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A Contribution to the Study of the Formation and Properties of Nephrotoxins.

Metchnikoff• has given the name cytotoxin to poisons of cellular origin having a specific and elective toxicity for the same cells from which they took their origin. The study of the cytotoxins has been pursued at first with regard to cells which live isolated in the organism, e.g. the cells of the blood. Thus Bordet•• Ehrlich and Morgenroth••• and Dungern•••• demonstrated that the blood serum of animals into which one had injected the blood of another species of animal acquires the property of destroying the red corpuscles of the blood of animals of the latter species, or, in other words, that a haemotoxin is developed in the blood of the injected animal. Pursuing the same line of investigation Metchnikoff••••• has obtained a leucotoxin and a spermotoxin. This latter has also been described by other observers. Dungern•••••• has obtained toxin which arrests the vibratile movements of the cilia of the tracheal epithelial cells. The preparation of artificial cytotoxins hurtful to cells which are easily isolated appears to present comparatively few difficulties and the authors who have

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• Ann. de l'Inst. Pasteur, Juin, 1900.
•• Do. Oct., 1898.
••• Berlin Kli. Wochen, 1899 (Nos.1 & 22) 1900, No.21.
•••• Munch. med. Wochen, 1899, Nos.13 and 14.
••••• Ann. de l'Inst. Pasteur, Oct. 1899.
•••••• Munch. med. Wochen, 1899, No.38

made researches on these subjects arrive at conclusions which agree at least in most particulars, and in the case of the haemolysins more particularly the studies have been so minutely and thoroughly pursued as to furnish a definite basis of knowledge. It is quite a different matter when we come to the consideration of cytotoxins hurtful to the specific cells of certain organs. The numbers of cytotoxins or cytolysins, as they are also called, now known and more or less fully studied, cover all, with few exceptions, of the tissues and organs of the body, but the greater number of them such as hepatotoxin, adrenaltoxin, nephrotoxin, etc. have either a doubtful existence or have yet to be proven to possess specific properties. In proving the activity and specificity of these cytotoxins very considerable difficulties have to be surmounted. In the case of the haemolytic sera the lysis of the red blood corpuscles "in vitro" demonstrates an activity which it is impossible to deny. Any such easy and conclusive method of proving the activity of the somatogenic cytotoxins is of course out of the question and with the exception of investigations undertaken to enquire into the taking up of Complement by the Immune-body, all the tests depend upon the reaction of the living cells within the body to the action of the cytotoxin. These reactions consist of degenerative changes in the tissues visible on microscopic examination and of symptoms of disease caused by the poison. Unfortunately in regard to both of

these, differences of observation and opinion give rise to great divergence. In the study of nephrotoxin, however, alteration in the urinary excretion might be supposed to yield valuable aid and the appearance of albuminuria and of tube casts might be reasonably expected in the event of injury to the renal tissue. It is probably on this account that nephrotoxin has been the most extensively studied of the somatogenic cytotoxins.

What strikes one in glancing over the fairly extensive literature of the subject is the claim of some of the investigators that much light is thrown, not only on the aetiology and pathology of nephritis but also upon the associated circulatory changes and uraemia, but on closer examination it will be painfully evident that the majority of the experiments have been conducted with very faulty technique and without the aid of careful bacteriological tests.

The endeavour has been made to prove the existence of three different varieties of nephrotoxins, viz:-

(1) auto-nephrotoxins or auto-nephrolysins

(2) iso-nephrotoxins or iso-nephrolysins

(3) hetero-nephrotoxins or hetero-nephrolysins.

In regard to (1), several French and Italian investigators claim that as a result of ligature of a ureter or of the renal vessels, toxic substances are absorbed by the blood from the degenerating kidney and these, operating through the blood stream, exercise a deleterious effect on the opposite kidney. The deleterious effect, they say, is due to the formation of auto-nephrotoxins.

(2) Iso-nephrotoxins are produced theoretically by injecting the kidney of one animal into another animal of the same species. The blood serum of animals in which attempts have been made to generate auto-nephrotoxins has been considered when injected into animals of the same species to be iso-nephrotoxic. The following methods have been used:-

(a) unilateral ligature of ureter.

(b) unilateral ligature of vessels.

(c) immunization of an animal against kidney of an animal of the same species.

(3) Hetro-nephrotoxins are generated by immunising an animal of species A with the kidney of an animal of species B. After a number of injections of varying amounts of kidney emulsion, repeated at intervals, usually of 7-10 days, a hetero-nephrotoxin appears in the blood serum of the injected animal and when this is injected into another animal of species B, various phenomena are observed according to different investigators, including albuminuria, progressive wasting and death. The kidneys show various grades of degenerative change including disappearance of cell granules, necrosis of cells, pyknotic changes, fragmentation and disappearance of nuclei, accumulations of round cells in the interstitial tissue and of the granular material and tube casts in the tubules. Some authors have described symptoms which they consider to agree with those caused by uraemia in man.

We shall now glance over the most important contributions

to the literature of the subject. Lindemann· states that he has succeeded in preparing an active nephrotoxic serum by injecting into guinea pigs an emulsion of rabbit kidneys.

Schültze·· has been quite unable to observe the nephrotoxic effect of the serum of rabbits which he injected with emulsion of guinea-pig kidneys.

Nefedieff··· performed several sets of experiments. He states that the serum of rabbits obtained by injecting into them emulsions of the kidneys of guinea pigs is highly toxic for these latter animals. He noted a slight albuminuria which corresponded to the lesions seen at the autopoy, viz: congestion of the capillaries and glomeruli, swelling of the epithelial cells which had become more or less granular and destroyed in certain tubules. This action according to him is specific for the serum of the normal rabbit injected in the same doses does not possess any general or local toxicity for the guinea pig. On the other hand he found that the serum of guinea pigs injected with emulsions of rabbits' kidneys only presented extremely feeble nephrotoxic properties. He is of opinion that the feebleness of these nephrotoxic sera depends on the fact that the kidney tissue is highly toxic and that it is impossible to inject into

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. Ann. de l'Inst. Pasteur, fev., 1900.
.. Deutsch med. Wochen, 1900, No.27.
... Ann. de l'Inst. Pasteur, 1901, p 17.

the animals used in the above experiments a sufficiency of kidney emulsion to produce a powerful nephrotoxic serum.

Nefedieff also investigated the effect upon the other kidney of tying one of the ureters of the rabbit and also after an interval the effect of the injection of the serum of such an animal into a normal one of the same species. He found that on examination of the other kidney of the animal that had one of its ureters tied extensive changes in the epithelium were met with. That of the straight tubules was atrophied whilst that of the convoluted tubules was extensively vacuolated and the nuclei had disappeared or were greatly modified. He attributes this change to the accumulation of toxic products in the blood which exercised their hurtful action on the functionating kidney. After the injection of the serum he observed a notable albuminuria and the kidneys of the animals presented all the appearances of a diffuse inflammation. The epithelium of the convoluted tubules was either necrosed or extensively vacuolated and the vessels of the interstitial tissue and of the glomeruli were very hyperaemic whilst accumulations of round cells were met with between the tubules.

Castaigne and Rathery', who laid great stress on the

. Toxicite de la substance renale et nephrotoxines.
Press medicale, aout, 1902.
Lesions experimentales du rein.
Arch. de med. exper., sept., 1902.

toxicity of the renal tissue obtained both an auto-nephrotoxic serum and a hetero-nephrotoxic serum. The latter they obtained by injecting rabbits with guinea-pig kidneys. The activity of this serum these observers hold to be proved by (a) the death of two animals after injection, (b) the occurrence of albuminuria, (c) constant diminution in weight, and (d) the presence of microscopic lesions of the kidneys.

Albarran and Bernard* followed up the last two observors by repeating and further elaborating some of their experiments. They also dwelt at length on the powerful toxic action of the renal parenchyma and adduced several experiments to prove their contention. On account of the difficulty experienced in preparing a nephrotoxic serum by injecting rabbits with guinea-pig kidneys they had recourse to animals of very different species. They subjected geese to series of injections of guinea-pig kidney emulsion. The geese did not stand the injections well but considerably better than rabbits. The serum, however, which they obtained even after massive doses of kidney substance appeared to be very slightly toxic for in order to kill a guinea pig, , injections of the nephrotoxic serum had to be repeated several times, and even then the guinea pigs did not die

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. Etude sur les cytotoxines renales.
Archiv. de med. exper., janvier, 1903.

unless the serum was derived from different geese. These observors differed from the preceding ones in that they refused to attribute either to kidney substance or to the serum of an animal injected with kidney substance any specific action. They found lesions practically identical when they injected into animals either emulsion of liver substance or the serum of an animal which had been subjected to a series of injections of liver substance.

They also repeated Nefedieff's experiment of tying one of the ureters in a rabbit and after a time injecting the serum of this animal into a normal one. The results were inconclusive, in only one of a series of four animals were signs of an epithelial nephritis visible. The results obtained on examining sections of the functionating kidney of the animal which had its ureter tied, were also inconclusive.

Bertenson* carried out various operative procedures on the kidney with the effect of producing hypertrophy, degeneration or inflammation of the opposite organ.

Ascoli and Figari** treated rabbits with series of injections of emulsion of dogs' kidneys: many of the rabbits

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* Contrib. a l'anatomie path. de l'hydronephrose exper. Bobn. Gaz. Bork. 1900.

** Nephrolysin. Berl. Klin. Wochen., 1902., No.24.

died. A few days after the injection of the serum of these rabbits into dogs, marked albuminuria (up to 10%) appeared with haematuria and cylinduria which lasted for weeks and even months. The general condition of the animals suffered but no oedema or uraemic symptoms were evident.

They also investigated the effect of ligaturing one ureter and also of removing a kidney and they found the injection of the serum of an animal treated thus into an animal of the same species caused a condition similar to that which they observed in their previous experiments but less severe. They consequently came to the conclusion that such a serum contains an isolysin. In the above experiments they considered that autolysins were present on account of (1) the protracted length of the cases, (2) serum of a dog which has received heterolysin when injected into other dogs caused albuminuria after a latent period, (3) serum of a rabbit with one ureter ligatured contains isolysin, also autolysin because urine is albuminous. (4) They also came to the conclusion that auto-nephrolysins exist because if one injects a dog A with heated serum of another dog B which has already been treated with heterolysin and then a week later inject heterolysin into dog A no result appears; if, however, the serum of the dog be heated before injection the morbid result is merely delayed.

These observers also noticed that when one of the ureters of a rabbit was tied, the animal developed marked

heart hypertrophy, especially of the left ventricle. They also noted a rise in the blood pressure curve in the dog after an injection of heterolysin, whilst in a control animal injected with normal rabbit serum there was no such change.

They also investigated the effect of injecting heterolysin subdurally in dogs comparing it with the effect produced on injecting normal rabbit serum. In the former case the results were very marked. Tonic and clonic spasms, paralysis and death occurred in a few hours whereas in the control animal no effect was noticeable.

No results of any histological examination of the kidneys are given.

Bierry. in a number of somewhat short and disjointed papers, has investigated the effects of treating rabbits with dogs' kidneys. His results agree with those of Ascoli and Figari in regard to the amount and persistence of the albuminuria induced by the injection of a serum, obtained as above, into dogs. He has also succeeded in immunising rabbits against dogs' kidneys by injecting the nucleo -albumins of these organs instead of the organs themselves.

. Recherches sur les nephrotoxines. C.r. de la Soc. de Biol. No.26, 1902, also No.13, 1903, and C.r. de l'Acad: des Sciences No.14, 1903.
Recherches sur les injections de sang et de serum cytotoxiques au chien.
C.r. de la Soc: de Biol: 1901.

