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EXPERIMENTAL AND PATHOLOGICAL ALBUMINURIA.

A contribution to the study of the source from which the proteid excreted in Normal and Pathological conditions of the kidney is derived.

by

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INTRODUCTION.

The view generally adopted regarding the nature of the proteid excreted in albuminuria, is that the kidney under certain pathological conditions, allows the proteids of the blood serum to pass into the urine, in other words, the proteid excreted is identical with the proteid of the blood.

There are however certain conditions where albuminuria appears in the urine in the absence of any demonstrable change in the kidney. For these cases an explanation has been attempted, by postulating changes in the proteid constituents of the blood, and these conditions have been grouped together accordingly, as haematogenous albuminurias. Analogies have been drawn with the albuminuria following the injection of proteids foreign to the organism, such as Egg white, caseinogen etc. It was thought/

thought, that the proteids of the serum might be changed in such a way as to make them behave in the same way as caseinogen, that is, to be excreted in the urine. Semmola¹ even maintained, that this passage of the proteid through the kidney was the primary cause of the pathological change taking place in the organ, and that he was able to produce experimentally a condition of large white kidney by the injection of proteids.

It is difficult to imagine how such a change in the serum proteids could be brought about. The recent work of Abderhalden² has shown, that the composition of proteids of the serum is kept remarkably constant, and quite independent of the chemical composition of the food proteid from which it is formed.

Many of these questions can be put to an experimental test. The most difficult problem is to investigate the nature of the proteid excreted in the urine. Up till recent years rough chemical tests were the only means of establishing the identity of these proteids, and the proteids of the blood serum. The precipitin reaction gives however a more delicate method, and allows too of deciding whether any essential changes in the serum proteids of patients with albuminuria have taken place. This method has been used before by several observers.

PRECIPITIN REACTION.

This reaction is based on the fact that if any proteid, e.g. the serum of one animal, is injected into an animal of another species (immune animal), the serum of this immune animal acquires the property of giving a precipitate with the proteid which has been used for the injection (homologous proteid).

The production of a precipitate is with certain exceptions dependent on the homologous proteid, in so far as, only the homologous proteids derived from animals of the same species are able to give this reaction. Thus, for example, if an animal, such as a rabbit, be taken and immunised against ox serum by several intraperitoneal injections, and the serum of this animal be obtained and tested against the ox serum with which it was immunised, a precipitate will be formed, showing that the serum of the rabbit had acquired the power of producing a precipitate with the proteid (homologous proteid) with which it was injected. But if, on the other hand, the immune rabbit's serum is tested against the sera of other species of animals, as the goat, human serum, etc., there will be no precipitate produced by the immune serum.

If one wishes to make use of the reaction for the study of the nature of the proteid which is excreted/

excreted in Albuminuria, two methods suggest themselves.

- A. Immunise animals against human blood serum, and test the serum of the immune animal against the urine of patients suffering from Albuminuria.
- B. Immunise animals against ^{human?} albuminous urine, or proteid prepared from the urine, and test the serum of the immune animal against human blood serum.

In both cases the formation of a precipitate would indicate, that the proteid of the albuminous urine, if not entirely, is at any rate partly, derived from the proteid of the human body. Both these methods have been used in the last few years by a number of observers.

Leclainche³ and Valee⁴ were the first observers to employ the precipitin reaction, for the identification of the proteid in albuminous urine. They investigated three cases of interstitial nephritis, the urine of which cases they tested against the serum obtained from an animal after injections of human albuminous urine. These two observers injected the human urine, but do not state whether they injected the globulin and albumin separately, after separating them out with half and/

and full saturation with Ammonium Sulphate respectively. In each case they observed a strong precipitate formation. They also experimented with a case of parenchymatous nephritic urine, which contained relatively more globulin, and which gave a much fainter reaction. The serum of an animal which had been immunised against human albuminous urine, gave in their experiments a precipitate with pleuritic exudate, but not with human blood.

Mertens⁵ and Zuelzer⁶ obtained similar results. The first named observer used the immune serum of rabbits treated, both with human serum, and human albuminous urine, and tested it against the urine of patients suffering from Albuminuria. In every case a positive result was obtained.

The observations of Zuelzer⁶ who immunised his animals with albuminous urine confirm those of Mertens.

Linossier⁷ and Lemcine⁸ also came to the same conclusions. They went a step further, and investigated the question, whether the proteid of the food could pass through the nephritic kidney. They fed nephritic patients on milk, and tested the urine against the serum of a rabbit immunised against ox serum. The formation of a precipitate indicated that some of the proteid of the milk had passed through/

through the kidney.

Ascoli⁹ fed patients and normal individuals on eggs, and tested the immune serum of rabbits immunised against egg white, against the proteid excreted in the urine after the egg diet. He obtained a positive result, and deduces from his experiments, that the excretion of proteid in the urine, occurs both in normal and pathological conditions of the kidney. Linossier⁷ and Lemoine⁸ think, that the excretion of proteid in the urine after a proteid diet, only occurs in pathological conditions of the kidney. It remains an open question, whether the passage of proteid takes place easier under abnormal, than normal conditions of the kidney.

The opinions of these observers already mentioned differ, however, in regard to the extent to which the precipitin reaction allows, of a distinction between different proteids of one and the same species. Some of them e.g. Leclainche³ and Valee⁴, claim to have been able to prepare a serum, which precipitated the albumin of the urine, but not the globulin.

