CYTOTOXIC SERUM PRODUCED BY THE INJECTION OF NUCLEOPROTEIDS.

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The phenomena of the production of antisera by the introduction of foreign proteids into the circulating medium of an animal are now so well known as to need no introduction. Such antisera have made possible the biological differentiation of proteids, and have furthermore indicated that the proteids obtained from different organs of the same animal species have individualities which, possibly, may be associated with the functions of those organs. The cytotoxic sera were among the first to be produced, and, as early as 1899, von Dungern suggested the possibility of using an epitheliolysin in the destruction of unrecognizable cancer cells that may remain in the tissues after excision by the surgeon. Since that time a considerable amount of work has been done, and from the claims made in the literature one is led to believe that cytotoxins have been produced for nearly all the various tissues of the body.

In nearly all of this work crushed tissues mixed with blood have been injected into some animal at intervals for varying lengths of time and the antiserum thus produced has been used to produce cytotoxic effect upon some member of the original animal species. A serum made in this way is not specific—that is, it causes changes in other organs than the one from which it was made. It invariably shows haemolytic and haemagglutinative properties, which is what should be expected when one considers that blood has been used in making the antiserum. The work which Pearce has done along this line indicates that much of the effect produced by such antisera may be explained by their haemolytic and haemagglutinative effects. In order to
obtain a serum more nearly specific, Pearce has washed out the organs by perfusing through the blood-vessels with salt solution, but he finds invariably that antisera produced by such washed organs have strong hæmolytic and hæmagglutinative properties in vitro; however, they do not always show the same behavior in vivo. Nevertheless, he finds that sera prepared in this way from pancreas and adrenal may cause degenerative changes in the liver and kidney. He says: “Some of these cytotoxic sera have no effect upon organs for which they are supposed to have a morphological affinity, but exert a powerful lytic action on other cells,” and he concludes that with the exception of the nephrotoxin “the various cytotoxins studied have no specific action in the morphological sense.” Pearce is fully justified in drawing these conclusions from the results of his experiments, and his work must be regarded as the most complete and thorough which has yet been done on the specificity of the cytotoxins. It is evident that to add to the subject as he left it requires some new method of attack.

The cytotoxic sera which Pearce made have been produced by the injection of washed organs, and the antibodies thus formed have been developed for the proteid constituents of the cells used for immunization. Without doubt, even in the washed organs there are proteids which closely resemble those found in the blood, together with others that have been built up into complexes characteristic of the particular group of cells used. There must be a physico-chemical basis in the liver, pancreas, and kidney cells which enables them to perform their varying functions. Some portion of the proteid constituents of the tissue must be characteristic for that organ. When we consider that the precipitin or biological test will distinguish between two proteids as closely related as serum globulin from the cat and from the dog, it seems reasonable to believe that two organs such as the kidney and pancreas must contain proteids that are sufficiently characteristic for those organs to be distinguished by one of the biological methods now in use for differentiation. The writer has undertaken the present work in the hope of being able to develop from certain of the proteid constituents of
various organs, cytotoxic sera having a higher degree of specificity than can be obtained with the full cells.

The accepted biological principle at the present time is that the nucleus is the most important morphological and physiological portion of the cell; and hence it follows that its chemical constituents must be those most necessary for nutrition. In accordance with this idea the writer has isolated nucleoproteids from various tissues and has produced antisera by their injection. The present paper deals with the methods of producing, and some of the results obtained with, such antisera.

On studying the literature one finds that few attempts have been made to obtain antibodies to nucleoproteids, and these attempts have been in nearly every case negative. Ide in discussing this point in 1902 said that from such negative results "one fears that it is very difficult to form antibodies against the most important substances of bacilli." His pupil Malengreau found it impossible to demonstrate an antibody after giving a rabbit nine large doses of thymus nucleohiston. He quotes Pick as being unable to show an antibody after giving ten doses of nuclein from the colon bacillus. More recently Galeotti has claimed to obtain an antiserum having considerable immunizing power for the anthrax bacillus by the injection of its nucleoproteids. Levene has made some experiments on the production of haemolytic sera from different constituents of erythrocytes which favor the belief that nucleoproteids may be effective in forming antibodies. The following substances were used by him: crystallized haemoglobin, aqueous extract of the red cells, residue from the alcohol-ether extraction, neutral sodium chloride extract, sodium bicarbonate extract. The results were all negative except for the bicarbonate extract, which gave a serum having a strong haemolytic power. The filtrates from the partial digestion of erythrocytes by pepsin-hydrochloric acid and by a sodium carbonate-trypsin mixture were used. The latter filtrate gave a serum having approximately the same haemolytic effect as the sodium carbonate extract; the filtrate from the pepsin digestion gave a very much weaker serum. From the well-known solubilities and behavior of the nucleoproteids on digestion one
can readily see that those extracts which gave the active serum must have contained compounds of nucleic acid.

