I. THE EXCRETION OF ANTIGEN.

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If egg-white is introduced parenterally into a rabbit, an albuminuria follows. At the same time the animal thus treated becomes immunised. It produces precipitins, which enable its serum to precipitate in the presence of the protein with which it had been originally injected. In this connection the protein is known as antigen. The combination of antigen and antiserum is not only characterised by a precipitate, it possesses also the faculty of binding the complement of fresh normal serum. The withdrawal of complement is tested for by adding sensitised red blood corpuscles, which, in the absence of free complement, do not become haemolysed.

Hamburger [1902] found that the albuminuria which follows injection of egg-white diminished with each re-injection, as the animal became more immune. Oppenheimer [1903] thought the excretion of albumin irregular in amount, bearing no relation to the degree of immunity or to the formation of precipitins. Cramer [1908] investigated this question, and found that the albuminuria did diminish with further injections of egg-white, but that the amount of albumin excreted depended still more on the stage of digestion. A fasting animal did not make use of the albumin injected into the peritoneal cavity as well as a digesting animal, unless it had received a previous injection of saline solution to induce a local leucocytosis. If the leucocytes took up the protein it was digested by the leucocytic proteolytic ferments and assimilated. Failing a local leucocytosis, it was not assimilated to the same extent and was excreted in much greater amount through the kidney cells. The amount excreted was always less than the amount injected. Cramer also examined chemically the albumin in the urine, and found that it gave every

characteristic chemical test of albumin and that in its behaviour to alcohol and ether it resembled egg-albumin and not serum-albumin. Yet it differed from the original egg-albumin in that it was incapable of forming a precipitate with anti-egg serum. Moreover, it did not give a precipitate with anti-rabbit serum. It was apparently egg-albumin which had lost its "species specificity."

There are two ways in which egg protein may be tested for specificity, first, *in vitro*, by precipitating with anti-egg serum, secondly, *in vivo*, by producing precipitin in the serum of the animal into which it has been injected. At Dr Cramer's suggestion, I took up this question in order to determine whether this excreted albumin was still antigen in the sense of being capable of producing antibody when injected into another rabbit, also whether, when mixed with anti-egg serum, it could bind complement.

THE EXCRETED ALBUMIN TESTED AS ANTIGEN *IN VIVO*.

The albumin was salted out of the urine by saturation with ammonium sulphate, was redissolved and reprecipitated, and was finally dialysed in parchment sacs to remove the precipitating agent. In spite of this treatment it retained a considerable amount of urinary pigment. The urine used for this purpose was that from Rabbit 1 of Group 1, taken on the first and second days after each of four successive injections of egg-white; its behaviour as antigen *in vitro* will be discussed later. After dialysis, this excreted albumin was thrice injected intraperitoneally into one rabbit, at five-day intervals. On the third occasion the injection was followed within a few minutes by convulsions and death, with all the appearance of anaphylaxis. Blood was taken from the heart immediately after death, and the serum separated from it contained no precipitin. A control rabbit, injected with egg-white which had been similarly precipitated with ammonium sulphate, and then dialysed, did produce precipitin, but of a very low titre, 1/100.

THE EXCRETED ALBUMIN TESTED AS ANTIGEN *IN VITRO*.

An investigation into the influence of excreted albumin upon anti-egg serum *in vitro* disclosed, in the first group of rabbits examined, a curious phenomenon.

Three groups of rabbits were used, each group in a different year, season of the year, and laboratory, under different feeding conditions; different

samples of serum were also used in the three groups of experiments. It is interesting that each group of rabbits excreted an albumin which differed from the albumin excreted in the other two groups in its properties as antigen.

Group 1 comprised three rabbits, four times injected, yielding twelve samples of excreted albumin. These samples all possessed the same properties as antigen. They failed completely to precipitate with anti-egg serum. When mixed with anti-egg serum, they exhibited the curious phenomenon of completely inhibiting it from precipitating with fresh egg-white. Although the mixture of excreted albumin and anti-egg serum did not precipitate, it bound complement.

