A TREATISE

on

THE EXCRETION OF PROTEIN IN THE URINE UNDER VARIOUS EXPERIMENTAL CONDITIONS.

Presented for the Ellis Prize.

by

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INTRODUCTION.

The assimilation of the protein foodstuffs is still one of the most obscure problems. Our knowledge of the fate of these substances practically ceases the moment they leave the intestinal canal. The discovery of erepsin together with the advances in our knowledge of the chemistry of amido-acids and polypeptides have directed the attention of recent workers mainly to these lower decomposition products of the proteins as the essential factors concerned in the assimilation of proteins. The appearance of these substances in the blood of digesting animals has been looked for by a number of observers, but has not yet been conclusively demonstrated. The only other possible alternative seemed to be then, that the assimilation takes place in the intestinal wall. Although no positive evidence for this view exists, it has been accepted by many physiologists. Acoording to this view the intestinal cells are differentiated from all the other cells of the organism in having the power to transform the protein foreign to the body into the protein of the organism.

By means of the intestinal ferments the protein of the food is broken up into its constituents, which are then built up again by the intestinal cells into entirely new substances, adapted to the chemical organisation/

organisation of the body, namely the proteins of the serum and lymph. Under normal conditions the cells of the organism derive their nourishment as far as proteins are concerned solely from these substances. In a very striking experiment Abderhalden has shown that the chemical composition of the serum proteins is kept constant, no matter what kind of protein is offered to the intestinal cells. If we accept the view given above we must conclude that the cells of the body never get to know what kind of food has been ingested. The intestine regulates as it were the metabolism of the body cells and warrants a uniform and constant composition of the tissues.

This modern view does not take into account the result of the experiments of Voit und Bauer, Eichhorst, Ozerny and Latschenberger and Nencki, Macfadyen and Sieber. These observers have shown conclusively that unchanged protein may be absorbed from the intestine and metabolised. Their conclusions are confirmed by the clinical experience of alimentation by the rectum. This view is further supported by the alimentary albuminuria which frequently appears after a meal in the urine of otherwise quite normal persons. Grainger Stewart and Lauder Brunton who were amongst the first the study this phenomonen

already/

already held that the protein excreted in the urine is the protein of the food. This question can be decided definitely only by means of the precipitin test. On my suggestion Dr. Stewart has examined the urine of three persons with healthy kidneys after a diet consisting of 12 raw eggs in each case. The chemical tests indicated traces of albumen in the urine, which by means of a suitable Anti-Serum was identified as egg white.

The albumen which appears in the urine in cases of alimentary albuminuria is of course not metabolised. On the other hand Voit und Bauer showed clearly that the protein absorbed unchanged from the intestine enters into the metabolism of the organism. An explanation of this apparent contradiction is offered by the experiments of Neumeister. He injected a number of different protein substances into animals and examined the urine. Some proteins e.g. egg white and caseinogen were excreted in the urine, while others such as the proteins of the serum and acid albumin from egg white did not reappear in the urine. He classified proteins accordingly as "animilable" and "non-animilable". Munk and Lewandowsky have shown however that even in the case of the non-animilable proteids e.g. caseinogen the quantity/

quantity excreted is very much smaller than the amount injected and that the difference between the two classes of proteids is not so much one of kind as one of degree.

The recent work of Friedemann and Isaac has further shown that that portion of the protein introduced "parenterally" - i.e. by other ways than by the intestinal canal-which is not excreted by the kidneys undergoes the same metabolic changes as protein introduced"enterally". But these facts do not reveal the nature of the conditions governing the excretion of one portion of the injected protein and the assimilation of the other portion. It seemed of interest to investigate further the fate of protein introduced parenterally in order to obtain some evidence as to the mechanism regulating the excretion of protein.

There is yet another aspect of this problem. The repeated parenteral introduction of proteins leads, in some animals, e.g. rabbits to a change in the serum, namely the formation of precipitins. The serum of an animal treated in this way will give a distinct precipitate in the presence of a small quantity of the protein, which has been used in immunising the animal. The formation of a precipitate/

precipitate takes place only in vitro. No observer has been able to produce the formation of a precipitate in vivo, however highly immunised the animal may have been, and Hamburger has shown that the protein injected circulates as freely in the blood of an immune animal as in that of a normal animal.