If the pedicle of a dog's kidney be tied leaving the ureter free and if, after an interval, a leucotoxic serum derived from the goose or rabbit be then injected into the animal marked albuminuria appears. From this observation he comes to the conclusion that the nephrotoxins are leucocytic poisons which, when the leucocytes are destroyed, pass into the circulation and exert their poisonous action.

In these experiments no mention is made of microscopic examination of the kidneys or bacteriological tests or tests of the haemolytic power of the sera employed. The sera obtained by Bierry were very feeble as a rule, and, except for the fact that they caused albuminuria, did not seem to possess any evident nephrotoxic properties.

Pearce• repeated most of the experiments of previous investigators and he was the first to employ proper controls and careful bacteriological tests. In regard to ligature of the ureter or vessels of one kidney, he came to the conclusion from the careful study of several cases that this procedure does not produce changes which differ in any way from those following unilateral nephrectomy, that is to say, that under normal physiological conditions, the degeneration of renal cells does not lead to the production of auto-nephrotoxins. In regard to the formation of iso-nephrotoxins, one may say his results were entirely negative. He attempted the formation

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• Univ: of Penna: Med: Bull: vol. xvi, p.217.

of hetero-nephrotoxins in three ways, viz: (1) the injection of the kidney of the rabbit into guinea pigs, (2) the injection of guinea pig kidney into rabbits (3) the injection of dog kidney into the rabbit. The serum produced by the first two methods gave unsatisfactory and inconclusive results. The results obtained by the 3rd. method were interesting. He came to the conclusion that the haematuria observed by Ascoli and Figari after the injection of this serum was really a haemoglobinuria depending on the presence of a powerful haemolytic immune body. The serum of animals treated with washed kidneys failed to produce haemoglobinuria but set up an intense albuminuria with numerous tube casts which persisted for many days. However, even with the serum derived from unwashed kidneys the persistence of the albuminuria for two or three months was never observed. Histological examination of the kidneys showed extensive granular degeneration of the convoluted tubules and fatty changes demonstrated by osmic acid limited almost exclusively to the loops of Henle Casts were seen especially in the collecting tubules. Glomeruli were much congested. The animals lost weight invariably.

Pearce treated the serum derived from unwashed kidneys with washed dogs' red corpuscles at 0°C. After an hour the serum was found to be only faintly haemolytic but he thought that the nephrotoxic serum had lost some of its strength. On this account in the remainder of his experiments he only used

thoroughly washed kidneys for purposes of immunization. A serum produced by this method never caused haemoglobinuria but its action on the kidneys was evidenced by severe albuminuria and abundance of granular and fatty casts. The histological changes were very similar to those produced by the serum from unwashed kidneys.

Pearce confirmed the observation of Bierry that the serum of dogs treated with nephrotoxic serum could, when injected into fresh dogs, cause renal disturbance evidenced by transient albuminuria and excretion of tube casts. The dogs whose serum gave positive result had had a series of injections of nephrotoxic serum.

Dogs were also treated by hepatotoxic serum and the effect on the kidneys was practically identical to that caused by nephrotoxic serum.

Pearce described a spontaneous nephritis in dogs accompanied by albuminuria with tube casts which he met with in 13 out of 64 dogs. Injection of the serum of these dogs into normal ones in some cases caused slight temporary albuminuria.

It was found that the serum derived from animals treated with the cortex of kidney was much more toxic than that derived from animals treated with medulla only.

Pearce was quite unable to corroborate the observations of Figari and Ascoli in regard to the rise of blood pressure

after the injection of heteronephrotoxin. All attempts at the production of chronic renal changes by injections of heteronephrotoxin or by tying a ureter were entirely fruitless.

In discussing the specifity of nephrotoxin, Pearce alludes to Morgenroth's views on the conflicting question of the specificity of the cytotoxins that specificity is a question not of cells but of acceptors; whence a given cell may be in chemical conformity with cells of a different organ or animal. As the hepatotoxin and haemolysin also excited an action upon the kidney their relation to nephrotoxin is further established.

Woltmann• has recently investigated the specificity of the cytotoxins. In his experiments geese were used for the production of the cytotoxins and the material inoculated into them was obtained from sheep. His general conclusion is that none of the cytotoxins can be regarded as really specific although nephrotoxin comes nearest being so. Marked congestion of the medullary portion of the kidney and pronounced cloudy swelling of the cortex were observed after inoculation with this toxin and with no other toxin were similar changes of the same severity found.

• Journal of Experimental Medicine., April, 1905.

METHODS EMPLOYED IN THE INVESTIGATIONS DESCRIBED BELOW.

We now proceed to give a short description of the various methods employed in the investigations which we have conducted with the object of throwing more light if possible upon the subject of the nephrotoxins.

Rabbits were the animals employed for immunization and they were all specially selected on account of their vigour. The rabbits were treated in the following manner with the kidneys of guinea pigs which had been bled to death from the carotid immediately before. The kidneys were removed from the guinea pig with ordinary aseptic precautions, andwithout washing, and placed in a sterilised watch glass, the weight of which was known. The capsules of the kidneys and any loose fatty tissue were then carefully removed and the weight of the organs ascertained. The kidneys were then transferred to a porcelain mortar and reduced to an emulsion. This process was much facilitated by snipping up the kidneys into small pieces with scissors before using the pestle at all, and also by having the mortar quite dry. The reduction of the kidney to a fine state of emulsion demands the expenditure of considerable time and labour and when large quantities were being treated a great saving of these two valuable items was effected by rubbing the coarse emulsion one or more times through a fine wire gauze tea-strainer. Sterilised normal salt solution was gradually added but not before the

kidney had been reduced to a fairly fine state of division. By these means a fine emulsion was obtained which however always contained coarse particles which subsided on standing. The emulsion was injected by means of an antitoxine syringe. It was found much more convenient to have the needle fitting to the syringe without a screw as, when large quantities had to be injected, the syringe was much more easily withdrawn for re-filling - the needle being left "in situ". The injection was made into the peritoneum in all cases and the site of injection was anywhere about the centre of the abdomen so as to be clear of the liver above and of the pelvic viscera below. Preparatory to making the injection, if the hair was very long, it was cut short over the selected area which was then rubbed with 5% lysol. In making the injection, the skin and muscle is pinched up between the finger and thumb of the left hand and the needle thrust boldly in with the right. The intestine is not easily wounded. On the other hand if the needle be not inserted right into the abdominal cavity and a large quantity of emulsion be injected, sloughing of the skin of the abdomen may occur from excessive distension of the subcutaneous tissue with emulsion. This accident is rather apt to occur as the muscle tends to jump from between the finger and thumb unless a good grip be taken. In the seven rabbits which were immunised with guinea pig kidney

emulsion slight sloughing of the abdominal wall occurred in two in the course of the injections but it was very limited and, after the slough separated, healing was rapid.

All the apparatus required in the above procedures is simple and easily sterilised by boiling. Nefedieff with apparatus somewhat similar to that described above found that all the animals treated either died or presented signs of abscess formation at the seat of puncture after the first and second injection. He attributed this result to aerial contamination of the emulsion during the process of passing it through the strainer and, in order to avoid this, he invented an elaborate press completely protected from the air and capable of sterilisation in an autoclave. The emulsion thus prepared was found to be sterile.

In the present investigation the immunised rabbits had the nephrotoxic power of their serum tested before being bled to death. Blood was withdrawn from the ear, centrifugalised and the serum was pipetted off. If it were desired to eliminate the haemolytic immune body in the serum it was mixed with excess of washed guinea pig red blood corpuscles and kept at $0^{o}C.$ for one hour. After this treatment the serum proved to be only very slightly haemolytic. If the serum from the rabbit appeared satisfactorily toxic when injected into a

guinea pig, the animal was bled from the carotid. The blood being allowed to coagulate was placed in a cool chamber till the serum separated when it was poured off and put up in sealed glass tubes. The serum was sterilised by placing the tubes in a stove at 56°C for one hour each successive day in a week. The nephrotoxic serum was injected into guinea pigs intra-peritoneally, the skin of the abdomen being sterilised by rubbing with turpentine, spirit andthen 5% lysol, the hair having previously been cut short. Guinea pigs dying after injection with nephrotoxic serum were examined as soon after death as possible, and to exclude bacterial infection of the peritoneum, films were made of the exudate which were stained with methylene blue or carbol thionine blue and Jenner's stain. Cultures were also made on agar-agar tubes. All animals were kept in cages, the bottoms of which were of wire netting beneath which were metal trays so that the urine was easily collected.

The urine of both the rabbits and guinea pigs was regularly examined by Heller's and the heat tests. The urine was cleared either by acidifying and filtering or, if that did not prove sufficient, by precipitating the phosphates with caustic potash solution, filtering and then slightly acidifying with acetic acid. The guaiac test was also regularly employed.

We shall consider the various phenomena observed in connection with the production of the nephrotoxic serum in the rabbit and its injection into guinea pigs under the following headings:-

I. The effect of the injection of guinea pig kidney emulsion into the rabbit, including:-

(A) Symptoms after injection.

(B) Changes observed in the peritoneal exudate.

(C) Changes seen in the rabbit's peritoneum and

(D) In the blood and in the various organs.

II. The effect of the injection of the nephrotoxic serum of the rabbit into guinea pigs, including:-

(A) Symptoms after injection.

(B) Changes seen in the kidneys and other organs.

(C) Changes observed after repeated small doses of nephrotoxic serum with the idea of inducing chronic changes.

And lastly, we shall consider briefly, under III, the results of some experiments made "in vitro" giving a short account of the procedure followed.

I. (A).

As stated above, 7 rabbits were treated intra-peritoneally with guinea pig kidney emulsion. The amount of kidney substance used in the injections was in all cases small at first and was gradually increased. To begin with, the dose varied from 1½ to 2 grammes of kidney substance emulsified.

This was increased so that animals getting their eighth injection would receive 8-10 grammes. The kidney of a medium sized, full-grown guinea pig weighs approximately 2 grammes. The injections were repeated as a rule every ten days.

Of the above 7 rabbits, 1 received four injections, 3 received eight injections, (one of these three received emulsion of guinea pig kidney, heated for one hour to 56°C), the remainder only received one injection.

The immediate symptoms after an injection of the emulsion were those of collapse, temperature sinking to 96°, respiration being accelerated, ears cold, appetite completely lost and urinary secretion greatly diminished. This condition of collapse, however, was recovered from in 12-20 hours and it was remarked that, after a series of injections, although the amount injected was greatly increased, the duration of these symptoms was much lessened and the animal began to feed sometimes in 3 or 4 hours. Loss of flesh at first was the rule but, later on, in the course of immunization, the animals usually put on flesh, fed well and appeared healthy. If the animal were pregnant when the injections were commenced, abortion shortly after the first injection appeared to be invariable and occured in three of the animals of my series. The animal however might become pregnant during the course of immunization and in this event pregnancy might not be interrupted by several large injections, thus one of my animals

had a litter of six healthy young about a week after the 8th. injection consisting of 10 grammes of kidney substance. None of the above animals succumbed after the injections and in only one was there any evidence of bacterial infection of the peritoneum. This animal after the first injection appeared very ill. A capillary pipette was passed into its peritoneal cavity and the fluid withdrawn contained large numbers of a bacillus morphologically identical with the bacillus coli communis. This animal wasted to an extreme degree but later began to put on flesh. It was not treated further.

