THE RETENTION OF FOREIGN PROTEIN BY THE KIDNEY.

A Study in Anaphylaxis.*

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This investigation was undertaken for the purpose of obtaining exact information in regard to two points of prime importance in connection with the physiology and the pathology of the kidney: (1) does a foreign protein tend to accumulate in the kidney; and (2) if so, is this tendency greater in the presence of a nephritis? The first problem is of importance mainly in connection with the etiology of nephritis; the second, in connection with the prognosis, treatment, and dietetics of nephritis.

It is obvious that the acute nephritides associated with acute infectious diseases (scarlet fever, diphtheria, etc.) are due either to the poison elaborated by the causal microörganism or to the products of tissue catabolism. Soluble bacterial and protozoan poisons as well as the poisonous products of tissue destruction act on the kidney cells, either in a specific selective way or through irritation as the result of long continued contact during a process of gradual elimination.

The latter has quite generally been assumed to be the correct explanation, but this assumption has no experimental or other data as a basis. Because the glomerulus is the usual seat of the renal lesion of scarlet fever, it is believed that the glomerulus is affected as the result of the elimination by the kidney of the toxins of scarlet fever. So also in the case of cholera and eclampsia, the epithelial lesion in the kidney tubules is supposed to be due to the elimination of the toxins of these diseases through the cells of the

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tubules. Likewise, in the experimental nephritides, the glomerular lesion due to arsenic and the tubular lesions due to potassium chromate are similarly explained.

On the other hand, it is possible that these various poisons may have a specific selective affinity for certain constituents of the various cells of the kidney, and may be "fixed" as it were in these cells, remaining within them appreciably longer than the period usually required for mere elimination.

The solution of this problem depends, therefore, on some method capable of demonstrating that a foreign protein introduced into the blood-stream is (1) equally distributed in the blood and organs; or (2) that it is present in greater amount in the kidney than in the blood; or (3) that it may be demonstrated in the kidney after it has disappeared from the circulating blood. These various possibilities may be tested by a method that is dependent on the phenomenon of anaphylaxis, and the suggestion that these problems could be solved by this method I owe to the papers of Vaughan and his associates[1] on the parenteral introduction of protein, and to the statement of these investigators that a foreign protein (egg-white) may be detected in the kidney of the rabbit after it has disappeared from the circulating blood.

Previously Wells had used the anaphylaxis reaction in the study of alimentary albuminuria, and more recently Van Alstyne and Grant have used it to demonstrate the absorption of albumin from the intestine without digestion. Wells found that guinea pigs can not be sensitized to egg-albumin with the urine of individuals receiving a diet of raw eggs, even when such individuals have an alimentary albuminuria, and concluded that ingested egg-albumin does not pass through the body unchanged, and that the albuminuria under such circumstances is due to the elimination, not of ingested foreign protein, but of serum proteins.

The use of the anaphylaxis reaction to detect foreign protein in the circulating blood or in the organs has been attempted, so far as

[1] Vaughan, V. C., Cumming, J. G., and McGlumphy, C. B., The Parenteral Introduction of Proteins, *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 16; Vaughan, V. C., Cumming, J. G., and Wright, J. H., Protein Fever, *ibid.*, 458.

I am aware, only by Vaughan and his associates, and by Van Alstyne and Grant.[2]

The latter injected into a vein of a dog 20 c.c. of a mixture of equal parts of egg-albumin and salt solution, and drew blood after one quarter of an hour, and after four, forty-eight, and seventy-two hours. The serum of each of the various samples and the urine collected during the first twenty-four hours were used to sensitize guinea pigs. At a later period an intoxicating injection of egg-albumin caused in each animal the characteristic symptoms of anaphylaxis, these being least marked in the animal sensitized with the serum drawn after seventy-two hours, only moderately severe in the animal receiving the forty-eight hour serum, but severe in all of the others, including the animal that received the urine.

In a second series of experiments Thiry-Vella fistulas were made at various levels of the intestinal canal, egg-albumin was placed in these, and blood was drawn at periods varying from one to three hours. The blood serum thus obtained was injected into guinea pigs and sensitized these animals to egg-albumin. Similar results were obtained with the urine of the first twenty-four hours.

