Some remarks on Ehrlich's side chain theory of disease and immunity, being a preliminary description of some yet uncompleted investigations on the side chain theory in some of its pharmakological pathological and therapeutic aspects, submitted as an essay in competition for the Milner Fothergill Gold Medal in Therapeutics by

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Recent progress in medicine has caused the pathologist to become less of a pure histologist and has obliged him to apply the methods of the pharmakologist to unravelling the problems of disease, and the pharmakologist on his part has been obliged to become more of a pathologist, microscopist and bacteriologist in his search for therapeutic agents. The fact that poisons alone cause the symptoms of many diseases was enough in itself to justify the pharmakologist being attracted to the field of investigation opened up by achievements of the bacteriologist. How much they have contributed to the study of the toxic origin of disease, and of improved methods of treating disease in accordance with modern conceptions, is evidenced by the work of Lauder Brunton, Fraser, Heymans, Pohl, to mention only a few pure pharmakologists, who have contributed to the study of what may be termed the newer pharmakology, not to mention the many, who, perhaps fearing the ousting of the older medicine by the new, have endeavoured to stem the tide which has carried us to our present conceptions of a definite rational serum therapeutics, and, at least in regard to the thyroid gland, of a rational organo-therapeutics, and the conception of ~~what~~ a, if yet only theoretical, promise of a rational cyto-therapeutics. Thus the pathologist may be justified in claiming the pharmakology of this field as a branch of pathology and the pharmakologist may justly retort that pathology in this narrowed sense is only a branch of pharmakology. Therefore although I feel some trepidation in submitting this essay in

competition for the Julius Rotherfill Medal, I feel also that I have some justification in humbly claiming for pharmacology and therapeutics a wider signification than was possible only ten years ago.

The somewhat scattered nature of the work forming the basis of this essay has arisen out of the extent of the field of investigation, and the difficulties which have been encountered while endeavouring to obtain an insight into the affinities in the body of the toxines of disease, of the toxalbumins and bodies producing antikörper and not producing antikörper, and into the nature of the difference in the actions of the two classes of bodies which produce and which do not produce antikörper, also into the difference between acquired tolerations e.g. to alcohol, morphine, arsenic etc. a condition always in its highest degrees resulting in excessive fatty degeneration and infiltration, and real immunity resulting in the production of the specific antikörper to the toxines of disease and the vegetable and animal toxalbumins. The various work which forms the basis of this paper can only be regarded as having afforded me a better orientation in what is practically a new field of enquiry, in a field of investigation which has suffered from what I presume to think has been the too hasty assumption of a fundamental difference in the nature of the actions in the body of the two classes of substances which produce and which do not produce antikörper.

3

It may render the connection between seemingly little related facts more readily comprehensible if this note be prefaced by a resumé of the recent advances in our knowledge of some of the processes of immunity.

Pfeiffer's[1] discovery that if the cholera vibrio be introduced into the peritoneum of a cholera immune guinea pig, or into the peritoneum of a normal guinea pig together with a little of the serum of such an immune animal undergoes immediate solution forced the conceptions of investigators to become of wider extent than when the problems of immunity were practically dominated by considerations dependent on Behring and Kitasato's[2] epoch making discovery and Metschnikoff's[3] phagocytosis theory. A still further broadening of the horizon we owe to Ehrlich[4] In a number of publications based on a mass of experimental evidence, the result of an unusual amount of work carried out in accordance with one general plan Ehrlich has sought to deepen our knowledge of the processes involved in the actions of the bacterial toxines and of the bacteria themselves in the body, and of the responses which the body makes to their presence. He has at least succeeded,

(1) Ein neuer Grundsatz der Immunität (Deut. med. Woch. 1896. No 7 and 8)

(2) Ueber das Zustandekommen d. Diphtherie-Immunität und d Tetanus Immunität bei Thieren (Deut. med. Woch. 1890. No 49)

(3) Etudes sur l'immunité (different memoires in Ann. Inst. Past.)
Immunität (in Weyl's Handbuch d Hygiene 1896) cf. Metschnikoff Inflammation.

(4) Die Werthbemessung des Diphtherie Heilserums, und deren theoretische Grundlagen Klin. Jahrbuch Bd. VI.
Ueber die Constitution des Diphtheriegiftes. (Deut. med. Woch. 1898. No 38)

4

in a theoretical way, in throwing rays of light on problems hitherto shrouded in impenetrable darkness. It is also well known Ehrlich has likened the cell to an organic chemical body in which there is a chemical nucleus and many side chains, through which alone the nucleus enters into outside relations (reactions). This is a purely hypothetical way of regarding the cell but a perfectly comprehensible one. Our relations, as bodies composed of a conglomeration of cells, with the outside world as regards food, respiration excretion etc. are purely chemical processes within our ken, and can be demonstrated by the exact processes of qualitative and quantitative analysis. Of the chemistry of what we vaguely call protoplasm or indeed of that of the albuminous bodies in general we know very little, but it certainly is generally accepted that the nutritive processes of the cell — assimilation and dissimilation — must partake of a chemical nature. Ehrlich goes further and seeks to obtain an intelligible conception of these chemical processes, which are beyond our ken. He conceives the existence in each cell of hypothetical side-chains concerned in the normal chemical life of the cell, maintaining its nutrition and relations with neighbouring and remote cells and fluids, the media being especially the blood serum and lymph. Of account of the chemical affinities which characterise them these side chains are liable to enter into many unions, indeed, to union with all bodies

5

possessed of correspondingaffinities and these bodies may on their part, by their union, disturb to a greater or less extent the normal life of the cell.

To many, but more particularly to the British medical man, this conception of metabolism and of disease as a disturbance of metabolism in the above special sense, may appear too purely hypothetical. My experience in Germany has brought me to the conviction that whereas the British graduate in medicine can well hold his own against his German colleague in pathology clinical medicine and therapeutics, as a chemist he is deplorably handicapped. This obliges a British graduate to become an ardent student of the theory of chemistry in order to follow intelligently the drift of the work which is being carried on in France and Germany. The more one becomes familiarised with this side chain theory of metabolism and disease, the less one becomes inclined to cursorily cast it aside. It is a ground principle in chemistry that definite atomic groups only enter into union with certain other atomic groups. The more complicated the combination be the more complicated become the conditions essential for the occurrence of the reaction between the combining groups. Pasteur[1] in his classical work on tartaric acid

(1) Compt. Rend. XLVI. 616 (1858) LI. 298 (1860)

6

and polarisation long ago showed that if the spores of Penicillium glaucum be introduced into a neutral solution containing ammonium racemate (i.e. both right and left-handed tartaric acid), during the development of this lowly organism the right handed tartarate disappeared and there remained a pure solution of the left handed tartarate. In the nutrition of the Penicillium the molecules of the right handed configuration are preferred and the left handed shunned. More recently similar facts have been ascertained for many bacteria. Pasteur explained this through the asymmetrical structure of the chemical molecule. Emil Fischer[1] in his studies on the sugars and sugar derivatives has drawn attention to the important bearing of stereo-chemistry on physiology and has shown that the configuration of the molecule is of the first importance in the alcoholic fermentation of the mono-saccharides, in the splitting by the enzyme emulsin, of the natural and artificial glucosides, in the action of the yeast enzyme on the polysaccharides, and has pointed out that it is not therefore to be wondered at if of two stereo isomers one acts strongly on our sense of smell, and the other not at all, since the relation between the configuration of the molecules and their efficacy as biological agents is a very definite one. Jf.

(1) Bedeutung der Stereochemie für die Physiologie (Ztschr. f. Phys. Chem. XXVI. p. 60, 1898-99.

(2) Die Chemie der Kohlenhydrate und ihre Bedeutung f. d. Physiologie (Hirschwald Berlin 1894)

cf. also Wedekind. die Grundlagen der Stereochemie. (Physikalische Zeitsch. I. 1900)

the function most essential to the maintenance of the life of the yeasts viz. nutrition has been shown to be more dependent on the geometrical configuration of the sugar molecule than on its composition – it is a matter of no moment whether the sugar be a monose or a hexose – has much more important a rôle the mere configuration of the molecules may play in the chemical processes of cell nutrition we are scarcely able to conceive. The albuminous bodies are also optically active and the conclusion is therefore justified that here again we have to do with a structurally asymmetrical molecule. This asymmetry of the molecule may play a very responsible part in reactions between the complicated groups concerned in the chemical processes of cell life and may give occasion for unions with all manner of substances of which the geometrical structure of the molecules does not too widely differ from that of normal food stuffs possessed often of very different composition. I have stated at some length the grounds which appear to have led Ehrlich to formulate his side-chain theory of disease and immunity. Ehrlich has nowhere given in extenso the line of reasoning which he has followed, and the foregoing is the only justification for it which I have been able to arrive at. Although with the present limitations of our knowledge one may not wish rashly to commit oneself to the unqualified support of Ehrlich's side chain theory, the immense assistance this hypothesis has rendered, perhaps in deepening our knowledge of the processes of immunity, but

certainly in stimulating investigation, justifies its careful study and its employment in explaining facts that without an application of the side chain theory would appear so insignificant as to seem to be without important bearing: but taken into consideration in the light of the side chain theory they acquire the little importance, and it may be their true significance, in assisting our comprehension of the problems of disease immunity and therapeutics.

It is probable that certain atomic groups — and I use the expression as applying to all pharmacologically active bodies — are only capable of exerting an action in the body when they find there other corresponding groups, and the conditions suited for a union with them. When such corresponding atomic groups, or suitable conditions fail in the body the substances introduced from without remain inert. There seems no justifiable reason for assuming that insusceptibility to an alkaloid has a different basis from insusceptibility to a toxine. Bodies may of course may be inert for other reasons, viz. non-absorption, or decomposition into other bodies, or because of their union with tissues or constituents of a tissue which afford of no opportunity (fat, cholesterin etc.) for an action being evidenced. It is obvious that the union of substances introduced from without, with the cells may have consequences of a trivial of a transitory or of a more or less permanent nature.