During the progress of the study which forms the basis for this paper, Bierry and Mayer reported the production of cytotoxic sera by the injection of nucleoproteids from the liver and kidney. This work has, it appears, been reported at the meetings of various societies in France, but a complete report has, to the writer's knowledge, not appeared, so that it is difficult to say how closely it resembles his own. Comments will be made on the work of Bierry and Mayer at appropriate places in this paper.

The nucleoproteids are, as is well known, compounds of nucleic acid with proteid; and when the name was first applied it was supposed that they were the proteids of the cell nuclei. It is by no means certain, however, that the usual method of preparation gives as a product those proteid-complexes which are characteristic of the cell nuclei. There is no doubt that the nucleic acid portion of the compound exists chiefly in the nucleus, but precise proof is lacking as to whether the proteid part of the compound comes from the nucleus or from the general cell protoplasm. The problem is not a simple one. Kossel believes that what we recognize as chromatin is nucleic acid combined with more or less proteid, together with some free nucleic acid. The smaller the proteid content of the combination, the more closely does it resemble nucleic acid; and we have reason to believe that the proteid content in the chromatin of the same cell nucleus may be a variable one. We know that nucleic acids can combine with varying quantities of proteids, and the character of these compounds varies with the amount of proteid thus combined. Pepsin digestion breaks off a considerable amount of proteid from the nucleoproteids, leaving the so-called nucleins, which are bodies richer in phosphorus and resistant to pepsin digestion, but still containing some proteid. Osborne believes that the combination of nucleic acid with proteids forms a salt, the character of which is given by the proteid, and that the conditions governing this combination are not radically different from those determining the combination of inorganic acids and
bases. Quite a variety of such salts or protein nucleates differing in the amount of proteid which they contain can be obtained from the same tissue. The nucleic acid probably exists in the cell combined with proteid, but we simply have no proof that the compound which we isolate by the ordinary methods is identical with the one preformed in the cell. From the extensive studies which have been made of the chemistry of the nucleic acids, we know that those obtained from the kidney, liver, spleen, and pancreas show considerable variations, so that, chemically at least, the nucleoproteid which we isolate from any given organ is not precisely identical with that from any other organ. We may reasonably expect, therefore, to obtain sera having a higher degree of specificity by inoculating into some animal of an alien species nucleoproteids derived from different organs.

Preparation of the Nucleoproteids.—The usual method was followed in isolating the nucleoproteids used for preparing the antisera described in these experiments. The organs were sliced thin and placed in jars of running water to remove as much blood as possible. Only the parenchyma of the organs was used, omitting the large blood-vessels of the liver and kidney, and after thorough washing the slices were ground to a fine pulp in a hashing machine, two volumes of water added, and the mass extracted in a cold room for twenty-four hours, chloroform being employed to prevent bacterial developments. In the later work physiological salt solution was substituted for water. After straining through cloth, the fine tissue fragments in the filtrate were removed by centrifugation. The powerful centrifuge used for this purpose gave a perfectly clear extract from which the nucleoproteids were precipitated by the addition of acetic acid up to a frank acid reaction. The precipitate was allowed to settle in large hydrometer jars in a cold room for twenty-four hours, when a large part of the supernatant fluid could be siphoned off. The precipitate was now centrifugated and repeatedly washed with physiological salt solution until the wash-water was perfectly clear. The washing was very readily accomplished by using the centrifuge, for which purpose it was found best not to run the machine too long so as to pack the precipitate too hard.
in the tubes. After thorough washing, the precipitate was sus-
pended in normal salt solution and a little sodium carbonate
added to dissolve it. Acetic acid was next added to acid re-
action and the resulting precipitate again thoroughly washed.
The process of dissolving, re-precipitating, and washing was
again repeated, the final precipitate being the substance used for
injection into animals. In some cases this final precipitate was
dissolved before inoculation in 0.5 per cent. sodium bicarbonate
solution and kept in the refrigerator. A little chloroform being
added, it was noticed invariably that a fine deposit settled out
after some weeks, a change probably due, in part, to the well-
known coagulating action of chloroform. The temperature of
the refrigerator was always very near 0° C. and the tubes were
occasionally congealed. In no case has such a solution been
kept for more than six weeks. A considerable part of the proteid
prepared for the work has been dried at 40° C. in the apparatus
already described elsewhere. This dried proteid has been found
to dissolve readily in a 0.5 per cent. sodium bicarbonate solution,
and the serum made from it is precisely similar in its action to
that made from the proteid kept in solution.