With one or two questionable exceptions, only in the case of the animal injected with guinea pig kidney emulsion, heated for one hour at 56 C was a slight transient albuminuria discovered. No tube casts were ever discovered.

In the face of such results the very toxic effects of the injection of kidney emulsion as described by several authors mentioned above are difficult to explain except as a result of bacterial infection.

(B) Two similar series of experiments were made with the object of investigating the character of the peritoneal exudate of the rabbit after the injection of guinea pig kidney emulsion. In each case two rabbits were used, one which had already received five injections of an average dose of about 3 grammes, and the other was a fresh animal. A dose of about 1½ grammes was then injected into each animal and at intervals

of 5, 10, 20 and 40 hours capillary pipettes were introduced into the peritoneal cavity and some of the fluid withdrawn. No difficulty was experienced in introducing the pipettes if, after the tiny piece of skin was snipped off, care was taken to remove also the tough fascia lying over the muscle. The fluid withdrawn was examined in film. Dried films were fixed and stained with Jenner's stain which proved very convenient and generally satisfactory. Very clear pictures were procured by fixing dry films in formol-alcohol and staining with haemalum and eosin or Ehrlich's haemotoxylin-eosin. The peritoneal exudate of the immune rabbit examined 5 hours after injection contained a number of red blood corpuscles but the polynuclear leucocytes, the granules of which were deeply stained were in great preponderance. A few endothelial cells were present and these varied considerably in size. The greater number were small, the nucleus taking up nearly all the cell space. In the larger ones small round vacuoles were arranged around the nucleus. In none of the polynuclears were there any definite appearances of phagocytosis of the kidney debris, and in no case was there the slightest evidence of the taking up of the renal nuclei either by the polynuclears or the endothelial cells. No remains of the kidney tissue could be distinguished.

The peritoneal exudate of the fresh rabbit was paler, more fluid and more abundant than in the case of the immune

animal. Practically speaking the only cells to be seen in the films were polynuclear leucocytes which occurred in great numbers. They were small in size, the nucleus staining darkly and occupying thegreater part of the cell; the granules were closely packed and stained deeply. A few renal nuclei and granular debris, presumably debris of renal tissue were visible. At this stage an endothelial cell was very rarely seen.

After 10 hours in the exudate of the fresh rabbit only an occasional endothelial cell was to be seen. The polynuclears, however, appeared larger. An occasional renal nucleus was to be seen.

In the fluid from the immune rabbit the endothelial cells were much more numerous and larger and showed numerous vacuoles of varying size. The polynuclears were large, open in texture as it were, and in some cases showed clear areas devoid of granules probably evidence of degeneration.

After 20 hours the fluid from the fresh rabbit showed large numbers of polynuclears and endothelial cells. The former varied very considerably in size, the smallest being those with the small, circular, separate darkly stained nuclei. These circular nuclei varied much in size in the same, and in number in different cells. They might number from 1-8; few of the cells had only one, many had two or more nuclei. The neutrophil granules in all these cells stained well. The

typical polynuclears varied considerably in size and in the largest the nucleus stained comparatively faintly. The endothelial cells or macrophags showed great activity and could be seen actively englobing the polynuclears whilst in their interior were numerous vacuoles, many of which contained polynuclears or their remains. In the interior of the macrophags the polynuclears appeared to lose their nuclei very soon, neutrophil granules remaining long after trace of the nucleus haddisappeared. No appearances were visible similar to what was seen on injection of emulsion of rabbit kidney into a fresh guinea pig. Here the circular separate nuclei of the degenerate polynuclears when englobed by the macrophags persisted as more or less symmetrically arranged dots long after all trace of the neutrophil granules had disappeared. The peritoneal exudate of the immune rabbit as compared with that of the fresh rabbit at this stage was scanty, thick and gelatinous and was with difficulty sucked into the capillary pipette. What struck one on examining films of this fluid was the comparative scarcity of polynuclears. Those present appeared to be considerably degenerated and in many of them there were comparatively few neutrophil granules, and these were often aggregated at one side of the cell. From some of the cells the neutrophil granules seemed to have practically disappeared, the protoplasm being particularly clear and glancing. Very few polynuclears were seen with the circular

separate nuclei described above. The macrophags were actively englobing the polynuclears.

A differential count showed that in the films from the fresh rabbit at this stage 84.2% of the cells were polynuclears 6% of these belonging to the group with the small, round, circular nuclei where as in the case of the immune animal the polynuclears were only 51.6% of the total cells. The remainder of the cells were marcophags, except a few cells with basophil granules.

After 40 hours the exudate from the fresh rabbit was much more viscous and scanty than before. On examining it in film a number of polynuclears were still to be seen and the macrophags still showed considerable activity.

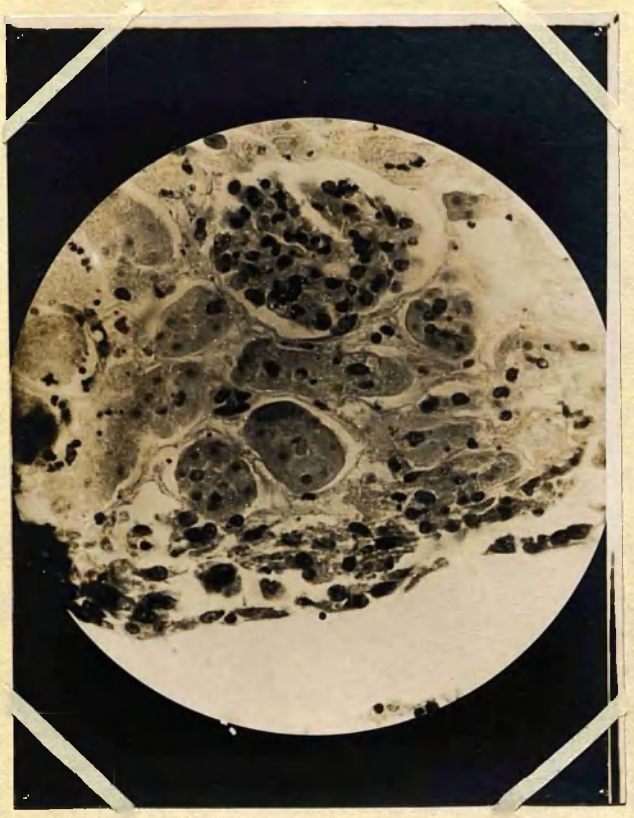
The fluid from the immune animal was extremely scanty, thick and gelatinous and it was with difficulty that the smallest quantity was secured. The macrophags were virtually the only cells present, and showed numerous vacuoles, but in only a very few of these were remains of neutrophil granules seen.

The process in the immune animal differs only slightly from that in the fresh animal except in its rapidity. In neither case at any time was there any phagocytosis of the renal cells or nuclei but the speedy disappearance of these from the exudate suggests the action of a ferment probably leucocytic in origin, and the subsequent absorption of the

products either by the combined action of the leucocytes, macrophags and lymphatics, or directly by the lymphatics. Some experiments had been previously undertaken to observe the action in the peritoneal exudate when rabbits' red corpuscles were injected into fresh guinea pigs and guinea pigs' immunised to rabbits' red blood corpuscles by previous injections. It was seen that the polynuclears greedily devoured the red blood corpuscles and were in turn eaten up by the macrophags, the process in the case of the immune animal differing only from that in the fresh animal in its greater rapidity. When the red corpuscles were injected along with the serum of an immune guinea pig into a fresh guinea pig the red corpuscles completely disappeared in some cases lysis evidently having occurred.

The injection into a fresh rabbit of guinea pig kidney emulsion along with the serum of an immune rabbit gave rise to appearances differing little from those observed in the case of an immune rabbit.

(C) Sections of tissues were as a rule fixed in perchloride of mercury, embedded in paraffin and stained with haemalum and eosine of haemalum and Van Gieson's stain. Sections of kidneys were also fixed in formol-alcohol or formol-Muller. To demonstrate the neutrophil granules in the polynuclears, Ehrlich's triacid stain, diluted with four times its own volume of water as recommended by Muir, was employed.



Section of Omentum of Rabbit.

48 hours after injection (high power) showing nodule of kidney tissue, the nuclei of the epithelium of the tubules of which are much faded. Large endothelial cells are seen in the midst of the tissue and the covering layer of endothelium is seen in process of formation.

On opening the abdomen of a rabbit killed 6 hours after an injection of guinea pig kidney emulsion a considerable quantity of free fluid was found which was of an opalescent reddish tint. A deposit of the coarser fragments of the emulsion was seen on the omentum, mesentery and on the surface of the gut, especially the caecum. Sections of the omentum on being examined microscopically showed large numbers of more or less distorted renal cells deposited on this structure; the nuclei were darkly stained but their edges were not clearly defined. The omentum was very hyperaemic and large collections of leucocytes, many of them non-granular, could be seen in the vicinity of blood vessels. These leucocytes appeared to force their way between the endothelial cells and in places seemed to cause an exfoliation of these.

When the abdomen of a rabbit killed 48 hours after the injection was opened little or no free fluid was visible. The coarser grains of the emulsion were seen scattered over the omentum, and these could be displaced on lightly touching them with the finger. The surface of the coils of intestine was remarkably clear of deposit. On closer examination there were evident between the coils of intestine, long, thick, opalescent gelatinous threads which, on being raised by the forceps, displayed branchings in every direction between the coils, and were apparently in connection with the mesentery. On examination of sections of omentum and of these whitish threads very

similar appearances were evident. The coarser grains of the kidney emulsion had settled down on the endothelium and when one of these little nodules was seen in section remains of tubules and glomeruli were often plainly visible. The epithelium of the tubules was necrosed and even at this early stage the nuclei still preserving their circular shape were seen as the merest shadows and at many places were invisible. The nuclei of the cells of the interstitial tissue seemed to be better preserved but in several places these too had disappeared. The endothelial cells of the glomeruli seemed to be the most resistent. Around the periphery of one of these little nodules a layer of endothelial cells was to be seen, one or more cells in thickness and evidently in process of forming a sort of capsule. The mode of formation of this layer seemed to be partly by deposition and partly by proliferation, although evidences of the latter were not striking.

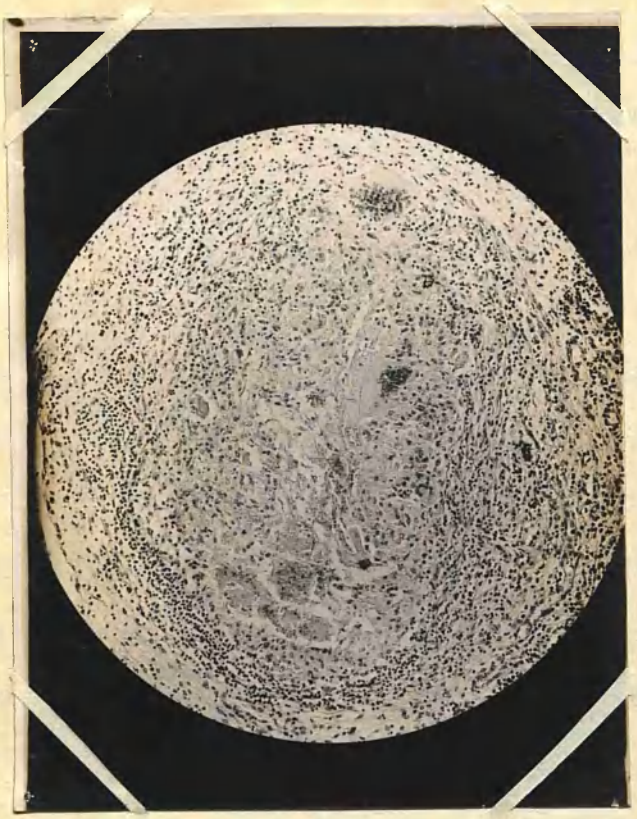
Besides grains of the above description others were seen of a much more homogeneous composition which did not show any suggestion of structure such as tubules or glomeruli and were merely masses of homgeneous necrosed tissue without any nuclear staining. These had usually a considerable number of endothelial cells scattered about in their interior, and, surrounded by their covering of endothelial cells, were often found superimposed on grains of the former description. These were presumably formed from the lighter elements of the emulsion.

