Acquired Immunity
and its application to
Serum Therapeutics.

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The possibility of protecting human beings against the attacks of microbes either by combating them or by destroying their noxious products, is a subject at present widely attracting the attention of medical men with regard to infectious diseases. Up to the present time the most successful instance is that of vaccination against Variola; still we do not know how vaccination acts in bringing about Immunity from Variola, or in rendering it mild should it be contracted.

The importance of this question of Immunity was not recognised till after the works of Behring and Kitasato (1) on Tetanus and Diphtheria, and since that time it has been shown that the serum of immunised animals is both preventive and curative in several organisinal diseases. How how is this Immunity produced, how does the human organism react to defend itself against pathogenic microbes?
Numerous theories have been put forward to explain the mechanism of acquired immunity, but at the present time, amongst the means of defence of the organism vaccinated against microbes and against their products, we have to deal with two only viz. the Cells and the Fluids. The former has been defended by Metchnikoff and the School of Pasteur, the humoral by Behring, Buchner and others of the German School.

The humoral theory may be considered from three different aspects:—(1) the fluids of the organism would be able to kill the microbes (bactericide property), (2) they would be able to take away from them their pathogenic properties (attenuating power), and (3) they would be able to act directly on the toxin secreted by the bacteria so as to neutralise its effect (antitoxic power).

Buchner in his latest work writes as follows: "the bactericide power of blood is due above all to the leucocytes which set free the alexines capable of destroying the microbes. When, in an infectious process, there is established an inflammation with a considerable accumulation of leucocytes, these cells intervene..."
intervene not only to enclique the dead microbes but also to set free the micro-
bicidal fluid." He also says that the microbes undergo an injurious influence 
from the natural fluids of the body, such as the aqueous humour and blood 
plasma. If these fluids destroyed the microbes, the organism remained unharmed and the dead microbes were simply car-
pried off by the leucocytes. If, on the other hand, the fluids were unable to de-
stroy the microbes, these develop freely and with impunity.

In attributing the bactericidal power to some products of the leucocytes, and in admitting that these cells are directed towards the microbe-invaded foci, Buchner approaches the Cellular 
interpretation of Immunity.

At the London Congress of Hygiene in 1891, Roux proved that Behring's discovery of the antitoxic power of serum was quite unable to serve as a base for a 
general theory of Immunity.

In Metchnikoff's laboratory at the Pasteur Institute, Heijme and Wassermann have made researches which prove that Immunity of man against Cholera, and of guinea-
hogs against choleraic peritonitis does
does not rest on an antitoxin power of the blood. Behring himself admits that immunity to Tetanus resides in the cellular powers of the organism. He distinguishes true acquired immunity as "active immunity" from transient or "passive immunity." The former is now attributed to Behring to a cellular function, while he adds that the passive immunity is only communicated to an animal by injection of fluid from a vaccinated animal, a view he considers as purely humoral, a mere matter of the transference of part of the acquired immunity to another animal, by means of sanguineous fluid.

This passive immunity is the prevention of infection by the injection of serum from an already immunized animal. A strong leucocytosis and general cellular reaction is produced; in other words, the cell elements are stimulated.

This idea of stimulation is confirmed by numerous researches e.g. Héfiff and Wassermann on Cholera Peritonitis in guinea pigs, Fraenkel and Sobaken on the same disease; and especially by the work of Izseff in Koch's laboratory, in which he says that to prevent cholera peritonitis in guinea pigs, it is sufficient
sufficient to inject them previously with liquids which stimulate the leucocytes e.g. ordinary nutrient bouillon, tuberculin etc.

Stern (of the German school) makes the following statement: — "the exclusively humoral conception of Immunity — at least in the great majority of cases — is insufficient. It is precisely the study of the action of the blood and humours fluids deprived of cells, which brings us to admit that Immunity more often depends on a change of the cells themselves or of their functions."

Immunity is thus reduced to the sensitiveness and activity of the cell-elements. Even Buchner, as already mentioned, accepts an important rôle for the leucocytes.

Emmerich of Munich speaks of leucocytes as "centres of active albuminoid substance which provoke cure." But neither he nor Buchner admit the enclosure and intracellular destruction of the microbes. This enclosure of the microbes however, by the phagocytes, appears to be accomplished with a rapidity quite remarkable.

Iverigo states that when a sufficiently large
large number of bacteria are injected into
the blood of rabbits, they are enclosed by
the cells immediately after the injection,
and the majority are found inside the
phagocytes in a few minutes.

