(emotional, &c.,) from the higher parts of the central nervous system, and probably also, as far as a diminution of its activity is concerned, by the influence of some portion of the nervous mechanism concerned in the execution of muscular effort. And, as far as we know, it appears to be mainly upon the mediation of the vagus centre that the most important changes in the cardiac rhythm, in so far as determined by nervous influences, are primarily dependent.

V. "On the presence of Urea in the Blood of Birds, and its bearing upon the Formation of Uric Acid in the Animal Body." By Sir ALFRED GARROD, M.D., F.R.S. Received May 2, 1893.

Some experiments upon which I have recently been engaged have yielded results which appear to be inconsistent with any explanation yet advanced of the mode of formation of uric acid in the animal body, and to necessitate a new theory of its formation. These results I propose to discuss in the present paper, but before doing so it will be well to refer briefly to the different views upon this subject which have been from time to time advanced.

Until the year 1847 it was commonly supposed that uric acid was formed in the kidneys themselves, none having ever been detected in the blood, but in that year I succeeded in demonstrating the presence of uric acid in the blood of gouty subjects, which led to the conclusion that uric acid was formed in certain other organs and tissues of the body, and was merely eliminated by the kidneys.

It is now generally supposed that in mammals urea is produced as the ultimate product of the metabolism of nitrogenised tissues, the formation of a soluble urate being an intermediate stage of the metabolism; but that in birds the nitrogen is eliminated in the form of urate of ammonium without having undergone the further change into urea. Many efforts have been made to explain why these changes should be so different in animals of these two classes. A view once very popular and supported by great authority was that the difference could be accounted for by the amount of oxidation in the system; for example, it was assumed that, as respiration in the cold-blooded animals is slow and imperfect, the uric acid is not broken up, but is eliminated in the form of urate of ammonium; whereas in hot-blooded animals, such as the mammalia, the oxidation processes are more active and urea is produced. Those who held this opinion had in mind only the mammal and the reptile; altogether overlooking the fact that the bird, which throws out uric acid in the same way as the reptile, has even hotter blood and a more active respiration than the mammalia themselves. Hence this view had to be aban-

doned, and the difficulty of explaining the above-mentioned changes remained as before.

Another explanation was that the occurrence of urea or uric acid is dependent on the character of the food, and even at the present time it is often assumed that a highly nitrogenised food, such as meat, leads to the production and elimination of a large amount of uric acid. The fallacy of this I showed in a paper read before this Society in 1886, in which I proved conclusively that mammals living on raw meat threw out infinitely less uric acid in proportion to their weight than small birds fed on canary seed and water. In that paper it was shown that the lion and the tiger eliminated probably less than a hundred thousandth part of their weight of uric acid per diem, whereas a small bird would eliminate as much as an eighty-fifth of its own weight. The amount of uric acid cannot, therefore, depend on the mere nature of the food.

There are, however, physiologists who do not consider it necessary to assume that urea must previously have existed as urate of ammonium, and some few have hinted that there may even be a synthesis in the production of uric acid. The whole subject is, however, in a state of great uncertainty, and, as Dr. Michael Foster has stated in his 'Text-book of Physiology,' "The whole story of proteid metabolism consists at present mostly of guesses and of gaps."

The difficulties in connexion with the formation of uric acid in the animal body have been before my mind for many years, and the aim of this paper is to suggest a solution which, in the first place, accords with facts which have been long established, and, in the second place, with others which I have recently discovered and proved experimentally.

It has always been difficult to me to believe that the metabolism of nitrogenised tissues should differ so completely in animals which are so closely allied in most other respects as the toad and the lizard, but which excrete their nitrogen, the one as urea, the other in the form of urate of ammonium, their diet being identical, and I think it will be found that the assumption that urea must be a product of the oxidation of urate of ammonium, has had much to do with the difficulties which have arisen in connexion with this subject.

Let us now consider certain points in the physiology of urea and uric acid, beginning with urea.

In mammalia, including man, it has been ascertained that urea is always present in the blood, and, though the quantity may be small, it is sufficient, nevertheless, to be measurable. I have recently obtained it not only from the blood of man, but also from that of the ox, the sheep, the goat, and the dog. And I have also confirmed a statement made by M. Picard that the blood of the renal artery is

much richer in urea than the blood of the renal vein, in the proportion, according to my experiments, of about three to one; of about two to one according to the experiments of M. Picard.

As far as I am aware, urea has never hitherto been detected in the blood of birds. Possibly it has never been looked for, as it would scarcely have been imagined that urea could be contained in the blood of an animal excreting only urate of ammonium. I have, however, recently found that the blood of birds contains urea in quantities not less than those found in the blood of mammals. The views I entertained on the formation of uric acid in the animal body led me to investigate this subject, as they necessitated the presence of urea in the blood of the uric-acid-excreting animals, as well as in the blood of those which eliminate urea only.