Schuetze¹⁰ states, that the immune serum produced by the injection of rabbits with the albumin of human muscle, gives a precipitate with this albumin, but not with the albuminous urine of human patients. Aschoff¹¹ found, that when he immunised animals with emulsions of/

kidney, and tested this immune serum against a solution of kidney proteids, he obtained a positive result. On testing this immune serum against serum proteids, the result was negative.

The whole problem to what extent one is able to distinguish between the chemically different proteids of one and the same animal, has been studied very extensively, and has called forth a voluminous literature. The statements of various observers are very contradictory, but the study of the literature leads one to conclude, that an absolute specificity for the various proteids of the organs of animals of one and the same species does not exist, and that if there is a difference, it is only one of degree. A very striking illustration of this view may be found in the following table by ¹² Nuttall, which has been constructed from the experiments performed by Graham, Smith and Sanger:-

Material	Anti Human No. I.	Anti Human No. II.	Percentages from mean of these two.	Anti Ox.	Normal Serum.
. Fresh Human Serum (2 days)	.0291 cc.	.0197 cc.	100%	-	-
I. Old Human Serum (8 months)	.0272 "	.0187 "	93.8%	-	-
II. Placental Serum (8 months)	.0150 "	.0112 "	54.5%	-	-
V. Pleuritic Exudate (2 weeks)	.0065 "	.0084 "	30.3%	-	-
. Hydrocele Fluid (9 months)	.0046 "	.0037 "	16.7%	-	-
I. Fluid of ovarian cyst. (9 months)	.0018 "	trace	6.1%	-	-
II. Amniotic Fluid (9 months)	.0009 "?	trace	3.0%	-	-

present

The quantity of homologous proteid^{is}, however, not stated, so that the degree of precipitin reaction, may be due to the differences in the amount of proteid present in the various liquids.

The contentions of observers who have claimed to be able to distinguish between chemically different proteids of the same animal, have not been made good, and the observations of most workers on this subject, and my own experience, convinces me, that such a distinction by means of the precipitin test can either, not at all, or only/

only under exceptional conditions be made.

Although most observers agree in the main point, that the proteid excreted in the urine gives the biological (precipitin test) reaction of the proteid of the human serum, the number of cases which they have separately investigated is comparatively small, and does not exceed a dozen. Moreover, they do not agree entirely in all their statements. It seemed therefore possible that a systematic investigation of a large number of cases, might reveal differences suggesting differences in the proteids which are excreted in different pathological and physiological conditions.

Another line of investigation, which I intended to follow was, to decide whether the albuminuria following after the ingestion of a large number of raw eggs, is due to an irritation of the kidney leading to the passage of the proteids of the serum, or whether the proteid of the food is capable under certain conditions to enter the blood stream from the intestinal canal unchanged.

According to Linossier⁷ and Ascoli⁹ it is excreted more easily by a diseased kidney, than by a healthy kidney.

Another series of experiments was undertaken, in order to compare the albuminuria produced experimentally/

experimentally, by injecting proteid foreign to the organism into animals, with that present in pathological conditions of the kidney, and to decide whether the presence of proteid foreign in the blood, is in itself sufficient to produce any lesions, and to cause a pathological albuminuria, in other words, whether only the injected proteid is excreted under experimental conditions, or whether as some observers have stated, more proteid is excreted than can be accounted for by the amount of proteid injected.

EXPERIMENTAL.

I will now relate in detail, the result of my experiments which I have obtained,

- A. By the immunisation of rabbits with Human Serum, injected intraperitoneally with the strictest aseptic precautions, and testing the immune serum of these rabbits against the Human Serum.
- B. By testing the immune serum of these rabbits, against the urine of patients suffering from albuminuria.

The Human serum was obtained from a patient Mrs. G., who was admitted to Hospital suffering from Eclampsia. On admission she had had several Eclamptic convulsions. Her whole body was very swollen and dropsical, and she was passing very little urine. Her urine was dark in colour, and contained a great amount of Albumin and urates. The amount of albumin as registered by Esbach's Albuminometer, was to the extent of twelve grains per ounce of urine passed per day.

Her condition was so grave, that venesection was performed, with a view to relieve her circulatory embarrassment. This was done on the following day after her admission to Hospital. The amount of blood/

blood withdrawn by this method, was to the amount of fifteen ounces.

The blood was collected in a sterile conical glass and allowed to clot, and stand for twenty four hours, after which time the exuded serum was drawn off. After I had obtained this serum, the first part of my experiments was begun with. ?

On the 9th November 1906 a rabbit was taken, and injected intraperitoneally with ten cubic centimeters of human serum, under the strictest aseptic precautions. On each succeeding fourth day, the rabbit was re-injected with a greater amount of serum:-

15/11/06	=	20 cc's.	Human Serum	injected.
19/11/06	=	20 cc's.	"	"

So that at the end of the injections, the animal had been injected with fifty cubic centimeters of human serum intraperitoneally. A period of ten days now elapsed since the date of the last injection of serum, and the day on which the animal was killed. The animal was killed on the 29th November, and the blood was collected under aseptic precautions. The defibrinated blood was centrifuged, and the serum siphoned off. The serum of the immunised rabbit was now taken, and tested against the human serum with which it was immunised.

The results of the experiment are as follows:-

One cubic centimeter of immune serum (undiluted), was/

was added to one cubic centimeter of human serum (undiluted), the result was Positive; an abundant precipitate being produced.

On testing with further dilutions of both immune serum and human serum, the following results were obtained:-

1 cc. Immune Serum	(dilution 1:10) +		
1 cc. Human Serum	"	1:1	= abundant ppc.
	"	1:10	= distinct "
	"	1:100	= " "
	"	1:1,000	= " "
	"	1:10,000	= negative.