Borrel finds the same thing in the case of Tubercle bacilli, of which a
great number are enclosed a few minutes after the intravascular inoculation.

I have seen his specimens showing this—in his laboratory at the Pasteur
Institute—and they clearly demonstrate that the microbes are very quickly
seized by the leucocytes and withdrawn by them from the direct action of the blood.

Even in the most toxic diseases,
phagocytosis plays a very important part, as established by Vaillant for
Tetanus and Gabrichewsky for Diphtheria.

In every infectious disease there is a
close relation between the infectious
germs and the ameboid cells of the
organism; and this relation is demon-
strated by the fact that each infection
causes a distinct leucocytic reaction
—followed by phagocytosis.

Perhaps the researches of Dr. Bich
and Kissen on the bactericide prop-
erties of serum of animals vaccinated
against
against the Vibrio Kletschnikovi afford the most remarkable and definite proof of the Bactericide power of the "humors". They show that this vibrio, which multiplies actively in the serum of the normal guinea-pig, perishes rapidly in the serum of the vaccinated animal, and also that this difference is the cause of the Immunity.

Now, is there any difference between what one observes in the living vaccinated animal, and in the serum in vitro?

This question is very complex and requires to be divided and its different factors taken up separately. We shall take up firstly the question as studied by Sanarelli. He says that in both cases the vibrios can multiply, and, transplanted into bouillon, assume there a pathogenic power superior or at least equal to that of the original culture.

Thus it becomes more difficult to attribute an attenuating power to the organic liquids of the vaccinated animal. Sanarelli however shows that the vibrios cultivated in the serum of vaccinated guineapigs are not attenuated, but conserve or even increase their virulence and take, by their new culture in bouillon,
a virulence almost always greater than they originally possessed.

Koch, working at Kochchola, with reference to immunity, finds that the serum of vaccinated and even hyperimmunized rabbits permits a very abundant culture of the organism of Kochchola, which is developed with absolutely normal form and aspect. He therefore concludes that bactericide or antitoxic power does not exist in the serum, and that no attenuation of the microbes in the serum has as yet been demonstrated.

On the other hand, we must admit a very well-marked action on the course of the disease in that the serum preserves "fresh" or "control" rabbits against fatal infection by the organism of Kochchola.

If, therefore, the preventive serum does not act on the bacteria and their products, the only other conceivable method of action is by its influence on the organism submitted to the treatment. It is not, then, the microbe, which is modified by the action of the preventive serum, but really the organism itself.

Preventive serum, in the case at least of the Kochcholas of rabbits acts by stimulating the phagocytes to super-activity,
activity, rendering them less sensitive to toxicines, and exciting them in their struggle with the bacteria.

The researches of Sanarelli, Gamaleia, Pfeiffer and Metchnikoff show that guineafogs completely vaccinated against the living vibrio are as sensitive to the toxicines as the nonvaccinated.

Sanarelli says that the animals cured at the end of a treatment by serum therapeutic do not acquire immunity to a new virulent injection, and do not furnish a serum endowed with preventive properties.

By a series of experiments he demonstrates the number of the white blood cells (which ought to be considered as most active phagocytes) in the bloodstream and in the local inflammatory exudate during the course of the disease. Guineafog No. 1 "control"

do No. 2 received injection of serum

At 11 o'clock each was inoculated with some amount of virus. First as to the edema, we observe that the role of leukocytes is almost entirely wanting in the case of No. 1; in No. 2 the number of leukocytes emigrated to the point of injection attained in five hours the considerable number of 32,000 per cubic millimetre. At nine the same evening when the leukocytes seemed to
to have attained its maximum intensity, the leucocytes in the adena of No. 1 were only three hundred per c.mm.; in No. 2, they attained the number of 188,000. We find similar results in the case of the blood.

In No. 1 the number of white cells decreases rapidly, immediately after the inoculation of the virus, and this persists until death. In the case of the other we find the contrary. A true leukocytosis is produced which lasts for days till cure is definitely effected. How is this general leukocytosis produced which is to exercise such a great influence on the result of the disease? Gabriëwsky and others have shown that leucocytes are endowed with a quite peculiar sensiveness with regard to the chemical substances of various kinds.

This chemical sensiveness or chemotaxis, is called positive, negative or indifferent, according as the leucocytes are attracted, repelled, or not at all influenced by the substances with which they come in contact.

Sanarelli has also stated that a notable leukocytosis always takes place after an inoculation of preventive serum.

