THE PHYSIOLOGY OF EXCRETION IN A BLOOD-SUCKING INSECT, *RHODNIUS PROLIXUS* (HEMIPTERA, REDUVIIDAE)

I. COMPOSITION OF THE URINE

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(*Received 13th June*, 1931.)

(With Four Text-Figures.)

CONTENTS.

It is the object of the present work to attempt a complete description of the process of excretion in a single species of insect, a description in which the anatomical structure of the excretory system and the histological changes during activity will be correlated with the chemical composition of the urine. Information of this kind is almost entirely wanting in the case of insects; yet it will be shown that in some respects they are so well suited to this type of investigation as to rouse the hope that such studies may throw light on the more general problems of secretory activity.

The insect chosen for this purpose is the blood-sucking Reduviid bug *Rhodnius prolixus*, an insect, which, besides being of convenient size (it is about 2 cm. in length) and being easily reared at all seasons of the year, presents the additional advantage of feeding on an absolutely constant diet, the composition of which is accurately known.

In this paper an account will be given of the composition of the urine at different stages after a meal.

GENERAL METHODS.

The methods employed in rearing and feeding *Rhodnius prolixus* in the laboratory have been described by Buxton (1930). For the present purpose, only adult insects have been used. After being fed to repletion with rabbit blood, these were kept for 24 hours at room temperature (about 18° C.) and thereafter at 23° C. in a humid atmosphere. The process of excretion is greatly influenced by temperature, and, except where otherwise stated, these conditions have been closely followed throughout.

For the collection of urine, each insect, with the wings held in a Mohr's clip, was kept suspended over a watch glass or hollow ground slide. The methods used in analysing the urine are described later.

GENERAL COURSE OF EXCRETION.

The weight of an adult *Rhodnius* varies from about 50 to 80 mg., and the quantity of blood taken at a single meal from 140 to 180 mg. Under the conditions employed, the complete digestion of this amount of blood requires five or six weeks.

Almost immediately after feeding, the insect voids the black residue of its previous meal; then, a few minutes later, a drop of cloudy watery fluid. For the next three or four hours it passes, at intervals of a few minutes, a perfectly clear colourless fluid; and then the passage of urine ceases.

On the next day it may pass a drop of cloudy fluid; or it may pass no more for three or four days or a week. The longer the appearance of this next drop is delayed, the greater is the proportion of sediment it contains; and if it does not appear for a week, it is in the form of a pultaceous mass which dries as a yellow powder.

Sometimes, after the first day, the urine is contaminated with haematin from the intestine, and this is always the case in the later stages of digestion; but frequently it contains no faecal material for a week or ten days, and in rare instances for a month, after the meal.

The frequency with which the excrement is discharged varies greatly in different individuals; some voiding a little every few days, giving as many as ten samples in a month; others producing no more than two or three evacuations in the same period. A complete stoppage is not uncommon, but is always fatal in a few weeks.

For purposes of description, it will be convenient to consider separately the excretion of clear fluid during the first few hours after the meal and the subsequent excretion of semi-solid material.

EXCRETION OF WATER.

To study the rate of excretion of water, the clear urine passed in the few hours following the meal has been collected in a graduated pipette. The fluid was allowed to fall upon a waxed slide from which it could be collected quantitatively without loss.

The results are given in Fig. 1, which shows the volume of urine in four insects plotted against time. In two cases (*A* and *C*) the rate of excretion was more rapid during the first half-hour, but in all cases the rate was more or less constant or

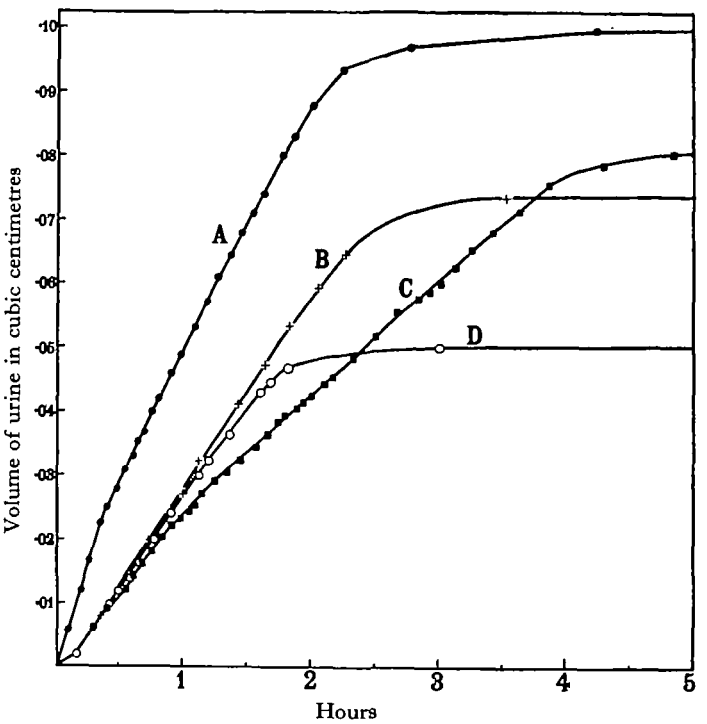

Fig. 1.

linear throughout the greater part of its course, and then the excretion ceased abruptly. It is interesting to note the great difference in frequency with which the drops of urine (as indicated by the points on the curves) were passed by these different insects.

