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Tissue Components of the Domestic Fowl

2. BLOOD UREA*

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The domestic fowl, besides being of great economic importance, is often used as an experimental animal. Pathological abnormalities of various organs which arise from time to time, even under the best husbandry, are rarely detectable except at post-mortem examination. The investigation of avian urine for pathological constituents is at present impracticable; blood examination and tissue biopsy afford the only approaches to diagnosis by chemical means. The flock of brown leghorns at the Poultry Research Centre is carefully isolated and is not subject to contagion from outside; nevertheless, organ dysfunctions, e.g. of the kidney, are sometimes encountered. The possibility that some aspect of blood analysis might lead to early detection of kidney dysfunction is under investigation.

The literature from 1923 to 1952 contains con-

flicting reports on blood urea of presumably healthy fowls; the figures quoted cover a remarkable range despite the view generally accepted by biologists that urea is found only in low concentrations in normal avian tissues (cf. Clementi, 1914, 1915, 1918, 1922, 1932; Hunter & Dauphinée, 1925; Pitts & Korr, 1938; Howell, 1939a).

In the literature results have been expressed in two ways: as 'mg. of urea' and as 'mg. of urea nitrogen'. As the former is quantitatively more than twice the latter, this is a matter of some moment, since papers exist where both modes of expression are used indiscriminately to cover the same or similar sets of values (e.g. Hogan, Shrewsbury & Kempster, 1929; Herrmann, 1946a, b). The collated summaries of Shimer (1937), Howell (1939a) and Sturkie (1954) contain errors arising from confusion of the two values.

This paper describes an examination of the blood-

* Part 1: Bell (1957).

urea content of healthy fowls and of a few birds proved by post-mortem examination to have been suffering from early pathological lesions of the kidney glomeruli. The Conway apparatus was used with urease which had been tested against possible interfering blood components.

METHOD

Both the healthy and sick birds were allowed free access to water and to the normal diet of the Poultry Research Centre (Bell, 1957). Blood was taken from the wing vein into citrate (2 mg./ml. of blood).

In blank determinations, the volatile base ('ammonia') released in appreciable amounts from the blood had, of course, to be measured. This 'ammonia' is of no significance *in vivo* since Conway & Cooke (1939) have shown that, in birds as well as in other terrestrial animals, it is impossible to detect ammonia in freshly shed blood. It originates in birds by enzymic deamination of adenine, guanine, guanosine, cytidine and cytosine after the blood is shed.

Urea determinations by diffusion (devised by Mr T. P. Bird)

The procedure of Conway (1947) for mammalian blood had to be modified only with respect to permitting titration of the ammonia formed by urease from the small amounts of urea in 1 ml. of avian blood. With ordinary microburettes this was found possible by acidimetry, iodate-iodide with thiosulphate being used (cf. Bang, 1916).

The iodate-iodide solution was prepared before each batch of titrations by mixing equal volumes of 2% (w/v) KIO_3 and 40% (w/v) KI. The concentration of KI is fairly critical; if too much is present it interferes with the blue colour of the starch indicator, which becomes reddish, and a satisfactory end-point cannot be obtained. If insufficient KI is present iodine is lost by volatilization.

(a) *Ammonia recovery.* Ammonium sulphate solution (1 ml.; N content, 0.0159 mg.) was placed in the annular space, and 0.5 ml. of 0.01 N- H_2SO_4 in the centre compartment, of a Conway no. 1 unit. Saturated K_2CO_3 solution (1 ml.) was added to the annular compartment and, after sealing the lid, the whole was incubated at 38° for 2 hr. Blanks with water in place of $(\text{NH}_4)_2\text{SO}_4$ were run simultaneously. The acid in the centre compartments was titrated iodometrically; 0.25 ml. of iodate-iodide solution was added to the centre compartment and mixed with a fine rod. After 1 min., to allow the reaction to come to completion, the iodine was titrated with 2 mN- $\text{Na}_2\text{S}_2\text{O}_3$, with one drop of 1% starch solution as indicator. Triplicate experiments gave a recovery of 100%.