A great part of this phagocytic work, which represents the best system of defence against the invasion of bacteria in the case
case of the guineapig treated symptomatically may therefore be due to the positive chemotaxis of the leukocytes.

The bacillus of the plague has been recently found both by Hitasato and Rome in the interior of phagocytes.

Heijer in his researches on influenza has found that at the outset of the illness the greater part of the microbes are free, but as convalescence approaches, their enclosure by leukocytes becomes more and more considerable. In Heijer's earlier papers, one notes that he inclines to the bactericide power of humours and declines to admit the active role of phagocytes. In his later ones, however, with Wassermann, he speaks of the intervention of phagocytes in the elimination of vibrios, but he thinks that these vibrios are not enclosed till after being destroyed by an extracellular factor still undetermined.

To elucidate this question Josseff and Heijer made a research in which they were convinced of the great extension and capital importance of phagocytic reaction in the choleraic peritonitis of guineapigs. They show that the remarkable resistance provoked by simple injection of bouillon, tuberculin, etc is caused by the stimulation of the phagocytes, which incorporate
incorporate the vibrios and so free the organism from these producers of poison. They thus attribute a considerable role to the phagocytes, but they distinguish between a passing resistance due to the injection of a substance like bouillon, and true acquired immunity provoked by vaccination with the vibrios and its toxic products. In the same journal, Steifer insists on the bactericide action of the fluid part of the peritoneal exudate of guineapigs hyperimmunized against the cholera vibrio. "When the living vibrio is injected into the peritoneum of these hyperimmunized guineapigs and a small quantity of the peritoneal fluid is drawn off ten to twenty minutes afterwards, there are seen very few leucocytes as well as a quantity of vibrios, immobile and transformed into small spherical globules. If the number of these globules diminishes, that of the leucocytes increases."

On these facts Steifer comes to the following conclusions with regard to acquired immunity: - after the injection of the choleraic vibrios into the peritoneum of the hyperimmunized guineapigs, the living cells, probably the elements of the endothelium, secrete a liquid which kills the vibrios and
and destroys them very quickly, the leucocytes only slowly intervene to play a purely secondary part.

This conception of immunity evidently comes in line with the cellular theories. He says in fact that the cells are irritated by the invasion of the microbes and thenceforth secrete a liquid injurious and fatal to them—a bactericide action of leucocytic fluid.

Koch repeated Heffer’s experiments and was assured of the truth of the fact that choleraic vibrios, in the peritoneum of hypervaccinated guinea pigs, remain living for several hours and that the peritoneal liquid rarely contained enclosed vibrios.

The plasma of the fluidate was then incapable of killing the microbes. This transformation of the vibrios into immobile globules requires examination.

Koch points out, with regard to Heffer’s experiment, that Heffer takes no account of the fact that the peritoneal cavity contains lymph rich in leucocytes; that on killing the animal one finds false membranes which cover the liver and mesentery, and which are composed of an enormous quantity of phagocytes. The leucocytes of the peritoneal lymph ought not to be wholly excluded as a factor in the transformation of vibrios.
vibrios into globules. When the peritoneal fluid is drawn off five minutes after the injection of vibrios, one is struck by the following phenomenon—the leucocytes are seen surrounded by a layer of vibrios in great part already transformed into globules. The red discs are not surrounded by any microbes. This fact shows the existence of a chemoattractive action of the leucocytes.

The vibrios are seen to have lost their mobility in the immediate vicinity of the leucocytes. A transparent zone between the stratum of vibrios and the surface of the leucocytes is also noted. There is then some production of liquid around the leucocytes, which may be a veritable secretion.

From these facts it is reasonable to suppose that the bactericidal power of the lymph rests in the leucocytes, which elaborate a substance capable of killing vibrios. Here is an analogy to ordinary stomach digestion, which is extracellular.

The theory of phagocytes applies itself—in spite of Heffers’ affirmations—to the choleraic peritonitis of guinea-pigs just as it applies itself to a great number of phenomena of resistance against the invasion of microbes in general. Metchnikoff believes in phagocytes not only as a means of microbial absorption and destruction.
but in the extension of the role of phagocytes without the enclosure of solid bodies.

The great sensiveness of these cells, with regard to the products of the microbes, leads us to infer some action of the leucocytes on the toxins.

Chatenay made a study of the leucocytic reaction of animals poisoned with bacterial products (tetanus and diphtheria). When death supervenes in a short time, the number of leucocytes diminishes. If there is a survival for twenty-four hours, there is a hyper-leucocytosis more or less pronounced.