Fig. 2 was derived from the same experiment as curve *A* in Fig. 1, and shows graphically the proportion which the volume of fluid excreted bears to the total fluid ingested. The block *A* represents the initial weight of the insect (78 mg.). The blocks *B* and *C* represent respectively the solid constituents (44 mg.) and the water (132 mg.) in the blood ingested (176 mg.). The block *D* represents the weight of the insect four hours later, and *E* the weight of water that has been excreted; the gap between *D* and *E* being loss of weight unaccounted for. It will be seen that the

water excreted was 101 mg. or 76·5 per cent. of the total fluid in the blood ingested. So that 23·5 per cent. of the ingested fluid, together with the water produced in metabolism, is all that remains available to the insect to accomplish the whole of its excretion during the next six weeks.

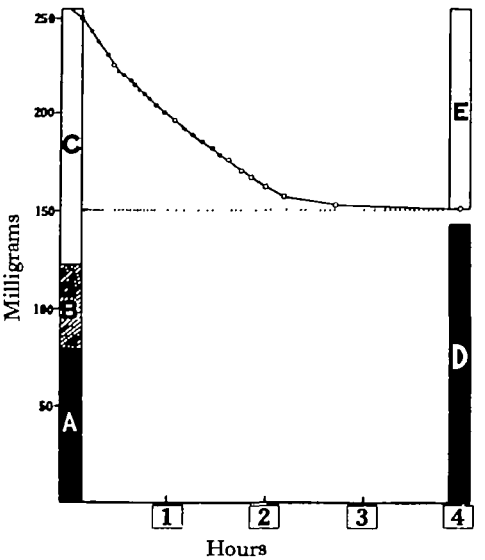

Fig. 2. Excretion of water by *Rhodnius*. *A*, initial weight of insect; *B*, solids and *C*, water in the ingested blood; *D*, weight of insect at end of four hours; *E*, water excreted.

CHARACTERS OF THE CLEAR URINE.

Specific gravity. The specific gravity of the clear urine, as determined by the method of Barbour and Hamilton (1926), was about 1·007 in four insects, and no difference in specific gravity between successive samples could be detected with certainty.

Osmotic pressure. This was measured by the vapour-pressure method of Barger (1904). Solutions of sodium chloride of known strength were used as standards and the values for osmotic pressure determined to the nearest 0·05 per cent. of sodium chloride.

The total clear urine of four insects gave values of osmotic pressure equivalent to 1·05 per cent. sodium chloride, 1·05 per cent. ($\Delta = 0{\cdot}68$), 1·0 per cent. ($\Delta = 0{\cdot}65$), and 0·95 per cent. ($\Delta = 0{\cdot}62$).

In two other insects the osmotic pressure of successive samples of urine was measured. The results are shown in Table I.

It will be seen that there is a tendency for the concentration to rise very slightly towards the end of the period, but that in general the clear urine is almost isotonic with the ingested blood; the Δ of rabbit plasma being 0·59, which is equivalent to 0·91 per cent. sodium chloride.

Table I.

	Osmotic pressure expressed as equivalent strengths of sodium chloride, to nearest 0·05 %				
Time after feeding (hours)	½	1	1½	2	2½
Insect 1	0·95	0·90	1·0	1·05	1·05
Insect 2	1·0	1·0	1·0	—	1·1

Reaction. As ordinarily collected, the urine is strongly alkaline (*p*H 9); but this is partly due to the loss of carbon dioxide to the atmosphere; for if the urine is mixed with indicator on a waxed slide (Wigglesworth, 1927) the instant it is passed, it is hardly ever more alkaline than *p*H 8·0. The following results were obtained with successive samples from one insect which was fed at 11.0 a.m.: 11.20, *p*H 7·8 (cresol red); 11.30, *p*H 8·0 (cresol red); 11.50, *p*H 7·8 (cresol red); 12.0 noon, *p*H 8·0 (thymol blue); 12.15 p.m., *p*H 8·1 (thymol blue); 12.30, *p*H 8·0 (cresol red); 1.20, *p*H 8·0 (cresol red); 1.45, *p*H 7·8 (cresol red). Probably the more acid figures (*p*H 7·8) represent most nearly the true reaction. It will be noted that there is no significant change in reaction during the excretion of the clear urine.

Total alkalinity. It is evident from the rapid increase in the alkalinity of the urine on exposure to the air that it contains a considerable amount of base in the form of bicarbonate. It is important to know what proportion of the total excess base in the food the amount lost in this way represents. For, as will be shown later, the urine contains no ammonium; so that any excretion of bicarbonate will serve to deplete the supply of fixed base available for excretion with uric or other organic acids.