The side chain theory on account of its general applicability must be regarded as knitting together and affording the most intelligible explanation of more of the facts of disease and immunity than any other explanation yet advanced. Its truth cannot be said to be established nor even its probability. There has been the period when pathology was studied purely macroscopically, during the first fifty years we have earnestly studied cellular pathology, and it may be that with the side chain theory, or some modification of it, as helpmate we now find ourselves embarked on the study of the microscopy of the microscope, if I may be allowed to use the expression, for many of the problems before us are as much beyond the elucidating power of the microscope as they are insoluble by methods of investigation purely chemical and physical - at least in so far as such methods are now available.

The study of the process of haemolysis has made us familiar with the exactly similar process of cytolysis, of which indeed haemolysis is only a special form convenient for study because the haemoglobin contents of the erythrocytes serve as a ready indicator of results which in other cases are only to be ascertained with the aid of the microscope. The study of haemolysis and cytolysis has again stimulated investigation into bacteriolysis, which process again, by the fact that bacteria are cells specially adapted to maintaining their existence in the living body, must be regarded as a very special form of cytolysis. It may be that the seemingly so similar processes of cytolysis and bacteriolysis

will as further investigation be found to present significant deviations from one another, due to the very special nature of the relationship existing between the bacteria and the living body. In many cases the evidence does not justify the assumption that all the facts of haemolysis and cytolysis hold also for bacteriolysis especially in their applicability to the prevention and cure of disease. The sources of error in Wassermann's[1] experiments in this connection are too apparent to call for comment.

Pfeiffer[2] was struck by the ferment like nature of the process he described, and held that in the serum of the immunised guinea-pig there was present a single body, comparable to a zymogen, which through the agency of the living cells of the peritoneum was converted into the bacteriolysine. Metschnikoff[3] showed that the bacteriolysis took place in vitro if a small quantity of the peritoneal exudate of a normal guinea pig was mixed with the serum of the immunised animal.

(1) Ueber neue Versuche auf dem Gebiete der Serumtherapie (Deut. med. Woch. p. 285. 1900)
Ueber die Ursachen der natürlichen Widerstandsfähigkeit (Deut. med. Woch. p. 4. 1901)

(2) loc. cit.

(3) Sur la destruction extracellulaire des bactériens dans l'organisme (Ann. Inst. Past. ix. p. 369. 1895)

Bordet[1] and Gruber and Durham[2] determined that the serum of the immunised animal was incapable to effect bacteriolysis in vitro provided that it was quite fresh, and Bordet also showed that when by keeping the serum had became inactive it was possible to again render it active by adding a small quantity of the serum of a normal animal. Bordet and Gruber and Durham, advanced the view that the bacteriolytic action was not due to the action of a simple body (Pfeiffer) but the consequence of the combined action of two bodies viz. a specific antikörper and a ferment-like substance present in normal serum, but which was only able to exercise an influence on the vibrio through the agency of the specific antikörpes. Gruber and Durham regarded agglutination as an essential preliminary to bacteriolysis. This conception of bacteriolysis had been prepared for, by the many years

(1) Les Leucocytes et les propriétés actives du serum chez les vaccinés (Ann. Inst. Past. IX. p. 462. 1895)
Sur le mode d'action des serums preventifs (Ann. Inst. Past. X. p. 193. 1896)

(2) Gruber and Durham. Eine neue methode zur raschen Erkennung des Cholera Vibrio und des Typhus bacillus (Mun. med. Woch. p. 285. 1896)
Gruber. Activ und passiv Immunität gegen Cholera und Typhus sowie über bacteriol. Diagnosis (Verhandl. XIV. Cong. f. inn. Med. p. 207. 1896.
Durham Theorie der activ und passiv Immunität gegen Cholera u. Typhus (ibid. p. 228)
—— The mechanism of the reaction to peritoneal infections (Jour. of Pathology. IV. p. 388. 1897.
—— On a special action of the serum of highly immunised animals. (Proc. Roy. Soc. 1896)

work of Buchner[1] on the bactericidal and globulicidal properties of normal serum. The bactericidal property of serum first described by Nuttall[2] and later worked out by Pfuppe and his pupils was regarded by Buchner as due to the action of a simple body which he named "alexine". Buchner opposed the supposed dual constitution of the body possessed of bacteriolytic or of the comparable globulicidal action (Buchner) of normal serum, on strange erythrocytes, and also the idea that an inactive serum could again be rendered active, the latter of course being an essential corollary to his maintaining the unity of the bacteriolytic body.

Belfanti and Carbone[3] have injected horses with rabbits blood found that the serum of the horse had thereby acquired a poisonous action previously absent, which killed rabbits. Bordet[4] carried this investigation further and having injected, Rats, immunised guinea-pigs with rabbits blood, he showed that the poisonous action of their

(1) Untersuchungen über die Bakterien feindlichen Wirkung des Blutes und Blutserums (Arch. f. Hygiene Bd X. p. 384. 1890) Weitere Untersuch. ü. d. Bakterienfeindlichen und globulociden Wirkungen des Blutserums (Ibid. XVII. p. 112.)
Ueber Immunität und Immunisierung (Centralb. f. Bakt. XV. p. 673. 1894.
Ueber Phagocyten Theorie (Munch. med. Woch. p. 1320. 1897.)

(2) Experimente über die bakterien feindlichen Einflusse des thierischen Körpers (Zts. f. Hyg. IV. p. 353, 1888.

(3) Produzione di sostanze tossiche nel siero di animali eterogeneo (Giorn. d. Acad. d. Med. Torino. 1898 No 8.

(4) Sur l'agglutination et dissolution des globules rouge par le serum d'animaux injectés de sang defibriné (Ann. Inst. Past. XII. p. 688, 1899).

serum in the body of the rabbit corresponded to a specific haemolytic action - previously absent - on rabbits erythrocytes in vitro. Bordet also showed that the action of this artificially produced haemolytic serum was exactly analogous to the process of bacteriolysis. An agglutinative action which he observed, he, in keeping with Gruber and Durham's conception of bacteriolysis, also regarded as a necessary preliminary to the haemolysis. Ehrlich and Morgenroth[1] have later shown that the agglutination is no part of the haemolysis, each is a distinct process, this I have many times confirmed. Agglutination and bacteriolysis appear also to be independent processes. Bordet also produced evidence that as in the case of bacteriolysis so here also two bodies played a rôle, one which was the specific antikörper (sensibilatrice) and more resistant to heat, and a second which was easily destroyed by heat, was present in normal serum, and which he regarded as being Buchner's alexine. The serum rendered inactive by heating to 55° C. during half an hour had its action again restored by the addition of normal guinea-pig serum, or of rabbits serum. Struck by the great importance of this work of Bordet's, Ehrlich and Morgenroth have subjected

(1) Zur Theorie der Lysinwirkung. (Berl. Klin. Woch. № 1899.) also Ueber Haemolysine ibid. № 22 1899: № 21 and № 3 1900 and № 10. 1901: Ehrlich. On Immunity with special reference to cell life. Proc. Roy. Soc. Mar. 22. 1900.

the processes of haemolysis to an exhaustive study. They showed in the first place that the haemolytic action exercised by a normal serum on the erythrocytes of another species was of the same nature as the process described by Bordet as occurring in the case of a serum obtained after specific immunisation. To avoid confusion only haemolytic sera obtained after a process of immunisation are here referred to. Thanks especially to the work of Ehrlich and Morgenroth (who also to Bordet[1], Buchner[2], von Dungern[3], Landsteiner[4], Metschnikoff[5], Moxter[6] etc. the study of haemolysis and of the analogous processes that can be demonstrated to occur when the serum of an animal that has been immunised

(1) loc. cit. and Agglutination et dissolution de globules rouges par le serum (Ann. Inst. Past. XIII. p. 273. 1900)

(2) Zur Kenntniss der Alexine sowie der specifisch bactericiden und specifisch haemolytischen Wirkungen (Münch. med. Woch. 1900. p. 277.)

(3) Globulicide Wirkungen des tierischen Organismus (Münch. med. Woch. 1899 No. 13)
Specifisches Immunserum gegen Epithel (Münch. med. Woch. 1899. No. 38)
Beiträge zur Immunitäts Lehre (ibid. p. 699. and 962, 1900)

(4) Zur Kenntniss d. specifisch auf Blutkörperchen Wirkenden Sera (Cent. f. Bakt. XXV. p. 546)

(5) For summary of views and conclusions of. Les poisons cellulaires (Rev. general de Science No. 1. 1901. p. 7.

(6) Ueber ein specifisches Immunserum gegen Spermatozoa (Deut. med. Woch. p. 61. 1900).

against tissues other than erythrocytes, e.g. kidney, liver, spermatozoa, ciliated epithelium etc. is allowed to act on these tissues in vitro has thrown a flood of light on some hitherto inexplicable processes.

Bordet, Buchner, Ehrlich and Gruber and Metschnikoff differ essentially in the views they take of the process of haemolysis, in consequence the literature is somewhat polemic and the subject confused by a multiplicity of names corresponding to the diversity of the hypothetical considerations. A serum without may be made to acquire a haemolysine by subjecting the animal to a process of immunisation against the blood corpuscles of another species. There then appears in the serum a haemolysine directed against the species of erythrocytes injected. Metalnikoff[1] has obtained the same result by feeding rats on blood of the horse This calls to mind the similar immunisation process employed by Ehrlich ~~and Fraser~~ in the case of ricin and abrin and Fraser[2] in the case of snake venom. The haemolysis is by all regarded as being the consequence of the combined action of two factors which exist side by side in the serum, and without the presence and the absorption of one of them by the specific inimical erythrocytes, are indifferent to one another.