It follows that the existence of precipitins in the serum is not capable of bringing about a fixation of the parenterally introduced protein and does therefore not seem to have any practical significance analogous to the formation of bacteriolysins, antitoxins, etc. This view is also in accordance with the result of the work of Moll and of Welsh and Chapman. These observers have shown that the homologous protein does not to any appreciable extent, if at all, enter into the formation of the precipitate in vitro. If the existence of the precipitins does not indicate an adaptation of the organism analogous to that taking place after the injection of toxins or bacteria or red blood corpuscles, one has to look to the fate of the protein introduced as giving indications of such a change. Curiously enough this aspect has been almost entirely neglected. Only two sets of observations on this point are on record, the results of which are diametrically opposed to each other./

other.

Hamburger stated that the albuminuria following upon injections of egg white into normal animals disappeared after the animals had received repeated injections of egg white. These observations were made only on a few rabbits and no details of the experiments are given. Oppenheimer who repeated these experiments immediately afterwards in greater detail was unable to corroborate Hamburger's statements. The excretion of coagulable proteid was quite irregular and he arrived at the conclusion that it stood in no relation to the formation of precipitins.

Although the problem is one of great interest, as Nuttall has pointed out, it has not been investigated again. Hamburger himself in his later papers bearing on the subject does not even mention this question and appears to have abandoned his standpoint in view of Oppenheimer's criticisms.

The object of my investigations was to study the excretion by the kidneys of protein introduced parenterally in normal and in immune animals, in order to decide whether the assimilation of protein is affected by the process of immunisation and to gain some insight into the mechanism of assimilation.

METHODS.

For these experiments full grown male rabbits of approximately the same weight (1800 - 2200 g) were used. The animals before subjecting them to an experiment were kept under observation for 2 - 4 days by examining the urine. Two animals were found suffering from pathological albuminuria, in one animal, a condition similar to Diabetes insipidus was observed. These animals were excluded.

The proteins used for injection were egg white, representing the so-called "non-assimilable" proteins of Neumeister, and ox serum as a representative of the "assimilable" proteins. The parenteral introduction of these substances was brought about by intraperitoneal injection. Under suitable precautions substances can be administered repeatedly and over a long period in this way, the animals remaining perfectly healthy. The amount of protein excreted in the urine collected every two days was determined by coagulating the protein with acetic acid and collecting it on a weighed filter paper which after washing the precipitate with alcohol and ether was dried and weighed.

The strength of the precipitin reaction was measured by determining the dilution of the homologous protein, (egg white or ox serum) which was able to produce/

produce a distinct precipitate in the serum to be examined.

On the excretion of ox serum and egg white by normal animals.

The injection of ox serum in quantities up to 15 c.c. did not produce any albuminuria. After larger doses of 20 - 30 c.c. serum the urine contained sometimes, but by no means regularly, traces of coagulable protein. The quantities excreted were however so small compared with those introduced that it was obvious that the protein had almost entirely been assimilated. No albumoses were found to occur in the urine.

After injections of egg white even in small doses coagulable protein appears regularly in the urine. Albumoses could not be detected. The amount excreted is very variable and depends, as will be shown later, partly on external conditions. In all the cases investigated the amount of protein introduced parenterally exceeded that excreted in the urine, as will be seen from the following examples.

1. Rabbit, weight 2020 g, received intraperitoneally
0,9040 g Egg white.
Excreted after 2 days. 0,2115 g /

Excreted after 2 days. 0,2115 g

" " 4 " 0,1240 g

Total 0,3355 g

After that period the urine was free from protein.

percentage of protein excreted. 37.11%

Assimilated 62.89%

2. Rabbit, weight 1990 g, received intraperitoneally 1,3560 g Egg white.

Excreted after 2 days. 0,7428

" " 4 " 0,1100

Total 0,8528

No more protein in the urine afterwards.

Percentage of protein excreted. 62.89%

assimilated. 37.11%

3. Rabbit, weight 1820 g. received intraperitoneally 1,3560 g Egg white.

Excreted after 2 days. 0,2820 g

" " 4 " 0,0500 g

Total 0,3320 g

No more protein in urine afterwards.

Percentage of protein excreted 25.22%

assimilated 74.78%

4. Rabbit, weight 1870 g. received intraperitoneally 1.5665 g Egg white.

Excreted after 2 days. 0,9096

" " 4 " 0,3205

Total 1,2301 g

No more protein in the urine.