To test this point, a number of *Rhodnius* were weighed before and after feeding, and as soon as the urine became clear, it was allowed to drop into a measured volume of 0·01 *N* sulphuric acid. At the end of six hours the acid, after being heated to boiling, was titrated with standard soda. Control experiments without any insects were also made; and the difference represented the total alkalinity of the urine.

A single experiment will serve to illustrate the results. A *Rhodnius* weighing 57 mg. took 155 mg. of blood, and the total alkalinity of the urine was equivalent to 0·07 c.c. of 0·01 *N* acid. Now the ash from 1000 gm. of blood contains 0·68 gm. of sodium not combined with acid[1]; therefore 155 mg. of blood will contain 0·01 mg. which is equivalent to 0·43 c.c. of 0·01 *N* acid. Thus, the excess base in the ingested blood, expressed as a volume of 0·01 *N* solution, was 0·43 c.c.; and of this about one-sixth (0·07 c.c.) was excreted during the first few hours, leaving 0·36 c.c. available for excretion with the organic acids.

This result is, of course, only approximate, but it will be seen later that the calculation is not without significance.

Chemical composition. On evaporation, the clear urine yields a mass of crystals, mixed with small amorphous granules, "dumb-bells" and "wheatsheaves."

[1] In the absence of precise analyses of rabbit blood, figures taken from Karl Schmidt's analysis of human blood have been used in this calculation.

Of inorganic constituents: it contains a considerable quantity of *carbonates*, effervescing actively with dilute acids. It is rich in *chlorides* (precipitated with silver nitrate in presence of nitric acid). It contains a small amount of *sulphate* (precipitated with barium chloride in presence of acetic acid); but no phosphate (method of Briggs, 1922). As to kations: it contains no ammonium (Nessler's test), but much *sodium* (precipitated with saturated potassium pyroantimonate) and *potassium* (precipitated with sodium cobaltinitrite in strong acetic acid). It sometimes contains a trace of *calcium* (precipitated with ammonium oxalate in presence of ammonium acetate), but no iron (prussian blue reaction) nor magnesium (tested with ammonium phosphate and ammonia after removal of calcium; also titan yellow test (Kolthoff, 1927).

Of organic constituents: it contains an appreciable quantity of *urea* (radiating needle crystals with xanthydrol and glacial acetic acid; also urease test), and *uric acid* (Folin's test; murexide reaction). It contains no protein (sulphosalicylic acid test; Millon's test; biuret reaction), no reducing sugar (Benedict's test), no creatine (Jaffe's test after hydrolysis with half-normal hydrochloric acid in a sealed tube at 140° C. for two hours), nor creatinine (Jaffe's test). It does not give a positive nitroprusside reaction (for acetone or aceto-acetic acid or reduced sulphydryl compounds), nor does it contain lactic acid (giving no colour with dilute ferric chloride).

COMPOSITION OF CLEAR URINE AT DIFFERENT STAGES AFTER FEEDING.

It was of interest to compare the chemical composition of the clear urine at different stages of its excretion, and this was done as follows. The time of feeding of each insect was noted and its urine collected in a watch glass during the next half-hour. The watch glass was then changed, and in this way half-hourly samples of urine were collected until the flow ceased. Since the urine is usually passed every few minutes, fairly even samples were obtained. The fluid was allowed to dry and then the various qualitative tests mentioned above were applied, using measured quantities of the reagents in each case. The intensity of the reaction, whether colour change or precipitate, could then be compared directly on the different samples and the relative values expressed by + signs.

The results are shown in Fig. 3, the + signs being expressed graphically in the form of blocks. For convenience in comparison experiments have been selected in which the secretion of fluid continued for at least three and a half hours. The chart does not, of course, give any indication of the proportion which the various constituents bear to each other, sodium and potassium chlorides far surpassing any other constituent. It is intended merely to give an approximate idea of the degree of variation in a given constituent.

It will be seen that the output of *uric acid* is high at first and then gradually falls until it amounts to a trace only. Doubtless this is due to the "washing out" of urates from the previous meal. Towards the end of the period, however, it begins

to rise again, showing that uric acid is already being produced from the new meal. These results were obtained by Folin's test, but they can be confirmed by microscopic examination of the dried urines. The amorphous granules, spheres, "dumbbells," etc., are numerous in the early and late samples, scanty in the middle period.

The excretion of *urea* is constant throughout. Probably it is derived largely, if not entirely, from the preformed urea in the ingested blood.

The *sulphate* excretion seems to run more or less parallel with that of uric acid, but the quantities are so minute that too much reliance cannot be placed on these results.

The *chloride* is more or less constant throughout; and the same is probably true of the *carbonate*.