(1) Metalnikoff. Ueber haemolytisches Serum durch Blutfütterung (Cent. f. Bakt. XXIX. p. 591. 1901).

(2) Immunisation against serpents venom. (Brit. Med Journ. 1895. also Nature 1896)

16

i. A factor somewhat resistant to heat. All four investigators agree that this factor acts directly on the red blood corpuscles, in regard to the nature of the action they differ. When it occurs artificially Bordet designates it "sensibilatrice". Ehrlich and Morgenroth name that which occurs in a normal haemolytic serum "zwischenkörper" and when artificially produced "immunkörper" i.e. intermediate body or immune body. In keeping with their purely chemical conceptions of these processes Ehrlich and Morgenroth propose to give to this factor the name "amboceptor." Buchner admits that in the artificially produced haemolytic sera there is present a specific "antikörper". Metschnikoff calls this factor "philocytase" implying thereby also its direct action on the erythrocytes. Müller[1] has recently employed the term "cupola".

ii A factor easily destroyed by heat, not producible by immunisation, normally present in serum. Ehrlich and Morgenroth maintain that it is quite indifferent to the erythrocytes themselves, unites on the contrary with the first factor, thereby causing haemolysis. Bordet and Buchner however hold that this their alexine acts directly on the erythrocytes which have been rendered susceptible to its action by the foregoing absorption of the "sensibilatrice" or "antikorper". Metschnikoff gives it the name "cytase" thereby implying that it acts directly on the erythrocytes etc. He therefore supports the view of Bordet and Buchner. All

(1) Ueber Antihaemolysine (Centralb. für Bakt. No 5 p. 175. 1901)

agree in regarding this factor as being of a ferment like nature. Buchner does not admit that the haemolytic action of a normal serum is exactly analogous to that of one obtained by immunisation. He maintains that in the case of the normal serum only the "alexine" is responsible for the haemolysis. The experiment of the elective absorption at 0°-3°C. by rabbits blood of the "intermediate body" present in normal goats' serum I have many times repeated in Professor Ehrlich's laboratory and have no hesitation in supporting the opinion that the process is quite analogous in the two cases. Bordet, Buchner, Ehrlich and Morgenroth, Metschnikoff are all agreed that the "intermediate body" antikörper or sensibilatrice enters into direct action with the erythrocytes, and Bordet has shown with the stroma of the erythrocytes. Regarding the mode of action they differ. Bordet and Buchner while granting that the "sensibilatrice" or anti-Körper is specific and is taken up by the erythrocytes do not admit that this union is of a purely chemical nature, further they regard the absorption as merely rendering the erythrocytes in a general way more susceptible to the action of their alexine. Bordet indeed calls the body "sensibilatrice" and the corpuscles that have been subjected to its action "globules sensibilatrices". According to their conception the erythrocytes are thereby so modified that they are in a mechanical way rendered more susceptible to the direct action of the alexine. The conception of a general heightening of the susceptibility of the erythrocytes to destructive agencies cannot be entertained

72.

for I have found that rabbits' erythrocytes saturated with the specific intermediate body or "sensibilitrice" contained in anti-rabbit serum (from guinea-pig) rendered inactive by heating to 55° C. for 30 min. are susceptible only to the addition of the addiment e.g. of normal guinea-pig serum and when tested in regard to ricin, crotin, abrin, cyclamin digitalin, solanin, saponin, Vetanolysine, and the haemolysine of goats serum the limits of the actions of these bodies are the same as in control experiments with normal erythrocytes. One can therefore only speak of a heightened susceptibility in a very limited sense.

The seemingly so simple experiment of haemolysis in the test tube requires for its successful carrying out, regard being taken of a great number of factors, and no little experience, so that experimentation in this field is by no means so simple as at first thought appears. During the time I have been privileged to work in Professor Ehrlich's Institute I have had the opportunity — partly for the purpose of study and partly in furtherance of other work on the affinities of the toxines etc. for the tissues — of repeating practically all the published experiments on haemolysis, some of them many times over. I need not go deeply in experimental details which would be but a repetition of what can readily be found elsewhere[1] but will only allude to a few points. Ehrlich and Morgenroth

(1) Ehrlich and Morgenroth loc. cit. Berl. Klin. Woch.

have shown that at 0°C – 3°C. the "immune body", "intermediate body" or "antikörper" is taken up by the erythrocytes so that it can by centrifugalising at this temperature be removed in union with them, and the centrifugalised fluid only contain the complement or addiment (Buchner's and Bordet's alexine) From the fact that the "addiment" or "complement" or "alexine" separated as above by centrifugalising, does not itself lead to the solution of the erythrocytes if these latter be added to it unless there be also added fresh "intermediate body" or "antikörper" the conclusion is drawn that the "addiment" "complement" or "alexine" is indifferent to the red cells, and it certainly proves that it is more indifferent to them than is the "intermediate body" or "antikörper". I have performed here a further experiment and after having added fresh erythrocytes to the centrifugalised "addiment", have again centrifugalised and have determined by now adding fresh "intermediate body" or antikorper and for the third time erythrocytes that at the second time of contact when only erythrocytes and addiment ~~solution~~ were present the addiment had not been absorbed by the erythrocytes for had this been so the addition of serum of guinea pig (immunised against rabbits erythrocytes), which had been rendered inactive by heating for half an hour to 55°C. would not of itself have been able to effect haemolysis.

To occasion haemolysis both components require to be present and the intermediate body or amboceptor must have been already taken up by the erythrocytes, which unless the latter absorption has taken place are indifferent to the "addiment or complement" or "alexine". Ehrlich and Morgenroth suppose therefore that the "intermediate body" or antikörper has two distinct combining atomic groups, one, which unites with the a corresponding atomic group of the "complement" or "addiment" or "alexine" after the other group has entered into union with a corresponding atomic group of the erythrocytes. They consider the processes here to be of a purely chemical nature, and the consequence of the satisfaction of chemical affinities. Ehrlich has assumed that in such a haemolysine there are present three haptophore[1] atomic groups of which two belong to the intermediate body and one to the addiment. The intermediate body links the addiment to the erythrocytes and therefore Ehrlich would give it the name "amboceptor." One of the haptophore groups of the "intermediate body" unites chemically with a definite atomic group of the erythrocyte. Thereby must be produced although Ehrlich and Morgenroth do not make the statement — a change in the combining affinity of the second haptophore group of the "intermediate body" which previously unable, is now able to combine with that "addiment" which without union of the intermediate body with the erythrocyte, had existed free in the serum

(1) Croonian Lect. Loc. cit.

side by side with the "intermediate body". Such changes in chemical affinity through combination are well known in chemistry. It may also be that through the union of the erythrocyte with the intermediate body a quite new combining affinity arises and hence new union with the complement occurs under conditions previously non-existent. It appears to me that a mere increase in the combining affinity of the haptophore group supposed to unite with the "complement" is all that is required and fits most suitably with Ehrlich's conceptions of the process.

The artificially (i.e. by immunisation) produced intermediate body or anti-körper represents according to Ehrlich, like any other antitoxine, a side-chain thrown off from cells, stimulated to the production of side chains in superabundance on account of these normally present having been taken up by union with atomic groups of the injected erythrocytes injected in order to produce the immunity. The atomic group of the erythrocyte which enters into union with the specifically produced "intermediate body" must be <u>identical</u> with the atomic group which by its union with the side-chains of cells had led to the reproduction, and over production of these side-chains, till appearing free in the serum they formed the "intermediate body" or anti-körper. von Dungern[1] in Ehrlich's Laboratory satisfied

(1) Beiträge zur Immunitätslehre loc. cit.

the red corpuscles of the ox for the specific intermediate body obtained by immunising rabbits against oxen blood, and found that such oxen corpuscles, of which presumably a definite atomic group had already been satisfied with the specific "intermediate body" or ambikorper had, when injected into rabbits, lost their power to cause the production of an Haemolysine i.e. of an "intermediate body" or ambikorper against oxen erythrocytes. If now the atomic groups which by its union in the body called forth the "intermediate body" were a different atomic groups from that which in the test tube unites with the "intermediate body" one could have expected the oxen corpuscles when treated as above to have lost to the production of the specific intermediate body or ambikorper just as well as if they had not been united with the intermediate body. The assumption that the atomic groups are really different is not tenable in accordance with the principles of the side chain theory. If on the contrary in accordance with the side chain theory, the atomic groups be one and the same the non-production of the specific "intermediate body" against oxen erythrocytes is quite intelligible. I have repeated this interesting experiment of von Dungern's and followed exactly his instructions. In two cases in which I examined the serum on the tenth day after injection I obtained a confirmation of his results and in three cases in which I examined the serum on the fifteenth sixteenth and eighteenth days a

contradiction, for the serum of these latter rabbits had acquired a specific haemolytic action against ox-en corpuscles. It would therefore appear that the saturation ~~of the~~ and indeed supersaturation of the erythrocytes with the specific intermediate body or "antikörper" does not render these erythrocytes inert in the body for only a delay occurs in the acquisition by the rabbits serum of the specific haemolytic action against the injected ox-en erythrocytes. This delay may be more apparent than real and due to the removal from the serum of addiment by the intermediate body injected, in which case the haemolytic action of the serum would ~~require~~ remain in abeyance until such time as ~~the~~ new addiment could be provided. This is a point which will easily admit of experimental investigation. In any case the production of a haemolysine by erythrocytes saturated with the specific antikörper when introduced into the body of the same species as that providing the specific antikörper, would seem to support the conception of mere mechanical absorption by the erythrocytes, and calls in question the assumption that the process ~~is~~ analogous to the reaction between toxine and antitoxine*. One would expect that the conditions in the body of the animal yielding the specific antikörper would be the best possible for the maintenance of any chemical union which occurred in vitro. If the production of the specific

* See also on page 35

antiamboceptor under the conditions just detailed be the consequence of a union in the body of an atomic group of the erythrocytes not identical with the group which in vitro unites with the specific intermediate body, then one of the essential requirements of the side chain theory is untenable. It may be ~~that~~ I have pointed out, what has previously passed unnoticed, that guinea-pigs, which have intraperitoneally received 10 to 30 cc rabbits blood, and rabbits that have received 30 cc oxen blood, so that on the 9th to 10th day their serum has acquired a specific haemolytic action are not necessarily immunised in the true sense of the term, for, if instead of being bled so early, the animals be left alone most of them die between the fifth and seventh weeks.