Kletchnikoff, in the fatal cases of rabbits poisoned by arsenic, mentions a hyper-leucoctysis. But in the rabbits accustomed to arsenic the same dose which causes a hypoleucocytosis and death of the control rabbits, produces a considerable augmentation of the leucocytes.

These experiments prove on the one hand the leucocytic reaction against poisons, and on the other hand that hyper-leucocytosis may be provoked by toxic substances.

Robert of Bopha introduced into the circulation of different animals a very soluble preparation of iron—not precipitated in alkaline media—and followed the circulation of the metal. He ascertained that the greatest part is arrested in the liver, spleen and bone-marrow.
bone marrow, and that a very small quantity is eliminated by the kidneys. The iron is absorbed by the leucocytes, the endothelial cells of the liver and the cells of the splenic pulp.

Samuel of Dorpat, working with soluble salts of silver, also shows the great role that the phagocytic elements play in the absorption and transport of metals.

In his paper of 1897, Behring thought he had ruined the cell theory and the phagocytosis of Metchnikoff, but the greater weight of evidence allows us to say that the prevention and therapeutics of infectious diseases by serum is a matter of a stimulation of the activity and resistance of the living cells of the organism, and that acquired immunity is the accustoming of the phagocytes to the microbic products.

This theory of immunity proposed by Metchnikoff does not deny that besides phagocytosis it is possible to have other means of protecting the organism, but it affirms that the phagocytic action is of all these means the most diffused and the most efficacious to give account of all the facts, and by it our acquaintance with microbic poisons and chemical vaccination has been made more clear.

Up to the present time we know only that the
the serum of Tetanus and Diphtheria are able to restrain the growth of the microbe and neutralise its products. In these diseases the bacillus lives only locally and the enemy which has to be fought is not the microbe invading the tissues in general, but a substance elaborated, so to speak, outside and penetrating throughout the organism. It is a general poisoning we have to treat or prevent.

By the subcutaneous injection into an animal of frequently repeated doses of the filtered chemical product (i.e. bacilli-free) of either of these bacilli, its system gradually becomes accustomed to their action, so that in time, a dose of toxine, which at the beginning of the process would have been fatal, can be borne with impunity.

Other infectious diseases, e.g. Cholera and Pneumonia furnish, on the contrary, protective serums which destroy the living virus but are powerless over the toxine.

We should not in these cases, speak of antitoxic serum but of antinfectious or antimicrobial serum.

"Turning to the question of production of immunity from typhoid fever, it was found that by several methods Fraenkel and Simmonds, Rontenesser, Todai and others
others were able to immunise certain animals. Grieger, Kitasato and Wassermann obtained the best results, and they showed that the serum of such immunised animals would, when injected beneath the skin or into the veins, confer immunity upon guinea pigs. Stern further found that the serum of a man who had had typhoid fever would prevent the disease in mice, whereas the serum of a man who had never suffered from the disease had no such power. Samarelli, Chantemess and Bidet confirmed these results. Thus the latter observers found that the animals rendered immune by the serum method obtained their immunity sooner, but retained it for a much shorter time than those animals which were rendered immune by other methods. Further, although they were immune against infection, they were not immune against intoxication—that is, against the action of the product of the bacilli. Again, in six cases of persons who had had typhoid fever—in one case twenty years previously—they found that in all of them the serum had an immunising effect upon guinea pigs and rabbits, whereas the serum of persons who had never had typhoid had no such constant effect.
Moreover, the serum of persons who had had typhoid fever, and also of persons in various stages of typhoid fever, was found to bring about recovery of rabbits and guinea-pigs which some hours previously had been injected with typhoid cultures.

The antitoxic power is a specific power having effect only on a single poison; antitetanic serum for example only acts on the poison of Tetanus; whereas microbicidal action may in certain circumstances be exercised over another and a different microbe e.g. symptomatic anthrax furnishes a serum which is active not only for its own microbe but for that of Septicemia.

Let us next examine how the blood of immunized animals becomes in its turn immunizing? There is formed in the blood a very active substance called antitoxine, and probably derived from the fixed cells of the tissues reacting under the influence of the toxin. The mechanism of its production is, however, still obscure. Behring affirms that this antitoxine is simply the transformation of the toxin. What is the origin of these antitoxines, or, as Kletschnikoff prefers to call them, Stimulines? Woodward's idea is that
"Materials contained in the blood serum can only be those introduced into it either from without or as the result of the vital activity of the cells of the body, the products of which are thrown into this fluid, and it is maintained, not only by Behring, Ehrlich and Buchner on the one hand, but also by Metchnikoff and his followers on the other, that the antitoxic substances, whatever they may be, found in the serum, must be the result of the action of the tissue cells, especially of the connective tissue and blood cell groups, stimulated or acted upon by these tissues introduced from without.