Percentage of protein excreted. 90.71%

" " " assimilated 9.29%

The results of these experiments showed that Neumeister's classification is not correct and that even in the case of the proteins classified by him as "non-assimilable" a variable, but nevertheless considerable amount is assimilated.

On the excretion of Egg white by immune animals as compared with normal animals.

In these experiments rabbits were immunised against egg white by repeated intraperitoneal injections. The amount of protein injected was kept constant from the beginning to the end of the immunisation and varied in different experiments from 10 c.c. - 20 c.c. of egg white. Each injection was performed only after the protein, excreted in consequence of the preceding injection, had disappeared from the urine.

The results which were obtained by comparing the amount of protein excreted by one animal in the course of its immunisation agreed with those of Oppenheimer. An animal would for instance excrete more protein after the fifth injection than after the second/

second or third or even after the first injection. The excretion of protein was as irregular as that observed in normal animals and did not appear to indicate any difference in the metabolism of normal and immune animals.

The experiments were then carried out on a larger scale. The protein excretion of two rabbits which had received previous injections of egg white was compared with that of a normal animal. All the animals were kept under the same external conditions and received at the same time an injection of 15 c.c. egg white.

Rabbit I. had received 10 injections of egg white previously.

Rabbit II. " " 2 " of egg white previously

Rabbit III." " no " " " " " "

After the injection of 10 c.c. egg white the total amount of proteins excreted was:-

by Rabbit I. 0,0769 g

" Rabbit II. 0,2010 g

" Rabbit III. 0,3355 g

Seven days after this injection the animals received another injection of 10 c.c. egg white, the urine of all the animals being free from protein.

In this series the amount of protein excreted was/

was by Rabbit I. 0,2711 g

" Rabbit II. 0,4220 g

" Rabbit III. 0,7843 g

It will be seen that in this last series the amount of protein excreted by each animal is higher than it was after the preceding injection. On the other hand the behaviour of the animals exhibits a certain regularity in so far as taking each series separately, the animals which had been injected previously (I and II) excreted less protein than rabbit III.

Similar results were obtained in a corresponding series of experiments.

These observations appeared to point to external conditions influencing in some way the excretion of egg white. Further investigation showed that the excretion varied with the time when the injection was performed and that these variations depended upon the stage of digestion, during which the protein was introduced into the peritoneal cavity.

Excretion of Protein by fasting and digesting animals.

In these experiments the amount of protein excreted by animals, which had received the injection after having been kept fasting for 24 hours was compared/

compared with the protein excretion in animals which had been fed after 24 hours fasting and had received the injection 5 - 6 hours after the meal.

In normal animals the following results were obtained.

After an injection of 10 c.c. egg white.

a digesting rabbit excreted 0,1640 g Protein

fasting " " 0,3927 g

After an injection of 15 c.c. egg white

a digesting rabbit excreted 0,8528 g Protein

fasting " " 1,2301 g

In immune animals the protein excretion is affected in the same manner by the stage of digestion during which the injection is given. This is illustrated by the following experiments.

A rabbit "A" which had received two previous injections of egg white received a third injection of 10 c.c. during digestion.

Another rabbit "B" which had been previously injected six times with 10 c.c. egg white received the 7th injection while fasting and the 8th injection during digestion. The amount of protein excreted in each case is given in the following table.

Rabbit A.

After 2nd injection.	Stage unknown.	0,1869 g Protein
" 3rd "	fasting	0,3404 g "
" 4th "	digesting	0,0600 g "

Rabbit B.

After 6th injection	Stage unknown	0,0891 g Protein
" 7th "	fasting	0,5930 g "
" 8th "	digesting	0,0694 g "

It is obvious that the stage of digestion exercises a profound influence on the excretion of the protein injected both in normal and in immune animals. The variations which are introduced by this factor are so great that an immune animal in the fasting stage may excrete more protein than a normal animal injected during digestion.

This fallacy must therefore be excluded, if one wishes to study the fate of egg white introduced parenterally both in normal and in immune animals. Comparable results can only be obtained by feeding the animals after a fast day and performing the injection 5 - 6 hours after the meal.