Those who have worked with nucleoproteids will appreciate
the difficulty of washing and filtering these substances; and
hence it is the writer's belief that the powerful centrifuge used
in his experiments has enabled him to get a pure product with
less change due to autolysis than has been the case with previous
writers. The whole process as here given can be completed in
thirty hours from the time the fresh organs are received, during
the larger part of which time the tissues are in the refrigerator.

Properties of the Nucleoproteids.—The fresh products prepared
in the manner described were found to exhibit the property,
characteristic for nucleoproteids, of causing intravascular clotting
of the blood when injected intravenously. Determinations of
phosphorus were made on two samples of each proteid prepared
at different times. The average results are given in the following
table:

<table>
<thead>
<tr>
<th>Nucleoproteid from</th>
<th>Phosphorus</th>
</tr>
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<tbody>
<tr>
<td>liver</td>
<td>2.3%</td>
</tr>
<tr>
<td>kidney</td>
<td>5.2%</td>
</tr>
<tr>
<td>pancreas</td>
<td>5.4%</td>
</tr>
</tbody>
</table>
From these figures it is evident that if one follows the scheme of Kossel and Lilienfeld, the final product might more properly be called a nuclein. The phosphorus percentages are much higher than are usually found for nucleoproteids. The method of preparation has evidently been such as to split off a large part of the proteid from its combination with nucleic acid. The amount of proteid which combines with the acid when the two are precipitated from a common solution varies with the concentration of the solution. The first precipitate caused by the addition of acetic acid does not completely dissolve on making the suspension alkaline, and in the succeeding operations of precipitation and washing, proteid is lost and the percentage of phosphorus is increased. This behavior has been well described by Bang. The final product is, therefore, in its phosphorus content more nearly comparable with the so-called nucleins than with the nucleoproteids, though it would seem that the essential difference between the two substances consists in the quantity of proteid combined with the nucleic acid. The fact that these bodies are so rich in nucleic acid leads one to believe that the production of antibodies may perhaps be caused as much by the acid portion of the compound as by the proteid.

The color reactions which these substances gave indicate also that only a small amount of proteid has remained in combination with the nucleic acid. The Millon, xanthoproteic, and biuret tests gave only feeble reactions. The Adamkiewicz test gave a good reaction; the Molisch, a very strong reaction.

Preparation of the Serum.—Rabbits only were used in making the serum, and because of the intravascular clotting caused by intravenous injection of nucleoproteids the solutions were always injected into the peritoneal cavity. White rabbits were chosen for the first and gray ones for the later experiments, but no difference could be detected in their reaction to the injections.*

The symptoms caused by the injections of nucleoproteids into the white and gray rabbits differed in no respect as far as could

* This difference in the clotting of the blood of white and gray rabbits has never been explained. Halliburton has found that artificial colloids when introduced rapidly cause intravascular clotting in gray rabbits, and, like the nucleoproteids, are without effect on albino rabbits.
be observed. Five injections were made at intervals of six days with gradually increasing quantities of proteid, the final injection being in many cases 0.2 gram, a quantity sufficient to kill several animals if introduced directly into the circulation. Eight days after the last injection the animals were bled. It was found very much better to exsanguinate the animal from the carotid at once than to bleed successively from the ear, because the animals do not stand repeated bleeding and injection well, and furnish a poor grade of serum under the latter circumstances.