The output of *sodium* is very high in the early samples but present only in traces later; whereas *potassium*, present at first in very small quantities, gradually increases in amount. This interesting distinction is doubtless due to the fact that the greater part of the sodium is contained in the blood plasma, whereas the

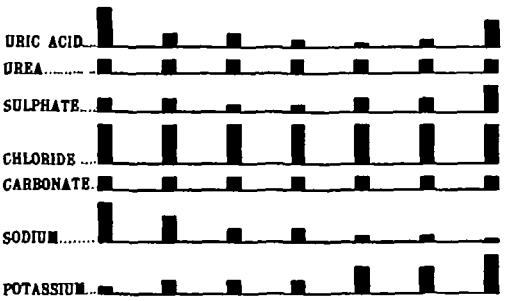

Fig. 3. Diagram showing course of excretion of chief urinary constituents, over half-hour periods for 3½ hours after feeding. The blocks roughly represent five grades of reaction: trace, ±, +, ++, +++.

potassium is for the most part confined to the corpuscles. It recalls the observation of Haldane, Wigglesworth and Woodrow (1924) that during experimental acidosis in man the loss of sodium in the urine precedes the loss of potassium, again presumably because the sodium is more readily available in the plasma and tissue fluids.

CHARACTERS OF URINE AFTER FIRST DAY.

To gain a true idea of the character of the urine after the first day, it must be obtained directly from the rectum by dissection. The rectum is a pyriform sac, which will be described in detail in another paper (Wigglesworth, 1931 *a*), containing, when it is well distended, about 10 to 12 c.mm. of fluid. If examined at the end of twenty-four hours, it is found to contain a clear faintly yellow fluid above a whitish sediment. The sediment is composed of the familiar uratic spheres, the structure and composition of which will be considered later. At the end of forty-eight hours, the sediment has greatly increased and the supernatant fluid is a deep

amber colour. Thenceforward the rectum becomes mainly filled with the uratic deposit.

Osmotic pressure. It has been possible to insert into the rectum a fine capillary tube with the tip ground to an oblique point, to draw off the clear urine overlying the uratic sediment, and to use the mixed fluid so obtained from a number of insects for osmotic pressure determinations. The methods of dissecting and manipulating the insects will be described in another place (Wigglesworth, 1931 *a*); the essential points are to allow some time for the sediment to settle out and to dry the surface of the rectum carefully with filter paper. The results are shown in Table II, one of the experiments on the urine of the first few hours being included for comparison. It will be seen that there is a great increase in the concentration of the urine after the first day. Unfortunately I have been unable to obtain enough clear fluid for estimation later than forty-eight hours after feeding.

Table II.

Number of insects used	Time after feeding	Osmotic pressure as equivalent strength of sodium chloride	Δ (by calculation)
1	4 hours	1·05	0·68
4	24 ,,	1·7	1·10
3	48 ,,	2·2	1·43

Reaction. The reaction of the later urines has also been determined by dissecting out the rectum, opening it upon a waxed slide, and mixing the contents at once with an indicator. Only insects with no haematin in the rectum were used. The results are shown in Table III, each of the figures being obtained from a different insect. Although there are considerable individual variations, it will be seen that the urine gradually becomes more acid until it is about *p*H 6·0.

Table III.

Time after feeding	*p*H and indicator used
3 hours	7·8 (see above, p. 415)
24 ,,	7·2 (B.T.B.); 6·6 (B.T.B.); 7·4 (B.T.B.); 7·4 (P.R.)
48 ,,	7·2 (B.T.B.); 6·8 (P.R.); 6·4 (B.T.B.)
3 days	6·6 (B.T.B.); 7·0 (P.R.); 7·2 (C.R.); 6·2 (C.P.R.)
10 ,,	6·0 (C.P.R.); 6·2 (B.T.B.); 5·8 (C.P.R.); 6·0 (C.P.R.)

B.T.B. = bromo-thymol blue; P.R. = phenol red; C.R. = cresol red; C.P.R. = chloro-phenol red.

Chemical composition. The chief constituent of the later urines is, of course, uric acid, and the precise form in which this is excreted will be discussed later. To demonstrate the other constituents the dried sediment was stirred with 0·5 to 1 c.c. of 1 per cent. acetic acid and allowed to stand for half an hour. Under this treatment the uratic spheres disappear, most of the uric acid crystallises out, and everything else, except any haematin present, goes into solution. On filtration this yields a perfectly clear fluid upon which the various tests already mentioned were performed.

Carbonates, which were tested for in the dried residue before extraction, are absent after the first day. *Chlorides* are present in traces, and *sulphates* and *phosphates* in appreciable amounts. Ammonium is absent, *sodium*, *potassium* and *calcium* present in small quantities; free iron is absent, *magnesium* absent or present in traces only. There are traces of *urea*, no protein, no reducing sugar and no creatinine. *Creatine* is present; acetone and lactic acid absent. Guanine (picric acid and potassium ferricyanide tests) is absent. The occurrence of other nitrogenous compounds, such as *amino acids*, will be considered later.

The yellow *pigment* in the urine is of unknown nature. In water it gives a yellow or amber-coloured solution with a slight green fluorescence. It was thought that it might be derived from preformed carotin in the blood of the rabbit; but it is insoluble in chloroform and gives no blue colour with concentrated sulphuric acid. It is insoluble in hot alcohol, differing in this respect from the "entomourochrome" of Veneziani (1904), and it shows no absorption spectrum. In all these general properties it agrees with the yellow pigment ("lepidotic acid") described by Hopkins (1896) in the wing scales of Pieridae, which is closely related to uric acid. An attempt was made to test this resemblance by conversion into the purple pigment ("lepidoporphyrin") by heating with sulphuric acid. This attempt was unsuccessful, but this may have been due to the very small amount of the pigment available.