C. Condit

The experiment clearly shows, that the animal was well immunised, and that a strong antiserum was formed, which when tested against human serum, gave a positive reaction with dilutions of human serum to the extent of 1 - 1,000.

The same immune serum was next tested against the urines of patients suffering from albuminuria, whose brief clinical history is as follows:-

CASE I. ALEXANDER Q.

He was admitted to hospital on the 28th November 1906, suffering from an attack of Acute Brights/

Bright's Disease. He complained of headaches, puffiness of the eyes, and pain in the back, with the passage of much less urine than he was accustomed to pass before his illness. His attention was drawn to the marked change in the colour of his urine. He was very dropsical about the lower extremities.

Examination of urine.

Colour = Dark red.

Specific Gravity = 1028.

Reaction = Acid.

Albumin was present.

Blood " "

Microscopic examination of the centrifuged deposit, revealed Tube and Epithelial casts.

The albumin as registered by Esbach's Albuminimeter, amounted to four grains per ounce per day.

CASE II. THOMAS K.

This patient was suffering from Chronic Bright's Disease. He was admitted on the 5th November, complaining of swelling of both lower extremities, with slight facial oedema. He complained of severe frontal headaches, and occasionally attacks of giddiness.

Examination/

Examination of urine.

Colour = Amber

Reaction = Acid.

Specific Gravity = 1032.

Albumin present and registered by Esbach's Albuminimeter, = twelve grains per ounce per day.

Microscopic examination of deposit revealed granular and hyaline casts.

CASE III. MRS. E. M.

She was admitted to Hospital suffering from Uraemia. She complained of severe pains in the head, drowsiness and stupor. There was marked oedema of the face, especially about her eyes. Oedema of the extremities was also present. Urine diminished in amount.

Examination of Urine.

Colour = Amber.

Reaction = Acid.

Specific Gravity = 1025.

Albumin was present to the amount of four grains to the ounce of urine passed per day.

CASE IV. MRS. G.

It was from this patient, that I was able to obtain the human serum for immunisation, the clinical features of whose case have been previously described in the text.

Physiological Albuminuria. DAVID M.

This case is one of interest. The above named, a young man, who, not in any athletic training, took part in a football match.

I collected his urine

- A. Before the match.
- B. At half time.
- C. At the end of the game.

These three samples of urine were carefully tested chemically for albumin. In both instances

- A. Before the match,
- B. At half time,

the samples of urine gave a negative result.

The sample collected at the end of the game, however, gave a distinct positive reaction by chemical investigation. The amount of albumin as registered by Esbach's Albuminimeter, was .4378 grs. per ounce of urine.

CASE VI. HARROLD S.

The urine of this person was taken to perform a control test. When tested chemically it gave no evidence of the presence of any abnormal constituents.

The results which I have obtained, by testing the immune serum against the albuminous urines of the/
the/

the patients just described are as follows:-

<u>PATIENT.</u>	<u>DISEASE.</u>	<u>AMOUNT OF ALBUMIN EXCRETED.</u>	<u>PRECIPITIN REACTION.</u>
I. Alex. Q.	Acute Bright's Disease	4 grs. per ounce	Positive.
II. Thos. K.	Chronic " "	12 " " "	"
III. Mrs. M.	Uraemia.	4 " " "	"
IV. Mrs. G.	Eclampsia.	6 " " "	"
V. David M.	Functional.	.4378" " "	"
VI. Harrold S.	Healthy individual	-	Negative.

In each of these experiments, one cubic centimeter of immune rabbit serum, was added to one cubic centimeter of human albuminous urine. All the albuminous urines gave a positive result.

In all these cases, the amount of albumin present in the urines, was registerable by Esbach's Albuminometer with the one exception that of CASE II. (Thos. K.)

The urine of this case was so loaded with albumin, (the patient passing twelve grains per ounce per day), that the precipitate formed in Esbach's Albuminometer after standing for twenty four hours, reached to a height above the mark "U" on/

on the Albuminimeter. It was not till after a dilution of the urine to the extent of 1 - 10, that the amount of albumin was registerable.

A very important fact was now observed, that when this undiluted urine thick with albumin, was tested against the immune serum, a Negative result was obtained:-

1 cc. Immune serum (1:1) + 1 cc. urine (1:1) =
No precipitate.

This result was clearly explained by the fact, that after the urine was diluted to the extent of 1 - 10, and then tested against the immune serum, a Positive result was obtained viz:-

1 cc. Immune serum (1:1) + 1 cc. urine (1:10) =
Distinct precipitate.

This showed clearly the fact, that in the undiluted urine the excess of homologous proteid, dissolved the precipitate.

A further observation is worthy of note in regard to the experiment with the urine of Case V. (David M.)

It will be remembered, that in this case, the urine was collected at three different periods of a football match,

- A. Before the game
- B. At half time
- C. At the end of the game,

and/

and in the first two instances A. and B., the urine was carefully tested chemically for proteid with a Negative result.

On testing the urine collected at B. (half time) with the immune serum, a Positive result was obtained. This demonstrates the fact, that, a Positive precipitin reaction can be obtained with a urine, which contains proteid to such a small extent, that it cannot be demonstrated by chemical investigation.

As observed with CASE VI. (Harrold S.), normal urine does not give a precipitin reaction with the immune serum.

My next experiments with the serum of rabbits, immunised with human serum, commenced on the 27th November 1906.