Round the blood vessels in the vicinity of these little nodules there were large collections of leucocytes, both granular and non-granular. The necrosed tissue of both forms of nodules was stained a light purple by Ehrlich's triacid mixture. No giant cells were seen at this stage.

When the abdomen of a rabbit which has received a series of injections of guinea pig kidney was opened about 10 days after the last injection no free fluid was found to be present. In the case of a rabbit which had received four injections of an average of about 3 grammes each of kidney substance small granules evidently of kidney substance were seen at intervals on the omentum and were intimately adherent as they were not displaced when touched by the finger. The only other abnormal appearance was that presented by four tags of tissue irregular and nodulated in outline which hung into the abdominal cavity from the anterior abdominal wall and probably marked the sites of puncture when the injections were given. They measured from ½-1 inch in length or a little more. A nodule about the size of a small bean which had been noticed during life in the subcutaneous tissue was found at the autopsy to correspond in position to one of these tags. During life and even when examined at the autopsy on account of its soft consistence and yellow caseating appearance it was considered to be some sort of caseating focus but, when investigated microscopically, it was at once evident that it was due to the presence of some kidney substance, which had escaped from the syringe into the

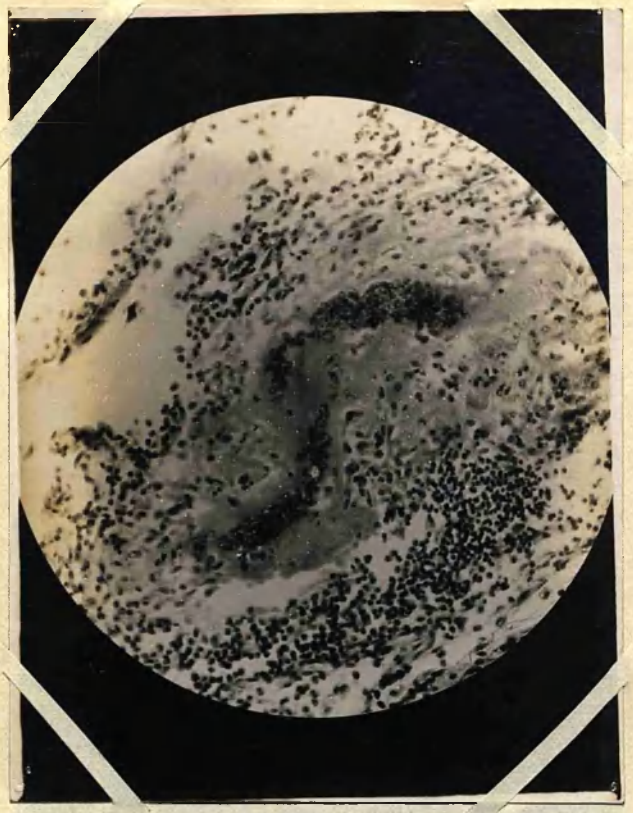
subcutaneous tissue, and also to the tissue reaction which it had caused. The description of the microscopic appearances will be given under the next heading.

On examining the peritoneum of one of the rabbits which had eight injections (the dose gradually increased up to 8-10 grammes of kidney substance at each injection) the appearances were much more striking. The inner aspect of the abdominal wall was covered at places uniformly over considerable areas, at other places in patches with shaggy greyish granulations. These formed, where present, a thick and velvety coating and showed in many places long pedunculated tags usually nodulated in their course and being almost invariably so at their free extremities. They measured up to one inch or more in length and in some instances with a long thin pedicle the small nodular extremity was extremely congested as if the process of absorption had proceeded too quickly at the base and had led to constriction of the vessels. The velvety granulations existed scattered over the caecum and its mesentery, on the anterior surface of the stomach where they might form a thin, uniform coating and even on the diaphragm, surfaces of the liver and spleen and on the broad ligaments. In one case very extensive adhesions had occurred between the abdominal wall and the caecum by means of a multitude of the projections above described; the adhesions were very soft and not at all fibrous and were very easily broken through. The omentum showed no fibrous change, for, although it had numerous tiny



Section of Omentum of Rabbit.

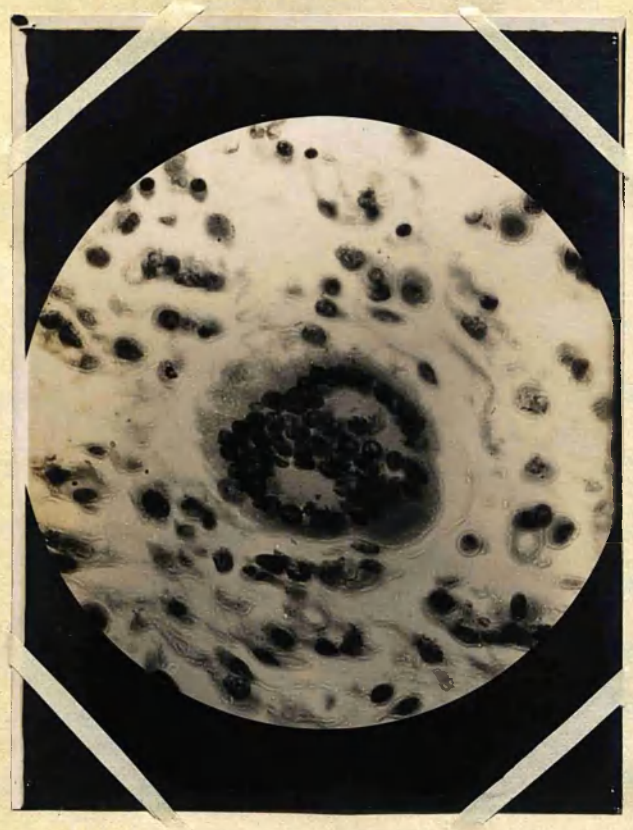
After a series of injections (low power) showing nodule surrounded by zone of leucocytes. Giant and epithelioid cells and remains of tubules and a glomerule are also seen.



Section of Omentum of Rabbit.

After a series of 4 injections (low power) showing a large giant cell with many nuclei in the centre of a small nodule in which the process of absorption is almost complete.

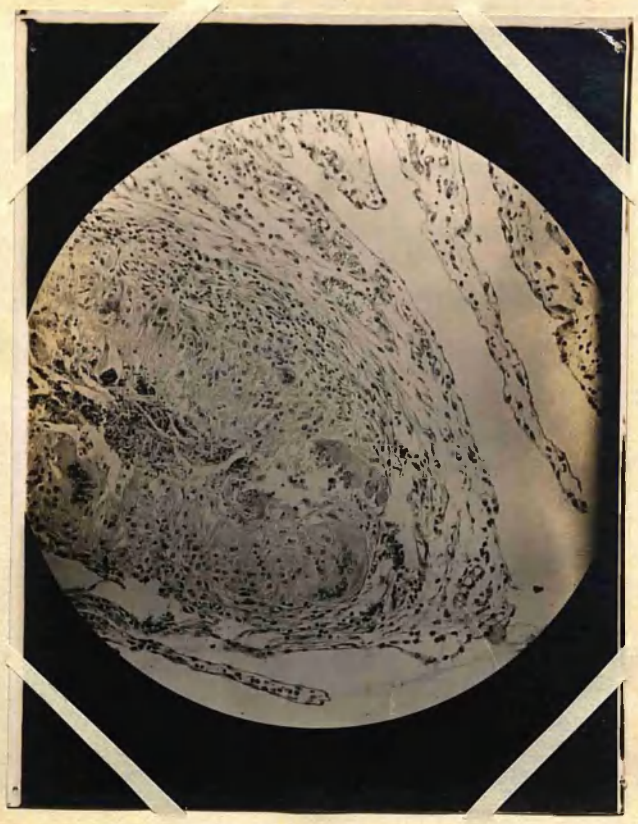
nodules in its substance and studded at intervals over it, on lifting it up it appeared as thin and diaphanous as usual between the little granules. Microscopic examination of an omentum as above described showed the nodular enlargements. These were circumscribed by fine open fibrous tissue with the fibres arranged concentrically and having large, clear, oval shaped nuclei. This covering was evidently formed from the endothelial cells and in it were numerous evidently newly formed blood vessels with thin walls. In the centre of the nodules in many cases were seen undoubted remains of kidney tissue, both tubules and glomeruli. The tubules as a rule were extremely shadowy in outline and the nuclei if seen at all were extremely faint. In a few exceptional cases the nuclei were much shrunken and distorted and stained darkly and in such instances the epithelial cells of the tubule seemed to separate from one another and stand out individually. Round this necrotic tissue and penetrating into it from all sides were numbers of large, elongated epithelioid cells arranged more or less radially in a fan-like manner. These cells had large oval nearly circular clearly staining nuclei with small chromatin dots and their protoplasm was faintly granular. As the process proceeded giant cells were formed in all probability from fusion of epithelioid cells because the nuclei were identical and no evidence of proliferation was seen. The giant cells might be seen either lying close to the fibrous



Section of Omentum of Rabbit.

After a series of 8 injections (high power) showing giant cell in process of breaking up.

capsule with long processes extending towards the centre of the nodule between the epithelioid cells or else directly in contact with the renal tissue and evidently actively engaged in its absorption. There might be several giant cells in one nodule and the number of nuclei might be very great and as a rule were arranged either in a clump or several clumps or quite regularly. Though many sections were examined in no case were any definite remains of renal cells seen either in the epithelioid or the giant cells. It appeared to be the case, however, that the more highly immunised the animal was to the kidney substance the more numerous and active were the giant cells. Outside the zone of epithelioid cells was a layer of undifferentiated leucocytes and among them especially where active absorption was going on were numerous polynuclear leucocytes which appeared to have migrated from the newly formed blood vessels already alluded to. The process of absorption completed, the epithelioid cells evidently fused with the giant cells which thus attained an enormous size with as many as a hundred nuclei or more, and lay in the centre of the nodule which had apparently contracted down simultaneously with the absorption of the kidney substance. The giant cells now attacked the cells of the nodule and several of the undifferentiated leucocytes could be seen in their interior. The leucocytes with the neutrophil granules disappeared and the giant cells diminished greatly in size. Some of the nuclei of the giant cells became much larger and clearer evidently a



Section of Peritoneal Tag from Rabbit.

(Low power) showing fine loose fibrous tissue, single covering layer of endothelium and a nodule with giant cells. Kidney tissue is seen being absorbed in the centre of the nodule.