There is one other feature of the urine that must be mentioned, although its significance is not understood. It has been observed that until about the fourth day the dried urine will not mix nicely with water, but breaks up into granular and flaky masses. After about the fourth day it mixes at once, to give a uniform suspension. This change is quite independent of any contamination from the contents of the gut.

COMPOSITION OF URINE ON SUCCESSIVE DAYS AFTER FEEDING.

As already mentioned, the different insects pass their urine at very different intervals, so that it is not easy to follow accurately the composition of the urine on successive days; but by collecting the excreta of a large number of insects, it has been possible to obtain samples at all stages after the meal and thus to piece together a consistent picture of the course of excretion.

Uric acid seems to be excreted at a pretty constant rate (about 0·5 mg. a day, see below) for three weeks or so after feeding, and then the rate of excretion falls off. *Urea* is present in small amounts in the urine passed at twenty-four or forty-eight hours after feeding, though never in such quantity as during the first few hours. After the first day or two it is present in minute traces only. *Creatine*, which is absent during the first day, is absent also in the next forty-eight hours or so. But then it appears and seems to increase in amount during the later stages of digestion. *Chloride* is present in fair amount during the first twenty-four or forty-eight hours, but thereafter only in traces. The *sulphate* excretion increases after the first day and then remains fairly constant, like the uric acid. *Phosphate* is entirely absent during the first twenty-four hours. It is present as a minute trace at the end of

forty-eight hours, and then increases, to remain constant or to fall off a little in the later stages of digestion. In one case an approximate estimation of the phosphorus output was made by Briggs' method, the results of which are shown in Table IV.

Table IV. *Phosphate excretion by* Rhodnius, *fed* 20. i. 31.

Date of passage of urine	Inorganic phosphorus in urine, in mg.	Output of phosphorus per diem, in mg.
22. i. 31	·0005	·00025
24. i. 31	·003	·0015
28. i. 31	·012	·003

Sodium and *potassium* are both present throughout in very small amounts. In view of the differences in the excretion of these two metals during the early stages of excretion, it was interesting to compare them on the later days. In the absence of ammonium, the potassium excretion may be judged by the quantity of precipitate with sodium cobaltinitrite under standard conditions; but owing to the presence of appreciable quantities of calcium in the urines after the first day, potassium pyroantimonate is of no use as a test for sodium, and therefore the recent method of McCance and Shipp[1] (1931) has been employed. A series of urines from a given insect were treated with 1 per cent. acetic acid and filtered as already described. Then aliquot parts were taken and treated respectively with sodium cobaltinitrite and uranyl zinc acetate according to the technique of McCance and Shipp. The precise values obtained are of no significance, but so far as the relative amounts are concerned it was found that during the first day or two, as in the latter part of the first day's excretion, the potassium output was relatively very high; but thereafter, though both metals were present in very small quantities, the ratio of sodium to potassium remained more or less constant, the output in the case of sodium being of the order of 0·015 mg. per diem.

Calcium, which is absent, or present in traces only, during the first day, occurs in much greater amounts during the next forty-eight hours. The excretion then falls to a very low level, although minute quantities are constantly present. *Magnesium* often does not appear in the urine until the very late stages of digestion, but it can always be demonstrated in urines passed three weeks or a month after feeding.

The general course of excretion of all the urinary constituents which have been recognised is summarised in Fig. 4. This chart is drawn up on the same lines as Fig. 3, but differs from that in being merely deduced from a number of observations on different insects and not based on individual experiments. The clear urine excreted in the first few hours is included with the first day.

[1] I am much indebted to Dr R. A. McCance for showing me the details of this method before publication, and for providing me with the necessary reagents.

COMPOSITION OF THE URATIC SPHERES AND THE FORM IN WHICH URIC ACID IS EXCRETED.

From a glance at Fig. 4 it is evident that by far the most important excretory function in *Rhodnius* is the elimination of uric acid, and that the greater part of this elimination takes place after most of the water in the meal has already been excreted. This problem must now be considered in more detail from the chemical standpoint, though its complete elucidation must be deferred until the anatomy and histology of the excretory system have been described (Wigglesworth, 1931 *a* and *b*).