It may be said, indeed, that the tissue cells, when feeling the necessity of protecting themselves against the action of a specific poison, become so modified that they give rise to the formation of a substance which directly antagonizes the action of that poison; and this substance, thrown into the blood, remains there for some time, accumulates in greater and greater quantities as larger and larger doses of the poison are thrown in, neutralizing the poison, whose power of doing damage to the system is thus checked at once. When one ceases injecting toxine into an animal, the antitoxine decreases little
little by little in the blood, as if the source from which it comes required renewal. A consequence of this hypothesis is that the quantity of antitoxine in the blood ought to be in proportion to the toxine introduced. If one frequently bleed animals without injecting them with fresh toxine, the provision of antitoxine ought to be rapidly exhausted.

Donz & Vaillard say this is not so, and they try to prove it by the following experiment. They vaccinate a rabbit against Tetanus, then they draw off from it, in successive amounts, a quantity of blood equal to or greater than the total volume which circulates in its vessels.

In spite of the complete renewal of the blood, they state that, at the end of the experiment, the antitoxic power of the serum is not sensibly lowered—an evident proof that the antitoxine is reproduced in the blood. The same authors have shown that there is no definite proportionality between the toxine injected and the antitoxine produced. Equal volumes of toxine were, in the space of two months, injected into two rabbits of same weight, in the one case in nine massive doses, in the other, in thirty three fractional doses.
In the latter a serum is produced twenty thousand times more active than in the former. It cannot then be a simple question of the mere transformation of the toxine, since equal quantities of this substance give rise to, according to these experiments, antitoxine which varies so enormously. It seems then indispensable to bring in here, as in all phenomena of this kind, a biological factor viz. the reaction of the organism which battles with the poison, as it battles elsewhere with the microbe by phagocytic digestion.

Each time the cells of the organism are subjected to the action of the toxine, they fortify themselves more and more against its influence. The repeated introduction of toxine permanently influences the cells which secrete the antitoxine. Stimulated by the toxine, the cells are put on their defense, probably in secreting, as certain animals do, a sort of protecting fluid, which they oppose to the effects of the poison. This Counterpoison is no other than the antitoxine, but it is to be noted particularly that the toxine does not furnish this substance. The toxine is only the Stimulant which promotes its secretion; its function, to speak
in chemical terms, appears to be strictly confined to a simple action of catalysis or presence.

How does the serum in its turn, introduced into another organism, succeed in protecting it?

The most simple explanation would be a chemical equation and to say that the antitoxine neutralises, in the living body, the toxin which it may meet there, just as a base neutralises an acid. What gives this hypothesis a certain amount of probability is that this purely chemical action seems to be accomplished _in vitro_.

When to tetanic toxin is added a certain amount of tetanus immunising serum, the mixture becomes harmless, as if the union of the two bodies had formed a compound as different from each of them as a salt differs from the two elements of which it is formed. The experiments of Roux and Vaillant have not been slow to contradict this too easy explanation, and to show that it is impossible to reduce the interpretation of this phenomenon to a simple chemical reaction.

They have shown that here again the principal part is played by the cellular
cellular activity, and that the function of the antitoxin is not to neutralize the poison but to rouse the cells to this guardian reaction, which prevents the poison from being harmful to the organism.

By the following experiment these observers have proved that the presence of antitoxin cannot be sufficient unless it is backed by the action of the tissues.

They inoculate at the same time, with a mixture of tetanic serum and toxine, a series of fresh guineafiggs and a series which have previously submitted to the action of different microbes e.g. cholera or coli communis. The first series resists, the second takes tetanus. The explanation is that the intact cells of the fresh guineafiggs have reacted successfully to the stimulato, while the cells remain indifferent in the case of the animals already impressed by the previous action of the other microbial products.

Another still more striking experiment of these observers demonstrates the insufficiency of the purely chemical actions to explain antitoxin effects. Though it was done on the venom of serpents, the analogy between venom and toxine is sufficiently close to allow the one term to be used for the other. Animals immunised against the venom
venom of serpents furnish an antitoxie 
serum for the same venom, just as in Tetanus 
or Diphtheria. Calmette, Phisalix and Ber- 
rand have shown this.