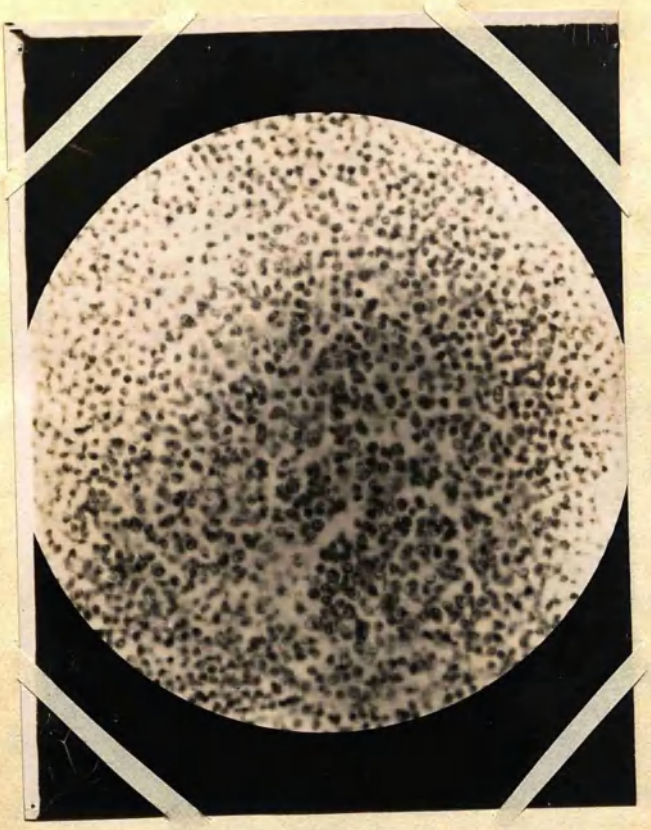
degenerative process, whilst from the periphery of some of the giant cells portions with nuclei appeared to break off and thereafter seemed to rapidly disappear. All that remained of the nodule was now a small giant cell, often with its few nuclei arranged circumferentially so that it greatly resembled a giant cell of a miliary tubercle, and surrounded by a small amount of fine fibrous tissue, in which the new formed vessels rapidly disappeared. The giant cell then appeared to break up into elements with a single nucleus which seemed to disappear quickly whilst the cells of the finely fibrous tissue appeared to melt away without leaving any trace of their existence. The absorption of these nodules was therefore absolutely complete and thus it was that the omentum preserved its wonted tenuity.

On examining a section of one of the peritoneal tags previously referred to it was seen to be a branching structure covered with a single layer of endothelium and composed of fine loose fibrous tissue in which were numerous thin walled vessels. The structure of the nodules in their interior which always contained remains of renal tissue and the mode of their absorption were practically identical with those in the omentum. Giant cells, epithelioid cells and leucocytes were present and were similarly arranged. In some of these appendages the absorption of the tissue at the pedicle and the obliteration of the vessels, ocurred so rapidly that marked

congestion and extravasation of blood from the thin walled vessels appeared at the tip.

(D) Under this heading we shall first of all take up the consideration of the changes which the Blood of the rabbit undergoes after the injections of the kidney emulsion. For the enumeration of the corpuscles the Therma-Zeiss haemocytometer was employed in the usual fashion. Dried films were fixed and stained with Jenner's stain or after fixing with formol alcohol were stained either with haemalum and eosine or Ehrlich's haematoxylin and eosine.

After a single injection of the emulsion the total number of leucocytes per cubic millimetre was found to undergo little change but the proportion of the polymorphs was found to increase slightly at the expense of the lymphocytes. After a few days however the normal relation of the lymphocytes to the polymorphs was re-established. After a series of injections had been given a distinct hypo-leucocytosis was noticeable after a further injection, the proportion of the polynuclears, however, being slightly increased relatively to the lymphocytes In a couple of days, however, there was a distinct leucocytosis the lymphocytes being much more markedly increased than the polynuclears and this condition reached its height in about a week when the leucocytes might number over 20,000 per cubic millimetre and the proportion of lymphocytes to polymorphs be over three to one. After this there was a gradual decline to



Section of the Spleen of the Rabbit.

After a series of 4 injections (high power) showing germ centre in Malpighian corpuscle.

the normal.

When the blood of an animal which had received a series of injections of guinea pig kidney emulsion was examined in film, one was struck at once by the marked polychromatophilia which the red corpuscles exhibited. There was also fairly well marked poikilocytosis. An occasional normoblast was to be seen whilst numbers of the polynuclears appaared to be very poor in granules, some parts of the protoplasm of these cells being apparently quite free of them.

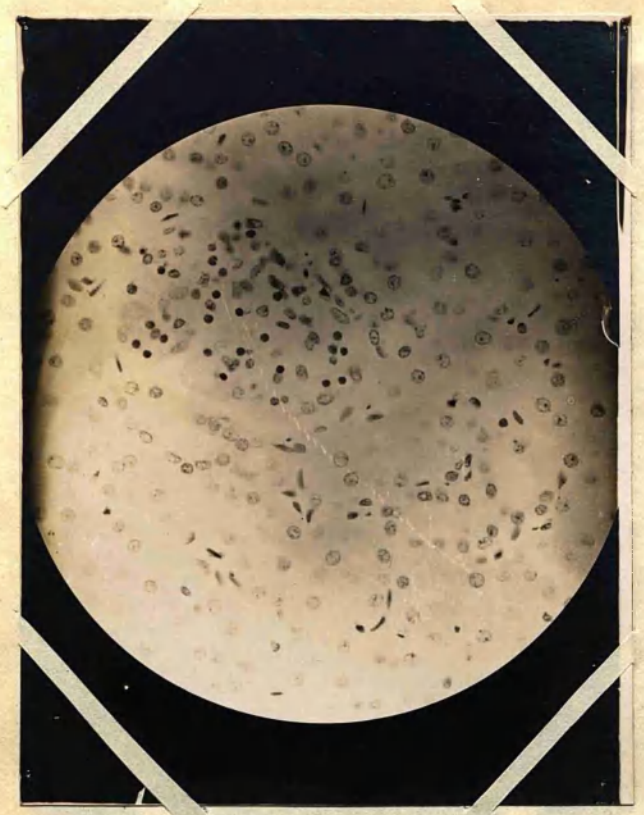
The red corpuscles were found to be reduced in number but never markedly so, the lowest record being 3,250,000 per cubic mm. In the case of the rabbit treated with the heated emulsion besides the polychromatophilia there was also visible in many of the red corpuscles a marked pyknotic change or basophil degeneration, the particles stained by the basic dye varying from the very finest andscarcely discernible up to large coarse ones. This degeneration was most marked in the discoloured corpuscles but the very fine granules were often seen in red corpuscles of quite the normal tint.

S P L E E N. When the rabbit was killed a short time after a single injection the pulp of the spleen was found on section to contain a large quantity of blood and the phagocytes in the pulp were seen to contain large masses of blood pigment which appeared to be more or less crystalline.

When the rabbit was killed about ten days after the last

Section of Spleen of Rabbit.

After a series of 8 injections (high power) showing edge of Malphigian corpuscle, and depleted pulp in the interstices of which are numerous red blood corpuscles.



Section of Kidney of Rabbit.

After a series of 8 injections (high power) showing no marked abnormal change. The swelling and granularity of the epithlium of the convoluted tubules is not well shown here.

of a series of injections the spleen was found to be invariably reduced in size and shrunken and there was little evidence of phagocytosis in the sinuses of the pulp. There was active proliferation of the germ centres which might come to occupy a large part of the interior of the Malpighian corpuscles whilst the differentation between the outer border of these bodies and the splenic pulp was to a great extent lost. Granular cells were not numerous. The pulp appeared at first to be unduly cellular but later on it was usually very depleted, the lymphocytes appearing to be greatly diminished in number.

LIVER - little change was distinguished.

KIDNEY - Nothing markedly abnormal could be distinguished on comparison with sections of a normal organ. The epithelium of the convoluted tubules, however, appeared to be unduly granular and swollen, a condition which also affected the epithelium of the loops of Henle but to a less degree. No change in the glomeruli or interstitial tissue after a series of injections was evident and there was no tendency to the separation of the epithelium in the form of casts. This agrees in most particulars with the appearances described by Ramond and Hulot•.

Subcutaneous nodules, caused by leaving particles of kidney beneath the skin and by the tissue reaction which the

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• Degenerescences exper. du foie et des reins d'originie cytotystique. C.r. Soc: de Biol:, 1901.

kidney substance gives rise to, were noticed during life in three rabbits. As many as three nodules were present in the subcutaneous tissue of the abdomen at one time. They were gradually absorbed and disappeared completely in three or four weeks. They were only examined histologically in one case.

On microscopic examination the nodule was seen to be encapsuled by fine loose fibrous tissue, the fibres of which were concentrically arranged. In the centre were obvious remains of kidney tissue in the form of glomeruli and tubules. The kidney tissue seemed to be better preserved than when met with in the omentum or in the peritoneal tags. The nuclei of the glomeruli were well marked and deeply and uniformly stained. The epithelial cells of the convoluted tubules were separated from the basement membrane and from each other and in the nuclei in places the normal arrangement of the chromatin could be distinguished. The tissue between the tubules showed large numbers of distorted darkly staining nuclei as if there had been some proliferation in the interstitial tissue. Otherwise the arrangement of the giant cells, which might reach a great size, epithelioid cells and leucocytes agreed precisely with what was seen in the omentum.

II. (A) Symptoms following injection of the nephrotoxic serum of the rabbit into guinea pigs.

When the guinea pig was put back into the cage after injection with nephrotoxic serum it would sit huddled up and showed decided indisposition to move, the hair, especially that about the neck, was inclined to stand on end and respiration was increased in frequency. In the cases where death took place comparatively rapidly the symptoms gradually increased in severity and twitchings of the hind legs, more or less rhythmical, set in with increasing weakness till the end. In the cases in which death did not take place for a longer period, a temporary abatement of the symptoms might take place and the animal might run about and even feed a little, in the intermission.

The urinary secretion in the fatal cases was always extremely scanty and it might be added as a corollary to the above that whenever an animal, after injection, was noted to pass any considerable quantity of urine, however grave the symptoms might be, the probability was strong that recovery would take place.

In the cases which developed peritonitis, diarrhoea was not uncommon.

The appended table will show at a glance the results obtained on injecting nephrotoxic serum into guinea pigs. Unless where otherwise stated the Complement had been removed from the serum by heat.

The following symbols are employed:- C - Complement.
M.H.D. - Minimum haemolytic dose.

No. of Guinea Pig.	Weight before Injection.	Weight at Death.	Time after inj. Died.	Amnt. of Serum Injected.	Minimum haemolytic dose of Serum.	Number of Injs. rabbit had from which serum was Derived.	Remarks.
1	481 grms.	438 grms.	At least 12 hrs; found dead in morning.	8 cc. (C. not removed)	0.6cc.	4	Slight sub-serous haemorrhages and haemorrhages into lymph follicles of caecum at autopsy. Slight bloody peritoneal exudate.
2	424 "	397 "	Killed in 24 hours.	4 cc. (C. not removed)	Haemolytic factor removed.	4	Evidence of peritoneal infection.
3	453 "	432 "	7 hrs.	2½ cc. (C. not removed)	Do.	7	Injected into subcutaneous
4	481 "	424 " on 2nd. day after inj.	Recovered	1 cc. (C. not removed)	0.2cc.	7	Injected into subcutaneous tissue; slight transient albuminuria after the injection but no casts.
5	424 "	-	21 hrs.	2 cc.	0.2cc.	7	Peritoneal and subpleural extravasation.
6	509 "	No loss of weight	dead next morning	4 cc.with 4 cc.normal rabbit serum	haemolytic factor removed	8	Evidence of peritoneal infection.