Group 2 consisted of three rabbits, four times injected, yielding twelve samples of excreted albumin. These all failed to precipitate with anti-egg serum, but only one of them exerted any inhibitory influence at all (and that only partial) on precipitation between anti-egg serum and fresh egg-white. They also were unable to bind complement.

Group 3 was represented only by one rabbit, once injected. This sample of excreted albumin precipitated with anti-egg serum, and, with it, bound complement.

The animals were placed in cages, and their urine tested as to quantity, reaction, percentage of urea, and freedom from albumin. They then received an intraperitoneal injection of 10 to 13 cc. egg-white. The urine of the first and second days after injection was examined similarly for quantity, reaction, urea, and albumin. The urine seldom contained more than a trace of albumin after the second day after each injection. The rabbits of the first group were investigated in very cold weather. They were small, about 1.5 kilos. in weight, were poorly fed on bran and water, and showed suppression of urine for 12 to 24 hours after injection. Those of the second group were investigated in hot weather, they were large rabbits above 2.5 kilos. in weight, and were well fed on corn and carrots. No consistent alteration could be observed in the reaction or quantity of urine passed during the day after injection, though there was a rise in the percentage of urea constantly present. The single rabbit representing Group 3 was investigated in spring, it weighed 2 kilos., was fed on corn and turnips, and the urine was increased in total quantity on the day after injection. On the day before injection it was as low as 120 cc., the day after injection 225 cc., the second day after, it rose to 375 cc.

Table from Group 1 showing Inhibition.

(By "immune" urine is meant the albuminous urine of a rabbit which had been injected with egg-white.)

						Precipitate
1.	0.2 cc. Anti-egg serum	+	0.2 cc. Normal urine	1		0
2.	"	"	+	"	Immune urine 1	0
3.	"	"	+	"	0.85 % NaCl	0
4.	"	"	+	"	1/10 Egg-white in NaCl	++
5.	"	"	+	"	" " normal urine	++
6.	"	"	+	"	" " immune "	+
7.	"	"	+	"	NaCl " + 0.2 cc. 1/10 Egg-white	++
8.	"	"	+	"	Normal urine + " " "	++
9.	"	"	+	"	Immune " + " "	0
10.	"	"	+	"	NaCl " + " 1/100 "	++
11.	"	"	+	"	Normal urine + " " "	++
12.	"	"	+	"	Immune " + " " "	0

In order to obtain this inhibition it was necessary to add the urine first to the antiserum, before the fresh egg-white (see Exp. 6). If excreted albumin and fresh egg-white were added together, as where egg-white was dissolved 1/10 in the immune (already albuminous) urine (Exp. 9), the inhibition was only partial.

Complement Binding Experiment.

(Two drops of fresh guinea pig serum were added from a capillary pipette to a series of tubes containing antiserum, and NaCl, urine, or egg-white. After one hour at room temperature 0.5 cc. red corpuscles 2 % were added with immune body. The tubes were then placed at 37° for one hour.)

						Haemolysis
1.	0.2 cc. Antiserum	+	0.2 cc. Normal urine			complete
2.	"	"	+	"	Immune "	trace
3.	"	"	+	"	0.85 % NaCl	complete
4.	"	"	+	"	Normal urine + 0.2 cc. 1/10 Egg-white	0
5.	"	"	+	"	Immune " + " "	0
6.	"	"	+	"	NaCl " + " "	0
7.	0.4 cc. NaCl		+	"	" " "	complete

Table from Group 2 showing Indifference.

Urine "Rabbit 2" of Group 2 exhibited after the first injection a partial inhibition which was not observed after later re-injections. As it was atypical of the group, the results obtained with urine from Rabbit 1 of the group will be represented.