Another experiment has yielded results which allow of their being interpreted in accordance with the requirements of the side chain theory that the atomic groups of the erythrocytes, which in vitro unites with the "intermediate body" must be identical with the group which causes the origin of the specific "intermediate body". If a rabbit of 1500 - 2000 gms be injected subcutaneously with 0,15 phenylhydrazin hydrochlorate and if after 17 hours the animal be bled the blood shows a changed character. On taking this blood in distilled water the stroma of the erythrocytes is readily obtained by centrifugalising, which may be three or four times repeated to remove practically all haemoglobin. More detailed examination of this stroma which took up indifferently acid and alkaline dyes, and gave

the reaction for virus, seemed to show that it had undergone a change of the nature of coagulation. Such stroma is able to absorb corrosive sublimate ($\frac{1}{10.000}$. $\frac{1}{100.000}$.) methyl green ($\frac{1}{2.000}$ - $\frac{1}{20.000}$) saponin sodium taurocholate, digitalin, tetanolysine (produce haemolysis) abrin, ricin (produce agglutination) so that 0,5cc of a 15% suspension of this stroma in normal saline added to the ~~Ab~~ already determined haemolytic and agglutinative doses of the above bodies protected the subsequently added 1cc 5% suspension of normal rabbits corpuscles from the actions of these bodies. A 5% suspension of normal blood corresponded to 15% suspension of the anaemic phenyl hydrazine blood. On the contrary the stroma did not have more than the faintest trace of protective action against the haemolytic actions of crotin, goat's serum and the serum of guinea pigs that had been specifically immunised against rabbits blood. After heating for 30 min. to 70°C. the protective capacity of this stroma remained quantitatively and qualitatively unaltered. I do not now enter into the question of what the difference, if any, may be in the absorption of the above named series of bodies by erythrocytes, nor do I intend to enter into a discussion of whether the absorption be merely mechanical or partake of the nature of chemical union but in keeping with Ehrlich assume that of the above named bodies those of them which have been proved to produce anti-Körper (abrin, ricin, crotin, tetanolysine + those anti-bodies against goat's serum, guinea-pigs serum) if they unite, as Ehrlich says, with definite atomic groups of the stroma

do not in all probability unite with one and the same group, but with different groups corresponding to their different combining affinities, and that of the phenyl-hydrazine stroma retained the groups (Ehrlich's receptors) capable of entering into union with abrin, ricin, and tetanolysine, it had apparently lost those capable of entering into union with crotin, and with the intermediate bodies present in the serum of the goat, and of the guinea-pig immunised against rabbits blood. It was therefore of interest to enquire how this stroma would react in the body of the guinea-pig as compared with normal rabbits erythrocytes. In vitro phenylhydrazine stroma was not able so to take up the special "anti rabbit" haemolysine of the guinea-pig, that it protected rabbits erythrocytes from the action of the serum of the immunised guinea-pigs. If this stroma when injected into guinea-pigs led to the production of a specific "anti-rabbit" haemolysine then its production was quite independent of the non-existence in the stroma of the atomic group able to unite with the "intermediate body" in vitro, and Ehrlich's side-chain theory which necessitates the assumption here that the atomic group of the erythrocytes which unites with the "intermediate body" in vitro must be the same as the atomic group of the erythrocyte which in corpore leads to the production of the "intermediate body, must

before. If on the contrary the stroma did not lead to the production of a haemolysine i.e. of a specific "intermediate body" then the absence of the action of the atomic group of the erythrocytes in vitro corresponded to the absence of its action in corpore, and the non-production of the haemolysine was possibly to be ascribed to the absence of that special atomic group of the erythrocytes which it has been assumed enters in vitro into chemical union with the "intermediate body". The experiment gave results in accordance with the latter statement. The immunisation of four guinea pigs with the stroma had not given rise on the fifteenth day to the production of a haemolysine directed against rabbits erythrocytes.

[In each test-tube there is contained 1cc of a 5% suspension of normal rabbits erythrocytes, to the erythrocytes added in a control series normal guinea pig serum and in a second series the serum of guinea pig that had received a total of 15 c.c. of 15% Phenyl hydrazine stroma intraperitoneally.

Control. Normal.

Serum.	1,0cc	Result.
	0,75	Complete haemolysis
	0.5	
	0.35	
	0,25	
	0.15	
1/10	1.0	trace of haemolysis
	0.75	no haemolysis
	0.5	
	0.35	
	0,25	
	0.15	

Stroma guineapig.

Serum	1.0	Result
	0,75	Complete haemolysis
	0.5	
	0.35	
	0.25	
	0.15	
1/10	1.0	
	0,75	trace of haemolysis
	0.5	? Spur
	0,35	no haemolysis
	0.25	
	0.15	

The serum of the stroma guineapig possessed a slightly stronger haemolytic action than the control. In order to determine if this could be regarded as being really due to the presence of

specific intermediate body" it was only necessary to add a little normal guinea pig serum, i.e. to add a little extra addiment for experimentation has shown that only "intermediate body" is produced in the process of immunisation against erythrocytes and also that it is produced in excess of the addiment present in the serum so that the full haemolytic power of a serum is not brought out till one has added excess of addiment. The addition of one drop of normal guinea pig serum did not produce the slightest change in the haemolytic power of the serum of the stroma guinea pig and the conclusion was therefore justified that no new "intermediate body" had been formed in the case of the animal, i.e. that in vitro and in corpore the stroma had lost its capacity to react in regard to union with and power to produce "intermediate body".

The mechanism of the abolition of the action of haemolysine must be of special interest. Ehrlich[1] has assumed that in such a haemolysine there are present three "haptophore atomic groups" two of which belong to the "intermediate body" and one to the complement. Therefore to each of these three an "anti-group" is conceivable. Some samples of horse serum do not possess any haemolytic action on rabbits corpuscles, but are able to render again haemolytic goats serum that has lost its haemolytic action by having been heated for half an hour to 55°C. In explanation of this it is assumed that the addition of the horse serum adds a suitable "addiment" to the "intermediate body" present in the inactive goats serum. If such a horse serum (i.e. addiment) be injected into goats, as in a process of immunisation, the antiserum obtained contains a body which is an "anti addiment" and which prevents the union of the complement with the "intermediate-body". The "anti addiment" does not interfere with the union of the "intermediate body" with the erythrocytes nor does it unite with that group of the intermediate body which unites with the "addiment" or "complement". It unites solely with the haptophore group of the addiment preventing the latter from uniting with intermediate body. The experiment is easily carried out by placing the different possible combinations in contact in the presence of erythrocytes and after centrifugalising determining in the presence of fresh erythrocytes the modification which has occurred. This

(1) loc. cit.

experiment I have repeatedly performed, indeed experiments performed by me under the direction of Professor Ehrlich were the first which established the existence of this "anti-addiment". Bordet has independently determined the existence of an "anti-alexine" and an "anti-sensibilatrice". Quite recently Müller[3] by immunising rabbits with ox's blood has obtained an antiserum in which there was present "anticomplement" or "anti-addiment", but also an "anti-intermediate body" preventing union of the intermediate body with the erythrocytes.

Ehrlich and Morgenroth assume that the analogy between an active toxine an active haemolysine, and the analogy between a toxine that has become weakened in toxicity, and a haemolysine rendered inactive by heating etc. are perfect analogies.* If this be true then reactions that hold good in the one case must also hold good in the other. It is practically proved by the work of Cobbett[1] Ehrlich and Fraser,[2] that toxine and antitoxine enter into direct chemical union, it is therefore to be expected that the toxine with which we are concerned in haemolysis viz. the foreign erythrocytes and the antitoxine viz. the "intermediate body" of the artificially produced haemolysine enter also into direct chemical union. This assumption

* cf. especially Ueber Haemolysine. Vierte Mittheilung. (Berl. klin. Woch. 1900. No. 31)

(1) cf. Cobbett's excellent summary in this connection. "The nature of the action of antitoxine" (Journ. of Path. p. 183 Aug. 1899

(2) loc. cit.

(3) loc. cit.