7	523	"	Little loss of weight	Dead next morning	4 cc.with 4 cc. heated normal rabbit serum.	haemolytic factor removed	8	Evidence of peritoneal infection.
8	509	"	-	Recovered.	4 cc.with 4 cc.normal rabbit serum.	Do.	8	Urine contained least trace of albumen.
9	594	"	571 "	Dead next morning	4 cc. and 4 cc. heated normal rabbit serum.	Do.	8	Exam. of films & cultures of peritoneal fluid negative.
10	339	"	322 "	Dead next morning	1 cc. with 1 cc. of normal rabbit serum.	haemolytic factor removed	8	Exam. of films & cultures negative: no appearances of peritonitis.
11	679	"	537 "	46 hrs.	4 cc.	Do.	8	Peritonitis due to bacillus coli communis.
12	509	"	-	Recovered.	4 cc.	Do.	8	Serum used was derived from the rabbit treated with heated kidney emulsion.
13	396	"	-	Do.	1½ cc.	Do.	8	Do ... do... do... do ...
14	551	"	453 "	74 hrs.	4 cc.	0.15 cc.	8	Slight albuminuria; subperitoneal haemorrhages.
15	509	"	467 " after 72 hrs.	Recovered.	1 cc.	0.15 cc.	8	Serum used was derived from the rabbit treated with heated kidney emulsion.

No. of Guinea Pig.	Weight before Injection.	Weight at Death.	Time after inj. Died.	Amnt. of Serum Injected.	Minimum haemolytic dose of Serum.	Number of Injs. rabbit had from which serum was Derived.	Remarks.
16	368 grms.	322 grms.	50 hrs.	3 cc.	0.15 cc.	8	Peritonitis due to a diplococceus.
17	481 "	424 "	76 hrs.	5 cc.	0.15 cc.	8	Bloody peritoneal exudate; albuminuria and haemoglobinuria.

Besides the animals enumerated in the above table, one guinea pig was injected with nephrotoxic serum derived from a rabbit which had had 4 injections. The minimum haemolytic dose of the serum was 0.4 cc, and before it was injected it was treated with excess of guinea pig kidney emulsion at laboratory temperature for over one hour. Collapse symptoms after the injection of 5 cc of the serum were very transitory and it was noted that there was very slight loss of weight although no figures were recorded. Two guinea pigs were injected each with 5 cc of normal rabbit serum and in each a well marked albuminuria was set up.

From consideration of the table it will be seen that, despite the fact that several of the animals recovered, a strong case is made out for the toxicity of the nephrotoxic serum. A noteworthy feature is that even in the cases which recovered a marked loss of weight resulted, and although this was also observed after injection of normal rabbit serum it was comparatively trifling.

Peritonitis is difficult to avoid.

Pearce (loc. cit.) in his series of 9 guinea pigs injected with nephrotoxic serum derived from the rabbit had 4 cases of peritonitis.

Albuminuria, except in the case of guinea pig No.8, where it was extremely slight and transient, was never dis-

covered when the haemolytic factor of the serum had previously been removed by treatment with guinea pig red corpuscles at 0°C. for at least one hour. And in all cases except guinea pig No.17, the albumen was a mere trace. No.17 was also the only guinea pig in which blood pigment was present in the urine. The explanation of the above facts is simple for, according to Copeman*, where haemoglobin is liberated in small quantity in the blood, albuminuria and not haemoglobinuria appears, the limit however is soon reached and then blood pigment is discoverable in the urine.

The method of estimating the haemolytic power of the serum will be discussed later. It is evident however that the nephrotoxic serum becomes strongly haemolytic and such a dose as guinea pig No.17 received might reasonably be expected to produce well marked results.

The power of the nephrotoxic serum did not seem to be increased or diminished by the presence or absence of rabbit complement or complementoid.

(B) In the case of guinea pigs killed by nephrotoxic serum from which the haemolytic factor had not been removed, it was noticed that on opening the abdomen the vessels in the abdominal walls and coursing over the viscera were unduly distinct, exceedingly minute ramifications being clearly seen.

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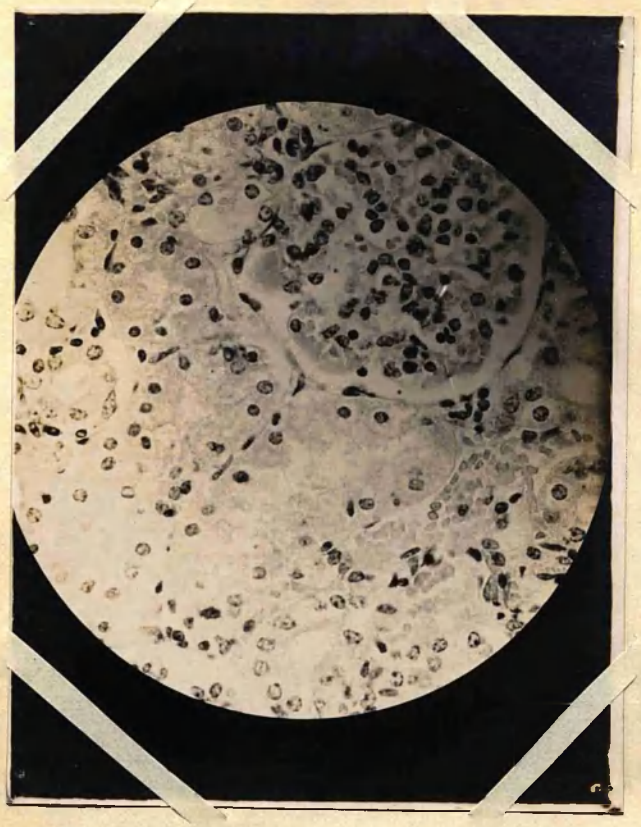
* Allbutt's System of Med: vol. v. art. haemoglobinuria.



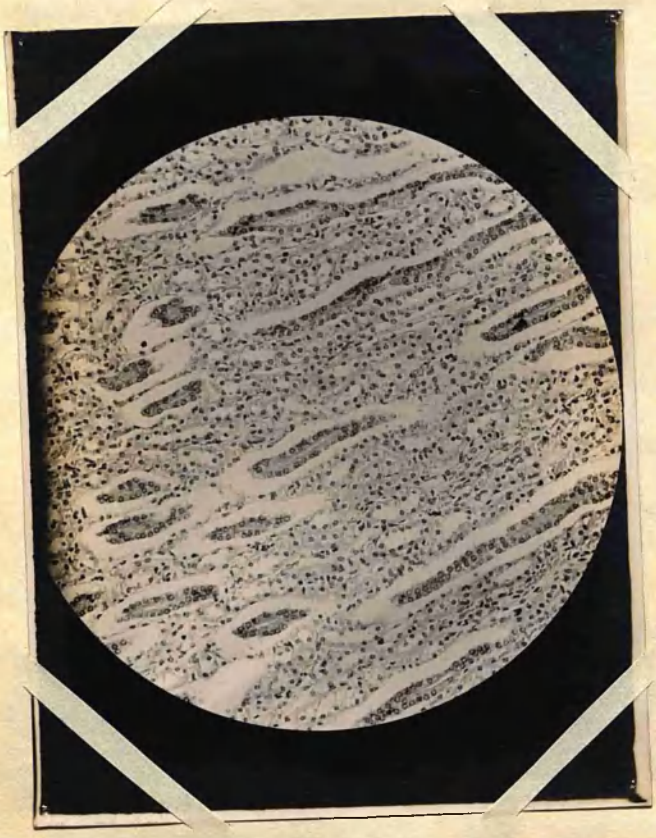
Section of Cortex of Kidney of Guinea Pig, No.1.

(High power) showing granularity and swelling and vacuolisation of the epithelium of convoluted tubule.

In such a case also there would be present a more or less blood stained fluid in the peritoneum and a number of subserous haemorrhages with haemorrhages into the lymphoid follicles of the caecum. The congestion of the viscera, moreover, was always specially marked.

When the haemolytic factor had been removed before injection, the fluid in the peritoneum was colourless - very pale straw - the blood vessels were not so distinctly marked out and there were no subperitoneal haemorrhages. The peritoneal fluid when examined microscopically was seen to contain in the former case numerous crenated and distorted red blood corpuscles but, in other instances, these were practically absent. Numerous endothelial cells and polymorphs were also seen, and in the former numerous cells of the latter variety undergoing digestion. The colourless peritoneal exudate noted above was found to contain a small number of polynuclears and endothelial cells.

K I D N E Y S. When examined as soon as the animal was dead after the injection, these organs were seen to be intensely congested. As a rule they were of a distinct chocolate colour with a variable amount of yellow mottling. The capsule was always readily stripped off without leaving any granularity. The kidney on section had a similar congested appearance. On microscopic examination it was found that the epithelium of the convoluted tubules was extremely granular and swollen and when the lumen was not completely filled up

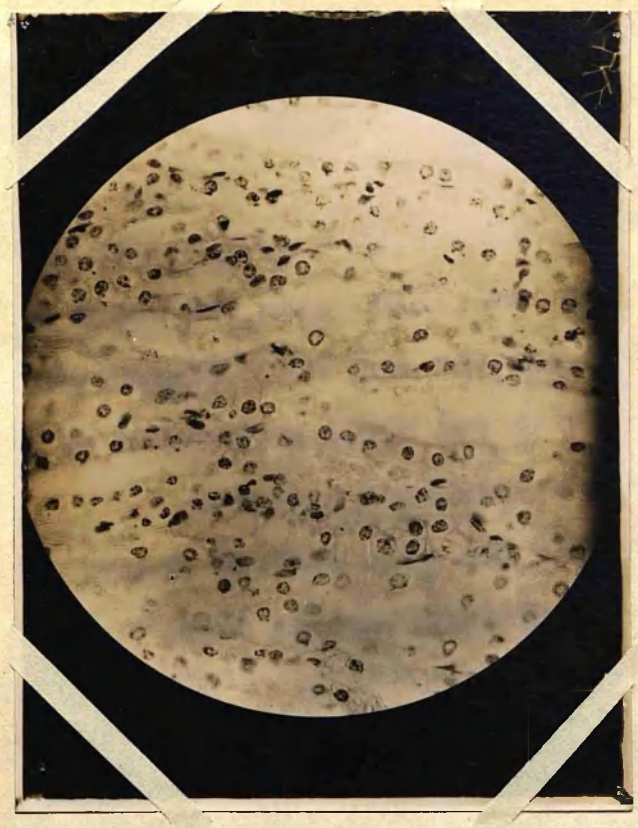


Section of Medulla of Kidney of Guinea Pig, No.1.

(Low power) showing separation of the epithelium of the straight tubules into casts.

with the swollen epithelium a large quantity of loose granular material was visible which seemed to be derived from the protoplasm of the epithelial cells which seemed to have lost all clearness of definition internally. As a result of the loss of granular material, the nuclei of the cells appeared at times to be situated in the centre of a clear space with perhaps only a small amount of granular protoplasm in the vicinity of the basement membrane. This change was also seen but to a less extent in the loops of Henle. In many of the epithelial cells but more especially those of the convoluted tubules there seemed to be disappearance of many of the nuclei. The changes noted above are common after injection of both kinds of serum but it was remarked that only when the serum still possessed its haemolytic power was there any decided and widespread tendency to the formation of epithelial tube casts in the straight tubules. These epithelial casts were never seen in the urine. Great congestion of the vessels, more especially of those running between the straight tubules, was constant. No definite changes were ever observed, either in the glomeruli or the interstitial tissue. When sections were treated with osmic acid, not a trace of fatty reaction was apparent in the convoluted tubules and only a few isolated granules were visible elsewhere, particularly in the loops of Henle.