In this way the following results were obtained:-

Amount injected.	Number of injections	Protein excreted after first inject.	last inject.
15 c.c.	5	0,3610 g	0,0490 g
15 c.c.	4	0,3172 g	0
15 c.c.	4	0,3134 g	0,1412 g
15 c.c.	6	0,4137 g	0,1450 g
15 c.c.	6	0,2682 g	0
15 c.c.	5	0,2796 g	0,0390 g
15 c.c.	5	0,2782 g	0

In the case of sixteen other rabbits which were immunised against egg white for other purposes by myself and by Dr. Stewart, the protein excretion in the urine after the first and last injection was determined by means of Esbach's albuminometer. In every case care was taken to perform the injections under the same conditions, namely 5 - 6 hours after a meal. Although Esbach's method does not give any accurate quantitative results, at any rate with the urine of rabbits, it indicates sufficiently well the striking difference between the excretion of protein by an immune rabbit and a normal rabbit respectively. Thus after injecting 15 - 20 c.c. egg white into a normal rabbit the Esbach tube is more than half filled with the precipitate, while after 4 - 6 injections the precipitate may fill the bottom of the/

the tube up to the markings ½ or 1 or it may be absent altogether.

No blood was taken from any of the animals under observations until the experiment was finished, as bleeding seemed to affect in some way the excretion of protein.

The extent to which the excretion of egg white can be diminished by previous injections is however not unlimited. The protein excretion gradually falls until the 4th - 6th injection. Then it becomes stationary and is not any more decreased by further injections.

In the case of ox serum no estimations were made as there is little if any protein excreted even by the normal animals.

These experiments establish the following facts.

1. That in rabbits egg white introduced parenterally can be partly assimilated, the amount assimilated varying with the stage of digestion, in such a way that a greater proportion is assimilated, if the substance is introduced at the height of digestion than if it is administered to a fasting animal.

2. That a rabbit after having received several injections of egg white acquires the power to assimilate more egg white, as illustrated by the diminished/

diminished excretion of protein. This holds good only, if the substance is administered at the height of the digestion. Such an animal, if it receives the protein while fasting may excrete more, i.e. assimilate less egg white than a normal animal at the height of digestion.

On the excretion of egg white after previous injections of saline.

The facts stated above indicate a change in the metabolism of immune animals. The question arises, what is the nature of this change and what are the factors concerned in it. From the fact that it becomes evident only under certain experimental conditions, we may conclude that it is not so much a general change affecting all the cells of the organism as a change affecting only those cells concerned in the mechanism of digestion. Further the difference between a normal and an immune animal is not one of kind but of degree, the difference in the protein excretion of a fasting normal animal and a digesting normal animal being comparable to that of a digesting normal animal and a digesting immune animal. It appears therefore that the change which distinguishes an immune animal is, at any rate partly, an exaggeration of the process which takes place in a normal animal during/

during digestion. These considerations led me to look to the leucocytosis taking place in the splanchnic area during digestion as the factor governing the excretion of egg white introduced into the peritoneal cavity.

In order to put this view to the test an experimental leucocytosis was produced in fasting animals by Metchnikoff's method of intraperitoneal injection of sterile water or saline. Egg white was introduced ten hours afterwards. The protein excretion was compared with two normal rabbits, which had been injected with the same quantity of egg white, the one during digestion, the other while fasting.

Out of four series of experiments on twelve animals three were in agreement with the view detailed above.

Injection of 20 c.c. egg white into three normal rabbits.

Total amount of protein excreted by	
digesting rabbit	0,8528 g
fasting "	1,2301 g
fasting " after saline	0,3320 g

In another experiment 15 c.c. were injected, and the excreted protein was determined only in the urine of the first two days.

The qualitative examination of the urine of the following/

following two days showed the urine of the fasting animal to contain by far the greatest amount of protein.

Amount of protein excreted by	
digesting rabbit	0,1786 g
fasting "	0,5712 g
fasting " after saline	0,2796 g

A third experiment gave similar results while in a fourth series the rabbit treated with saline excreted slightly more protein than the fasting animal.

On the mechanism of the assimilation of protein introduced parenterally.

These experiments justify the conclusions, which have been arrived at on theoretical grounds and are in agreement with the observations of Michaelis and Oppenheimer, which were confirmed by Hunter. These observers found a leucocytosis to take place after intraperitoneal injections of proteins, the number of leucocytes increasing after each injection. Thus Hunter's figures show a leucocytosis of 10,000 after the first injection rising gradually to a leucocytosis of 30,000 after the sixth injection. At each successive inoculation there are therefore more leucocytes available to deal with the protein which has/

has been introduced into the peritoneal cavity. The increase in the number of leucocytes is however not unlimited; it becomes stationary after 4 - 6 injections. The excretion of protein shows as we have seen, a corresponding behaviour.

