their elaboration of chemical poisons. I hope to give the proof of the above sometime soon.

Secondly It produces immunity, of how long duration I cannot say as far more elaborate experiments are required to determine this point.

Thirdly. The dose varies from a $\frac{1}{5000}$ to a $\frac{1}{750}$. If one dose is not sufficient and in few cases it will be sufficient a second should be given slightly larger than the first and so on until no reaction is got after the injection. The first dose I hold should not be more than a $\frac{1}{5000}$ at most.

I do not pretend that these experiments are conclusive or nearly conclusive but I hold that they carry out the theory on which I was working which was that Diphtheria being a parasitic disease was curable by the death of the microbe causing it and by administering the antidote to the poisons produced by it (the microbe). This cannot so far be done by the chemical poisons of the Diphtheritic bacillus itself but the body may be rendered unsuitable to its development by the action of the Erysipelas virus or products, just as an animal is rendered immune to Antharax by the bacillus prodigiosus or its products. These facts I think render the subject just treated well worthy of further study and I hope soon to be in a position to make experiments on a much more extensive scale.