A mixture in convenient proportions 
of venom and immunising serum injected 
into animals is without any effect whatever. 
But heated to 70° C. this mixture gains 
violenct activity. The heat has destroyed the 
power of the serum and left the venom un-
altered, which proves that the two substances 
were not by any means combined, but existed 
side by side in the mixture, ready to ex-
ercise on the cells the beneficial or harm-
ful action which each of them preserved 
intact.

Klein agrees with Behring and Roux that 
antitoxine is produced by a stimulation 
of the tissues of the animal by the gradually 
increasing injections of toxe.

He is not a supporter of the phago-cytie 
theory in relation to the production of ac-
quired Immunity, and he expresses him-
self in the following terms.

"Behring has clearly established that 
the blood-Serum of an immunised animal 
owes its antitoxie power to chemical substances; 
that these are present dissolved in the blood, 
as such, and not as cellular bodies; that
their action takes place outside (in vitro) as well as in the body of an animal; and that, therefore, the production of acquired immunity, i.e., the presence of antitoxin in the blood, has nothing and can have nothing to do with mechanical phagocytosis as advocated by Kletchnikoff and his pupils. The antitoxic serum acts antagonistically against the toxin in corpse or in vitro on lines and principles determined by quantity, so that the whole theory of mechanical phagocytosis of Kletchnikoff has become superfluous as far as acquired immunity is concerned. In the case of acquired immunity produced by tonic injections the action of phagocytes is out of place because no bacteria are introduced; the curative action of antitoxic serum in tetanus and diphtheria deals with the neutralization of toxin that had been formed and is circulating in the tissues of the infected body and therefore also in this the swallowing up and destroying of bacteria has no place. The manner in which in acquired immunity the antitoxic condition of the blood is brought about is not known. Let us study the process of cure in an animal treated by serumtherapeutics. Sanarelli was able to draw off after several
several days, virulent vibriosis from the bodies of animals treated by the preventive serum. We cannot then attribute cure to a bactericide or attenuating power of serum. He was also able to eliminate the possibility of an antitoxic power of the serum itself. There is then nothing left us but the cell elements. The best method of studying their intervention in all its phases is the examination of what happens in that limited area in which is the local process of infection and of cure. For this purpose Janarelli experimented with two guinea pigs under the following conditions

No. 1. Control guinea pig inoculated with the virus.
No. 2. Guineapig inoculated with the virus after previous injection of therapeutic serum.

No. 1 dies. No. 2 continues well. In No. 1 the invasion takes place rapidly, without hindrance, and without meeting cellular reaction. All the tissues become the field for abundant multiplication of vibrios, the leucocytes are rare or absent and in a short time the inflammation is generalized and ends in death.

In No. 2 there appeared in the vicinity of the point of inoculation masses of mobile cells which seemed to form an obstacle to the spreading of the vibriosis and enclosed great numbers of them.
These phagocytes advance as far as the centre of the adenomatous region and, as they continue their work of destruction, there are no more vibrios to be seen in 24 hours. The animal shows no signs of suffering either local or general.

It has often been stated that lowering the temperature of the body notably diminishes the functional activity of the leucocytes. Pasteur and Metchnikoff have shown, most especially in case of fowls, naturally refractory to anthrax, that they may be killed by this disease if their temperature were sufficiently lowered, death depending on the lessened energy of the leucocytes, and as the animal finding it was unable to liberate itself from the pathogenic microbes. Tanarelli has made observations on the influence of cooling the body with regard to cure by serum therapeutics. He made experiments on two guineapigs under same conditions as before but with temperature lowered by their being placed in bath of cold water. Not only did the preventive serum not manifest any effect on the course of the disease, but the animal treated by it succumbed much sooner than the control. These experiments showed
showed that preventive serum does not influence the injured organism by some action on the bacteria but really by a specific excitation of the cellular activity; and that animals, rendered ill by the infection, but susceptible of cure, infallibly succumb if they are deprived of the help of the phagocytes.

The conclusions he came to were:

1) that the serum of vaccinated animals is endowed with eminently preventive properties.
2) the inoculation of this serum into animals always succeeds in preventing the disease.
3) the serum stimulates the cellular activity by provoking a concourse of leucocytes in the general circulation and to the point of inoculation.
4) the destruction of microbes in the organism treated by preventive serum is always by the phagocytes.
5) the cooling of the body paralyses the action of the phagocytes, which do not react any more to the stimulation of the preventive serum and the organism treated inevitably succumbs to the infective process.