(b) *Urea-recovery tests.* Urea solution (1 ml., 0.0443 mg.) was placed in the annular space along with 1 ml. of M/15 phosphate buffer (pH 7.38) and a small knife-point of urease powder (L. Light and Co.); 0.5 ml. of 0.01 N- H_2SO_4 was placed in the centre compartment and, after sealing, the whole was incubated at 38° for 15 min. Saturated K_2CO_3 solution (1 ml.) was added to the fluid in the annular compartment and, after mixing, incubation was continued for 2 hr. The residual acid in the centre compartment was then determined as in (a) above (average titration, 1.72 ml. of 2 mN- $\text{Na}_2\text{S}_2\text{O}_3$), the blank experiment having water in place of urea solution (titration 2.48 ml.). The difference of 0.76 ml. is equivalent to 0.0456 mg. of urea, i.e. 103% recovery.

Under the conditions of (b) above no measurable volatile base was yielded by the enzyme alone, the buffer alone and the buffered enzyme with allantoin and with L-arginine.

(c) *The method applied to blood.* The annular space was charged with 1 ml. of blood, 1 ml. of buffer and a small knife-point of urease; 0.5 ml. of 0.01 N- H_2SO_4 was placed in the centre compartment. The subsequent procedure was as for (a) above. The blank contained 1 ml. of water instead of blood.

RESULTS

The values for the blood urea of presumably healthy birds are given in Table 1. Among the older hens no differences were found between the laying and non-laying conditions. Also recorded are measurements made on birds found to have early glomerulonephritis (Table 2).

DISCUSSION

A summary of previous results and methods, and of the present experiments, is given in Table 3. Wide variations in the urea levels, and their ranges, cannot be attributed solely to the use of particular methods. It is accepted that urease will quantitatively, and specifically, decompose urea, other ammonia-forming enzymes and their substrates being absent (Howell, 1939*b*; Damodaran & Sivarajakrishnan, 1946); gross errors must therefore be sought in some point of technique other than the initial enzymic treatment. In the methods of Folin & Wu (1919), Karr (1924), Leiboff & Kahn (1929) and Kibrick & Skupp (1950) the ammonia formed is measured by nesslerization, which Folin & Wu, however, stated to be unsatisfactory in the presence of either amino acids or peptones. Referring to urea nitrogen, Folin & Wu also say 'whether one finds... 3 instead of 2 mg. in 100 c.c. of chicken

Table 1. *Blood urea of normal fowls*

Sex	Age	No.	Urea (mg./100 ml.)			Urea N (mg./100 ml.)		
			Range	Mean	S.D.	Range	Mean	S.D.
Female	1 month*	6	1.4-3.4	2.38	0.76	0.65-1.59	1.11	0.37
Male	1 month*	8	1.4-4.6	2.73	1.00	0.65-2.15	1.28	0.47
Female	1-5 years	14	1.7-3.9	2.69	0.79	0.79-1.82	1.23	0.37
Overall total		28	1.4-4.6	2.64	0.79	0.65-2.15	1.23	0.37

* Hsu & Combs (1952) reported a range of urea nitrogen of 3.8-18.5, mean 9.8, mg./100 ml. of blood for birds in this age group.

Table 2. *Blood urea of hens with defective glomeruli but normal tubules*

Bird no.	Age (years)	Urea (mg./100 ml.)	Urea N (mg./100 ml.)	Pathology*
1	4	2.4	1.12	Atherosclerosis. Proliferative changes in glomeruli
2	4	2.8	1.30	Metastasizing adenocarcinoma of oviduct. Low-grade glomerulosclerosis
3	3	3.8	1.77	As for 1
4	3	2.9	1.35	As for 1
5	2	1.9	0.89	No vascular lesions. Low-grade proliferative glomerulonephritis
6	2	3.5	1.63	As for 5
7	2	1.9	0.89	No vascular lesions. Acute diffuse proliferative glomerulonephritis
Average		2.7	1.28	

* Reports kindly provided by Dr W. G. Siller.

Table 3. *Published values for urea (all expressed as urea nitrogen) in fowl blood*

Use of Nessler's reagent is indicated by (Ne).

Urea N (mg./100 ml.)

Authors	Method	Urea N (mg./100 ml.)		
		Highest	Lowest	Average
Scheunert & Pelchriz (1923)	Folin & Wu (1919)	16.9	7.3	(Ne) 12.1
Hayden (1929)		3.6	1.2	(Ne) 2.5
Hogan <i>et al.</i> (1929)		5.4	2.3	(Ne) 3.8
Hayden & Brunett (1930)		7.5	5.1	(Ne) 6.6
Hayden & Sampson (1931)		4.5	2.1	(Ne) 3.3
Gonzago (1935)	Karr (1924)	2.7	2.0	(Ne) 1.3
Heller & Pursell (1937)	Folin & Svedberg (1930)	5.0	2.2	3.6
Dyer & Roe (1934)	Leiboff & Kahn (1929)	2.5	1.0	(Ne) 1.5
Herrmann (1946a)		—	—	(Ne) 8.9
Herrmann (1946b)		—	—	(Ne) 19.0
Hsu & Combs (1952)	Kibrick & Skupp (1950)	18.5	3.8	(Ne) 9.8
Pitts & Korr (1938)	Van Slyke (1927)	1.7	0.0	—
Howell (1939a)	Howell (1939b)	—	—	0.7
Present work	Conway (1947), modified	2.2	0.7	1.2