The way in which the protein is dealt with by the leucocytes may be either by phagocytosis the white corpuscles ingesting the protein and elaborating it intracellularly; or extracellularly by means of proteolytic ferments excreted by the leucocytes. If the second alternative were correct we should expect to find hydrolytic decomposition products of proteins in the urine. I have been unable to find any evidence of the existence of albumoses in the urine after the injection of egg white or ox serum. Munk and Lewandowski also record negative results in that respect and Friedemann and Isaac found the nitrogen distribution in the urine after an injection of protein to be quite normal.

The statement made by Vernon that almost every organ in the body is capable of secreting erepsin would, if correct, also throw light on the fate of protein introduced parenterally. In conjunction with Dr. Lochhead I have re-investigated this subject by determining directly the ereptic power of glycerine extracts/

extracts of kidney cells. According to Vernon this organ secretes even more erepsin than the intestine itself. Our results have shown that glycerine extracts of kidney, do not possess any ereptic power and that Vernon's results are due to certain fallacies.

These negative results with glycerine extracts must however not be taken as contradicting the numerous statements concerning the presence of proteolytic ferments in various organs. An unfortunate confusion has arisen between intracellular ferments and secreted ferments. It is now well known that proteolytic ferments are present in practically every cell. But the sphere of activity of these ferments which are intimately bound up with the protoplasm of the cell, lies entirely within the cell. They cannot be extracted by means of glycerine and exhibit their activity in vitro only after a mechanical distruction of the cell. The function of these ferments is therefore essentially different from that of the ferments of the digestive glands. For the purpose of the present investigation it is necessary to bear in mind, that besides these glands no other organ has the power of digesting protein extracellularly in vivo.

We may conclude then that the assimilation of egg white introduced parenterally is due to protein being taken up by the leucocytes while that portion of the egg white which is not assimilated by the leucocytes, is acted upon by the kidney as a foreign body and excreted. In the light of this view the apparently paradoxical behaviour of egg white, as a substance partly "assimilable" and partly "non-assimilable" is easily explained.

The increase in the number of leucocytes by itself is however not sufficient to account for the greater power of assimilation possessed by immunised animals. A factor of a distinctly specific nature comes also into play. This is evident from the following experiment.

A rabbit after having received three injections of ox serum was injected while digesting with 15 c.c. egg white. The amount of protein excreted was as high as that of a normal rabbit under similar conditions. A second experiment gave similar results. Although the injections of ox serum produce a considerable increase in the number of leucocytes, this increase alone is apparently not sufficient to bring about a greater assimilation of egg white and it appears necessary to assume a specific adaptation of the organism towards the protein introduced.

The formation of precipitin and its relation to the assimilation of the parenterally introduced protein.

We have already seen that the assimilation of egg white is independent of the presence of precipitins in the serum. An animal, which after repeated injections of egg white is highly immune as evidenced by the marked precipitin reaction of the serum, may under certain experimental conditions (fasting) excrete more protein than a normal animal.

The alternative question whether the formation of precipitins is dependent upon the assimilation of the protein is answered by the experiment also in the negative. If namely, rabbits are immunised by repeated injections of egg white when they are fasting and the precipitin reaction of their serum is compared with that of animals which have received an equal number of injections while digesting, no difference comparable to the difference in the excretion of protein has been observed.

I have also been unable to establish a simple relationship between the strength of the precipitin reaction and the amount of homologous protein used for immunising the animals. The formation of precipitins/

precipitins is evidently a very much more complex process than the assimilation of the injected protein, and one where the idiosyncrasy of the individual animal plays a large part. Rabbits which receive an equal number of injections containing the same amount of protein under the same external conditions frequently yield sera differing widely in the strength of the precipitin reaction. Although it cannot be denied that the amount of protein injected influences in some way the formation of precipitins, it is neither the sole nor the most important factor concerned in this process.

More positive results were obtained when this complex process was studied in its initial stages, by determining the strength of the precipitin reaction ten days after the first injection and varying the conditions under which the injection was performed.

With egg white the results obtained are given in the following table where observations made simultaneously on animals of approximately equal weight are grouped together in series.

Injection of 15 c.c. Egg white.