The experiments of Vaughan and his associates may be divided into two groups. In the earlier experiments they used for the sensitization of guinea pigs the blood of rabbits obtained a few hours after the feeding or injection of egg-albumin.

In one experiment they found that one and a half hours after the intravenous injection of egg-albumin (50 c.c. of 1:1 solution) the blood failed to sensitize guinea pigs; in a second, failure was noted after two hours; and in a third, after two and a half hours.

In a fourth experiment blood drawn one hour after the injection of egg-albumin sensitized, while that drawn after two, three, and five hours failed to sensitize; on the other hand, all the extracts of brain, kidney, spleen, and liver, made six hours after the injection of egg-white, possessed a sensitizing power which was most marked in the extracts of the kidney and liver.

In a fifth experiment after the egg-white had disappeared from the circulating blood the animal was transfused with one liter of salt solution and samples of fluid were taken from the heart in amounts of 2 c.c. These had the power to sensitize as did also extracts of liver, spleen, and muscle; extracts of brain and kidney failed to sensitize.

In a later communication it was shown that extracts of skin, kidney, brain, liver, spleen, intestine, and stomach from rabbits killed twenty-four and forty-eight hours after intravenous injection of egg-albumin are capable of sensitizing guinea pigs, but that similar extracts from animals killed after seventy-two hours do not have this power. The symptoms of anaphylaxis are, the writers note, most marked in guinea pigs receiving extracts of kidney and spleen.

It was this difference between the results at forty-eight and seventy-two hours and the marked reaction in guinea pigs receiv-

[2] Van Alstyne, E. V. N., and Grant, P. A., The Absorption of Albumin without Digestion, *Jour. Med. Research,* 1911, xxv, 399.

ing extracts of the kidney that suggested the use of this method of sensitization for the purposes of the present investigation.

METHODS.

The usual procedure has been to inject intravenously a group of three, four, or five rabbits with a foreign protein, to chloroform the animals at intervals of twenty-four hours, and then to inject their blood or the extracts of their organs into the peritoneal cavity of guinea pigs. After two or three weeks these guinea pigs received either intravenously or intraperitoneally an injection of the same kind of protein originally injected into the rabbits.

Egg-albumin and horse serum have been the substances used in most of the experiments. The egg-white has been diluted with three parts of physiological salt solution, except in some of the earlier experiments in which a dilution of equal parts was tried; but as this dilution frequently caused sudden death, it was abandoned for the dilution of one part to three. With the latter dilution no immediate ill effects have been seen, though very rarely an animal died on the second or third day after the injection.

All injections of egg-white and serum were made in the ear vein, forty cubic centimeters of diluted egg-albumin (ten cubic centimeters of egg-white) and ten cubic centimeters of undiluted horse serum being the amounts injected. All animals were killed by chloroform except when it was necessary to obtain the blood or to wash the organs with salt solution. Under the latter circumstances, ether anesthesia was always used. Extracts were made by grinding the organs in a mortar with sand, adding twenty cubic centimeters of salt solution, and allowing the mixture to stand at 33° to 35° C. for eighteen to twenty-four hours. The fluid was then removed by centrifugalization. The extracts thus obtained were injected into guinea pigs in amounts of five to eight cubic centimeters, and defibrinated blood in amounts of five cubic centimeters. All extracts for sensitization were injected intraperitoneally.

The intoxicating dose was given in the early experiments both intraperitoneally and intravenously; in the later work, injections were given into the jugular vein only. The amount of egg-white used for an intraperitoneal injection was five cubic centimeters of a 50 per cent. (1 to 1) dilution, and for intravenous injection four

cubic centimeters of a 25 per cent. (1 to 3) dilution. Horse serum was given by these two methods in doses of five cubic centimeters and one to two cubic centimeters respectively.