As in most other insects, as well as in birds and reptiles, the uric acid occurs in the form of minute spheres, 3 or 4μ in diameter, with a distinct radial striation. As observed by Sirodot (1858) in insects and Meissner (1868) in birds, if these spheres are treated with dilute acetic or hydrochloric acid, they rapidly disappear,

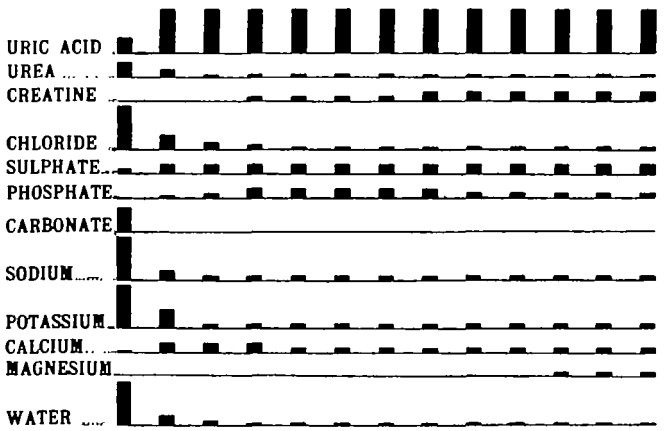

Fig. 4. Diagram showing course of excretion of chief urinary constituents during thirteen days after feeding.

leaving no trace, and crystals of uric acid separate out. On treatment with distilled water the same thing happens, but more slowly. If treated with sodium hydroxide the spheres dissolve at once; but if the alkali is very dilute they leave behind a diaphanous stroma or husk, which itself eventually dissolves. This husk is more readily seen if the spheres are treated with dilute ammonia, when their uratic contents quickly dissolve and reappear in the form of amorphous granules. Meissner observed a similar husk or stroma (Gerüst) in the uratic spheres of birds.

The nature of this stroma is uncertain. Meissner (1868) and Ebstein and Nicolair (1896) supposed it to be composed of protein; but we have already seen that there is no protein in the urine of *Rhodnius*, nor, according to Szalagyi and Kriwuscha (1914), does the urine of birds contain the smallest trace of protein. If the residue of the urine, after treatment with dilute ammonia, is dried and treated with Millon's reagent, there is active effervescence around the ammonium urate, but the husks of the dissolved spheres are unaffected, nor do they show any coloration. Doubtless the husks are composed of some material adsorbed on to the

spheres from the urine or secretory cells, but this material does not appear to be of a protein nature.

Turning now to the form in which uric acid is present in the spheres: Meissner concluded, from the observations with dilute acids and alkalis described above, that in the urine of birds the greater part of the uric acid is free and not combined with any base at all; and Szalagyi and Kriwuscha (1914), on analysis of the urine of hens, found that not more than 10 per cent. of the uric acid could be combined with base.

Another view of the composition of the spheres is that put forward by Bence Jones (1862), according to which they are composed of sodium or potassium "quadriurate," that is, a double salt made up of a molecule of uric acid combined with a molecule of an acid urate. This theory was examined by Kohler (1910), who concluded that there was no such body as "quadriurate," which is merely a mixture of uric acid and the acid salt in proportions varying enormously with the conditions under which crystallisation occurs. In the case of snake urine he found that not more than 16·7 per cent. of the uric acid present could be combined with base. Incidentally Kohler observed that under certain conditions his mixtures of acid sodium urate and uric acid would separate out in the form of spheres and that when these were placed in water they would disappear and be replaced by crystals of uric acid, just like the natural spheres in the urine of *Rhodnius*. This property was supposed by Bence-Jones to be characteristic of "quadriurate."

There are no quantitative observations on these lines in the case of insects, where the spheres are usually stated to consist of sodium or ammonium urate; but it is probable that here also, in many cases, most of the uric acid is free. Thus in the case of *Rhodnius* we have seen that the urine contains no ammonia, and therefore any uric acid in the form of salt must be combined with one of the fixed bases. But in studying the total alkalinity of urine during the first day's excretion (p. 415) we have seen that the total excess base in the blood ingested at an average meal was equivalent to no more than 0·43 c.c. of 0·01 *N* acid, and of this only 0·36 c.c. remained at the end of the first few hours. This amount of alkali, in the form of acid urate, will combine with 0·60 mg. of uric acid. But it will be shown later (Table V) that the excretion of uric acid is at the rate of 0·5 to 0·6 mg. per diem for about three weeks after feeding. Thus, the amount of base available is equivalent to only a single day's excretion of uric acid. It is evident, therefore, that most of the uric acid must be free.

In order to test this question directly, advantage has been taken of the observation by Sôrensen (1908) that, in the presence of formaldehyde, uric acid will dissolve very readily and titrate quite sharply as a monobasic acid. The procedure was as follows. A sample of the semi-solid urine, uncontaminated by faecal material, was dried over sulphuric acid and weighed. It was then dissolved in 0·4 c.c. of 20 per cent. neutralised formaldehyde and 2·0 c.c. of distilled water, and titrated with 0·01 *N* sodium hydroxide with phenolphthalein as indicator. From this figure the free uric acid can be calculated. The solution was then made up to a known volume and the total uric acid estimated by Benedict's method on an

aliquot part. The presence of formaldehyde interferes somewhat with the colour production by this method; therefore, as a standard, pure dry uric acid was weighed out, dissolved in the same quantity of formaldehyde, and titrated in the same way as the test specimen. This procedure served as a control for the titration, which gave values exactly equivalent with the uric acid weighed out. The results with three samples of urine are given in Table V.

Table V.