The human serum employed for immunisation in this case, was obtained from a patient Mrs. M., who was admitted to Hospital suffering from Uraemia.

Her previous history relates, that she suffered from very severe headaches, for about fourteen days before her admission. A day or two before she came to Hospital, her face was very oedematous, her sight was dimmed a little, and she suddenly lost her power of speech. She was very drowsy, and difficult to rouse./

rouse. There was no abdominal ascites, nor was there any previous history of renal troubles. She was passing very little urine.

Examination of Urine.

Colour = Amber

Reaction = Acid.

Specific Gravity = 1024.

Albumin present to the extent of 4 grs. per ounce per day. Granular and hyaline casts were present in the deposit. This patient was venesected on the second day after her admission to hospital, and the blood was collected in the manner previously described in the text, and the serum decanted off.

The serum obtained, the experiments were now commenced with. A rabbit was taken, and injected intraperitoneally with 20 cubic centimeters of human serum. The animal was re-injected on each succeeding fourth day, with the same amount of serum viz:-

1/12/06 = 20 cc. Human serum injected.

5/12/06 = 20 cc. " " "

After an interval of ten days, dating from the day of the last injection, the animal was killed on the 15th December.

The/

The blood of the animal was obtained in the same manner, as in my previous experiment. It was not allowed to clot, but defibrinated by stirring with a sterile rod, and after the fibrin was removed, the remainder of the blood was centrifuged, and the serum siphoned off.

This animal's serum was now taken, and tested against the human serum, with which it was immunised, and a Positive reaction was obtained.

1 cc. Immune serum (1:1) + 1 cc. Human serum (1:1) =
Precipitate.

On experimenting further with dilutions of both sera, it was found that, one cubic centimeter of immune serum diluted to 1-10, when added to 1 cubic centimeter of human serum in the dilutions of 1 - 10,000, gave the following results:-

1 cc. Immune serum (1:10) +

1 cc. Human serum	1:1	=	Negative
"	"	"	1:10 = Abundant Precipitate.
"	"	"	1:100 = Distinct Precipitate.
"	"	"	1:1,000 = Slight precipitate.
"	"	"	1:10,000 = Negative precipitate.

This experiment shows, that during the process of immunisation with human serum, a strong precipitin was produced in the animal's serum, giving positive reactions/

reactions with dilutions of human serum 1-10 to 1-1,000, a result similar to that of my first experiment.

The same immune rabbit^{serum} was now tested against some further fresh cases of patients suffering from albuminuria. The list of cases, with a few details concerning their Clinical history is as follows:-

CASE I. AGNUS N.

was a patient admitted to hospital, suffering from the effects of Aortic Incompetence. He complained of Great Dyspnoea on exertion, palpitations, and great dropsy about the feet and ankles.

There was no abdominal ascites.

Examination of urine.

Colour = Pale Amber

Reaction = Acid.

Specific Gravity = 1024.

Albumin was present to the extent of .218 grs. per ounce of urine per day. No casts present in deposit of mucus.

CASE II. ALEXANDER R.

This patient was another individual, suffering from Aortic Incompetence, and exhibited the same symptoms as the patient previously described.

Examination/

Examination of urine.

Colour = Amber.

Reaction = Acid.

Specific Gravity = 1022.

Albumin was present and registered with Esbach's Albuminimeter .218 grs per ounce per day. No casts were present in the deposit of mucus.

CASE III. JOHN C.

was admitted to hospital, suffering from Acute Bright's Disease. Previous to his admission, he complained of swelling of the feet and ankles, which gradually became worse. His face was oedematous, especially about the eyes. He suffered from severe frontal headaches. He noticed his urine change in colour, and he was passing a very small amount of urine daily.

Examination of urine.

Colour = Smokey.

Reaction = Acid.

Specific Gravity = 1028.

Albumin and blood present.

Numerous Epithelial and Tube casts present.

Albumin amounted to 3.9 grs. per ounce per day.

CASE IV./

CASE IV. ALEXANDER H.

This patient was an old standing case of Chronic Bright's Disease.

He was very dropsical, and complained of severe frontal headaches, giddiness and Dyspnoea.

Examination of urine.

Colour = Amber.

Reaction = Acid.

Specific Gravity = 1016.

Albumin was present to the amount of .218 grs. per ounce. Granular and hyaline casts present in the deposit.

CASE V. JOHN B.

He was admitted to hospital as a case of Chronic Bright's Disease, complaining of swelling of the face and legs. Eight weeks before admission, he suffered from severe headaches, occasional attacks of giddiness. His legs were very dropsical.

Examination of urine.

Colour = Pale Amber.

Reaction = Acid.

Specific Gravity = 1018.

Albumin was present to the amount of 4 grs. per ounce per day. Granular and hyaline casts were present in the deposit.

CASE VI./

CASE VI. DAVID D.

He was another case of Chronic Bright's Disease. Two years previously, he suffered from dropsy of the lower extremities. His face was oedematous on admission, and he was troubled with frequency of micturition.

Examination of urine.

Colour = Pale Amber.

Reaction = Acid.

Specific Gravity = 1008.

Albumin present to the amount of .218 grs. per ounce per day. Granular and hyaline casts present.

CASE VII. HUGH S.

Another case of Chronic Bright's Disease. Fifteen months previously, he had an attack of Acute Bright's Disease with cedema of lower extremities, with marked abdominal ascites present.

Examination of urine.

Colour = Amber.

Reaction = Acid.

Specific Gravity = 1026.

Albumin present to the amount of 10 grs. per ounce per day. Granular and hyaline casts present in the deposit.