The varying degrees of sensitization, as presented in the tables, are indicated by the following signs:

+ + + + = acute fulminant anaphylaxis with death and typically insufflated lungs at autopsy.
+ + + = marked symptoms,—severe respiratory distress, "bucking," convulsions, and prostration, but with eventual recovery.
+ + = moderate attack,—spasmodic cough, respiratory distress, "bucking," and a tendency to lie prone.
+ = "scratching," cough, slight "bucking," slight respiratory distress.
0 = no effect.

The following table confirms Vaughan's single observation as to the presence of egg-albumin in the kidney twenty-four and forty-eight hours after injection, and its absence (or failure to sensitize) after seventy-two hours; it also shows the results of control experiments.

SERIES I.

Egg-White.

The rabbits were killed one, two, three, and four days after intravenous injection of 10 c.c. of egg-white diluted with salt solution. Guinea pigs received intraperitoneally extracts of the kidneys of each rabbit, and after three weeks had elapsed they received intraperitoneally an intoxicating dose of 5 c.c. of a mixture of equal parts of egg-white and salt solution, or intravenously 4 c.c. of a 1 : 3 dilution.

No. of animal.	Extract.	Method of injection.	Result.
Guinea pig 1	24 hrs.	Intraperitoneal.	+++
Guinea pig 2	24 hrs.	Intravenous.	++++
Guinea pig 3	48 hrs.	Intraperitoneal.	+
Guinea pig 4	48 hrs.	Intravenous.	++
Guinea pig 5	72 hrs.	Intraperitoneal.	0
Guinea pig 6	72 hrs.	Intravenous.	0
Guinea pig 7	96 hrs.	Intraperitoneal.	0
Guinea pig 8	96 hrs.	Intravenous.	0
Control 1[3]		Intraperitoneal.	0
Control 1		Intravenous.	0
Control 2[3]		Intraperitoneal.	0
Control 2		Intravenous.	0
Control 3[3]		Intravenous.	++++

[3] Control 1. Normal guinea pigs.

Control 2. Guinea pigs that had received, twenty-eight days before, extracts of normal rabbit kidney.

Control 3. A guinea pig that had received, three weeks before, an extract prepared from normal rabbit kidney and 1 c.c. of egg-white.

SERIES II.

Horse Serum.

This series is like series I except that horse serum was used instead of egg-white. The rabbits were killed one, two, three, and four days after receiving intravenously 10 c.c. of normal horse serum. Extracts of the kidneys of each animal were injected intraperitoneally into guinea pigs, and after three weeks each guinea pig received intravenously 2 c.c. of the same horse serum, or 5 c.c. intraperitoneally.

No. of animal.	Extract.	Method of injection.	Result.
Guinea pig 1	24 hrs.	Intraperitoneal.	+++
Guinea pig 2	24 hrs.	Intravenous.	++++
Guinea pig 3	48 hrs.	Intraperitoneal.	++
Guinea pig 4	48 hrs.	Intravenous.	+++
Guinea pig 5	72 hrs.	Intraperitoneal.	++
Guinea pig 6	72 hrs.	Intravenous.	++++
Guinea pig 7	96 hrs.	Intravenous.	0
Control 1[4]		Intravenous.	0
Control 2[4]		Intravenous.	0
Control 3[4]		Intravenous.	++++

In series II the kidney extract of the third day still retained power to sensitize.

At this point, it seemed desirable to determine whether bacterial proteins were retained by the kidney in a form capable of sensitizing guinea pigs. The work of others on sensitization to tuberculin and to pneumococcus protein offered some hope of successful results, and, moreover, the use of bacterial proteins, which are factors in the production of nephritis, would, it was obvious, allow more definite conclusions concerning their possible retention in man, than would studies with egg-albumin and horse serum.

Rabbits were therefore given intravenously tuberculin in the form of O. T. bovine,[5] a tuberculin[6] prepared without heating, mallein,[7] mallease,[8] and pneumococcus protein.[9] A vegetable protein, gliadin,[10] was also used.

[4] Control 1. Normal guinea pig.

Control 2. A guinea pig that had received, three weeks before, an extract of normal rabbit kidney.