The first serum tested was made by the injection of calves’ thymus nucleo-histon; and this product proved to be a particularly good one. The precipitin reaction, which was used as a means of determining the presence of an antibody, was positive, a fact which stands in contradiction to the findings reported by Ide. I was, however, able, by using the antiserum in fairly strong concentration, say 1 to 2 or 1 to 5, upon a solution of the nucleohiston, to obtain a definite reaction. The nucleohiston used for this purpose was free from the ordinary nucleoproteid of the thymus gland. An interesting result was obtained with this antiserum by testing it with nucleoproteid from the calves’ thymus, for the latter proteid gave in the same concentrations a very much better precipitin reaction than did nucleohiston. This behavior should not be considered surprising. We know that nucleohiston has quite different solubilities* from nucleoproteid; we are also not justified in deciding on the basis of the precipitin reaction alone whether an antibody has been formed. The common statements that albumoses and peptones form no antibodies are based upon precipitin experiments; and it seems not unreasonable to offer the conjecture that if the inoculations were made with peptone, the mother proteid of the peptone might yield evidence of the presence of an antibody in giving this reaction. The formation of hæmolytic and agglutinative sera by the injection of bile and urine points in this direction, for the bile contains cleavage products only of the corpuscles and yet it

* In trying precipitin reactions with nucleohiston the precaution must be taken to make all the dilutions of serum with 2 per cent. sodium chloride solution, for it is precipitated by physiological salt solution.
produces an agglutinative serum which is scarcely weaker than that yielded by the blood itself.

Upon the basis of the precipitin reaction as the test, the statement can be made that definite antisera have been produced for the nucleoproteids of the liver, kidney, and pancreas of the dog. With the stronger concentrations of antiserum, that is, 1 to 2 and 1 to 5, the reaction shows clearly in one hour, while the more dilute solution requires frequently that the tubes be left in the incubator overnight. The difference in the reaction between immune and normal serum was perfectly sharp and definite.

The antiserum made in this way did not give a precipitin reaction with the blood-serum of the animal which served as a source of the nucleoproteids. That is to say, the antiserum made from kidney nucleoprotein of the dog did not give a precipitin reaction with normal dog serum. It may be remarked that more striking results were obtained by using freshly prepared proteid for the precipitin reactions; but for making the antiserum, as already stated, no difference was observed whether the dried or the fresh proteid was used. In one case the proteid used for making the inoculations was dried in an oven, which unintentionally reached a temperature of 53° C. for some hours. The product, which was ground very fine in a mortar, did not dissolve in 0.5 per cent. sodium bicarbonate solution and upon injection did not yield an active serum. In the other cases a new oven was used, the temperature of which did not reach above 40°, and no difficulty was experienced in dissolving the proteid.

The writer has attempted to demonstrate cytolytic action of these sera following the method adopted by Flexner and Noguchi in their study of snake venom, but without results. Definite agglutinating action of the serum on suspensions of finely pulped gland was, however, observed. The suspension used in testing this agglutinating action was made by grinding fresh gland finely in a porcelain mortar, mixing with physiological salt solution, and straining through gauze. When the filtrate so obtained is centrifugalized it is found that the sediment is separated into layers, the finer portions being at the top. This finer portion is readily poured off from the coarser fragments below, and
when thoroughly shaken with salt solution the suspension obtained settles very slowly if left to itself or when normal serum is added; immune serum, however, causes it to flock together and settle out much more rapidly. This agglutinating action is, except in strong concentrations, 1 to 1, or 1 to 2, specific. In dilutions of 1 to 4 the flocking can be distinctly seen in one minute.

The question as to whether the serum prepared in the manner described has haemagglutinative and haemolytic properties is one of considerable importance in deciding the manner of its action, particularly in view of the statements already made by Pearce. All the antisera thus far made are markedly haemagglutinative and haemolytic in vitro in dilutions up to one to five, but in dilutions of one to twenty or above, there is no difference to be detected between the immune and the normal serum. In no case has haemoglobinuria followed the administration of a serum to an animal. In such quantities as would be used as a therapeutic dose, no blood effects in vitro different from those produced by normal serum were to be seen. The writer is, therefore, unwilling to believe that the toxic effect of this serum, when employed in the living body in such concentrations, as were used in these experiments, depends upon the haemolytic and haemagglutinative properties which it shows in vitro.