						Precipitate
1.	0.2 cc. Anti-egg serum	+	0.2 cc. Normal urine	1		0
2.	"	"	+	"	Immune urine 1	0
3.	"	"	+	"	0.85 % NaCl	0
4.	"	"	+	"	Normal urine + 0.2 cc. 1/10 Egg-white	++
5.	"	"	+	"	Immune " + " "	++
6.	"	"	+	"	NaCl " + " "	++
7.	"	"	+	"	Normal urine + " 1/100 "	++
8.	"	"	+	"	Immune " + " "	++
9.	"	"	+	"	NaCl " + " " "	++

Complement Binding Experiment.

(Method as above.)

					Haemolysis
1.	0.2 cc. Antiserum	+	0.2 cc. Normal urine		complete
2.	"	"	+	" Immune "	"
3.	"	"	+	" 0.85 % NaCl	"
4.	"	"	+	" Normal urine + 0.2 cc. 1/10 Egg-white	0
5.	"	"	+	" Immune " + "	0
6.	"	"	+	" 0.85 % NaCl + "	0

Table from Group 3 showing Unimpaired Antigen.

					Precipitate
1.	0.2 cc. Antiserum	+	0.2 cc. Normal urine		0
2.	"	"	+	" Immune "	++
3.	"	"	+	" 0.85 % NaCl	0
4.	"	"	+	" Normal urine + 0.2 cc. 1/10 Egg-white	++
5.	"	"	+	" Immune " + "	++
6.	"	"	+	" 0.85 % NaCl + "	++
7.	"	"	+	" Normal urine + " 1/100 "	++
8.	"	"	+	" Immune " + "	++
9.	"	"	+	" 0.85 % NaCl + "	++

Complement Binding Experiment.

(Method as above.)

					Haemolysis
1.	0.2 cc. Antiserum	+	0.2 cc. Normal urine		complete
2.	"	"	+	" Immune "	0
3.	"	"	+	" 0.85 % NaCl	complete
4.	"	"	+	" Normal urine + 0.2 cc. 1/10 Egg-white	0
5.	"	"	+	" Immune " + "	0
6.	"	"	+	" 0.85 % NaCl + "	0

These experiments indicate that albumin may be excreted by the kidneys, after intraperitoneal injection of egg-white, in three conditions, as regards the precipitin reaction. In Group 3 its antigen properties were unimpaired in every respect. In Group 1 while chemical properties were retained, the power of precipitating was lost; in view of the inhibition, the albumin may be described as having retained its haptophore affinities. In Group 2 chemical reactions being again unchanged, both precipitating and haptophore properties were lost. It is no easy matter to explain the occurrence of these three conditions of albumin in urine. Michaelis [1903] states that an excess of antigen inhibits the precipitation, though Chapman [1910] finds that excess of antigen does not affect the reaction in either direction. In any case this explanation does not meet the present case. The inhibition was sometimes exerted by urine containing only a trace of albumin, and not by

urine containing much. Also, as has been already stated, fresh egg-white, dissolved in albuminous and inhibitive urine, while increasing the total protein present, diminished the inhibition, which was always greater where the inhibiting urine was added first to the antiserum than where both forms of protein were added together.