Ehrlich and Morgenroth make and at the same time point out that when Bordet assumes that the action of this intermediate body or antikörper is only mechanical rendering the erythrocytes "globules sensibilisées" he finds himself later when speaking of "anti-alexine" obliged to admit the probable pure chemical nature of the reaction between "alexine" and "anti-alexine" that is between complement or addiment and anticomplement or antiaddiment, and thereby comes into confusion if not also into contradiction. Wolff[1] in supporting the views of Bordet goes so far as to regard the intermediate body or sensibilisatrice as analogous to a mordant in staining or dyeing, and on this basis would deny all possibility of the haemolytic ~~action~~ phenomena being based on the satisfaction of chemical affinities. Wolf however forgets that whether the mordant finds its way into the bacterium, tissue or cloth, in accordance with physical or chemical laws the union of the mordant with the dye is of a pure chemical nature. I have already referred to the non-confirmation of v. Dungerns statement that erythrocytes saturated with the specific "intermediate body" are deprived of their capacity to produce

(1) Contribution à l'étude des sérums antihématique (Ann. Inst. Past. 1900. p. 297

"intermediate-body". The production of fresh intermediate body can hardly owe its origin to the union which had taken place in vitro again being dissolved in the erythrocytes plus intermediate body being introduced into the living animal of the species which had produced the intermediate body, for, if this union be a process of immunity then to be in keeping with our conceptions of all conditions, those in the body should be the most favourable for a firm union between the erythrocytes and the intermediate body directed against them.

The facts which have been ascertained ~~in~~ regarding haemolysis and cytolysis and especially the experiment of v. Dungern's already referred to have an important bearing on the therapeutic and prophylactic employment of antitoxic sera. We now know that all manner of tissues and fluids of different or indeed of the same species are capable (some eg. thyroid are not capable) of producing specific antikörper, and that some of the antikörper thus produced lead again to the production of what are really "anti-antikörper". A cycle of reactions of immense possibilities is thus brought to our knowledge. In practice we are not able to employ an antitoxic serum of human origin but only such as are provided by animals of widely removed species. We know that the passive immunity produced by injecting antitoxic sera is very transitory and the

question at once arises, whether an antitoxic serum of foreign origin injected into the body may not give rise to reactions of a nature prejudicial to its therapeutic value as an toxine neutralising agent in corpore, of course not in vitro. Indeed in the light of our present knowledge that the "intermediate body" or antikörper of a haemolysine gives rise to an anti-intermediate body it were not strange if its apparent analogue the antitoxine, especially that of a strange species, should also give rise to an "anti-antitoxin", the existence of which would explain the transitory nature of passive immunity and also account for the non-success in the employment, especially when prophylactically used in other species, of many antitoxic sera. It is evident that the inefficacy of anti-bacterial that is bactericidal sera, may now be shown to depend on the production of anti-intermediate body preventing the conference of immunity.

I have tried to settle this question in regard to the most important antitoxic serum that of diphtheria in the following manner. The result has however as yet been by no means decisive owing to the large number of experiments necessary and the large share played by uncontrollable factors.

In the case of a diphtheria bouillon the minimal lethal dose according to Ehrlich's standard was determined

for guinea pigs of in every case exactly 250 grms weight. Thirty-one experiments placed the at 0,00775. Next Ehrlich's two limits Lo and Lt were determined with like exactness. The highest dose of toxine which when added to one immunity unit just produced no recognisable reaction was by thirty seven experiments placed at 0,25. The Lt dose that quantity which together with one immunity unit killed a guinea pig of 250 grms on the fourth day was 0,45. If now the Lo mixture be injected into an animal of 250 grms it causes no reaction, and its repeated injection if the mixture be really a neutral one should also cause no reaction. I have performed concomitantly the following two series of experiments.

I Four guinea pigs of 250 grms received Lo. and at intervals of four days during which they progressively increases in weight the injection was repeated. In two of the animals after the third injection a slight transitory infiltration appeared and within twenty-four hours after the fourth injection all four animals died, the post mortem appearances being typical of acute Diphtheria. This seemed to indicate that at the fourth time of injection something happened which allowed of an acutely lethal dose of toxine being forced out of its union with the anti toxine contained in the Lo mixture. In a second series the same result was obtained. I have repeated at a later date (during January) this experiment in seven other series

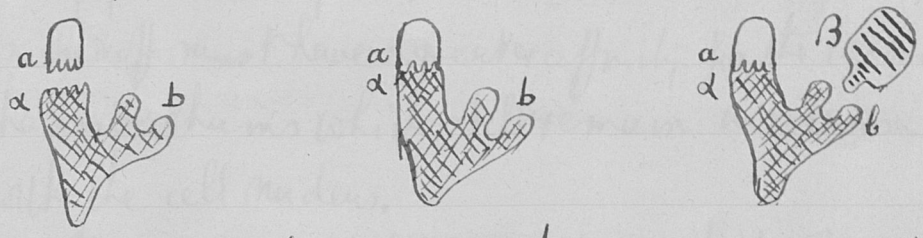
of four animals in only two cases has there been a slight transitory infiltration at the fourth time of infection.

ii The problem presented for elucidation was by no means an easy one. The amount of toxine set free was an acutely toxic dose. The question, was it due to cumulative action of a slight excess of toxine at each time of injection? was put out of count by the injection of four times the simple Lo mixture, which showed itself harmless. The Lo mixture was also Lo for younger animals of 230 grms. If it were due to cumulative action then it could only be explained by the rapidly repeated injection having produced a degree of heightened susceptibility, such as that noted by Fraser in the case of snake venom, that the death resulted from quantities of toxine which in their turn were very much less than the minimum lethal. The sudden development of the illness also spoke against cumulative action. That the union of toxine in vitro, was in corpore simply dissolved was not entertainable, because there was then no reason why the dissolution of this union should only make itself evident after repeated injection. The serum of animals repeatedly injected with Lo was found to be devoid of antitoxic action. Therefore the analogy of the union of toxine with antitoxine, and that of "intermediate body" (or anti Körper) with the erythrocyte did not hold i.e. an essential requirement of Ehrlich's side-chain theory is proved untenable. (cf also page 16)

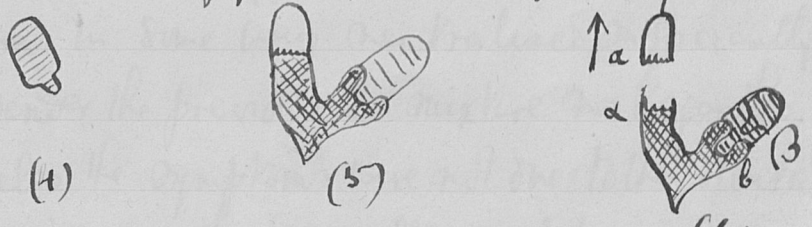
the only possible explanation seemed to be through some agency influencing the antitoxine in the Lo mixture, whereby, if this were not destroyed in part or wholly, it preferred to exchange its union with the toxine for another union, or, in virtue of its entering into a second combination its affinity for toxine was so diminished that toxine was set free. As the phenomena did not present themselves till after repeated injection they could only have their origin in a reaction taking place in the body and it was conceivable that this might occur in the following manner. One can conceive of the antitoxine as being a side chain pushed off from a cell, and therefore with a possibility of having at least two combining groups, one directed to union with the diphtheria toxine, and a second whereby it was previously in union with the cell which pushed it off. The pushed off side chain may be more complicated; but for the explanation of the present problem the assumption of the only two combining groups as necessary viz. one which unites with the toxine and a second which may or may not be the one which formerly united the side chain to the cell nucleus. One may construct the following scheme, which be it understood is purely hypothetical.

Figure (1) represents the toxine with its combining affinity a capable of uniting with the combining affinity α of the

(1) (2) (3)



antitoxine. and b represents the second affinity of the antitoxine. The figure (2) represents the union of the toxine with antitoxine i.e. the state of affairs in the Lo mixture. When the Lo mixture is introduced into the body it is conceivable that the affinity b meets with a corresponding affinity, and that the reaction is of such a nature that in the case of certain cells' sidechains with corresponding affinities B enter into union with b and new sidechains of the form B are produced in excess and in proportion are pushed off from their parent cells, these side chains would have the form indicated in figure (4). The consequence of this would be



(4) (5) (6)

the union of group b of the antitoxine with the pushed off side chain B instead of with the cells as formerly (fig 5) and it is quite intelligible that the union of group b with B should result in such a diminution of the affinity between a and α that

the toxine (fig. 6) was again set free on the occurrence of this union. Its a ground principle of the side chain theory of immunity that side-chains which have been pushed off must have a greater affinity for the toxine than side chains which still remain in relation with the cell nucleus.

The experimental elucidation of the problem offered great difficulties. The existence of an reaction involving antitoxine could only be determined by the occurrence of a modification in the previously determined reaction between toxine and antitoxine i.e. by means of the "Lo." mixture. When two guinea pigs were injected 3 times at intervals of four days with an immunity unit and at the fourth interval when they weighed 250 grms with "Lo." this neutral mixture produced an infiltration which appeared within 24 hrs was increased on the second day and then disappeared. This proved that the antitoxine was in some way neutralised sufficiently to ~~allow~~ render the previous Lo mixture no longer Lo. Therefore also the symptoms were not due to the dilution of the mixture in the body allowing of toxine becoming again free, nor to excretion of the antitoxine, but either to destruction of antitoxine or the formation of an antikorper acting on the antitoxine and setting up the toxine free. The following experiment was performed.