The most interesting results obtained with the immune sera were caused by toxic action upon the living animal. The toxicity being determined, the next step was to ascertain whether the serum is specific and could produce lesions in one organ without affecting injuriously other organs of the body. Various cytotoxins were studied in order to determine this question; but in this communication the results obtained by the injection of liver, kidney, and pancreas nucleoprotein cytotoxins only will be considered. The animals chosen for the inoculations were small adult fox-terriers, which had been kept under observation for some time previous to inoculation; the urine, moreover, was tested to make sure that no functional derangement existed.

The serum was given in some cases intraperitoneally in small and repeated doses, and in others intravenously in one large dose.
The small doses consisted of injections of from one and one half to two cubic centimeters in dogs weighing from five to seven kilos; the large doses consisted uniformly of two cubic centimeters per kilo. The injection of the serum, whether given one way or the other, is followed by a complex of symptoms which may be called a reaction. The animal vomits, and often defecates and urinates; the temperature may rise one degree in the course of the first hour, and there is marked chill followed by a period of sleep. Similar effects, though of slight degree, are produced by normal serum. They cannot be ascribed, I think, to immediate injury of a specific organ, but probably are nervous in origin. No peritoneal lesion was found in any case.

Effects on Kidney, Liver, and Pancreas.—The nephrotoxin made from nucleoprotein causes an acute degeneration of the kidney epithelium, but I have thus far failed to find that it causes those degenerative changes in other organs that have been noted by previous writers as a result of the injection of nephrotoxins. There is, however, almost complete agreement among recent investigators of the subject that of all the cytotoxins nephrotoxin comes nearest to exerting specific action. Pearce says that when prepared from washed kidneys the serum is specific for the kidney and does not affect other organs, "with the exception of the liver, which in some animals exhibited extensive granular degeneration." Woltmann, who evidently made his nephrotoxin from washed kidney, says: "The nephrotoxin perhaps comes nearest to being specific since macroscopically and microscopically marked congestion of the medullary portion of the kidney and pronounced cloudy swelling of the cortex were observed. Similar changes of the same severity were found in no other serum, and it appears that the renal toxin had a specific, perhaps merely irritating, action on the kidney itself."

The following experiment illustrates the action of my serum. A fox-terrier bitch of six kilos body-weight was kept in a cage for three days for observation. The animal was in a perfectly healthy condition as far as could be determined, and examination of the urine showed the kidneys to be sound. On April 8, twelve cubic centimeters of nephrotoxic serum were injected
into the femoral vein, using morphine and cocaine as anesthetics. The animal showed the usual behavior toward the serum. The urine was collected daily, but no albumin appeared until April 12, when a trace was found by using the acetic acid and potassium ferrocyanide test. The albumin increased in quantity daily, the animal remaining normal in behavior until April 14. On April 15 she ate very little and later in the day vomited; the following day she refused to eat. The urine on this day solidified in the tube when heated and analyses showed that 53 per cent. of the total nitrogen excreted in the urine was in the form of albumin. Abundant granular and hyaline casts also were found. The animal was very sick, the rectal temperature being 98°F. in contrast to 102°F. previous to the inoculation. On April 17, the rectal temperature had fallen to 95°F., and since it was evident that the animal would die shortly, chloroform was administered. The autopsy showed the liver to be slightly congested, but otherwise normal; spleen, normal; kidneys, swollen and pale yellow with punctate hemorrhages and obscured markings. About fifty cubic centimeters of clear, straw-colored fluid were in the peritoneal cavity.

The histological examination* of the tissues showed the following conditions: The liver shows a few vacuoles uniformly distributed in the cells and slight congestion of blood-vessels. No marked degeneration and no necrosis occur. The impression given by the section is that the organ is in a normal condition with no pathological change. The pancreas, spleen, and lymph nodes are normal.

The kidneys are very much congested, and on microscopical examination the blood appears to be agglutinated, though this appearance may be, and probably is, an artifact. Much cast matter exists in the tubules of the cortex and medulla, and many tubules are filled with blood. The tubules in places are considerably dilated, especially where there are casts. The tubular cells are separated by spaces, somewhat shrunken, often split

* The writer is very much indebted to Dr. Martha Tracy for mounting the tissues for histological examination, and to Dr. James Ewing for the description of the histology cited in this paper.
lengthwise, and eroded. The nuclei stain poorly and some mitotic figures are seen. The glomeruli are normal. Five additional dogs to which this serum was given showed practically the same lesions.