On comparing the appearances noted above with what was visible on examination of sections of normal kidney it was found that a degree of granularity of the epithelium of the



Section of Medulla of Kidney of Guinea Pig, No.3.

(High power) showing absence of the tendency to the formation of tube casts. The largeness and the undue clearness of some of the nuclei are noteworthy.

convoluted tubules was present normally with slight vacuolisation of the protoplasm in places but these never reached the extreme condition noted above.

S P L E E N. Seemed to be pretty constantly and deeply congested. The pulp was filled with red blood corpuscles undergoing rapid destruction and the large phagocytes of the pulp were either loaded with red blood corpuscles or large masses of yellow, more or less crystalline, pigment.

Proliferation of the germ centres seemed to be active but these never reached any considerable size.

L I V E R. Was noticed to be invariably congested. Both the inter- and intra-lobular veins were greatly distended and in some cases this distension had been so great that rupture had taken place and extravasations of blood could be seen compressing the liver cells. Even when no rupture of the vessels took place, the liver cells often appeared compressed from the congestion of the capillaries.

Many of the liver cells were vacuolated and seemed to have lost much of their granular protoplasm. There was a slight degree of fatty change.

(C). Three guinea pigs were inoculated with a series of small injections of nephrotoxic serum, either subcutaneously or intra-peritoneally. The result in all cases was a marked diminution in weight. Although the injections were small and

only given every ten days, or at greater intervals, the animals did not seem to get accustomed to the treatment. One of the three died shortly after 2 injections of 1 cc of the serum, the other two after three injections. There was no evidence of peritonitis. Traces of albumen were occasionally found in the urines after the injections but no permanent albuminuria was induced. On examination of the kidneys and the other organs, no changes of a chronic character were seen, the appear ances visible being very like those described under (B).

III. Under this heading we shall briefly describe the methods followed and the results obtained in some experiments conducted "in vitro".

These experiments can be divided into two groups, viz:-

(A) Experiments undertaken to demonstrate the action of the nephrotoxic serum on kidney cells.

(B) Experiments to demonstrate (a) the haemolytic power of the nephrotoxic serum and (b) the amount of Complement taken up by the Immune body when in contact with kidney cells.

(A) Guinea pig kidney emulsion was allowed to stand in contact with nephrotoxic serum both in the presence of guinea pig and rabbit Complement. Thin sections of guinea pig kidney were also placed in similar media and pieces were removed after treatment for varying times, fixed in perchloride of mercury, and embedded in paraffin. All these tests were conducted with controls in which normal rabbits' serum was employed.

In no case could it be said that any distinct action of the serum on the kidney cells or tissue was evident.

(B) The experiments described below are based largely on the results of researches carried out by Muir*, latterly in association with Browning, on the interactions of the constituents of the haemolytic sera. In this description the following symbols used in the papers just referred to are employed.

I.B. - Immune body. R -red blood corpuscles.

C. - Complement.

M.H.D. - Minimum haemolytic dose.

1 D. - 1 haemolytic dose.

I.B. rabbit v. ox signifies an Immune body derived from the rabbit which acts on the constituent of some fluid or tissue of the ox.

For the investigations the following are requisite:-

(1) 5% suspensions of ox and guinea pig red blood corpuscles in .8% saline.

(2) 10% emulsions in the same medium of guinea pig kidney, liver, brain, spleen, etc. andof rabbit kidney.

(3) Haemolytic I.B. obtained from the rabbit by injecting it with washed ox red blood corpuscles. M.H.D. of the I.B. employed was 0.0015 cc.

(4) Pipettes measuring respectively tenths and hundredths of a cubic centimetre.

(5) Centrifuge, test tubes, incubator at 37°C. .8% saline, etc, etc.

. On the action of haemolytic sera, Lancet, Aug., 15th., 1903. On Immunity, B.M.J., Sept., 10th., 1904.
With Browning. On the combining properties of Serum complements and complementoids. Proc. Roy. Soc., June, 9th., 1904.
with Browning. On chemical combination and toxic action as exemplified in haemolytic sera. Proc. Roy. Soc. Decr., 1st., 1904.

In the haemolytic experiments after the various constituents have been added to one another in the test tubes, these are placed in the incubator at 37°C. and shaken every ten minutes or so in order to facilitate the special action.

The I.B. acts more energetically and rapidly with guinea pig C. than with rabbit C. as the former contains more C. molecules and when the former is used one hour in the incubator is sufficient. When rabbit C. is employed, however, more time must be given. Lysis, moreover, is quicker and more decided when I.B. and red blood corpuscles are allowed to interact some little time before C. is added.

When lysis is incomplete in any tube its amount can be ascertained by centrifugalising, pipetting off the supernatent fluid from the unlysed corpuscles which have been deposited, laking these with a volume of water equal to the original volume of the suspension of red corpuscles, and comparing the tint with that obtained by laking the deposited corpuscles of a corresponding volume of the original suspension. In this way the percentage of lysis can be accurately noted. Complete lysis may conveniently be regarded as unity, and the various proportions can be described as decimals of this.

(a) In determining the haemolytic power of the serum, rabbit C. was at first employed but as a good deal of lysis occured in the control tubes owing to the presence of the natural I.B. its use was abandoned for that of guinea pig C.

When I.B. is used with guinea pig C. instead of rabbit C. against guinea pig red corpuscles, its dose is increased ten times.

A series of 4 tubes were put up with .2, .3, .4 and .6 cc. of the nephrotoxic serum respectively in each. 1 cc. of suspension of guinea pig red corpuscles was added to each tube and thereafter 2 doses of C. Complete lysis occurred in the 4th. tube; in the 1st., 2nd., and 3rd., it was incomplete, M.H.D. of the serum therefore was .6 cc.

The M.H.D. of the serum from a rabbit which had had 7 injections was similarly found to be .2 cc. and from one which had had 8 injections .15 cc.

From these data we can estimate precisely the haemolytic power of the various doses of the nephrotoxic serum injected into the guinea pigs.

(b) The following is the principle, applicable to all the specific cytotoxins, upon which the following experiments were performed.

Two similar series of tubes are taken, each tube of which contains a certain fixed amount of kidney emulsion and similar increasing doses of C. To each of the tubes of one series the same amount of I.B. is added. The tubes are placed in the incubator and well shaken at intervals so as to allow combination to take place between the kidney substance, the I.B. and C. After a certain time, the tubes are removed, and the fluid centrifugalised from the solid particles of the

emulsion. The fluid from each tube is then added to the corresponding tube in two similar series, each tube of which contains 1 cc. 5% suspension of ox red blood corpuscles sensitised previously with the appropriate dose of haemolytic I.B. The second series of tubes are then placed in the incubator. As soon as a free dose of C. is present in any of the tubes, lysis of the ox red blood corpuscles takes place, and the number of doses of C. taken up in combination with the kidney substance and the nephrotoxic I.B. is apparent. The control series is necessary in case kidney substance by itself leads to the taking up of C. This might be graphically represented thus. Where K = Kidney emulsion, x has its algebraic significance, dotted line represents an hour's interval.

K + x doses of C + I.B. (nephrotoxin)	K + x doses of C.
.....	.....
Add fluid to	Add fluid to
ox R + I.B.	Ox R + I.B.
.....	.....

The first procedure in these experiments was to ascertain the M.H.D. of the C. used.

1 cc. of the suspension of ox red blood corpuscles would be placed in each of a series of 6 tubes. 4 D. of the I.B. rabbit v. ox R. would be added to each tube, that is .06 cc. of I.B. diluted ten times for the sake of convenience.

* The nephrotoxic serum used in these experiments was derived from a rabbit, bled from the carotid ten days after the last of a series of 8 injections of kidney pig kidney. This serum was also employed in injecting guinea pigs, Nos. 16 and 17 (vide Table).

In the description of the experiments, which follows, I.B. is the symbol which represents this nephrotoxic serum.

Several of the experiments were repeated during the week required for the sterilisation of the serum but no appreciable difference in the results was noticed, showing that the Immune Body or Bodies were not affected by the temperature employed.

The serum of the rabbit injected with heated guinea pig kidney emulsion, and already referred to, was also tested. The results obtained by it were practically identical with those hereinafter recorded.

By referring to the paper alluded to "On chemical combination and toxic action as exemplified in haemolytic sera", we find the average dose of guinea pig C. when used with 1.B. v ox against ox red blood corpuscles is .025 cc. Consequently into the .6 tubes a series of doses would be put, e.g.

·01 ·015 ·02 ·026 ·03 ·04 cc.

The 1.B. was always put in before the C. for reasons already given.

Tubes would be then placed in the incubator and frequently shaken. At the end of an hour the exact M.H.D. would be easily seen. M.H.D. of rabbit C. was similarly ascertained.

We shall now proceed to the consideration of the different individual experiments.*

E X P E R I M E N T I.

M.H.D. of guinea pig C. = .02 cc. Two series of 6 tubes each.

Front row: Each tube contained 1 cc. 10% guinea pig kidney emulsion and 0.1 cc. I.B. with the following doses of guinea pig C.

·02 ·04 ·06 ·08 ·1 ·15 cc.

Back row: Each tube contained 1 cc. of the kidney emulsion and the following doses of guinea pig C.

.02 .03 .04 .05 .06 .07 cc.

These tubes were treated as above described and the

fluid added to sensitised ox red blood corpuscles. The result showed a remarkable difference in the two series. In all the tubes without the I.B., lysis had taken place and was complete except in the 1st. in which it was .8.

In all the tubes with I.B. practically no lysis had taken place. In further experiments with guinea pig kidney, the control series was omitted as it was evident that kidney by itself did not lead to the taking up of C. ?

E X P E R I M E N T II.

Another series of tubes was then proceeded with, containing the same amounts of kidney emulsion and I.B. but with the following doses of guinea pig C.

.15 .2 .25 .3 .35 .4 cc.

Practically no lysis was evident in this series either.

E X P E R I M E N T III.

A third series with same amount of kidney emulsion with .01 cc. I.B. and the following doses of guinea pig C.

.02 .04 .06 .08 .1 .15 cc.

The result showed only slight lysis in 5th. and 6th. tubes.

E X P E R I M E N T IV.

A fourth series with kidney emulsion and .001 cc. I.B. and the same doses of guinea pig C. as in the last experiment showed lysis in all, pretty marked in the last three tubes

although in none was it complete, in last about .8 lysis.

EXPERIMENT V.

A fifth series with same doses of kidney emulsion and I.B. and the following amounts of guinea pig C. (the guinea pig C. was found to become weaker M.H.D. - .025 cc.)

.15 .2 .25 .3 .35 .4 cc.

Result showed first tube nearly completely lysed, 2nd. completely so.

EXPERIMENT VI.

A sixth series with kidney emulsion omitted but with .001 cc. I.B. and the following doses of guinea pig C.

·02 ·04 ·06 ·08 ·1 ·15 cc.

Lysis was however found to be practically complete in the 1st. tube. This experiment was undertaken with the idea of proving the absence of an anti-C. in the nephro-toxic serum.

What strikes one in looking over these results is the difference of the action to what is seen in the investigations of the haemolytic Immune bodies whose action is prompt and definite. Here on the other hand lysis begins early in the series but comparatively enormous doses of C. have to be added to cause complete lysis.

When the tubes were left over-night there was always in those tubes in which lysis had been incomplete a marked diffusion of the haemoglobin at the bottom of the tube pointing

to the fact that the C. was not strongly combined.

E X P E R I M E N T VII.