Control 3. A guinea pig that had received, three weeks before, an extract prepared from normal rabbit kidney and 1 c.c. of horse serum.

[5] Products of the Pennsylvania Live Stock Sanitary Board, procured through Dr. John Reichel.

[6] Procured from Dr. John Reichel of H. K. Mulford and Co.

Extracts of the kidneys of rabbits which had received respectively twenty-five cubic centimeters of a 2 per cent. solution of gliadin in 0.1 per cent. sodium hydrate, the protein of six billion pneumococci, ten cubic centimeters of tuberculin, and five cubic centimeters each of mallein and mallease, all failed to sensitize guinea pigs to the respective proteins.

The disappointing results of these experiments necessitated a return to the use of egg-albumin and horse serum as the most satisfactory agents to be used in settling the fundamental points of the investigation. The most important of these points was to determine the distribution of the egg-white in the body of the injected rabbit. Does a foreign protein tend to accumulate to a greater extent in the kidney than in other organs, or is it present in the kidney in the same concentration as in other organs; in other words, is the content of foreign protein of an organ dependent merely upon the amount of the circulating blood present in that organ? This point was determined by the experiments summarized in series III and IV, representing the injection of egg-albumin and horse serum respectively.

SERIES III.

Comparison of Sensitizing Power of Blood, Liver, and Kidney after the Intravenous Injection of Egg-White.

Rabbits received 10 c.c. of egg-white diluted with 30 c.c. of salt solution, and were killed after twenty-four, forty-eight, and seventy-two hours, respectively. Blood and extracts of liver and kidneys were injected into guinea pigs. After three weeks each guinea pig received intravenously 4 c.c. of a 1:3 egg-white dilution or intraperitoneally 5 c.c. of a 1:1 dilution.

Guinea pigs.	Time.	Blood.	Liver.	Kidney.	Mode of injection of intoxicating dose.
Group 1	24 hrs.	0	+	++	Intraperitoneal.
Group 2	24 hrs.	++	+++	+	Intravenous.
Group 3	48 hrs.	+++	+	0	Intraperitoneal.
Group 4	48 hrs.	+	++	0	Intravenous.
Group 5	72 hrs.	0	0	0	Intraperitoneal.
Group 6	72 hrs.	0	0	0	Intravenous.

[7] Products of the Pennsylvania Live Stock Sanitary Board, procured through Dr. John Reichel.

[8] Procured from Dr. John Reichel of H. K. Mulford and Co.

[9] Prepared by Dr. J. A. Kolmer according to the method of E. C. Rosenow, Pneumococcus Anaphylaxis and Immunity, *Jour. Infect. Dis.*, 1911, ix, 190.

[10] Product prepared by Drs. T. B. Osborne and H. G. Wells, and obtained through the latter.

SERIES IV.

The same as series III except that horse serum (10 c.c.) was used instead of egg-white.

Guinea pigs.	Time.	Blood.	Liver.	Kidney.	Mode of injection of intoxicating dose.[11]
Group 1	24 hrs.	++++	+	++	Intravenous.
Group 2	48 hrs.	++	+	+	Intravenous.
Group 3	72 hrs.	+	0	++++	Intravenous.

These experiments give somewhat irregular results, but in general they indicate that sensitization is dependent on the egg-albumin content of the blood rather than on a retention of egg-albumin in the tissues of the organs. The irregularities in both series are difficult to explain, but such irregularities are not infrequent in sensitization experiments.

To settle the question of whether the egg-white was merely present in the blood or whether it was fixed by the cells of the organ, the experiments summarized in series V were undertaken. In these the sensitizing power of the blood of a rabbit was compared with the same power of extracts of the washed and unwashed kidneys.

The method was as follows. Under the ether anesthesia a rabbit was bled from the carotid artery to secure five cubic centimeters of defibrinated blood. The abdomen was then opened, and the vessels of one kidney were ligated, and the other kidney was washed out with about 1,000 cubic centimeters of physiological salt solution through a cannula in the aorta. Guinea pigs were sensitized with the blood from the carotid and with extracts of the two kidneys.

SERIES V.