Period of collection of urine, in days	Weight of dried urine, in mg.	Total uric acid		Free uric acid		Uric acid output per diem, in mg.
		Mg.	% of dried urine	Mg.	% of total uric acid	
13	9·2	7·75	84	7·3	87	0·59
3	2·4	1·84	77	1·65	89·5	0·61
6	4·7	3·0	64	2·48	83	0·50

It will be seen that 64 to 84 per cent. of the dried urine was composed of uric acid, and of this from 83 to 89·5 per cent. was free. The daily excretion of uric acid varied from 0·50 to 0·61 mg.

There are two possible sources of error in this method. In the first place there may be other acids[1] besides uric acid which are being titrated; and in the second place there may be other substances which titrate as acids in the presence of formaldehyde.

The presence of other free acids is certainly not a source of error; because if the dried urine is shaken up with water instead of formaldehyde and a drop of 0·01 *N* soda added, it is rendered alkaline at once, and it only becomes acid again very slowly as the uric acid dissolves.

The second possibility is more serious, because although ammonium salts and proteins have been shown to be absent, amino acids (notably leucine) are frequently stated to occur in the urine of insects. The possibility of these being present in quantity has been tested as follows. A sample of the dried urine free from faecal matter was divided into two lots, each of which was weighed. One lot was dissolved in neutral formaldehyde and titrated with 0·01 *N* soda to *p*H 8·5 with phenolphthalein as indicator. The other lot was treated with 1 c.c. of 1 per cent. acetic acid, filtered after standing, and the residue washed with a further c.c. of acetic acid. The filtrate was then adjusted to *p*H 8·5 by addition of soda. When neutralised formaldehyde was then added to this mixture it certainly became very slightly more acid, but it required only a drop or so of 0·01 *N* soda to bring it back to the original *p*H. Thus, 1·2 mg. of dried urine in presence of neutral formaldehyde required 0·85 c.c. of standard soda to titrate it to *p*H 8·5. 1·8 mg. of the same sample treated with acetic acid and neutralised to *p*H 8·5, on subsequent addition

[1] Hollande and Cordebard (1926) describe an unrecognised acid in large amounts in the excreta of the clothes moth (*Tinella biselliella*), but, in spite of their assertions to the contrary, it seems very probable that this is uric acid; and if it is reckoned as such, analysis of their figures shows that 86 per cent. of the uric acid in the excreta is in the free form.

of neutral formaldehyde, required 0·08 c.c. of 0·01 *N* soda to bring it back to *p*H 8·5. Therefore, 1·2 mg. would have required 0·05 c.c. Hence $\frac{0{\cdot}05 \times 100}{0{\cdot}85}$ or 5·9 per cent. of the formaldehyde titration was due to amino acids and not to free uric acid. This is not a very material error, and if the necessary correction be made in the results given in Table V, the figures for free uric acid in the three samples become 82·5 per cent., 85 per cent. and 79 per cent. of the total uric acid.

It is almost certain from these observations that by far the greater part of the nitrogenous excretion of *Rhodnius* is in the form of uric acid. But the urine contains a little creatine, a trace of urea, and probably some amino acids; and in order to eliminate the possibility of these, and possibly other unrecognised nitrogenous compounds, being responsible for a substantial part of the nitrogen excretion, estimations have been made, on the same samples of urine, of the uric acid nitrogen (by Benedict's method) and the total nitrogen (by the Kjeldahl method, according to the technique of Myers (1924)).

The results, which are given in Table VI, indicate that only some 8 or 10 per cent. of the nitrogen is not in the form of uric acid. Unfortunately this result is barely outside the experimental error of the methods employed; but at least it serves to show that uric acid is by far the most important vehicle for the elimination of nitrogen.

Table VI.

Weight of dry urine, in mg.	Total N	Uric acid N	Uric acid N as percentage of total N
2·5	0·538	0·495	92
1·6	0·410	0·368	90

DISCUSSION.

Urinary constituents fall roughly into two categories: (i) substances which are preformed in the food and, not being required by the organism, are eliminated unchanged in the urine; and (ii) the final products in the metabolism of the assimilated materials.

In *Rhodnius*, the former category comprises the metals sodium, potassium, calcium and magnesium; the chlorides, carbonates, and, to a small extent, the phosphates and probably urea. It will be seen at once from Fig. 4 that the elimination of this group of substances is accomplished almost entirely during the first day, and that most of the water in the meal is utilised for this purpose.

The discharge of much clear fluid soon after feeding is characteristic of nearly all blood-sucking insects and there has been much speculation as to its nature. It has often been regarded as "serum" separated in the intestine from the blood corpuscles. But Lester and Lloyd (1928) showed clearly that in the case of the tsetse fly (*Glossina*) it is produced by the Malpighian tubes, and the present work has shown that in *Rhodnius* (and this is probably true of other blood-sucking insects)

it is a salt solution, more or less isotonic with the ingested blood, which serves for the rapid elimination of the unwanted salts in the diet. About 75 per cent. of the water in the meal is got rid of in this way, and it can be shown by calculation that this volume of isotonic salt solution will contain nearly all the salts in the blood.