Experimental condition	Protein excreted after first 2 days	Highest dilution of egg white capable of producing a distinct precipitate.
	Series I.	
Digesting	0,1457 g	1 : 100
Saline	0,4120 g	1 : 10
Fasting	0,8119 g	1 : 10
	Series II.	
Digesting	0,1786 g	1 : 100
Saline	0,2796 g	1 : 10
Fasting	0,5712 g	1 : 10
	Series III.	
Digesting	0,2900 g	1 : 10
Saline*	0,4901 g	1 : 10
Fasting	0,1251 g	1 : 100

It will be seen that highest degree of immunity is accompanied by a minimal excretion of protein. The series marked * is the abnormal condition referred to on page 19 , where the fasting animal excreted less protein than either the digesting or the "saline" animal. It is interesting to note that here the abnormally small excretion of protein is reflected in the abnormally high degree of immunity.

With ox serum the following results were obtained.

Injection of 20 c.c. Ox serum.

Series I.

Experimental condition	Protein excreted after first 2 days	Highest dilution of Ox serum capable of producing distinct precipitates.
Digesting	Trace	1 : 1000
Fasting	0,0164 g	1 : 50
Saline	0	1 : 1000
	Series II.	
Digesting	Trace	1 : 100
Saline	0	1 : 1000
Fasting	Trace	1 : 50

These results confirm those obtained with egg white. They show that the condition produced by digestion or injection of saline is most favourable to the formation of precipitins.

Although a causal relation between the assimilation of the injected protein and the formation of precipitins does not exist, it is evident that the same mechanism which is responsible for the assimilation of parenterally introduced protein is also concerned in the formation of precipitins. In other words the formation of precipitins is due to the activity of the leucocytes.

This idea, which is in accordance with the teaching/

teaching of Metschnikoff is of course by no means a new one and has suggested itself to many observers. But no experimental evidence has yet been adduced in favour of it with the single exception of a preliminary note by Kraus and Levaditi. These observers examined the extracts of various organs of immunised animals with regard to their power of forming a precipitate in the presence of homologous protein. The omentum was the only organ giving a positive result. Kraus who repeated these experiments later in conjunction with Schiffmann failed to obtain positive results with the extract of any of the organs examined. He therefore abandoned his view and by a process of exclusion came to consider the endothelial cells of the bloodvessels instead of the leucocytes to be the source of the precipitins.

On the nature of the protein excreted and on the limitations of the precipitin reaction.

In discussing the experiments given above it has been assumed as a matter of course that the protein excreted is identical with or at least derived from the protein injected. This has also been the standpoint taken up by all the investigators including Neumeister and Munk and Lewandowsky who have studied the/

the fate of the injected protein. But if the protein excreted after injections of egg white is examined by means of the precipitin test for egg white, no precipitate is formed. This result is obtained both with the urine containing the protein and with the protein separated from the urine by means of full saturation with ammonium sulphate and freed from the salt by dialysis. Only once with a very powerful antiserum, which indicated egg white in a dilution of 1 in 200000, was a positive although very faint precipitin reaction obtained. Similar observations were made by Friedemann and Isaac.

One would expect then the excreted protein to be the protein of the serum or at any rate of the cells of the animals injected. But if it is tested by an antiserum against rabbit's serum negative results are again obtained. Further if animals are injected with the protein prepared from the urine in the manner given above, no precipitins against this substance are formed in the serum of the animals. These results were obtained with three rabbits and two guinea pigs. In the case of the guinea pigs the serum of the treated animals was also tested against normal rabbits serum. A precipitate was not formed.

In order to exclude the possibility that the preparation/

preparation of the protein from the urine might have introduced a fallacy, the following experiment was performed.

Egg white on the one hand and the urine of a rabbit which had 20 c.c. egg white on the other hand were fully saturated with ammoniumsulphate. The protein which separated out was dialysed, until the ammoniumsulphate had been removed. The two protein solutions were then brought to the same concentration of protein and two rabbits were immunised by an equal number of injections of these solutions. The ~~urine~~ serum of the rabbit which had received the protein prepared from egg white gave a distinct precipitate with egg white and the protein prepared from it. The serum of the other rabbit did not give a precipitin reaction either with egg white, or with the protein prepared from rabbits' urine or with the urine itself.
The following table gives the result obtained in these experiments.

Rabbit immunised against protein from egg white.

Immune serum + egg white	+
" " + urine after injection	0
" " + protein from urine	0

Rabbit immunised against Protein from urine.