Egg-White. Comparison of Sensitizing Power of Blood and of Extracts of Washed and of Unwashed Kidney.

Guinea pigs.	Time.	Blood.	Unwashed kidney.	Washed kidney.	Mode of injection.
Group 1	24 hrs.	++++	+++	+	Intravenous.
Group 2	48 hrs.	+++	+++	+	Intravenous.
Group 3	72 hrs.	++	++	0	Intravenous.
Group 4	96 hrs.	0	++	0	Intravenous.

[11] In this and in most of the experiments that follow, the intraperitoneal administration of the intoxicating dose was abandoned on account of the more definite results obtained by the intravenous method.

These results are conclusive. In the normal animal the power of sensitization depends on the egg-albumin content of the blood and not upon a peculiar fixation of the foreign protein in the kidney. It does not necessarily follow that the same is true for all protein substances, but these observations tend, nevertheless, to support the view that injury to the kidney by a particular substance is coincident with the period of elimination of that substance by the kidney, and not to a fixation in the parenchymatous cells.

Another problem, however, remained. Is a foreign protein eliminated as readily by the diseased kidney as it is by the normal kidney? This problem could perhaps have been more exactly solved if the experiments had been made on animals with a chronic renal lesion, but the difficulty of producing the latter with any degree of constancy compelled the use of animals with acute lesions due either to uranium nitrate or to potassium chromate.

The results obtained with three different groups of rabbits suffering from nephritis due to uranium nitrate are combined in the table that follows.

SERIES VI.

The Sensitizing Power of Kidney Extracts from Animals that Received Egg-Albumin After Developing a Uranium Nephritis.

Each rabbit received 0.0075 or 0.015 gm. of uranium nitrate subcutaneously. Twenty-four hours later, when coagulable protein was demonstrable in the urine, each received in the ear vein 10 c.c. of egg-albumin diluted with 30 c.c. of salt solution. The other procedures were the same as in the earlier experiments.

No. of animal.	Time.	Mode of injection.	Result.
Guinea pig 1	24 hrs.	Intraperitoneal.	+++
Guinea pig 2	24 hrs.	Intravenous.	++++
Guinea pig 3	48 hrs.	Intravenous.	0
Guinea pig 4	48 hrs.	Intraperitoneal.	0
Guinea pig 5	48 hrs.	Intravenous.	0
Guinea pig 6	48 hrs.	Intraperitoneal.	0
Guinea pig 7	48 hrs.	Intravenous.	++++
Guinea pig 8	72 hrs.	Intraperitoneal.	++
Guinea pig 9	72 hrs.	Intravenous.	++++
Guinea pig 10	72 hrs.	Intravenous.	++++
Guinea pig 11	72 hrs.	Intravenous.	++++
Guinea pig 12	72 hrs.	Intraperitoneal.	+
Guinea pig 13	72 hrs.	Intravenous.	++
Guinea pig 14	96 hrs.	Intraperitoneal.	0
Guinea pig 15	96 hrs.	Intravenous.	0
Guinea pig 16	96 hrs.	Intravenous.	0

These results are in general similar to those obtained in normal animals, but there is here a greater tendency to sensitization by extracts made as late as the third day (compare series I, III, and V). An acute nephritis due to uranium nitrate apparently lengthens slightly the period during which the kidney (or blood?) may retain a foreign protein. The difference is, however, slight. No explanation is at hand for the curious negative results in four of the five experiments with forty-eight hour extracts.

In another experiment, potassium chromate was used as the renal irritant, and the guinea pigs were sensitized as in series V with blood and with the extracts of washed and unwashed kidneys.

SERIES VII.

Comparison of Sensitizing Power of Blood and of Extracts of Washed and Unwashed Kidneys from Animals that Received Egg-Albumin After Developing a Chromate Nephritis.

Each rabbit was injected subcutaneously with 0.3 gm. of potassium chromate, and on the following day, after coagulable protein had been demonstrated in the urine, received in the ear vein 10 c.c. of egg-white diluted with 30 c.c. of salt solution. Blood was drawn and the extracts of washed and unwashed kidney were made after 1, 2, 3, 4, and 5 days. All intoxicating doses were given intravenously.