CASE VIII./

CASE VIII. ANDREW M.

Also a case of Chronic Bright's Disease. He complained of swelling of the legs and feet, general weakness, headache, and dimness of vision.

Examination of urine.

Colour = Amber.

Reaction = Acid.

Specific Gravity = 1018.

Albumin present to the amount of .218 grs per ounce per day.

CASE IX. THOMAS C.

This patient was admitted as a case of Purpura Rheumatica. He complained of pain in the joints, and muscles, great sweatings, and bilious vomiting. His body was covered with a rash.

Examination of urine.

Colour = Pale Amber.

Reaction = Acid.

Specific Gravity = 1025.

Albumin was present to the amount of 1.3 grs. per ounce per day. No casts were present in the deposit.

CASE X.

CASE X. GEORGE C.

This was a healthy individual, whose urine was taken to perform a control test. There was no abnormal constituent present in the urine when tested chemically.

The results which I obtained by testing the immune rabbits serum against the urines of the series of albuminuria cases just described are as follows:-

<u>PATIENT</u>	<u>DISEASE.</u>	<u>AMOUNT OF ALBUMIN EXCRETED.</u>	<u>PRECIPITIN REACTION.</u>
I. Angus N.	Aortic Incompetence	.218 grs. per ounce	Positive.
II. Alexander R.	" "	.218 " " "	"
III. John C.	Acute Bright's Disease	3.9 " " "	"
IV. Alexander H.	Chronic " "	.218 " " "	"
V. John B.	" " "	4 " " "	"
VI. David D.	" " "	.218 " " "	"
VII. Hugh S.	" " "	10 " " "	"
VIII. Andrew M.	" " "	.218 " " "	"
IX. Thomas C.	Purpura Rheumatica	1.3 " " "	"
X. George C.	Normal Individual	-	Negative.

In all these cases with albuminous urines, a Positive result was obtained, confirming the results of my first experiments.

The next series of experiments which I performed, were with rabbits immunised with Albumin and Globulin separated out from human albuminous urine.

The urine in this case was obtained from a patient Janet R., who was suffering from Chronic Bright's Disease, and excreting in her urine, proteid to the amount of 2.5 grs. per ounce per day, as registered by Esbach's Albuminimeter.

A litre of the urine was taken, and half saturated with solid Ammonium Sulphate, which precipitated the Globulin. The globulin was separated out by filtration, and the filtrate was fully saturated with Ammonium Sulphate, till all the albumin was precipitated. The albumin was collected in the same manner as the globulin by filtration.

Both these ingredients of the urine were removed from the filter paper, and dissolved in as little tap water as possible, and dialysed in parchment tubes, till all the ammonium salt was got rid off.

The remaining two solutions of globulin and albumin, (which gave all the chemical reactions for proteids), were now taken and used for the purpose of immunisation.

On/

On the 23rd October a rabbit was taken, and was injected intraperitoneally with twenty cubic centimeters of globulin. On each succeeding fourth day it received another injection of the same amount:-

27/10/06 = 20 cc. of globulin.

31/10/06 = 20 cc. " "

till it had been injected in all to the amount of 60 cubic centimeters of globulin.

Ten days later the animal was killed, and the blood collected, and centrifuged, and the immune serum decanted off.

This animal's serum was now taken and tested against the globulin with which the animal was injected.

The result was a Positive one, a distinct precipitate being formed viz:-

1 cc. Immune Serum (1:1) + 1 cc. Globulin (1:1) =
precipitate.

With dilutions of the immune serum, and globulin, to the extent of 1-10 of each, a Negative result was obtained viz:-

1 cc. Immune Serum (1:10) + 1 cc. Globulin (1:1) =
Negative.

1 cc. Immune Serum (1:10) + 1 cc. Globulin (1:10) =
Negative.

This experiment clearly shows that, during the process of immunisation with globulin, an anti serum was/

was produced in the rabbit, which, when tested in its undiluted state with the human globulin, gave a Positive reaction. On further dilutions of both anti serum and globulin, the reaction was Negative, showing that although immunisation did occur, the immune serum was not of a powerful nature.

This result is easily explained by the fact, that, the amount of proteid injected, was much smaller than in the experiments where serum had been employed.

Further the immune serum was now tested against human blood serum, which was obtained from a patient suffering from Eclampsia, when a Positive reaction was again obtained.

1 cc. Immune Serum (1:1) + 1 cc. Human Serum (1:10) =
precipitate.

A series of cases of patients suffering from Albuminuria was now taken, and their urines tested against the rabbits immune serum.

Before stating the results of the experiments, I will give a short clinical history of each individual case, some of which have been experimented with previously in the text.

CASE I. JANET R.

This patient was suffering from Chronic Bright's/

Bright's Disease. It was from her urine that the globulin and albumin used for immunisation were obtained. She was very dropsical, and showed all the clinical features of the Disease.

Examination of urine.

Colour = Pale Amber.

Reaction = Acid.

Specific Gravity = 1016.

Albumin present to the amount of 2.5 grs per ounce. Granular and hyaline casts were present in the deposit.

CASE II. THOMAS K.

Whose clinical history, and condition of his urine, have been previously described.

CASE III. JOHN G.

This was another case of Chronic Bright's Disease. He was admitted with oedema of the face and lower extremities.

Examination of urine.

Colour = Dark.

Reaction = Acid.

Specific Gravity = 1025.

Albumin present to the amount of .4 grs. per ounce. Hyaline and Granular casts present in deposit.

CASE IV. MRS. G.

This was a case of Eclampsia previously described in the text.