Immune serum + egg white	0
" " + urine after injection	0
" " + protein from urine	0

Guinea Pig immunised against Protein from urine.

Immune serum	+ Egg white	0
" "	+ urine after injection of egg white	0
" "	+ protein from urine	0
" "	+ rabbit's serum	0

According to its chemical character this substance is identical with egg white. Not only does it give all the typical protein reactions, but in its behaviour towards alcohol and towards hydrochloric acid it closely resembles egg white and is quite unlike the proteins of the serum.

In this instance then the chemical tests disagree with the precipitin test. It might be urged, that the protein in the urine is not chemically identical with the protein injected. But the following observation shows that the precipitin test is not a reliable indicator of chemical similarities or dissimilarities. The serum of a rabbit, which has received repeated injections of egg white, does not give a precipitate with crystallised egg white although an abundant precipitate is obtained with ordinary egg white. I am informed by Dr. A. Hunter that he has failed to obtain precipitins in the serum of animals treated with crystallised egg white. The analogy between crystallised egg white and the protein/

protein excreted in the urine seems to be complete and the conclusion must be drawn that substances, which chemists are accustomed to look upon as identical, show differences in their behaviour towards the precipitin test.

These results raise the wider question whether the precipitin test is really an expression of chemical differences. If this view is correct then there should be a specific precipitin for everyone of the various proteins of an organism. This has indeed been stated to be the case by Leclainche and Vallée, Mertens, Leblanc, Schutze and others. But the more careful work of recent observers has refuted these statements. (Nuttall, Oppenheimer and Michaelis, Levene and others)

I have investigated this problem by immunising rabbits and guinea pigs against the cells of the liver, kidney or testis of mice. The organs were washed entirely free from blood, by perfusing the mice immediately after death with sterile saline. The organs were then made into a fine emulsion and injected. The serum of the animals, which had received 4 - 6 injections of such emulsions, besides possessing haemolytic properties gave a distinct precipitate with mouse serum.

The protein excreted in the urine after injections of/

of ox serum gives the biological test for ox protein and if separated from the urine and injected into rabbits gives rise to precipitins in the serum of these animals. The same holds good for the protein in the urine of human persons suffering from various forms of pathological albuminuria, as Dr. Stewart has shown on a large number of cases. In his investigations the urine was tested against the serum of rabbits which had been immunised separately against the albumin and globulin of human serum. The strength of the precipitin reaction whether for globulin or albumin was however found to be quite independent of the amount of protein present in the urine. A urine containing little protein frequently gave a very much stronger reaction than a urine containing twice or ten times as much protein. This result was not due to an excess of protein being present and dissolving the precipitate, as the precipitin reaction in urines rich in protein decreased further in strength if the urine was diluted. Nor was it due to the presence of inhibitory substances in the urine, because the precipitin reaction could be obtained without any difficulty, if human serum was added to the urine, the strength of the reaction corresponding to the amount of protein added. If the supposition is correct that the precipitin test is indicative of chemical difference, we are led/

led to conclude that the protein excreted in clinically similar forms of albuminuria varies a great deal in its chemical composition and is as a rule not identical with the proteins of the serum. We would also have to assume that e.g. the globulin of ox serum is more similar to the serum albumin or the liver globulin of the ox than it is to the globulin of the sheep's serum or that the difference between crystallised egg albumin and egg white is greater than that between the globulin and albumin of the serum of an animal of the same species.

The classical work of Nuttal and of Uhlenhuth has shown that the precipitin test distinguishes with certainty the protein substances of different zoological species. It has been assumed as a matter of course that this is due to chemical differences and the existence of a specific arrangement of chemical groups in the homologous protein has been postulated as the cause of the reaction. No experimental proof has yet been adduced to justify this assumption. On the contrary the various attempts to establish this view have led to contradictory results for the explanation of which subsidiary hypothesis had to be advanced.

Our results show that substances which are chemically similar if not identical, behave differently towards/

towards the precipitin test and vice versa. It seems to us more in keeping with the facts to make the precipitin reaction depend upon the presence in the homologous protein of a substance which is not an integral part of the protein molecule as Obermager and Pick suggested in 1902. Another possible explanation, which also agrees with the facts but for which I have not yet been able to produce direct experimental evidence, is that the precipitin reaction is due to the physical state of the homologous protein. In fact in a more recent paper Obermayer and Pick have abandoned their former view and consider the physico-chemical conditions of the homologous protein to be some of the factors determining the precipitin reaction.