In a research on Immunity against the
the Pneumococcus Isaëff concludes that he cannot admit the existence of an antitoxic power of the blood in the case of animals vaccinated against the pneumococcus, and that the serum of such animals does not possess the property of neutralising the toxins of this microbe either in vitro nor in the organism. He shows how the toxins of Fraenkel's pneumococcus provoke a more energetic reaction in the case of the rabbits vaccinated against this microbe than in the corresponding control ones; that the pneumococcus inoculated into a vaccinated rabbit conserves its pathogenic powers for only eighteen hours and its vitality for about forty-eight hours after inoculation.

He finally concludes that in the immunity acquired against the pneumococcus, phagocytosis plays a rôle of the highest importance. What is the nature of the products prepared by the microbes and which play such an important rôle in the production of acquired immunity by their stimulating action on cells and which cause the phagocytes to become accustomed to these products?

In the actual state of our knowledge we have no means of deciding definitely as to the
the nature of toxine and antitoxine. The properties of these two substances are very similar. The physical or chemical agents which alter or destroy the one, alter or destroy the other. The toxine of Diphtheria, for example, has been compared to a diastase, but we do not know with any chemical definiteness what a diastase is. In some ways, however, this toxine does comport itself like a diastase; firstly, by its energetic action in imponderable quantities; secondly, by its properties being destroyed at high temperatures and lastly, loss of activity if medium becomes acid.

This bacillus-free toxine is able to set up all the general symptoms of diphtheria, including the paralytic sequelae.

Sidney Martin says the microbe produces an enzyme which is absorbed and acts on the proteins of the body, producing two poisonous substances, albumose and an organic acid which act on nervous system and cause the asthenia, albuminuria, etc.

As soon as a mixture of antitoxine serum and corresponding toxine is made, it is most febrile for animals. When one adds gradually increasing quantities of toxine to a given volume of Serum, a time arrives when its antitoxic power vanishes. The liquid contains free toxine and gives the disease to animals injected with it.
Antitoxin serum holds at the present time a high position on account of the immense services it has already rendered in the Cure of Diphtheria.

The serum of animals immunised against diphtheria is the only one which has, up to the present time, been employed to any extent, but those of pneumonia, tetanus, cholera are also therapeutic and preventative. They are not yet employed on a large scale because some rather delicate points in their study still remain obscure; or, as in the case of antitetanic serum, the disease is only recognised with certainty when the poison is already too far advanced for the antitoxin to be efficacious. By the time the first symptoms of tetanus show themselves the toxine has already acted on the cells of the tissues and poisoned them, so that they will not react to the strongest antitoxic serum.

In the case of Diphtheria, the presence of the false membrane precedes the poisoning and acts as a danger signal before the toxine has had time to influence the tissue cells immediately. Happily the false membrane is easily accessible to view. If it were developed in a place inaccessible to all means of
of clinical investigation, the disease would only be characterised by the signs of diphtherial poisoning and then it would be too late to intervene with serum. In this case antidiphtherial serum would not be a more certain remedy than the antitetanics.

Calmette in a very recent work affirms that the serum of animals vaccinated against the venom of serpents shows a most energetic antitoxic power. His experiments were made on the venoms of 4 species of serpents, and he avers that all these venoms are influenced in the same manner by the same physical and chemical agents, and all lose their toxicity when heated to 100°, and all may be attenuated under the influence of certain agents and used for vaccination. He brings out a very interesting fact when he says that antitetanic serum is not deprived of action over the venom of serpents; the serum of a healthy horse mixed with venom does not prevent it from acting, whereas the serum of a horse immunised against tetanus renders inoffensive the venom to which it is added. Still there is very little resemblance between the venom of serpents which kills by asphyxia in a very short time, and the poison of tetanus which only shows
shows its action after a period of incubation. This fact is almost sufficient to destroy the hypothesis of the specific nature of each antitoxic serum. Though we do not know exactly the chemical composition of what we in our ignorance call toxins, we know that they come within the group of the albuminoids and that they display, like certain alkaloids, a stability to which they owe their energetic action on the animal organisms.

Let us now rapidly review the principal facts in the study of preventive serums. We have seen there are two theories which attempt to explain the phenomena of acquired immunity, the humoral theory of Buchner, Behring and their school, the Cellular, so ardently upheld by Katschmoff and the school of Pasteur.