The results of the experiments are as follows:-

1 cc. Immune Serum +		<u>Reaction.</u>
1 cc. urine of	Case I. (Janet R.) =	Positive.
1 cc. " "	Case II. (Thomas K.) =	"
1 cc. " "	Case III. (John G.) =	"
1 cc. " "	Case IV. (Mrs. G.) =	"

In my next experiment a rabbit was taken and immunised with the albumin which had been prepared from the urine used in my previous experiment.

(Patient Janet R.)

The rabbit was injected intraperitoneally on the 24th October with twenty cubic centimeters of human albumin. On each succeeding fourth day, it was re-injected with twenty cubic centimeters of human albumin, viz:-

28/10/06 = 20 cc. Albumin injected.

1/11/06 = 20 cc. " "

so that in all, it received sixty cubic centimeters of albumin the same amount as was injected in the previous experiment with globulin.

Ten/

Ten days later the animal was killed, and the blood collected, and centrifuged, and the serum decanted off. The immune serum was now tested against the albumin with which the animal was immunised, and the following Positive results obtained:-

	<u>Reaction</u>
1 cc. Immune serum (1:1) + 1 cc. Albumin (1:1)	= Precipitate
1 cc. " " (1:10) + 1 cc. " (1:1)	= "

With dilutions of both immune serum and human albumin to the extent of 1-10 of each, a Negative result was obtained.

1 cc. Immune Serum (1:10) + 1 cc. Albumin (1:10) =
Negative

This was the same result which I obtained with the same dilution of globulin in my previous experiment.

Further this immune serum was now tested against the same series of albuminous urines, as were used in the experiments with globulin with the following results:-

1 cc. Immune Serum (1:10) +		<u>Reaction.</u>
1 cc. urine Case I. (Janet R.)	=	Precipitate
1 cc. " Case II. (Thomas K)	=	"
1 cc. " Case III. (John G.)	=	"
1 cc. " Case IV. (Mrs. G.)	=	"

Conclusions.

Both of these experiments with albumin and globulin from albuminous urine, conclusively corroborate the results which I have obtained, from the immunisation of rabbits with the serum of human patients suffering from Albuminuria. They further show that the Proteid excreted under pathological conditions is identical with that excreted under physiological conditions and does not appear to be different in any way from the proteids of the serum.

FEEDING HUMAN BEINGS WITH EGG WHITE.

There is however another condition in which proteid appears in the urine, while the individual may apparently be quite healthy. After an excessive proteid diet, proteid appears in the urine.

Ascoli investigated this condition by means of the Precipitin test. He has given to two nephritic patients with albuminous urines, and also to normal persons, a raw egg diet, and he finds, that in both cases, the urine gave a positive reaction with the serum of a rabbit immunised against egg white.

He states, that in the pathological cases, the reaction between the immune serum and the albuminous urines was stronger, than the reaction with the urines of normal persons.

I have repeated these observations, and as the amount of egg white, which may be excreted in the urine is necessarily very small, it is of special importance, that the serum of the animal immunised against egg white, should be a very powerful immune serum. The immune serum which I employed, gave a distinct precipitate with egg white in a dilution of 1-100,000.

The details of my experiments are as follows:-

A rabbit was taken and injected with ten cubic centimeters of egg white intraperitoneally on the 7th December 1906. On each succeeding fourth day, the animal was re-injected with ten cubic centimeters of egg white:-

11/12/06 injection of 10 cc. Egg White.

15/12/06 " " 10 cc. " "

19/12/06 " " 10 cc. " "

until the day of the last injection, 23rd December 1906, when it was injected with 20 cc. of Egg white. Thus in all, the animal had been injected with 60 cc. of egg white.

Seven days after the last injection the animal was killed, and the blood was collected in a sterile conical glass, allowed to clot, and stand for twelve hours, and the exuded serum decanted off.

This/

This immune serum was now tested against the egg white with which the animal was immunised, and the following results obtained:-

1 cc. Immune Serum (1:10) +	<u>Reaction.</u>
1 cc. Egg White 1-10	= Faint Precipitate.
1 cc. " " 1-100	= Very faint precipitate.
1 cc. " " 1-1,000	= Abundant precipitate.
1 cc. " " 1-10,000	= Abundant precipitate.
1 cc. " " 1-100,000	= Faint precipitate.

In all dilutions from 1-10 to 1-100,000 a visible precipitate was produced, showing that the immune serum was very powerful.

Having obtained such a powerful serum, I further investigated Ascolis' observations, by testing this serum against the urines of several nephritic patients whom I fed on raw eggs viz:-

The white of four raw eggs was made into egg flip, and given to the patients for their breakfast. The same number of eggs was given for their luncheon. Their urines were collected before the egg white diet, and four hours after the last egg meal.

The patients were:-

CASE I.	Thomas K.	Chronic Bright's Disease.
CASE II.	Hugh S.	" " "
CASE III.	Peter L.	" " "
CASE IV.	James S.	Waxy Disease of Kidneys.

The details of whose clinical history, with the exception of Case IV., have been previously stated in the text.

The result of this experiment is as follows:-

1 cc. Immune Serum + 1 cc. Albuminous Urine.

<u>PATIENT.</u>	<u>URINES BEFORE EGG DIET.</u>	<u>URINE AFTER EGG DIET.</u>
CASE I. (Thos.K.)	Very Faint.	More marked.
CASE II. (Hugh S.)	" "	Marked.
CASE III. (Peter L.)	" "	Abundant.
CASE IV. (James S.)	" "	Abundant.