blood is as yet of comparatively small consequence'. Peters & Van Slyke (1932), referring to nesslerization of deproteinized blood filtrates, state: 'Other constituents of blood filtrates apparently increase the intensity of the colour produced.' Connerty, Briggs & Eaton (1955) have emphasized that the colour produced by direct nesslerization of protein-free blood filtrates is not stable and that the optical density increases steadily until turbidity is produced. This point was in fact made by Scheunert & Pelchriz (1923), who obtained very high values for the apparent urea (Table 3). Turbidity is especially likely to develop in warm weather (Levey & Reid, 1952). Appreciable quantities of amino acids are present in fowl-blood filtrates (Dunn, 1949; Salander & Fisher, 1956; Bell & Gross, unpublished work). Interference by organic vapours is another comparatively unrecognized hazard in nesslerization.

The method of Folin & Svedberg (1930), which involved distillation and titration, should be accurate, but it gave high results in the hands of Heller & Pursell (1937) and Shimer (1937); the last-named also used Karr's (1924) method without differentiating the results of the two methods.

Direct chemical hydrolyses (Leiboff & Kahn, 1929; Kibrick & Skupp, 1950; Owings & Mandel, 1951) would also include ammonia from amides other than urea; the amide content of avian blood is not known.

The results of the present work are only slightly higher than those obtained previously by urease-fermentation (Howell, 1939a, b) and urease-manometry (Pitts & Korr, 1938); nesslerization has given comparable results only at the hands of Dyer & Roe (1934) and Gonzago (1935). Clementi (1932), by the xanthidrol method checked by urease, also found values within the range 0.2 mg./100 ml. The present author is convinced that this is in fact the range in the healthy domestic fowl and that much higher values are due to technical faults. The blood urea may be raised in some diseases, as Howell (1939a) has found. Pitts & Korr (1938) conclude that urea is excreted solely by the glomerulus, about 25% being re-absorbed by the tubules, but no elevation has been found in the pathological cases examined by the writer, which comprised birds showing limited proliferative and sclerotic changes in the glomeruli (Table 2).

The origin of avian-blood urea is not yet established. Clementi (1914, 1915, 1918, 1922) and Hunter & Dauphinée (1925) were unable to show the presence of arginase in avian liver; kidney, however, contains the enzyme. The latter authors suggest that the role of kidney arginase may be to produce the ornithine required to synthesize ornithuric acid. It is hoped to examine this possibility. Whether avian kidney, *in vivo*, does in fact generate the blood urea is not known; mammalian blood is free from ammonia, yet mammalian kidney hydrolyses glutamine to give NH_4^+ ion, which is apparently entirely lost to the urine. Avian urine also contains NH_4^+ ion. Since mammalian and avian metabolic systems need not necessarily follow the same pathways, sources of avian urea alternative to arginine may exist. Krebs & Eggleston (1939) have studied a *Corynebacterium* which can convert into urea the grouping

contained in creatine, creatinine, hypoxanthine, uric acid, allantoin, arginine and citrulline. *Pseudomonas* spp. and a mould possess similar capacities (cf. Nimmo-Smith & Appleyard, 1956; Appleyard & Woods, 1956). All these substrates are known constituents of fowl blood, except citrullin and allantoin. Circumstantial evidence for the presence of the latter in fowl urine has been offered by Coulson & Hughes (1930). Enzymic mechanisms first encountered in micro-organisms are often subsequently discovered to exist among the metazoa.

SUMMARY

1. Measured by urease in the Conway apparatus, the urea-nitrogen content of healthy fowl blood averages about 1.2 mg./100 ml., in reasonable agreement with some, but not all, previous investigations. Very high values which have been reported in the literature, e.g. 12–19 mg. of urea nitrogen/100 ml. of blood, are believed to arise from technical failures.

2. No elevation in blood urea was found in a number of birds which had been suffering from early glomerulonephritis.

3. The origin of avian blood urea is briefly considered.

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