In four guinea pigs the "Lo" dose was injected at intervals as before. At the time when the injection should have been made for the fourth time two of the animals were bled, the defibrinated blood at once centrifugalised and the serum employed as follows. To one immunity unit (Ehrlich) was added 1,5 cc of the serum the mixture remained ¼ hour in the incubator at 39°C. Then the Lo dose of toxine was added and the whole injected into a guinea pig of 250 grms weight. To the Lo mixture itself there was added 1,5 cc of the serum and after ¼ hour in the incubator this was injected into a second animal of 250 grms. To control this experiment the above two procedures were repeated with the addition of 1,5 cc of normal guinea pig serum, and the "Lo" mixture alone was injected into another animal. In the case of the first two animals a considerable infiltration appeared which was absent where the toxine-antitoxine Lo mixture had been treated with normal serum. The infiltration was therefore not due to toxine having delayed the union of toxine with antitoxine. The "Lo" mixture was really neutral. In another series of experiments four guinea pigs of 250 grms received at intervals of 4-5 days respectively at each injection one, two three and four immunity units three times repeated and when the fourth time for injection came round they received the Lo mixture. The animals then

all weighed over 300 grms and showed only a very slight infiltration. Another series in which quite young guinea-pigs of 200 grms received respectively at intervals of 4 to 5 days one, two, and four immunity units respectively, four times repeated, and when they had attained 250 grms the Lo mixture all died acutely within 24 hours. This seemed to place beyond doubt the existence of an "anti-antitoxine" which by its union with the antitoxine caused the latter to pass out of union with the toxine. It is obvious that "this "anti-antitoxine" would arise in some such way as that previously described and that it could not be characterised by separating the toxine from the antitoxine by interposing itself between the combining affinities of these two bodies, for such an occurrence must have given rise to phenomena of quite a different nature. The following and what was expected to be decisive experiment was made. [Three large guinea pigs of 560, 856, and 950 grms received on three successive days in the peritoneal cavity injections of serum equivalent on the whole to 41 immunity units. The last injection was on Jan. 26th. on April 9th the animals were bled. The serum obtained was employed in the following way. Five times the minimal lethal dose (~~this large dose was chosen because of spontaneous weakening of the toxine~~)

was mixed with 1cc. 2cc. 3cc of serum and injected into three animals of 250 gms each. All died therefore the serum possessed little anti toxic power. One immunity unit was mixed with 1cc. 2cc 3cc of the serum of the passively immunised guinea pigs, and the mixture placed for half an hour in the incubator at 37°C. Then to each was added the Lo virus and the mixture injected into three guinea pigs of 250 gms. In the case of the animal that received the largest ~~dose~~ quantity of fluid in the evening it was very feverish and there was at the end of 24 hrs a moderate infiltration, in 48 hrs this had practically disappeared. The animals remained for a day almost stationary in weight but showed no other sign of illness. This experiment was controlled by a similar series in which normal serum replaced that of the passively immunised animals. In this control series no infiltration occurred with the largest quantity of fluid. The result was somewhat disappointing. It was anticipated that the addition of the serum of the passively immunised animals to the Lo mixture would have had as a consequence the death of the guinea pig into which the mixture was injected. It may be that animals do not react so well to antitoxine. The experiments above detailed cannot be held to establish with certainty that an anti toxic holding serum when injected

into an animal of a strange species gives rise to reactions having for consequence a prejudicial influence on its value as a toxine neutralising agent. But the results at any rate point to the advisability of a more thorough investigation of the point which may have a very important bearing on the method of employment of antitoxic sera for therapeutic and for prophylactic purposes, especially the employment of repeated small doses instead of one large dose. Of course these experiments are no argument against serum therapeutics in Diphtheria, they simply point out a possible factor which must be taken into account.

Unfortunately owing to the carelessness of the laboratory servant in not attending to the ice-box during the vacation, the toxine used has undergone a great weakening of its toxicity which is now less than 0,01. This may explain the absence of a fatal issue where looked for; owing to the liberation of non-poisonous modifications of the toxine with a lesser affinity for the antitoxine than that possessed by the toxine. In any case on the present data it is impossible to carry the investigation further, for this purpose a new toxine and fresh determinations of its minimal lethal, Lo and L+ dose are required and this means a great sacrifice of animals. In repeating this experiment it will be advisable

to use a quite fresh diphtheria toxine so free as possible from toxoid, and also tetanus toxine which may offer more favourable conditions than those presented by diphtheria toxine. Dr Jules Renaux has informed me that the repeated injection of tetanus toxine more than saturated with antitoxine, i.e. with excess of antitoxine, has in his hands had fatal issue in the case of three rabbits. This would point to the same state of affairs in the case of tetanus where the lesser success of tetanus antitoxine may also find an explanation.

It may here be remarked that if one follow the teaching of the side chain theory to its logical conclusions a toxine should produce an antitoxine i.e. finished off side chains, and this antitoxine for its part should be possessed of an atomic group which when injected again into an animal will unite with side chains and the produced "anti-antitoxine" ought again to have the combining affinities and therefore properties characteristic of the original toxine. This however is not the case so that here again the side chain theory is found at fault.

The difficulties I have encountered in endeavouring to arrive at a more accurate knowledge of the distribution and affinities of the toxines in the body have led me to make yet a further application of the study of haemolysis. It was soon apparent in repeating Wassermann's experiments on tetanus toxine, that the evidence Ehrlich advanced regarding the division of the toxines in accordance with their affinities for the tissues in the body into monotrope and polytrope was utterly inconclusive and often quite unreliable. The experiments in this connection need not now be detailed suffice it to say that the use of broken down tissues as Wassermann employed them was calculated to lead to fallacious conclusions. Owing to the difficulty of the subject it was obviously of advantage to study in the first place toxic bodies the absorption of which by a tissue could readily be demonstrated by the loss of the action which their solutions manifested in vitro. No tissue can be so readily obtained isolated and worked with under conditions so little deviating from those inside the body as erythrocytes. There is also the advantage that no preliminary fine division of the tissue is necessary. Further as I was able to divide the bodies experimented with into the two classes of those which produce and those which do not produce antikörper I felt justified in discarding Wassermann's methods and striking out in a way of my own. The following bodies have been more especially studied, corrosive sublimate

the glucosides cyclamin, saponin, solanin, cyclamin, digitalin various colours especially methyl green, and new victoria green, the vegetable toxalbumins ricin, abrin, crotin, various haemolytic sera normal and artificial, and the bacterial toxine tetanolysine. In the above series of bodies are contained bodies which produce and which do not produce antikörper, and also such as act chemically on albuminous bodies, some which are assumed do not, and some of which the action is quite unknown.

In all cases rabbits erythrocytes freed from serum by centrifugalising 3 or 4 times in 0,85% saline were used. The haemolytic action of the glucosides takes place in such feeble concentration that it is impossible to regard their action as being based on osmose. Regarding the mechanism of the haemolytic action of the glucosides Perles[1] basing his opinion on observations of the haemolytic process with the microscope has asserted his belief that an erosion of the stroma was the cause of the haemolytic action of solanin. By one who has observed the haemolytic process with the microscope will assent to the difficulty of acquiring an accurate conception of the process by this means. An insight into the mechanism was obtained in the following way. It was determined that the relation between the corpuscles and the amount of glucoside necessary to effect solution was a simply quantitative

(1) Beiträge zur Kenntniss der Wirkungen des Solanins (Arch. f. Exp. Path. u. Pharmak.

one, i.e. a multiplum of the solvent dose of glucoside dissolves the same multiplum of the standard quantity of blood (1cc 5% suspension). It was observed that the quantity of glucoside which exactly dissolves the standard quantity of blood has after complete laking lost absolutely its haemolytic action. Fresh blood added subsequently to complete laking settles down without undergoing haemolysis as shown by a comparison of the colour index of the superjacent fluid before and after the addition of the second quantity of blood, be this blood of the same or of a different species of animal. Instead of using normal red cells a modification of the method devised by Bordet in his investigation of the nature of the action of haemolytic sera was employed. The red blood corpuscles were washed free from serum and then laked in distilled water so as to give a 5% solution corresponding with 5% suspensions. The laked blood was supplied with 0.85% sodium chloride. When to 1cc of this laked blood there was added the exact solvent dose of the glucoside and after half an hour there were added normal corpuscles the latter settled down without haemolysis of them taking place. The glucoside had been rendered harmless in the laked blood just as it had previously been shown to lose its haemolytic power during the haemolysis of normal red cells. It was now to be determined whether this loss of action was due to stroma or the haemoglobin. 5cc of blood was laked in 5% dilution

and immediately supplied with 0.85% sodium chloride, then centrifugalised during half an hour in a high power centrifuge, and the fluid separated. The fluid containing the Hb (taken from the topmost layer) when used as a medium for dissolving the glucoside showed itself quite as indifferent as normal saline and gave comparable results. The stroma obtained was not in great amount, to it 5 c.c. of normal saline was added, and 1 c.c. of this stroma suspension was able to protect normal red cells against four times the solvent dose of solanin chloride. From this experiment one may conclude that the haemoglobin in the haemolytic action of solanin hydrochloride plays a passive rôle that on the contrary the action of the glucosides is on the stroma. The same holds good for the actions of all the above named bodies; in the case of the haemolytic sera while I can fully confirm Bordet observation that the action is on the stroma and not on the haemoglobin there was the following difference to be noted. The protective action of stroma was for haemolysine relatively ~~very~~ weak e.g. 3 doses of a suspension of stroma sufficed to protect completely against 2½ solvent doses of Cyclamin and protected partially up to 10 solvent doses, the same amount only protected slightly against one solvent dose of goats serum and not at all against 3 solvent doses. If one be inclined to admit that the stroma no doubt killed by the taking would have its combining capacity for the haemolysine weakened or in part

destroyed this intelligible that the living stroma of the normal erythrocytes should be less protected from the haemolysine than from the glucoside. The weak protective action of the stroma against the haemolysine as compared with the glucoside would also seem to point to a difference in the nature of the absorption of the glucoside and the haemolysine.

Corrosive sublimate will be admitted by all to enter into chemical union with albuminous bodies. For a protocol of an experiment with corrosive sublimate showing both its coagulating and dissolving properties I must refer to page 57. Sufficient here to remark that a solution of corrosive sublimate 1/10.000 in normal saline added to equal parts of a 5% blood suspension (rabbit), to 5% solution in normal saline of laked blood, and to a 5% solution in normal saline of haemoglobin freed from stroma, produces in all cases a precipitate which soon leaves the supernacent fluid clear. This concentration coagulates. If the dilution however be 1/20.000 in all three cases no precipitation occurs but the 5% blood suspension is laked and all three tubes after 24 hours remain free from precipitation. The albuminate formed is soluble. The advantage of having in the series a body acting chemically is obvious.