On the assimilation of the protein of the food.

The conclusions arrived at in this investigation have some bearing also on the assimilation of the proteins of the food under normal conditions. The complete breaking down of the proteins of the foodstuffs into their constituents in intestinal digestion is believed to be a necessary preliminary condition both for the absorption of these substances and for their re-synthesis to the proteins specific for the organism.

In/

In the introduction sufficient experimental evidence has been quoted to show that unchanged protein can be absorbed from the intestine. Additional evidence in favour of this view has been adduced more recently by the work of Friedlander and Waymouth Reid. The permeability of the intestinal wall for colloidal substances has also been demonstrated by Friedenthal, who found that colloidal silicic acid introduced into the intestine was absorbed.

The numerous attempts to obtain some direct experimental evidence in favour of a re-synthesis of the products of protein digestion into the protein of the organism have as yet failed. Schryver, to quote only one example, compared in a large number of experiments on fasting and digesting animals the coagulable and incoagulable nitrogen of the serum and of the intestinal mucous membrane, without finding any difference. A critical discussion of the whole question is given by Leathes in his lectures on "Problems in Animal Metabolism " and is summed up by him very tersely as follows (p.141) "Till recently, it was believed that proteins were absorbed mainly if not entirely as albumoses and peptones, that these substances were converted by a synthetic change carried out in the intestinal mucous membrane into the coagulable proteins found/

found in the blood; that these blood proteins supplied the needs of the body and were the material used for all tissue repair. We have gradually learnt that the first of these articles of belief requires considerable modification, we have to recognise that the second remains purely hypothetical and that, therefore, the third is little in anything more than a preconception."

The results of this investigation have shown that protein that has passed through the intestinal wall can be assimilated, that this assimilation is brought about by the leucocytes ingesting the protein and that this mechanism of assimilation works under the most favourable conditions after a meal. It seems difficult to avoid inferring that this process actually takes place after the intake of a meal, although,of course, not to the exclusion of other processes such as the absorption and assimilation of albumoses, peptones and amido-acids. After a meal, especially if the food has been cooked, the protein passing through the intestine will not be the native protein, but the acid or alkali albumin formed from it.

The passage of protein through the intestinal wall has been believed to be an abnormal process against/

against which the organism protects itself by the formation of precipitins and by the excretion of the protein in the urine. We have seen however that the protein excreted represents only a part of the amount which has been allowed to enter into the organism, the other part having been assimilated. We know further that the precipitin reaction is a phenomenon restricted to some species and that dogs for instance, which do not produce precipitins are nevertheless capable to assimilate parenterally introduced protein. In the case of rabbits we have shown that the existence of precipitins in the serum would not afford any protection against the protein introduced, if such a protection were needed.

It is possible that the albumoses formed in intestinal digestion are assimilated also by the agency of the leucocytes, as Hofmeister suggested more than 25 years ago. Some experiments which I performed three years ago at the suggestion of Dr. E. F. Bashford throw some light on this question. An intraperitoneal injection of 20 c.c. of a 20% solution of "Witte Peptone", I invariably found to be fatal for rabbits weighing 1800 g and less. But if the animals are treated at first with small injections of 15 c.c., gradually increasing doses can be given until after/

after 6 - 8 injections 20 c.c. can be injected with impunity. The organism must therefore have acquired the power of dealing with the albumoses introduced by the injection. In two animals the treatment was continued for a period of five and six months respectively, the amimals receiving in toto 55 g and 70 g respectively. Although most observers failed to obtain a precipitin reaction against the albumoses of "Witte Peptone" the prolonged treatment of these two rabbits produced a distinct precipitin reaction of their serum. This observation confirmed Bashford's statement, who by an equally prolonged treatment produced precipitins in the serum of rabbits and goats.

There is therefore such a close analogy between the behaviour of albumoses and that of proteins introduced into the peritoneal cavity that a similar process may be assumed to take place in both cases. It is now possible to obtain more conclusive information on the fate of the albumoses. The results of this investigation have shown that quantitative observations on the amount of protein excreted under certain experimental conditions afford a useful method for investigating the fate in the organism of the substances used for injection. A similar series of observations on /

on animals receiving injections of albumoses may be expected to give valuable information on the function of the leucocytes in the assimilation of albumoses.

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