Experiments similar to the preceding were now performed with 10% liver emulsion of guinea pig instead of kidney.

Two series of 6 tubes each.

Front row: Each tube contained 1 cc. 10% guinea pig liver emulsion and .1 cc. I.B. and the following doses of guinea pig C.

.02 .04 .06 .08 .1 .15 cc.

Back row: Each tube contained 1 cc. 10% liver emulsion, no I.B. and guinea pig C. as follows:-

·02 ·03 ·04 ·05 ·06 ·07 cc.

In front row, lysis was incomplete in all; in the last tube lysis amounts to .75. All the controls were lysed completely except the first 2, and the amount of lysis in the first is .9, in the 2nd. as near as possible complete.

E X P E R I M E N T VIII.

As liver substance of guinea pig alone did not lead to the taking up of guinea pig C., further controls were not used. This series contained the same amount of liver emulsion and the same dose of I.B. as above and the following doses of guinea pig C.

.15 .2 .25 .3 .35 . cc.

Result showed well marked lysis in all but not quite

complete in last tube. So we see that, here too, there is a great gap between the beginning of lysis and its completion.

EXPERIMENT IX.

A further series was put up with 1 cc. 10% guinea pig liver emulsion and .001 cc. I.B. with the following doses of guinea pig C.

·02 ·04 ·06 ·08 ·1 ·15 cc.

Lysis was complete in 3rd. tube, almost complete in 2nd.

EXPERIMENT X.

The next experiments were performed with 10% emulsion of brain of guinea pig in the usual way.

Front row: Contains 1 cc. of the brain emulsion .1 cc. of I.B. and the following doses of guinea pig C.

(M.H.D. of guinea pig C. - .025 cc.)

·025 ·05 ·075 ·1 ·125 ·15 cc.

Back row: Similar but without I.B. Result showed complete lysis in all the controls except the 1st. where the amount unlysed was fractional. In front row there was a notable taking up of C., lysis in the last tube of the series being about .5.

EXPERIMENT XI.

Continuation series with .1 cc. I.B. and guinea pig C. as follows:-

·15 ·2 ·25 ·3 ·35 ·4 cc.

E X P E R I M E N T XII.

Another series with .01 cc. I.B. and the following amounts of guinea pig C.

·025 ·05 ·075 ·1 ·125 ·15 cc.

In the last two series lysis was incomplete in all but very well marked in the last tubes.

E X P E R I M E N T XIII.

Yet another series with all the constituents similar except the I.B. of which .001 cc. was now employed.

More variety was met with in the result of this experiment than usual. As much as twelve doses of C. had to be added on one occasion in order to get one dose of C. free, but the usual result was that a free dose over was found after the addition of about 4 doses of C.

E X P E R I M E N T XIV.

1 cc. of 10% emulsion of guinea pig spleen was then employed with .1 cc. I.B. and the following doses of guinea pig C. (M.H.D. = .03 cc.)

·03 ·06 ·09 ·12 ·15 ·18 cc.

With a control series with similar doses of C. and no I.B.

It was found that in neither the control series nor in the series with I.B. was there practically any taking up of C., the only tube unlysed being the first tube in the series

with I.B. and the amount unlysed was merely fractional.

EXPERIMENT XV.

Front row: Contained 1 cc. 10% emulsion of rabbit kidney, .001 I.B. and the following doses of guinea pig C. (M.H.D. - .03 cc.)

.03 .06 .09 .12 .15 .18 cc.

Back row: Similar to above but without I.B. The result showed that rabbit kidney alone led to the taking up of C., for it was only in the 4th. tube of the control series that lysis took place. The result in the front row was precisely similar. Accordingly it is evident that in the rabbits' kidney there do not exist receptors for the I.B. under investigation.

EXPERIMENT XVI.

Two series of tubes, similar to those employed in last experiment, except that rabbit C. was used instead of guinea pig C., were put up. The dose of rabbit C. corresponded to those of guinea pig C. thus (M.H.D. - .1 cc.)

.1 .2 .3 .4 .5 .6 cc.

Lysis was incomplete in the first 4 tubes, complete in the 5th. of both series.

The rabbit kidney therefore appears to cause the taking up of rabbit C. but the I.B. seems to be inert in this respect in the presence of rabbit kidney.

E X P E R I M E N T XVII.

A like result to that obtained in the last two experiments was got when rabbit liver emulsion was employed instead of rabbit kidney emulsion with the same amount of I.B. and similar doses either of rabbit or guinea pig C.

E X P E R I M E N T XVIII.

Experiments were then performed with similar amounts of the I.B., viz: .001 cc. with emulsions of guinea pig kidney, liver and brain, but instead of using guinea pig C. as before, rabbit C. was employed.

In none of these experiments was anything approaching complete lysis obtained, even after the addition of very large doses of rabbit C.

E X P E R I M E N T XIX.

A 10% dilution of the I.B. was then left in contact with washed guinea pig red corpuscles at 0°C. for two hours and then recovered by centrifugalising. The results obtained by the use of the treated I.B. with guinea pig kidney emulsion and guinea pig C. were exactly similar to those got with the untreated I.B. The inference of course is that the taking up of C. was not due to the activity of the haemolytic I.B.

E X P E R I M E N T XX.

In order to find out whether it was the same I.B. which led to the taking up of C. both in the presence of kidney and

brain substance, the following experiment was performed. 1 cc. of 10% dilution of I.B. was allowed to remain in contact for 2 hours with the deposit obtained by centrifugalising 5 cc of 10% guinea pig brain emulsion, and the tube was frequently shaken. After 2 hours I.B. was recovered by centrifugalising and .001 cc. of it added to each of 6 tubes containing 1 cc. of 10% guinea pig kidney emulsion and the following doses of guinea pig C. (M.H.D. = .03 cc.)

The result showed a free dose present when 5 doses had been added whereas, with the untreated I.B., a free dose was only obtained as a rule after the addition of 7 doses.

Though this result cannot be described as convincing it points in the direction of the I.B. being identical in the two cases.

E X P E R I M E N T XXI.

The following experiment was performed in order to discover what affinity the I.B. possessed for guinea pig kidney substance in the absence of C.

6 tubes, each containing 1 cc. 10% guinea pig kidney emulsion and .001 cc. I.B. were placed in the incubator for 2 hours and frequently shaken. The contents of the tubes were then centrifugalised and washed, the fluid and the washings from each tube being added to the corresponding tube in a similar series, each tube of which contained 1 cc. of the guinea pig kidney emulsion only. Guinea pig C. was then added in the

following doses (M.H.D. = .02 cc.):-

.02 .04 .06 .08 .1 .15 cc.

After treatment with sensitised ox red corpuscles the result showed practically complete lysis in the 6th. tube, lysis incomplete in the others.

The deposit in the first six tubes left after washing and centrifugalising had the same ascending doses of C. as above added and the final result showed that the amount of lysis in this series was practically the same as that obtained in the preceding series.

As this result, together with the results obtained when rabbit C. was used, pointed to the possible existence of a natural I.B. in normal rabbit serum it was deemed advisable to investigate this.

E X P E R I M E N T XXII.

On treating 1 cc. of the guinea pig kidney emulsion with .001 cc. I.B. of heated normal rabbit serum and ascending doses of guinea pig C. somewhat variable results were obtained with different sera, but, on an average, 3 or 4 doses of C. were taken up. On keeping the tubes overnight, well marked diffusion of the haemoglobin was apparent at the bottom of the incompletely lysed tubes.

E X P E R I M E N T XXIII.

6 tubes each with 1 cc. guinea pig emulsion and .01 cc.

of normal rabbit serum, heated for one hour to 56°C., were allowed to stand in the incubator at 37°C. for one hour and frequently shakem. The contents were then centrifugalised and washed and the fluid added to another similar series of tubes containing 1 cc. of guinea pig kidney emulsion and ascending doses of guinea pig C. as follows (M.H.D. = .02 cc.)

·02 ·04 ·06 ·08 ·1 ·15 cc.

The result showed that lysis was incomplete in all and the amount of lysis in the various tubes was practically identical with that in a similar series of tubes with the same dose of untreated heated normal rabbit serum.

When the deposit of the first 6 tubes after washing and centrifugalisation was treated with ascending doses of guinea pig C. similar to those above enumerated, a marked difference was evident. Complete lysis in this series occurred quite early. It was therefore evident that the natural I.B. against guinea pig kidney substance, which exists in normal rabbit serum, cannot be separated to any considerable extent by placing it in contact with guinea pig kidney emulsion alone. The results of Experiment XXI, however, go to prove that this natural I.B. is not the only I.B. present against the guinea pig kidney in nephrotoxic serum.

E X P E R I M E N T XXIV.

Quantities of guinea pig kidney emulsion were treated with I.B. and C. The amount of C. added was very considerably

less than what ought to have been taken up, as calculated from Experiments IV and V. This mixture was kept in the incubator for over 2 hours and frequently agitated. The fluid was then centrifugalised off and tested for C. which could invariably be separated. Numerous experiments were made with the kidney emulsion and I.B. in great excess but, without exception, C. could be dissociated.

E X P E R I M E N T XXV.

Four series of 6 tubes each.

Each tube contained 1 cc. of guinea pig kidney emulsion and

1st. Series had .0005 cc. I.B. in each tube.
2nd. " " .001 " " " " "
3rd. " " .002 " " " " "
4th. " " .005 " " " " "

Each series contained the following ascending doses of guinea pig C. (M.H.D. = .02 cc.)

·02 ·04 ·06 ·08 ·1 ·15 cc.

Result after being treated with sensitised ox red blood corpuscles showed that in the first series lysis was as near as possible complete in the 6th. tube. The 2nd. series showed lysis in the 6th. tube, approximately equal to that in the 5th. tube of the 1st series, and again, the lysis in No.6 of the 3rd. series was equal to that in No.5 of the 2nd. series.

In the 4th. series comparatively little lysis was evident, except in the 5th. and 6th. tubes and the amount of lysis in No.6 was about equal to that in No.4 of the preceding series.

These results show a well marked difference from what one would expect in the case of haemolytic Immune bodies where the amount of C. taken up corresponds within limits to the dose of the haemolytic I.B. Here we see that doubling the dose of I.B., which causes the taking up of 5 doses of C. only leads to the taking up of approximately another dose of C.

S U M M A R Y

of Results shown by the Experiments on the Taking up of

C O M P L E M E N T

by

N E P H R O T O X I C S E R U M.

I. The presence of a strong natural I.B. against guinea pig kidney substance in normal rabbit serum, which unfortunately, proved a very disturbing influence in the investigation; the probable existence also of natural Immune Bodies against guinea pig liver and brain in normal rabbit serum.

II. The fact that this natural I.B. evinces only slight affinity for guinea pig kidney substance in the absence of C.

III. The presence of an I.B. generated in the serum of a

rabbit by the injection of guinea pig kidney emulsion. This I.B. does not seem to differ much from the natural I.B. in the strength of its combining properties except that it can apparently be separated by kidney substance in the absence of C.

IV. The presence in large number of receptors in the guinea pig brain and liver identical with those in guinea pig kidney and their practical absence in spleen.

V. The marked difference in the properties of these Immune Bodies as compared with the haemolytic Immune Bodies evidenced by -

(a) The early commencement of lysis and the delay in its completion till many more doses of C. had been added possibly indicating the small size of the molecule.

(b) The fact that doubling the dose of I.B. led to the taking up of nothing approaching double the dose of C.

(c) The loose state of combination in which the C. existed.

.....oOo.....

J. WOTHERSPOON.