In the case of bodies possessed of an agglutinative action there is no difficulty in separating the fluid from the agglutinated red cells after completion of the action. After completion of haemolysis however many hours

centrifugalising yields no sediment. This leads to the inference but does not exactly prove that the stroma has been dissolved. If however stroma be obtained and to the cloudy suspension there be added solutions of a glucoside e.g. cyclamin in haemolytic dose, or corrosive sublimate in haemolytic dose the cloudiness disappears which proves conclusively the solution of the stroma. When the erythrocytes have undergone haemolysis their affinity for the above named bodies is not satisfied, for although dissolved they take up far more haemolysine, glucoside, methyl green, nicin, abrin than suffices for haemolysis or agglutination. The ~~absorption~~ haemolysis occurs at an early stage of the absorption of these bodies and is by no means an indication of the saturation of the stroma by them. Subsequently to my having studied this particular theme Ehrlich and Morgenroth* have more particularly studied this connection with haemolysis from the point of view of controverting Bordet's contention that the absorption of "intermediate body" is purely mechanical. At a later date I shall return to this subject when I have performed some further experiments. It has been shown that a long series of so called "blood poisons" are absorbed by the stroma of the erythrocytes when allowed to act on these in vitro. How far there was justification for the assumption that these seemingly specific actions also occurred in the body became necessarily a matter for enquiry.

*Berl. Klin. Woch. No 10. 1901

Ricin has been asserted by Robert[1] and his pupils to act specifically on the erythrocytes in the body and in vitro, and to this agglutinative action they have ascribed the death of the animals in consequence of thromboses. Ehrlich has supported this view and made a particular application of it. On the assumption that the action is purely on the erythrocytes Ehrlich[2] has based his statement that the quantitative neutralisation of toxine by antitoxine (ricin-antiricine) in vitro runs quantitatively parallel with the neutralisation of ricin inside the body. In four cases I have injected into the ear veins of rabbits 0,1 mgrm ricin. Death occurred in 24 hrs. There was to be found no evidence of primary thromboses in the liver lungs or kidney. In some other cases Dr C. Levaditti at my suggestion undertook to study more fully the changes which I noted in the marrow. In a series of cases of chronic poisoning Dr Levaditti could find no evidence of primary thromboses where thromboses occurred it was secondary to other necrotic changes. Hexner therefore seems to be correct in his description of the pathological changes occurring in ricin poisoning, and Ehrlich and Hexner wrong. Ehrlich's experiment on the quantitative neutralisation of ricin and anti ricin remains a demonstration of the comparability of the neutralisation in vitro and in

(1) Arbeiten des Dorpater pharmakologischen Institutes Bd viii
(2) Zur Kenntniss der Ricin Toxinwirkung (Fortsch. d. Med. Bd xv. p. 41. 1897.
(3) The pathology of hexalbumin intoxication. Johns Hopkins Reports, vi. 1897.

corpore but its signification must be modified in accordance with the facts of the action of rain in corpore, From the facts just stated I conclude that when rain acts on the red cells alone in vitro an agglutinative takes place immediately because an otherwise unattainable quantity of rain reaches the erythrocytes. For the intravenous injection of corrosive sublimate, tetanolysine, methyl green, cobra, I have also been unable to obtain a manifestation in the body of the actions occurring in vitro on erythrocytes. In the case of cobra venom, the glucosides cyclamin, digitalin, saponin, solanin hydrochlorate I have, and also in the case of the haemolytic sera. It is therefore evident that the assumption of analogies in the affinities of bodies acting in vitro and in corpore is not always justifiable. Another aspect of the matter is of interest just now. The constituents of serum and the erythrocytes are capable of entering into combinations which may be soluble or insoluble and evident. In the case of corrosive sublimate we have both forms of combination. In the case of the action of one serum on another serum or the action of a strange serum or of a specific immune serum on erythrocytes we may have phenomena of similar nature e.g. the agglutination action of the serum of guinea pig immunised against rabbits blood, on rabbits erythrocytes etc. and we have the more fully studied haemolytic action. The precipitation

of a serum by an antiserum has recently been utilised by Uhlenhuth [1] as means of specifically distinguishing human and other species of blood from one another. His work has been confirmed by others. It cannot therefore be doubted that sera may act on sera or on erythrocytes in vitro, in the two ways of producing soluble and insoluble combinations, the first corresponds to haemolysis and the second to agglutination. Toxine and antitoxine in vitro presumably unite chemically with one another and the combination remains soluble. But this is not all, for Gruyers [2] has shown that various other bodies eg. Witte' peptone if injected into the body result in the production of antisera eg. antipeptone which in vitro gives a precipitate with peptone. If this reaction in vitro be comparable with the action of the antipeptone in the body then the injection of peptone into the ear vein of such an immunised rabbit should result in death from embolism. Two rabbits of which one had during two months received intraperitoneally a total of 70cc 20% Witte's peptone intraperitoneally and the other 59 c.c. of yielded a serum which precipitated in 20% Witte peptone solution. The serum was obtained by bleeding from the ear veen. The injection however of 5cc and 10cc of peptone solution (20%) into the

(2) Ueber immunität gegen Proteide (Centl. f. Bakt. XXVIII p. 237. 1900.)

(1) Weitere Mittheilung über meine Methode zum Nachweise von Menschenblut (Deutsch. med. Woch. No 17 Apr. 25. 1901) Gives full references to other literature

ear vien gave rise to no symptoms in either case
the explanation being probably that in the body the
the peptone and "antipeptone form a soluble combination
Thus again a difference between actions in corpore and
in vitro has been shown to occur.

Reflexion on the haemolytic action of a serum on
the erythrocytes of another species, or of the serum of an
animal which by a process of immunisation has
become endowed with an artificial haemolytic action
has led me to seek the explanation in the possibility
of the possession by the reacting serum and erythrocytes
of affinities which can be satisfied by mutual
reaction. Further consideration of the matter has led
me to think that it may be permissable to assume that
the blood serum of any one species of animal is the best
preservative of the erythrocytes of that species, that
the serum and erythrocytes are in normal conditions
practically neutral as regards one another, and that the
basis of this indifference of serum and erythrocytes
to one another is to be found in their being possessed
of similar chemical affinities, or at any rate of
affinities which cannot be satisfied by a mutual
reaction. Reflexion on the above mentioned differences
between actions in corpore and in vitro will make it
no matter for surprise if in the case of bodies
having an action on erythrocytes these be found to be

This will also the case when two strange sera in contact produce a "precipitine" or "coaguline" etc. reaction.

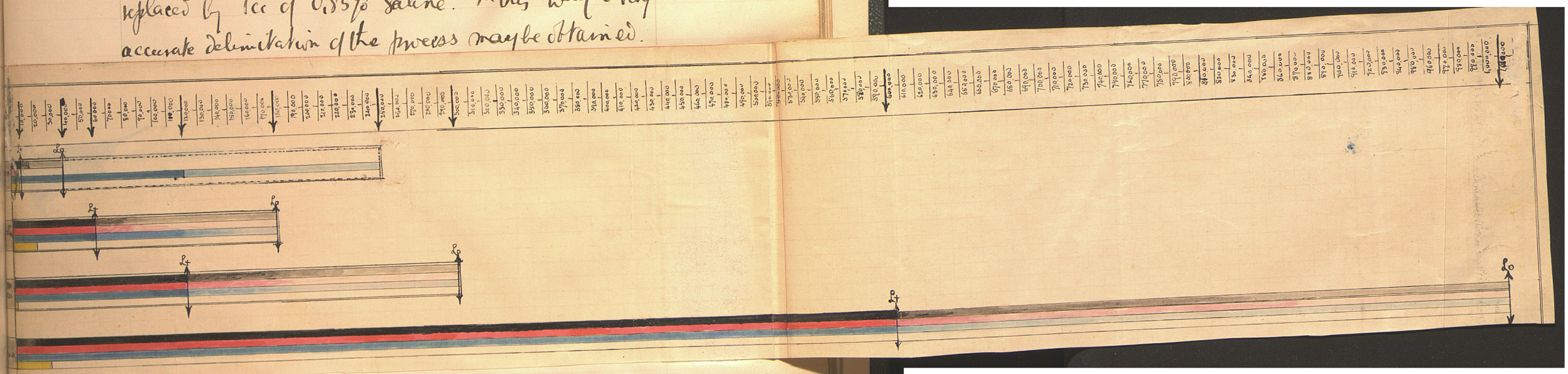
a difference in the actions of these bodies when they are allowed to act on erythrocytes alone i.e. washed free from serum and on erythrocytes in the presence of serum. Whether the modification in the action of agents having an action on erythrocytes by the presence of serum, is in all cases to be ascribed to the presences in the serum of naturally occurring anti-körper (specific) in accordance with the present day tendency has on these grounds seemed to me to be questionable The matter was also brought to my notice during an investigation I undertook at Ehrlich's suggestion into the assertion by Pohl of the successful production of a body fulfilling the requirements of "anti-solanin". Pohl observed that the addition of serum weakened the haemolytic action of solanin acetate, and at once assumed that the disappearance of the haemolysis was due to a reaction of the same nature as that between toxine and antitoxine. The fallacy of this assumption I have demonstrated elsewhere[1]. ~~Pohl has~~ Hedon[2] having repeated Pohl's experiments concluded that the protective action of serum is a purely physical one, although Pohl whose results I can here confirm had shown that mere increase of the concentration of the mixture of glucoside and erythrocytes by the addition of gum arabic, sugar

(1) Ueber Blutimmunität. (Arch. de Pharmacodyn. 1901. p. 101.)