As the question of the existence of urea in the blood of different animals is of so much importance in connexion with the subject of this communication, I have taken special pains to determine not only that it is present, but also in what relative amounts, and the methods I have made use of have been as follows:—

In all my experiments I made use of dried blood, which form was by far the most convenient for my purpose. It was thus obtained:—Wide-mouthed bottles were half filled with absolute alcohol, and the blood as it flowed from the animal fell directly into the bottle, after which the spirit and the blood were intimately mixed by shaking. This mixture was then dried on a water-bath, reduced to a very fine powder, and kept in air-tight bottles. In making experiments, I assumed that one part of dried blood equalled five parts of liquid blood.

To determine the amount of urea I adopt the following method. The dried blood is treated with about six times its weight of distilled water, very slightly acidulated with oxalic acid, and this is heated in a water-bath to about 75° C. for at least six hours. The next day the residual blood is again treated in the same way with fresh acidulated water and again heated. The fluids poured off are then filtered and evaporated to dryness. Any urea abstracted from the blood is now in the form of the sparingly soluble oxalate of urea. If any oily matter is found in the residue of the evaporated fluids, this can easily be removed by washing with potassium naphtha, in which the salt of urea is totally insoluble. The residue is next treated with distilled water and a small quantity of the carbonate of barium (sufficient to cause a slight alkaline reaction) is stirred up with it. By this means the urea is set free and the oxalic acid fully neutralised.

After again evaporating to dryness, the residue is boiled with absolute alcohol, by which means the urea (together with small traces of other substances) is dissolved; and when this has been filtered into a large watch glass and evaporated spontaneously the urea is obtained in a solid form.

If this is again dissolved in distilled water and the solution introduced into a small ureometer filled with the solution of hypobromite of sodium, the quantity of nitrogen given off can be readily determined and the amount of urea in the solution can be deduced from it. 1 gramme of urea equals 0·46 gramme of nitrogen, or 372·7 c.c. In practice, however, 354·3 c.c. have been obtained.

If, on the other hand, we wish to crystallise the salt and directly weigh the amount, this is best effected by adding a few drops of nitric acid to the urea solution, so causing the formation of nitrate of urea. This can be weighed, after purification, by redissolving it in absolute alcohol.

The fact that the residue left after evaporation is undoubtedly nitrate of urea is proved by the characteristic form of the crystals (either simple rhomboids or aggregated varieties of these), or by a microscopic test which I have adopted of adding a drop of the hypobromite of sodium to the crystal under the microscope, and seeing the innumerable bubbles of nitrogen which are given off.

The following table shows the amount of urea found in a hundred parts of the blood of different mammals and birds:—

Sheep....	0·029 per cent.	Fowl	0·025 per cent.
Sheep....	0·025 ,,	Turkey ...	0·026 ,,
Ox.....	0·022 ,,	Duck	0·029 ,,
Ox.....	0·021 ,,	Turkey ...	0·024 ,,
		Fowl.....	0·026 ,,
		Duck	0·020 ,,
		Fowl.....	0·026 ,,
		Goose	0·020 ,,
		Turkey ...	0·022 ,,

It will be seen that, if we compare these results, the amount of urea in the blood of the mammal and of the bird is practically identical.

It has been found that in normal human blood the urea varies from 0·020 to 0·040 per cent. My results, obtained from the different animals above mentioned, are all within these limits.

When urea is introduced into the stomach of a mammal, it is eliminated by the kidneys in an unchanged form. This has repeatedly been shown to be the case, and the fact is not questioned by physiologists. On the other hand, when urea is introduced into the stomach of a bird, or other uric-acid-excreting animal, it is not thrown out in the form in which it is introduced; but there is, instead, an increased formation of uric acid. This has been shown to be the case by H. Meyer and M. Jaffé.*

Next with regard to uric acid.

* In the 'Berichte d. Deutschen Chem. Gesellschaft,' vol. 10, 1877, p. 1930.

Uric acid is not found in the blood of healthy mammals. Man, however, is, to some extent, an exception to this rule, for uric acid is often found when the deviation from good health is scarcely perceptible. This I showed in a paper in the 'Medical Chirurgical Transactions,' in 1848. Observations are usually made on the blood of animals which are killed for food, and these are probably young and healthy; whereas, when observations are made on man, the chances of his being in perfect health are diminished, since it is usually illness which brings his condition under notice.

The blood of healthy birds, instead of containing uric acid, as would naturally be supposed, is usually quite free from that substance, except when it has been introduced into the system through the stomach, or by injection. In a paper read before this Society in 1884, I showed that the blood of the duck was, for the most part, free from any detectible uric acid, and, in a paper read in 1848, I showed that the blood of a healthy pigeon was entirely free from it. I have recently had occasion to examine most minutely the blood of the goose, the fowl, and the turkey, and have found that, although fairly rich in urea, no uric acid could be separated from it. We may, therefore, conclude that birds may throw out uric acid by the kidneys, although it is not present in their blood.

Perhaps it will be desirable to mention here that uric acid introduced into the stomach of the bird as a soluble urate is absorbed into the blood, which becomes so saturated with it that it can be crystallised out with the greatest ease.