Guinea pigs.	Time.	Blood.	Unwashed kidney.	Washed kidney.
Group 1	24 hrs.	++++	++++	++++
Group 2	48 hrs.	+++	++++	++
Group 3	72 hrs.	++	++	+
Group 4	96 hrs.	++++	+++	+
Group 5	108 hrs.	0	++++	0

Here we have definite evidence of a persistence of the egg-albumin in the blood, as is shown by the sensitizing power of the blood and of the extracts of unwashed kidney on the fourth day, a period greater by twenty-four to forty-eight hours (series I, III, and V) than that in the normal animal. The discordant result with the unwashed kidney extract of the fifth day cannot be explained, but it is in keeping with the irregular results seen in other series (series III, IV, and VI). These irregularities are due in all probability to the variations in the susceptibility of the guinea pig to sensitization, but may possibly be due, also, to variations in the power of the rabbits to eliminate a foreign protein.

This variation in the results is the one weak point of this investigation. However, the results, in general, agree, and a comparison of normal animals with those having nephritis shows that in nephritis there is a delay in elimination of about twenty-four to forty-eight hours, and this delay is too constant to be due to experimental errors in the method employed.

DISCUSSION.

This investigation is based on the assumptions that parenterally introduced protein is removed in part by the kidney unaltered, is in part destroyed (digested?) within the tissues, and that the power of the blood or of an extract of the kidney to sensitize against a certain protein is evidence of the presence of that protein in the blood or kidney used for sensitization. With this goes the assumption that until the protein is completely digested or removed, it is being eliminated by the kidney, and that therefore the period of its persistence in the kidney can be determined by sensitization tests. That the egg-albumin is actually eliminated by the kidney and occurs in the urine in a form and in sufficient amount to sensitize guinea pigs is shown by the following experiment.

Ten c.c. of egg-albumin diluted with 0.85 per cent. salt solution were injected into the ear vein of a rabbit and six hours later the urine of the animal was obtained by catheterization. Each of three guinea pigs received 2 c.c. of this urine intraperitoneally. After twenty-four hours the rabbit was again catheterized and the same amount of urine was injected into each of a group of three guinea pigs. Nine days later one guinea pig of each group received in the jugular vein 1 c.c. of egg-albumin diluted with salt solution. The disturbance which resulted was of slight severity and of doubtful significance. At the end of three weeks, however, a similar treatment of the four remaining guinea pigs resulted in typical symptoms and the death of all the animals. On autopsy they showed the characteristic lungs of anaphylaxis. There can be no doubt, therefore, that in the rabbit the intravenous introduction of egg-albumin is followed by its elimination by the kidney in a form capable of causing sensitization. No attempt has been made to determine how long the sensitizing power of the urine persists.

From other experiments, however, there is considerable evidence that the larger part of the injected egg-albumin is eliminated with the urine during the first forty-eight hours after injection. Thus, in the urine of the first day, and to some extent in that of the

second day, there appears on heating either a solid clot or a heavy, coarse, flocculent precipitate which settles rapidly and is quite different from the fine, granular, slowly settling precipitate of the serum proteins of nephritis. After forty-eight hours little or no precipitate is obtained. The possibility that the coagulable protein is in part composed of native serum albumin cannot be denied, but the prompt disappearance of coagulable protein after forty-eight hours is not characteristic of any known acute experimental nephritis giving so large a precipitate in the first twenty-four hours. As normal urine has the power through its non-coagulable protein content, according to Wells and others, to sensitize guinea pigs to serum proteins, the anaphylaxis reaction for such proteins cannot be used as a differential control and has, therefore, not been attempted in this investigation.

It has been assumed, therefore, that most of the coagulable nitrogen of the urine after intravenous injection of egg-albumin is indeed egg-albumin, and in order to determine the rate of its elimination, for comparison with the sensitization tests, determinations of the total nitrogen of the egg-white mixture injected, and of the coagulable protein of the urine, have been made. The following experiment is illustrative.