Another possible explanation of the loss of precipitating power, and of acquisition of inhibitory influence might lie in the action of the urea. Limbourg [1887] found that urea has a very powerful solvent action upon protein. Spiro [1900] observed that fibrin and coagulated egg-white were dissolved by urea at 37° , and could not be recoagulated by the application of heat as long as the urea was present. If however the solution of fibrin in urea was dialysed and the urea thereby removed the coagulability of the protein returned. Pick [1902] found that saturated urea solution (50 % in water) prevents the precipitin reaction through its action upon the serum protein. This fact I am able to confirm in the concentration, 50 %, which Pick used. I could find, on the other hand, no influence whatever in the case of 5 % urea. Egg-white dissolved in 0.85 % salt solution containing 5 % urea, did not lose as antigen in the slightest degree. If 5 % urea in salt solution was added to anti-egg serum in equal proportions no inhibition to the action of egg-white could be detected, other than by diluting the serum by one-half, an action in which it was equalled by control salt solution. It may be stated that although the percentage of urea in the urine rose after injection, it on no occasion rose as high as 0.5 %. It is possible that albumin may be subjected to a high percentage of urea in the kidney tubules, especially where suppression of urine occurs. Egg-white was therefore treated with an equal quantity of saturated urea solution at 0° , at room temperature, at 37° for 24 hours, and at 56° for two hours. The egg-white was then diluted to 1/10 in salt solution, making 1/10 egg-white in 5 % urea solution. No impairment of antigen properties could be detected. Egg-white diluted 1/10 in saturated urea solution, and after 24 hours treatment diluted to 1/100 in salt solution, did not appear to differ in any way from the control experiment with 1/100 urea solution. This result agrees with Spiro's observation that the solvent action of urea does not consist in any permanent change in the protein; if the urea is removed either by dialysis or dilution to an innocuous concentration, the solvent action disappears. This makes it clear that the action of urea in the kidney tubules is not responsible for the change in the albumin excreted in the urine. The concentration of the urea passed, under 0.5 %, was too low to inhibit precipitation.

The urine normally contains pepsin and trypsin [Sahli, 1885]. Pepsin and trypsin act for some time on protein changing its physical condition before a point is reached at which chemical protein reactions are changed or lost. Klug [1902] has found for pepsin, and Roberts [Oppenheimer, 1909] for trypsin, that increase of temperature, at least between such limits as 0° and 40° , has an accelerating influence upon protein digestion. Now the albumin of Group 2, where the antigen was most affected, was excreted in very hot weather. On the other hand, the weather was warmer in the third case (in spring), where the albumin was unimpaired, than in the first, where the albumin had lost its precipitating power, and where there was much cold and frost. It must be mentioned that toluene and thymol were placed in the urine bottles.

The first step in protein digestion is the most difficult. This means that protein digestion is unlikely to take place in "layers"; that is to say, when a part of the protein present has reached a certain stage, the succeeding stages are rapid, so that all stages of protein digestion may be present coincidentally.

I treated egg-white with 0.3 % trypsin in N/100 NaOH, and found that this enzyme had little or no action in 24 hours even in the presence of urea on antigen properties. Pepsin had a far greater action. I used 1/500 Ray's pepsin in N/50 acid, testing the treated egg-white in 0.5 hour, 1 hour, 1.5 hours, etc., after treatment at 37° , and room temperature. The antigen properties were quickly weakened, but a trace of precipitating power was left until the chemical reactions for albumin had also disappeared. When the egg-white was shown by chemical tests to be largely digested, the precipitating power was almost gone.

From these results it appears improbable that the deterioration of specific egg-white can be ascribed either to the action of the urea, or of pepsin and trypsin present.

Haswell Wilson [1909] has found that albumin excreted in human urine in disease is constant in its action as antigen in the complement binding test, but he was dealing with diseased kidneys, whereas in the rabbits under investigation the kidneys were normal. I am unable to find an explanation of the change of specificity in this albumin, other than that of a physical nature, such as Cramer has indicated. Cramer points out that these results are incompatible with the generally accepted view that the power of protein to act as antigen is dependent on a specific chemical group in the protein molecule. These facts, according to him, make it necessary to abandon this view and to assume that the property of protein to act as antigen either

depends on the physical state of the protein molecule, or that it is due to a substance which is always associated with protein in protoplasm without forming part of the protein molecule, and which can be separated from it without altering the purely chemical properties of the protein.

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CONCLUSIONS.

Egg-white introduced parenterally into a rabbit is excreted unchanged in chemical properties, but may be in three possible variations as regards specificity:

1. It may retain partial properties as antigen, binding complement in the presence of anti-egg serum, not causing precipitation but capable of exercising an influence over antibody so as to prevent it precipitating with fresh antigen.
2. It may be excreted in a non-specific form completely indifferent as antigen.
3. It may retain full antigen properties.

This variation in specificity of excreted albumin points to a physical rather than a chemical explanation of the precipitin reaction.

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