Uric acid thus introduced into the system is not thrown out by the kidneys, as these organs appear to be incapable of eliminating it, and this applies not only to the bird but to the mammal also. This fact has been proved by the researches of Zabelli.

Having considered these facts, the correctness of which is capable of being fully established, we are justified, I think, in coming to the following conclusions:—

First. That in mammalia and other urea-excreting animals the metabolism of the nitrogenised tissues results in the formation of urea as an ultimate product; that an appreciable and measurable amount of this substance is always found in their blood, and is constantly being excreted by the kidneys; and, further, that any cause leading to the decrease of this excretion produces an augmentation of the urea in the blood. It necessarily follows from this that, as stated above, the blood of the renal artery is richer in urea than the blood of the renal vein.

Second. That in birds and other uric-acid-excreting animals the metabolism of the nitrogenised tissues is exactly the same as in mammals, and that urea is the ultimate product of this metabolism; that urea is always present in their blood, in quantities not less than

in mammalian blood, and that the urate of ammonium is a subsequent product of the union of urea with some other principle or principles, glycine probably being one of them. Consequently, it is not necessary that uric acid should be present in the blood of uric-acid-excreting animals: in health, in fact, it is not detectible. When it is present, its presence is a result of its having been absorbed after formation in the kidneys or elsewhere.

With regard to the first proposition but little need be said, as most physiologists will agree with the statements it contains. With regard to the second, the case is very different, as the views therein enunciated are totally at variance with those which are held at the present time. The statement that the ultimate metabolism in the uric-acid-excreting animals is identical with that which takes place in mammals requires for its establishment the constant presence of urea in their blood. And I was led to seek for urea by my views on the production of uric acid, and from a recollection of having obtained urea from the blood of a turkey some few years ago. If no urea exists in the blood of birds, my theory falls to the ground. In this paper, therefore, I have laid special stress on the presence of this principle and the relative amounts contained in the blood of the two classes of animals discussed.

The statement that urate of ammonium is synthetically produced from this urea can easily be shown to be not only possible, but very probable; as likewise can the fact that it is not produced within the blood itself, but is formed, at least chiefly, in the kidneys. We have already spoken of the changes which take place when urea is introduced into the system of birds, whether by the stomach or otherwise, and certain experiments which I have made tend to indicate that glycine is, at any rate, one of the principles which plays a part in the building up of urate of ammonium. In the case of small birds, feeding on canary seed and water, the throwing out of the white urate of ammonia was observed hour by hour, and the quantity noted. When the seed was taken away—water only being left—the amount of the urate rapidly diminished. The exhibition of sugar did not increase the urate, but when a mixture of urea and glycine was given the amount of the urate was rapidly increased, whereas urea given alone at this period of the fasting produced little or no effect in the production of urate of ammonium. I do not, however, lay too much stress on these observations, as they were not sufficiently numerous, nor were the amounts weighed with sufficient accuracy.

From this theory it follows also that, though there is no need for uric acid to be present in the blood of animals which secrete it, the presence of urea is absolutely essential.

One of my observations is to this effect:—The blood of a goose

being examined, urea was crystallised out from it. Search was then made for uric acid, but no trace of this substance was discovered, and yet the renal secretion consisted of urate of ammonium.

It has been often said that the reason why uric acid is not found in the blood in any appreciable quantities is owing to the rapidity of its excretion by the kidneys, but this statement should certainly apply to urea, which, as I have shown above, is always found in the blood.

The existence of uric acid in the blood may be looked upon, therefore, as a morbid phenomenon. With birds, and especially those kept for domestic purposes, as, for instance, caged birds or ducks, the water they drink is frequently strongly impregnated with soluble urate, and this, when taken into the alimentary canal, is absorbed into the blood. I was enabled to crystallise uric acid out of the water of a duck pond, as I stated in a paper read before this Society in 1886. On the other hand, I have examined the blood of many ducks without being able to detect a trace of uric acid.

In the human subject, in which the average quantity of uric acid found per diem is small compared with the formation of urea, the blood appears, nevertheless, to be frequently contaminated by its presence, not, however, in the form of urate of ammonium, in which it is doubtless thrown out by the kidneys, but in the form of biurate of sodium, in which shape it exists also in morbid deposits—the so-called chalk-stones of medical authors.

When uric acid is not introduced into the blood by the alimentary canal, its presence must, according to my view, be accounted for by its absorption into the blood from the kidneys after its formation in these organs, and the salt is necessarily changed by the blood from urate of ammonium to biurate of sodium; whereas, according to the old view, it had to be assumed that urate of sodium was converted into some superurate of ammonium, which I believe all chemists would regard as an impossibility.

In conclusion, I may remark that the facts and deductions brought forward in this paper must prove, if established, not only of interest to the physiologist, but must aid the pathologist in the investigation of several diseases and be of value in devising methods for their treatment.

The Society adjourned over the Whitsuntide Recess to Thursday, June 1.