On April 26, 1912, a rabbit received in the ear vein 40 c.c. of a 1:3 mixture of egg-albumin. The nitrogen of 40 c.c. of this mixture, as estimated by the Kjeldahl method, was 0.191 gm.

Nitrogen in coagulable protein of urine of the first period of 20 hrs... 0.096 gm.
Nitrogen in coagulable protein of urine of the second period of 28 hrs. 0.0441 gm.
Nitrogen in coagulable protein of urine of the third period of 24 hrs.. 0.0054 gm.
Nitrogen in coagulable protein of urine of the fourth period of 24 hrs. 0.005 gm.

Other observations follow:

I. Total nitrogen in 40 c.c. of egg-white dilution (10 c.c. egg-white). 0.178 gm.
Nitrogen in coagulable protein of urine of the first 48 hrs..... 0.094 gm.
Nitrogen in coagulable protein of urine of the second 48 hrs..... 0.017 gm.

II. Total nitrogen in 40 c.c. of egg-white dilution..... 0.177 gm.
Nitrogen in coagulable protein of urine of the first 48 hrs..... 0.106 gm.
Nitrogen in coagulable protein of urine of the second 48 hrs..... 0.004 gm.

It is impossible to say that the coagulable protein of the urine does not contain serum protein, but its prompt decrease after forty-eight hours favors the supposition that it is mainly egg-albumin.

If this interpretation is correct, considerably more than half is eliminated within forty-eight hours and only very small amounts during the third and fourth days; the remainder is, presumably, digested or otherwise changed in the animal tissues. Thus the evidence concerning the elimination of egg-white obtained in this way supports the results of the sensitization experiments, for the stronger anaphylaxis was always obtained with the first and second day extracts, and the weaker reactions and the failures with extracts representing later periods.

In connection with the question of possible sources of error in these experiments another question arises—that of the effect of foreign protein on the normal kidney. If egg-albumin is toxic to the cells of the kidney, the experiments here described as demonstrating elimination in normal animals are really experiments on animals with injured kidneys. Vaughan, it may be remembered, believes that the kidneys of animals receiving egg-white are seriously injured. In the present investigation histological examination of the kidney was made only when an animal died before the regular period set for making extracts. In several instances a definite exudate about the glomerular tuft, markedly distending Bowman's capsule, has been seen. Whether this represents an exudate of serum albumin or of the foreign egg-albumin coagulated by the fixing agent we have found no means of determining. The prompt disappearance (after forty-eight to seventy-two hours) of coagulable protein from the urine has, however, seemed to indicate that the kidney is not seriously injured by a single injection and that the fine granular precipitate in the glomerular spaces is, therefore, in all probability egg-albumin.

SUMMARY.

Extracts of the kidneys of normal rabbits prepared one, two, three, and four days after the intravenous injection of egg-albumin and horse serum have the power to sensitize guinea pigs to a second injection of these proteins. The sensitization by first and second day extracts was constant and intense, that by the third day extracts was less marked and sometimes was not evident, and that by the fourth day extracts was only occasional, and when present was always weak.

Comparative studies of the power of the blood, liver, and kidney to sensitize, indicate that this sensitization depends on the content of foreign protein in the circulating blood and not upon its accumulation or fixation in the tissues of an organ. This opinion is supported by other experiments in which the sensitizing power of the blood and of the extracts of unwashed kidneys was compared with the sensitizing power of extracts of washed kidney.

The weak sensitizing power of washed kidney extract is taken as evidence that foreign proteins of the kinds used are not held in the tissues of the kidney, and if these results may be applied to nephrotoxic proteins, it follows that nephritis is not due to selective and persisting fixation of a protein by the renal cells, but is due to the action of such protein merely during the process of its elimination.

In experimental acute nephritis of the type due to uranium nitrate, the power of sensitization to egg-albumin is prolonged for twenty-four hours, and in the chromate type for forty-eight hours, thus indicating that in nephritis, of the acute type at least, the elimination of a foreign protein is delayed.

Attempts to study by the same methods the elimination of vegetable and bacterial proteins have failed.