Again, for the excretion of Egg White by the Normal kidney I have, intentionally, taken the urines of not absolutely healthy individuals, but of patients suffering from a disease other than albuminuria, and, having as far as can be judged, a normally functioning kidney. I did this because I believed, that the external conditions, lying in bed, etc., are more comparable to those obtained in the cases of the patients suffering from nephritic conditions, than if absolutely normal healthy persons were taken. The cases which I have taken for this experiment were fed on ^araw egg diet, and their urines collected in the same manner as the nephritic cases previously described.

The/

The patients were:-

	<u>Disease.</u>
CASE I. John H.	Fibro Sarcoma of Pharynx.
CASE II. Charles R.	Neurasthenia.
CASE III. Peter M.	Phthisis.

The urines of these three cases before the egg diet were perfectly normal.

On the addition of one cubic centimeter of immune serum to one cubic centimeter of their respective urines, the following results were obtained:-

<u>PATIENT.</u>	<u>URINE BEFORE EGG DIET.</u>	<u>URINE AFTER EGG DIET.</u>
CASE I. (John H.)	Slight turbidity	Abundant ppc.
CASE II. (Chas R.)	-	" "
CASE III. (Peter M.)	Turbid	" "

No marked difference in the strength of the reaction was observed in these two series of experiments.

The conclusion which I draw from this, is that both under normal and pathological conditions, the egg white appears in the urine, if an excessive egg white diet is administered. I am however not able to confirm Ascoli's conclusion, that such a passage of proteid takes place more easily in pathological than/

than in normal kidneys.

The results which I have obtained from my experiments which were described first in the text, namely:-

- A. Immunisation of a rabbit with human serum and testing the immune serum against the human serum.
- B. Immunisation with proteids separated from the human albuminous urines and the immune serum tested against these proteids, have led me to contrast the same conditions, which have been observed in animals.

If rabbits are immunised against egg white, considerable proportion of the egg white, (giving all the chemical reactions for proteids) is excreted in the urine as coaguable proteid. The urine of these rabbits gives the precipitin reaction with the serum of an animal immunised with egg white, but not with the serum of a guinea-pig immunised against rabbits serum, showing thereby, that the excreted proteid is derived solely from the injected egg white. Nevertheless, the behaviour of the proteid excreted after injection of egg white, is abnormal. Solutions of this proteid will not give a reaction, even although a solution of egg white of the same concentration will give a positive result. Only with a very powerful/

powerful immune serum is it possible to obtain the precipitin reaction.

Further, if the proteid is separated from the urine of the animals, after egg white injections, and injected into other rabbits, no precipitin is formed in the serum of these rabbits. This indicates, that some change has taken place in the proteid during its passage through the organism. The significance of this has, however not been investigated.

This is shown by the following experiments:-

In the first instance, two rabbits were taken and immunised with egg white, and their serum was obtained in the usual manner. The urines of both animals were collected after their injections and each urine gave a positive reaction for proteid when tested chemically. The urines contained coaguable proteid in abundance.

If the proteid was isolated from the urine and dissolved in saline it gave the following typical proteid reactions.

1. Biuret or Piotrowski's Reaction.
2. Millons Reaction.
3. Xanthoproteic Reaction.
4. Adamkiewicz's "
5. Liebermann's "
6. Heat Coagulation.
7. Precipitation by concentrated Mineral Acids.

The/

The serum of the immunised animals was now tested against their respective urines, and in each case the result was a negative one viz:-

1 cc. Immune Serum (1:1) +		<u>Reaction.</u>
1 cc. Urine of Rabbit I.	=	0
1 cc. " " " II.	=	0

The urines of several other animals when injected with egg white gave a similar result.

Thinking, that this negative result might be due to a weak anti-serum for egg white, I decided to repeat this experiment with as powerful a serum as possible.

A rabbit was taken and injected intraperitoneally with egg white. In all, the animal had six injections, four days intervening between each injection of egg white. Seven days later the animal was killed, and its serum was collected in the usual manner previously described.

how much?

This serum, when tested against dilutions of egg white to the extent of 1 - 200,000, gave a positive reaction, showing that the anti-serum was a very powerful one.

Two other rabbits were taken, and injected intraperitoneally with egg white on two occasions. Their urines were collected, and contained coaguable proteid in abundance, when tested chemically.

The/

The immune serum was now tested against the urines of these two animals and a positive result obtained.

1 cc. Immune Serum (1:1) +	<u>Reaction.</u>
1 cc. Urine of Rabbit I.	Marked Precipitate.
1 cc. " " " II.	" "

In the second instance a rabbit was taken, and immunised with proteid separated from the urines of rabbits immunised with egg white.

This proteid was separated out by full saturation with solid ammonium sulphate, filtered off, and the proteid dissolved in as little water as possible. The proteid solution was next dialysed till it became salt free. The rabbit was injected with fifteen cubic centimeters of this proteid on two occasions.

how much?

After an interval of ten days dating from the last injection, the animal was killed, and its serum was obtained in the usual manner.

This serum was now tested,

- I. against the solution of excreted proteid,
- II. against egg white,
- III. against the urine of rabbits receiving injections of egg white,

with/

with the following results:-

1 cc. Immune Serum (1:1) +		Reaction.
1 cc. proteid excreted	=	0
1 cc. egg white (1:10)	=	0
1 cc. urine of immune rabbit	=	0

This experiment was again repeated with the same results.