There are, however, certain exceptions. Thus, the calcium does not appear in appreciable quantities until a day or two after feeding, and the magnesium is often retained until very late in digestion. The reason for this is not entirely clear; but it was found by Bishop, Briggs and Ronzoni (1926) that, in the blood of the honey bee larva, the calcium and magnesium content is far higher than in mammalian blood, being 1·5 times higher in the case of calcium and eight times higher in the case of magnesium. If the blood of *Rhodnius* has the same sort of composition[1] as that of the bee larva, this might account for the temporary retention of these substances, for there is almost no blood in the fasting insect, but a fair volume of blood during the first few weeks after feeding.

The same argument may apply to the phosphate, which is absent from the urine during the early stages of digestion, for the phosphate content of bee blood is ten times greater than is that of mammalian blood. But phosphorus belongs also to the second category, for most of the phosphorus in blood is in organic form, as lecithin, nuclein, etc., and will therefore only be liberated during the katabolism of these substances.

The question of urea is interesting, because in most animals, of course, urea is an important member of the second category of excretory substances. In *Rhodnius*, however, almost the whole of the urea occurs in the urine in the earliest stages of digestion, which suggests that it is derived from the preformed urea in the blood of the rabbit. The occurrence of urea in the urine of insects is for the most part very ill substantiated, resting only on a few very old observations on crystal structure. There is, however, one curious exception. Babcock (1912) records an analysis of the dried excreta of the clothes moth (*Tinea pellionella*) which was said to contain 17·57 per cent. of urea. Unfortunately, no details of the methods employed are given; but certainly urea would seem to be a most unfavourable vehicle for nitrogenous excretion in an insect feeding on so dry a diet. More recently Hollande and Cordebard (1926) have found 0·4 per cent. of urea in the excreta of the clothes moth (*Tinella biselliella*), but their analysis, they point out, was made on excrement which had been lying about for four years.

The second category of excretory substances, in *Rhodnius*, comprises creatine, sulphate, uric acid and perhaps amino acids. Ammonia is absent.

The significance of creatine is not understood, but it is almost certainly an end-product of metabolism in the insect, and not derived from the food.

Sulphate, of course, is the end product of the cystine component of the blood protein, and since blood is poor in cystine the sulphate excretion is naturally very low. It is worth noting how rapidly it appears after the new meal has been ingested (Fig. 3); this is a familiar observation in mammals.

[1] Quantitative analysis of the blood of *Rhodnius* has not been attempted, but it is easy to demonstrate the presence of magnesium in a very small drop of it by the titan yellow test.

The part played by amino acids in the nitrogenous excretion of insects is a subject needing further investigation. There are many old records (Kölliker, 1858; Schindler, 1878) of the occurrence of numerous "leucine spheres" in the Malpighian tubes of insects, but these require chemical confirmation. Such crystals are certainly not conspicuous in *Rhodnius*, and the chemical evidence (formol titration) suggests that amino acids take only a very small share in the excretion of nitrogen.

The absence of ammonia from the urine calls for comment. Ammonia has often been recognised in the excreta of insects, notably in the clothes moth by Babcock (1912), Schulz (1925) and Hollande and Cordebard (1926). In these cases it was in such quantities as to suggest that the uric acid was present as an ammonium salt, but the possibility must also be considered that the ammonia was required to combine with the large amount of sulphate[1] derived from a diet of keratin, and that the absence of ammonia from the excreta of *Rhodnius* is correlated with the low sulphur content of its food.

The main nitrogenous constituent of the urine is uric acid, and it has been shown that only some 10 to 20 per cent. of this is in the form of urate. The mechanism by which the highly insoluble free acid is excreted, in the comparative absence of water, will form the chief problem of the histological investigation that is to follow (Wigglesworth, 1931 *a* and *b*).

SUMMARY.

An adult *Rhodnius* will ingest from two to three times its weight of blood at a single meal, and about three-quarters of the water in this blood is excreted as a clear fluid during the next three or four hours.

This fluid is alkaline (*p*H 7·8), more or less isotonic with the blood (sp. gr. 1·007; $\Delta = 0{\cdot}62$–$0{\cdot}68$), and serves for the elimination of most of the sodium and potassium chlorides in the meal. It also contains urea, bicarbonate, sulphate and uric acid.

After the first day, the urine gradually becomes acid (*p*H 6·0–6·5) and much more concentrated, and contains a yellow pigment. Uratic spheres appear and increase in number until the urine is semi-solid. The urine now contains only traces of sodium, potassium, chloride and urea. There are small amounts of calcium, magnesium, phosphate, sulphate, creatine and probably amino acids. There is never any ammonia.

Almost all the nitrogen is excreted as uric acid. This is in the form of minute spheres with radial striation, in which about 80 to 90 per cent. of the uric acid is free; the rest, presumably, as sodium and potassium acid urate.

[1] It may be recalled that Meissner (1868) showed that the ammonia in the urine of birds was all in the soluble fraction, and not in the uratic spheres.

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