In some cases from 350 to 500 cubic centimeters of bloody serum were found in the peritoneal cavity at the autopsy. If the animal was killed before the process had reached such extreme conditions the peritoneal cavity was free from fluid. There seems to be no doubt from these findings that this nephrotoxin sets up an acute degeneration of the kidney tissue, from which certain secondary changes in the liver might possibly be expected to result, but there was no evidence in these experiments that any of the lesions were caused by haemolytic or haemagglutinative properties in the serum.

Regarding hepatic toxin, Pearce states that after its injection "the only change observed was an increased granulation of the liver cells with occasionally slight fatty transformation," while its chief action was to produce more or less extensive fatty metamorphosis of the kidney. Similar conclusions on the nonspecificity of hepatotoxin were reached by Woltmann.

With the serum made by injecting liver nucleoproteids I have produced very serious lesions of the liver, apparently without causing injury to other organs. I shall give an illustrative case: a fox-terrier bitch of 3920 grams body-weight was kept under observation for six days previous to inoculation. The urine was normal in every respect. On May 2 intraperitoneal inoculations of hepatotoxin were begun. These were of two cubic centimeters each, and were made on the following dates: May 2, 3, 5, 10, 12, 13. The animal weighed 4125 grams on May 15 and for some days had been quite sick, but its condition was not so serious as that of other animals undergoing similar treatment, and was far less serious than the condition of the animal described in which the nephrotoxin had been injected. There was no indication at this time of severe hepatic lesions. At no time had the urine showed the least trace of albumin or sugar. The animal was killed by chloroform on May 15. The autopsy made one half hour after death showed the kidneys, spleen, and
pancreas to be normal. There was no fluid in the peritoneum, and all the organs appeared healthy except the liver, which was curiously mottled and showed an evident fatty condition. The following is a brief report of the histological examination of the tissues made by Dr. Ewing:

The liver, which is altered pathologically, shows areas one fourth to one half the size of the lobules in which the liver cells are invisible or missing, or the tissue is substituted by a mixture of necrotic liver cells, leucocytes, red blood cells, and detritus. Many of these areas appear to surround central veins. The liver cells in general show intense granular and fatty degeneration, and congestion, most marked in and about the necrotic foci where the red cells appear to be more or less fused. The fusion of red corpuscles may be a post-mortem change. About the hepatic veins round-cell infiltration exists.

The kidney shows nothing worthy of note except an accumulation of large round cells about the glomeruli. The tubules, normal in size and not dilated, contain a slight granular coagulum.

The spleen is normal.

It is evident, therefore, that this serum has caused a profound change in the liver, but has not damaged other organs. I do not think it probable that the specific action on the liver is to be attributed to the hemolytic and hemagglutinative effects of the serum. The difference in the action of this serum and that of Pearce and of Woltmann is undoubtedly to be explained by the fact that in its preparation it has been possible to eliminate from the tissue injected a large amount of extraneous material, as, for example, bile, which previously was included and which undoubtedly influenced the qualities of the serum.

The next experiment was made with a nucleoproteid anti-pancreatic serum. Pearce has found that an antiserum from the pancreas "did not produce hæmoglobinuria but a persistent albuminuria with fatty degeneration of the kidney. The strongest dose given caused also well-marked focal necroses of the liver with evidence of red-blood-cell thrombosis. No change in the pancreas cells could be demonstrated."

The following example shows the action of the serum made
from pancreas nucleoproteids: A white fox-terrier bitch was kept under observation for some days to be sure that she was in healthy condition. On May 25, using morphine and cocaine as anaesthetics, 15 cubic centimeters of the pancreas serum were injected into the femoral vein. The animal soon recovered and lived in a perfectly healthy condition until June 3, when she refused food. On the next day, June 4, she had marked indigestion with diarrhoea, which steadily grew worse, and on June 5 she was so badly off that she was chloroformed. At no time had there been any albumin in the urine, but beginning on May 30 the urine gave a partial reduction of Fehling's solution, which condition continued till the end of the experiment. By a partial reduction I mean the formation of an abundant green precipitate, but not the typical red deposit of cuprous oxide. The autopsy showed the internal organs to be in a normal condition except the pancreas, which was softer and redder than usual. The stomach and intestine contained a small amount of black, slimy material similar to that vomited and defecated during the preceding 48 hours. The urine taken from the bladder at the autopsy contained no albumin, but gave the usual green precipitate with Fehling's solution. The histological examination of the organs by Dr. Ewing showed the liver, kidney, and spleen to have been quite normal. Of the pancreas it must, I regret, be recorded that the fixation of the tissues was imperfect, thus making the study unsatisfactory. No explanation can at present be offered for this failure.