It might however be possible, that the precipitation of the proteid from the urine, might have affected the power of the proteid to produce a precipitin in the animal's serum, or that the amount of proteid injected, was too small for the production of an immune serum. In order to exclude this possible fallacy, I decided to repeat the experiments, having at the same time a perfect control, by injecting another rabbit with proteid prepared from fresh egg white, in the same manner as the proteid was precipitated from the urine, and by taking special care, that both solutions of proteid had the same concentration.

This last precaution was observed by evaporating the proteid solutions in vacuo (after they had been rendered salt free by dialysis), until an estimation by Esbach's Albuminimeter showed, that they had the same/

same concentration.

The repeated experiments are as follows:-

Two Rabbits A. and B. were taken.

Rabbit A. was injected with the proteid precipitated from egg white in the manner already described. The animal had two injections viz:-

6/12/06 = 15 cc. injected intraperitoneally.
10/12/06 = 15 cc. " "

On the 19th December nine days after the last injection, the animal was killed, and its serum obtained. The serum was tested against the solution of egg white proteid with which it was immunised with the following results:-

- 1 cc. Serum of Rabbit A. (1:1) + 1 cc fresh egg white (1:10) = abundant precipitate.
- 1 cc. Serum of Rabbit A. (1:10) + 1 cc. fresh egg white (1:10) = faint precipitate.
- 1 cc. Immune Serum (1:1) + 1 cc. proteid from urine (1:1) = no precipitate.
- 1 cc. Immune serum (1:10) + 1 cc. proteid from urine (1:1) = no precipitate.
- 1 cc. Immune serum (1:1) + 1 cc. proteid ppc. from egg white = precipitate.

Rabbit B. was now taken and injected intraperitoneally with the proteid separated out from the urine, on the same dates and with the same amount of proteid injected as in the case of rabbit A. The animal was killed nine days later, and its serum obtained/

obtained and tested against the solution of the proteid used for immunisation.

1 cc. Immune Serum (1:1) + 1 cc. fresh egg white (1:10)
= no precipitate.

1 cc. Immune Serum (1:10) + 1 cc. fresh egg white (1:10)
= no precipitate.

1 cc. Immune Serum (1:1) + 1 cc. proteid from urine (1:1)
= no precipitate.

1 cc. Immune Serum (1:10) + 1 cc. proteid from urine (1:1)
= no precipitate.

1 cc. Immune Serum (1:1) + 1 cc. ppc. from egg white
= no precipitate.

Another experiment which was performed, was the immunisation of a guinea-pig with the serum of a rabbit, and testing the serum of the guinea-pig, against the proteid excreted in the urine of a rabbit, after injections of egg white.

A guinea-pig was taken, and injected intraperitoneally with fifteen cubic centimeters of rabbit serum on four different occasions, an interval of four days elapsing between each injection. Seven days after the last injection, the animal was killed, and its serum obtained in the usual manner. At the same time, other rabbits were being injected intraperitoneally with egg white, and their urines were collected, and contained excreted proteid in abundance, when tested chemically.

The serum of the guinea-pig was now tested against/

against these urines when a negative result was obtained viz:-

Reaction.

1 cc. serum of guinea-pig + 1 cc. rabbit's
urine. = Negative.

The amount of proteid excreted in the urine of the rabbits injected with egg white was measured, and in no case was there more proteid excreted than injected. The albuminuria ceased four to five days after the last injections of egg white, with one exception, where one animal continued excreting proteid and at the same time passing a large amount of urine, suggesting symptoms of Diabetes Insipidus. This animal was killed, and post-mortem examination revealed diffuse abdominal adhesions, so that in this experiment, there had probably been some septic infection, produced by the injections, affecting the kidney. These results show, that the excreted proteid is not the rabbit's serum, but is part of the proteid injected.

After the animals, which had been used for the injections of proteid, had been killed, their kidneys were taken and examined microscopically, and in no case was there any evidence of a marked pathological change having been induced by the excretion of proteid during the injections.

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S U M M A R Y.

The investigation of the urine of eighteen patients suffering from different forms of albuminuria, shows that in all these cases, the proteid excreted gives the biological test (precipitin reaction) for the proteids of the human organism. The same result was obtained in one case of functional albuminuria in a normal individual. The precipitin reaction gives evidence of the presence of proteid in the urine, when the chemical tests fail.

After a diet of one special proteid (raw eggs) is given to nephritic patients; their urines give the biological test both for the proteids of the human organism, and for the ingested proteid of the food. In individuals with healthy kidneys, a diet of one special proteid is also followed by a slight albuminuria, the urine giving only the biological test for the ingested proteid of the food.

In the seven cases investigated, there was no evidence to show, that more of the proteid of the food is excreted by nephritic patients than by normal persons, as has been stated to be the case. The experiments show, that after an excessive proteid diet/

diet more proteid of the food enters the blood stream, and is excreted by the kidney. By its passage through the healthy kidney, it does not set up an irritation of that organ, leading to the excretion of the proteids of the serum.

Experimental albuminuria can be induced by injecting into animals proteids foreign to the organism, e.g. the white of egg, or the serum of a different species of animals. The proteid excreted in the urine gives the biological test for the proteid injected, but not for the serum of the animal experimented upon. The amount of proteid excreted is always less than the amount used for injection. The kidneys of the animals, which had received injections of proteid over a period varying from three weeks to three months, did not show any marked pathological changes, when examined microscopically, comparable to those met with in nephritic conditions. These experiments show, that the passage of proteid, foreign to the organism, does not set up an irritation of the kidney, leading to the passage of the proteids of the serum, and confirm the conclusion arrived at, as the/

the result of my experiments on human beings.

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