No antitoxine has been isolated and its chemical composition gauged or ascertained. The antitoxines being found in the blood in a correspondingly abundant quantity as the animals have received a greater quantity of toxin, the partisans of the humoral theory, and notably Buchner of Munich, think the antitoxine is derived from the toxin by a transformation in the animal organisms. This hypothesis goes well with the similarity of properties between...
between the two substances and also with the fact that the antitoxine diminishes little by little in the blood of animals which have ceased being injected with toxine. But as already mentioned Roux and Vailllard have shown that one may draw off from a rabbit immunised against tetanus, a volume of blood at least equal to that which circulates in its body without sensibly diminishing the antitoxin power of its serum.

The antitoxin is, then, reproduced in the blood without the necessity of introducing fresh toxine, and in consequence it cannot be the transformation of this last.

After careful study, one is inclined to admit an action of the serum on the cells rather than on the toxine and that immunity does not reside entirely in the antitoxin power of the fluids or humours. According to Buchner, Behring and the partisans of the humoral theory, immunity would be due above all to the presence in the animal humours, of particular products to which Buchner has given the name of Alcaires, and which would destroy the toxines, or be opposed to their effects. Now an objection
is immediately presented here and Buchner himself has been obliged to recognise that the humours do not prevent the germination of spores and do not exercise any bactericide power over young bacteria—the result of these spores. According to the Munich school, the microbes would be killed at first, in virtue of this bactericide power of the humours, and then the leucocytes intervene to carry off the dead bacilli. Their rôle would only be quite secondary—while Metchnikoff makes them play by far the most important part. Quite recently Hankin has slightly modified Buchner’s idea, in admitting that the Alexisins were the special products of secretion of these leucocytes characterized by the presence of Eosinophile granules.

This amounts to a concession made to the cellular theory; but this opinion does not hold in presence of the fact that the invertebrates and some of the lower vertebrates have no cells with Eosinophile granules and that in their case the phenomenon of phagocytosis and of intracellular digestion of microbes takes place as in higher vertebrates.

Buchner, however, is not after all so very far from accepting the cellular theory, judging from what he said at
at the Buda-Pesth Congress in September 1894.

"The action of the antitoxines does not consist in a direct destruction of the bacterial poisons by their contact with them, but it is produced in the organism which plays the part of inducing a diminution of the sensitiveness of the adjacent parts to certain poisons and so becomes capable of resisting them."

Metchnikoff thinks that the serums excite the phagocytic cells, we have already mentioned the nonspecificity of antitoxic serums. Serums put into play cellular action; the tozone acts as a stimulant to the cells which secrete the antitoxine.

Granted the cellular theory, we can explain the following: the antitoxic substance only exists in the serum, the clot does not contain it; the other humours of the animal organism possess the same properties as the serum, e.g. the aqueous humour and especially the milk which appears to play the principal part in the hereditary transmission of immunity; the urine and the saliva contain feeble proportions of antitoxine.

The antitoxic substance does not seem to be concentrated in any one organ more than another; it is met with in small
Quantity throughout and nowhere in such large quantity as in the blood. The part played by the leukocytes is to deprive the blood of toxic substances which it may contain. The leukocytes seize insoluble substances and fix the soluble, as has been demonstrated in the case of mineral and organic poisons. Experimental proof has been furnished by a series of inoculations of progressive and regular hyperleucocytosis in animals inoculated by the toxins of tetanus and diphtheria, by venoms. All these considerations go to substantiate more strongly the cellular theory.

There is no infectious disease which has profited more from the progress of Bacteriology than diphtheria; its pathology made clear by the discovery of the constant presence of a specific bacillus, its diagnosis rendered accurate by the microscope or by cultures, its clinical aspect explained by the presence of a poison; surgical interference transformed by the employment of tracheotomy, the internal treatment revolutionized by a method which promises everything. But yesterday we could do nothing more than retard the mechanical asphyxia by opening the trachea or...
try the vague treatment which empiricism or fashion suggested. But what is still more surprising than the number and rapidity of these conquests is the logic which has guided their acquisition. In all the recent history of Diphtheria one does not find a chance discovery or theory; all is deduced with precision, and facts have been added to facts, from the discovery of the bacillus, up to the climax of successfully combating it.

To render an animal immune, then, it is sufficient to accustom it slowly to the action of a toxic substance, the nature of which we are still ignorant. The serum of this immunized individual becomes a vaccine in its turn, not only preventive but also curative, capable of arresting a disease already commenced. This mode of protection is evidently immensely superior to the direct immunisation obtained by injecting the toxic itself, and is too slow to be of use in the treatment of an acute disease. On the other hand, the action of the serum — this second-hand product — is immediate, and the immunity which it confers is temporary.

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