The findings in this case are interesting, in spite of their inconclusiveness, in showing that a serum made with pancreatic nucleoproteid may act injuriously upon the pancreas without, at the same time, disturbing other organs. Another dog to which the pancreatic serum was given showed similar symptoms, and there was a similar difficulty in obtaining satisfactory sections of tissue for examination. In all of this work the Müller-formol fixing fluid has been used.

A point of considerable interest is seen in the latent period between the administration of the serum and the first appearance of the symptoms indicating action on some particular organ.
This period varies from four to ten days, depending upon the manner of giving the serum and to some extent upon the animal. When the nephrotoxin has been given intravenously albumin appears in the urine in most cases on the fourth day, but at that time it appears as a mere trace only and requires a delicate method to bring it out. The quantity increases rapidly until the eighth or the ninth day, by which time the kidneys have been so badly injured as to place the animal beyond recovery. If the serum is given in small daily doses intraperitoneally, the effects are delayed for some days, apparently until there has been a sufficient accumulation of the toxin. When injected into the peritoneum, the quantity of serum needed to produce toxic effects is greater than with intravenous injection, and should the treatment be stopped after two or three doses have been given intraperitoneally the animal may show no lesions at all, which would indicate that the organism possesses some degree of resistance to the toxin. What has been said of the nephrotoxin holds equally true for the liver and pancreas toxins.

The results which are given in this paper are based upon five experiments in which nephrotoxin, three in which hepatotoxin, and two in which pancreas antiserum were administered. They provide, it seems to me, strong indications that cytolytic sera having a high degree of specificity can be prepared from the nucleoproteids of tissues. Such a thing as absolute specificity under all conditions has never been demonstrated and probably never will be, but it does, nevertheless, seem possible, in the light of these results, to make a serum which will act primarily on a given organ. The demand that a serum, in order to be called specific, shall limit its injury to one organ only is unreasonable on the basis of the well known altruistic relations of the viscera.

Bierry and Mayer note the following in regard to the specificity of the serum which they made from nucleoproteids of the kidney: “L'examen histologique fait par M. Aug. Pettit a montré que les reins présentent des lesions considérables et systematisées affectant les glomérules et les cellules des tubes sécrétaires, mais en même temps d'autre organs (foie, centres
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nerveux) sont atteints. L'effet du serum nephrotoxique n'est donc pas rigoureusement spécifique.” These statements were not seen by the writer until after much of his work had been done. The results that have been obtained thus far with the nephrotoxin have not confirmed the work of Bierry and Mayer in this respect. The liver has in some cases been slightly congested as in the case cited, but no degenerations occurred. It has been the experience of nearly all investigators that the nephrotoxin is more specific than the hepatotoxin, but Bierry and Mayer have found the reverse to be true. In the following words they describe the lesions produced by their hepatotoxin made from nucleoproteids of the liver: “L'action des injections se traduit par l'apparition de lésions histologiques dont l'examen a été publié ici même par M. Auguste Pettit et l'un de nous. Ces lésions consistent en dégénérances graisseuse, vacuolaire et granuleuse du cytoplasma des cellules hépatiques. Les autres organes (rein, pancréas) ne sont pas lésés.” In the absence of details it is impossible to do more than quote their statements without trying to effect a reconciliation.

In much of the work on cytotoxins the chemical side of the subject has been lost in the maze of pathological considerations involved. The cell has been treated as though it was a definite chemical molecule; but it is my opinion that the time has now arrived when we can well afford to cease dealing with such complex factors as the intact cell and try to find out the details of this interesting matter. The work is being continued in the hope of answering some of the many questions which it has suggested, and I hope to contribute further facts regarding the manner of the action of these sera, together with some practical applications which they may have.

It is a pleasure to record my appreciation of the kindness of Dr. Geo. A. Wallace and Dr. R. A. Hatcher, who furnished me with most of the organs used in this work.

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17 and 18 refer to the same article as No. 15.

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