(2) Sur l'action des glucosides et les conditions du milieu qui la favorisent ou l'empechent (Compt. Rend. Soc. Biolog. Aug. 4. 1900.

or well beaten egg albumin does not influence the haemolyses. Egg albumin had no protective action. The protective action of the serum is not abolished by heating it to 95° C. during an hour or even when it is coagulated by boiling a short time. The protective action of serum has been determined for the following glucosides, cyclamin, saponin, digitalin, solanin hydrochlorate. Many hundreds of experiments have been performed the results of which may be briefly stated as follows. The addition of any blood serum provided it does not itself have a haemolytic action interferes with the haemolytic action on rabbits erythrocytes of the four glucosides tested, but the serum of the rabbit itself has the strongest protective action than has the serum of a strange species, eg. horse guinea pig, or ox when possessed of haemolytic action first heated to 55° C for half an hour. The less the haemolytic power of the glucoside on the erythrocytes the less in proportion is the protective action of the serum. The protective action of the serum is no specific one but applies to the haemolytic action of the glucosides in general and of some alkaloids of which the haemolytic action has been determined. The statements made may be best illustrated by the following chart constructed from the results of experiments made with the same specimen of

rabbits blood on the same day. The method is the same as that employed by me to show the action of alkali and acid[1] on the haemolytic action of the glucosides In a long series of tubes each containing 1cc of 5% rabbits blood in 0,85% saline and free from serum there was placed 1.c.c. of serum and then 1cc of the various concentrations of the glucosides was added. In a corresponding control series the serum was replaced by 1cc of 0,85% saline. In this way a very accurate delimitation of the process may be obtained.



In the above chart the figures at the top give the concentration of the glucoside present in each test tube. The black indicates the haemolytic action on erythrocytes freed from serum, absolute black indicates complete haemolysis shaded black indicates partial haemolysis. Yellow indicates complete haemolysis after the addition of serum. Red indicates the effect of the addition of 1cc. $\frac{1}{1000}$ Normal HCl Blue $\frac{1}{1000}$ NaOH; an influence is only present in the case of Solanin HCl[1]

(1) Cor. ref. (Arch. de pharmacodyn. p. 107. 1901)

If one study the action of serum on the actions of corrosive sublimate, aethyl green, Tetanolysine, ricin, crotin, and haemolytic sera one obtains similar results which I may illustrate in the case of a body which unites chemically viz corrosive sublimate, and in the case of ricin and Tetanolysine. For the other bodies the protocols are exactly the same.

Corrosive Sublimate has an agglutinative (coagulative) and a haemolytic (solvent) action. The modification produced by the presence of serum is best shown as follows.

In each test tube 1 c.c. of 5% rabbits blood freed from serum, in 0,85% Saline. To the Control series is added 0,3 normal saline and to the other series 0,3 rabbits serum.

Sublimate Control +0,3 Saline		Sublimate + 0,3 Serum	
1/1000 1,0	complete agglutination	1/1000 1.0	complete agglutination
0,75	no haemolysis.	0,75	no haemolysis
0,5		0.5	moderate haemolysis
0.35	trace of haemolysis	0. 35	complete haemolysis
0,25	weak - moderate haemolysis	0,25	
0,15	complete haemolysis	0,15	
1/10,000 1.0	"	1/10,000 1.0	almost complete trace of sediment
0,75	"	0.75	strong haemolysis
0.5	"	0.5	moderate haemolysis
0,35	almost complete, trace of sediment	0.35	
0,25	strong haemolysis amount of sediment increases.	0.25	0 no haemolysis.
0,15		0.15	
1/100.000 1.0		1/100.000 1.0	
0.75		0.75	
0,5		0.5	
0.35		0.35	
0.25	moderate	0.25	
0,15	little	0.15	
1/1.000.000 1.0	V	1/1.000.000 1.0	
0.75		0.75	
0.5		0.5	
0.35	trace	0.35	
0.25	0	0.25	
0.15	0	0, 15	

From the above experiment it is seen that in series 2 with sufficient concentration no agglutination and no haemolysis action occur presumably on account of this being hindered through the presence of serum, the unavoidable explanation is that in the presence of serum a portion of the sublimate unites with it, hence the diminution in the agglutinating, and haemolytic action on the erythrocytes. The range of complete haemolysis remains the same. It is simply due to a different distribution in reality to a sharing of the corrosive sublimate between the erythrocytes and the serum. (cf. also experiment on page 48)

For Ricin the experimental conditions remaining the same the following similar result is obtained.

Ricin + 0.3 Saline (Control)		Ricin + 0.3 Serum	
1/1000	1.0	1/1000	1.0
	0.75		0.75
	0.5		0.5 — complete agglutination
	0.35		0.35
	0.25 — complete agglutination		0.25
	0.15		0.15 — almost complete
1/10,000	1.0	1/10,000	1.0
	0.75		0.75 V
	0.5 — almost complete		0.5 — trace of agglutination
	0.35		0.35
	0.25 V		0.25
	0.15 — trace of agglutination		0.15
1/100,000	1.0	1/100,000	1.0
	0.75		0.75
	0.5		0.5
	0.35 — 0		0.35
	0.25		0.25
	0.15		0.15

Exactly the same appearance is presented as in the case of

corrosive Sublimate.

For Tetanolysine a similar protocol is the following here however owing to the Tetanolysine being only feebly haemolytic is necessary in order to bring out the same character to use 1cc of serum instead of 0,3.

Tetanolysine + 1cc Normal Saline (Control) . Tetanolysine + 1cc serum

1/10	1.0	complete haemolysis	1/10	1.0	complete haemolysis
	0,75			0,75	
	0.5			0,5	almost complete
	0.35			0,35	
	0.25	almost complete haemolysis		0,25	
	0.15			0,15	
1/100	1.0	V	1/100	1.0	V
	0,75			0,75	
	0,5			0.5	trace of haemolysis
	0.35			0,35	
	0.25	trace of haemolysis		0,25	
1/1000	0,15			0,15	
	1.0		1/1000	1.0	
	0,75			0,75	
	0,5	no haemolysis		0.5	no haemolysis
	0,35			0.35	
	0,25			0,25	
	0,15			0,15	

In the case of Tetanolysine the conditions are indistinguishable. Ehrlich[1] has stated that there is present in horse serum a true antitoxine directed against Tetanolysine. It is not necessary to detail the other cases which all present the same features, and apparently are due to a condition of things indistinguishable apparently from one another both in the cases of the bodies which do (nicin abrin crotin Tetanolysine, haemolysines) and the bodies which do not (Sublimate methyl green the glucosides) not produce antikörper. Jan

(1) Gesellschaft der Charité-Aerzte. (Berl. Klin Woch. 1898. № 12)

forced to the conclusion that the modification of action is in all cases simply the result of a sharing of the various bodies between the erythrocytes and the serum, of course there may be a special constituent of the serum and of the erythrocytes. To this conclusion I had come before Ransom[1] working with saponin only obtained results which have led him to think that saponin unites with the cholesterin in the stroma of the erythrocytes and that the protective action of the serum is due absolute presence of cholesterin. If this be so then the cholesterin, which is not present in the stroma as if in a mere mixture, is a chemical group extracted out of the cell complex much in the way that Ehrlich regards his side chains as being taken up, and the problem remains why if the action of saponin on the erythrocytes is merely the extraction of cholesterin, this body is not reproduced in excess to produce immunity with cholesterin as an "anti. körper" I have elsewhere shown[2] that saponin produces an antikörper, and no immunity. In any case the assumption eg. by Ehrlich that in normal serum an anti. toxine against tetanolysine is present, and the assumption of the existence there of many other anti. toxines to bodies having an action on the erythrocytes in vitro

(1) Saponin und sein Gegengift (Deut. med. Woch. No 13 March 28. 1901.

(2) Ueber Blutimmunität. l.c.

is probably not justified, especially as the actions in vitro, in the cases in which these antitoxines are supposed to occur do not present themselves also in corpore. The explanation of the protective action of serum is that it is but an expression of the distribution of the agent between the erythrocytes and the serum, and an indication of the distribution of the agents between the tissues which in the body also claim their share and so large a share it may be that as in the case of ricin, notwithstanding the affinity of ricin for the erythrocytes in vitro in the body the other tissues claim all the ricin injected intravenously and the erythrocytes do not suffer. The conclusion from the foregoing experiments that the attempt to divide the toxines and pharmakological active agents into the two groups of monotrope and polytrope has come too early for in none of the cases examined Tetanus toxine, Tetanolysine, ricin, crotin, has the alleged monotrope affinity found confirmation.

Since it has been shown that of the above bodies which produce anti-bodies some of the actions in vitro on the erythrocytes are not evidenced in the body, one will also be inclined to doubt if it be really the case that the antitoxine produced in the body and able to protect

the erythrocytes in vitro is really a product of the "ill cells" or of the other cells not ill. Ehrlich has assumed that the antitoxine is a product of the cells which have been made ill by their union with the toxine.

In conclusion I would only state that the stage at which I am in my work has led me to doubt very much in the tenability of the side chain theory of disease and immunity, and also to protest against the requirement of the side chain theory that only those bodies & which produce "antikörper" enter into chemical union with the tissues.

Except where I have made an express statement to the contrary the work described is solely my own, as also is the conception of the plan of the work and the method of carrying it out.

C. J. Bashford. M.B.

Pharmakologisches Institut
Berlin. April 26th 1901.