CLXIII. DECOMPOSITION OF URIC ACID IN BLOOD.

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GAROT [1926] showed that the uric acid content of dog's blood falls considerably after digestion with "pancreatin"; this effect he ascribed to the presence of uricase in the blood of these animals. Flatow [1926] found that uric acid undergoes oxidation if added to blood, and that this process is greatly accelerated by passing air through the mixture. The uricolytic agent in this case was, according to this author, a substance which he termed "uricoid."

Up to the present, no incontrovertible evidence has been adduced for the existence of uricase in the blood of any mammal, and the nature of the substance producing uricolysis is therefore still problematical.

Biltz and Schauder [1923] and Piaux [1924] showed that a number of substances exist which catalyse the oxidation of uric acid, the most important being such metals as iron, copper, manganese. Since an iron-containing substance, haemoglobin, is present in blood, the possibility arose that this was the factor responsible for the oxidation of uric acid in blood.

Experiments will be described in this paper having as their object the elucidation of the nature of the catalyst in question and of the action of haemoglobin upon uric acid.

EXPERIMENTAL.

A. *Determination of uricolytic agent.*

Horse blood was collected at slaughter-houses, and potassium oxalate added immediately to prevent clotting. It was used generally within about 6 hours of shedding.

The uricolytic powers of whole blood, blood-plasma, erythrocytes, boiled erythrocytes, stromata and haemolysed corpuscles free from stromata were determined separately. Two types of determination were made. In one, uricolysis was determined in bottles through which a feeble stream of oxygen was passed, whilst in the other determinations were made in stoppered flasks. In all cases 50 cc. of plasma, erythrocyte suspension, etc., were taken, and 20 cc. of lithium urate solution together with 20 cc. of water were added,

with thymol as disinfectant. The reaction mixtures were placed in a thermostat at 37°, and their uric acid content determined at different intervals of time.

The solution was freed from protein by the method of Folin and Wu, 100 cc. of 10 % sodium tungstate and 100 cc. of 2/3 *N* sulphuric acid being added. Uric acid was determined in the filtrate using the colorimetric Folin-Denis method, 12 cc. of filtrate being taken for each determination. The results obtained by this colorimetric method were confirmed by determination of purine-nitrogen using the method of Krüger and Schittenhelm.

The results given in Table I show that blood-plasma possesses no uricolytic power, since even after 48 hours the uric acid content did not diminish by more than 6.8 %, and this diminution may be ascribed to adsorption. In the presence of erythrocytes uric acid undergoes oxidation. An examination of the figures obtained shows that at first adsorption takes place; during the first two hours no difference in the content of uric acid can be observed, after which it undergoes continual destruction, so that after 48 hours about 73 % is oxidised.

In order to establish whether the agent responsible for this oxidation is a catalyst or a ferment, the above experiments were repeated with erythrocytes previously heated to 100° for 10 minutes. The results (Table I) show that their uricolytic power is in no way diminished by this treatment, whence it follows that blood contains a thermostable uricolytic agent.

Whilst crude uricase is inactivated by such treatment, it has been shown by Przylecki (unpublished work) that highly purified preparations of uricase are similarly thermostable. In order to verify whether this ferment is present, the procedure employed by Przylecki was applied to the separation of uricase. The product so obtained was inactive, whence it would appear that uricase is absent from mammalian blood.

The effect upon velocity of oxidation of passing a feeble stream of oxygen through the reaction mixture was next studied. The results obtained (cf. Table I) showed a distinct acceleration of reaction, since only traces of uric acid remained after 48 hours.

We have, therefore, established that erythrocytes contain some thermostable non-enzymic substance which energetically oxidises uric acid in the presence of oxygen. The question remains whether this substance is present in the stromata or the contents of the erythrocytes. Accordingly, a suspension of haemolysed erythrocytes was centrifuged, the supernatant liquid passed through a Berkefeld filter, in order to ensure the removal of fragments of stromata, and uric acid added to the filtrate. Only traces of uric acid remained after 48 hours (Table I). This result was confirmed by determining purine-nitrogen using the method of Krüger and Schittenhelm. The purine-nitrogen content fell in 48 hours from 25.5 mg. to 4.9 mg. (see Table III). Parallel experiments on washed stromata showed only absorption of uric acid, amounting after 48 hours to 10.5 %, after which no further fall in uric acid content could be observed (Table I).

Table I.

	Original uric acid content mg. in 2 cc.	5 mins. Uric acid content mg.	30 mins.		1 hour and 2 hours. Uric acid content mg.	4 hours		24 hours		48 hours		72 hours
			Uric acid content mg.	Loss %		Uric acid content mg.	Loss %	Uric acid content mg.	Loss %	Uric acid content mg.	Loss %	
Plasma	...	No change	0.795	6.68	No change	1.019	12.55	0.852	26.86	0.333	71.41	Uric acid content mg.
Catalyst	...	"	0.793	6.92	"	1.019	14.08	0.862	27.31	0.299	75.21	"
Mean	...	"	0.794	6.8	"	0.96	14.2	0.843	25.13	0.303	73.08	"
Fresh erythrocytes	...	No change	1.107	4.97	No change	0.999	13.64	0.852	26.43	0.311	72.23	"
Boiled erythrocytes	...	No change	1.107	4.97	No change	1.019	12.53	0.803	31.07	0.320	71.84	"
Mean	...	"	1.107	1.68	"	0.862	23.52	0.666	40.9	0.284	74.8	"
Erythrocytes with oxygen	1.153	—	1.107	4.47	—	0.966	16.71	0.803	30.87	0.334	71.82	"
Intracorpuseular fluid	1.127	—	—	—	—	0.367	68.17	0.372	66.99	Traces	—	"
Stroma 0.8 g. per portion	0.55	—	—	—	—	0.369	67.58	—	—	—	—	"
Mean	0.372	—	—	—	—	0.928	18.54	0.534	2.9	0.495	10	No change
Haematin 0.5 g. per portion	1.14	—	—	—	—	0.361	3.06	0.361	3.06	0.331	11.02	No change
Mean	0.461	—	—	—	—	—	—	—	2.98	0.463	10.51	No change
										Traces	—	"

The uricolytic agent is therefore present exclusively in the intracellular fluid, and it appears probable that it may be haemoglobin. This conclusion is further supported by experiments conducted on purified crystalline haematin, in the presence of which uric acid was similarly quantitatively oxidised.

B. Influence of alcohols, of methylene blue and of potassium cyanide upon the oxidation of uric acid in blood.

Butyl and propyl alcohols were added in various concentrations to solutions of haemolysed blood containing uric acid. The results, given in Table II, show that oxidation is inhibited to an extent depending upon the concentration of alcohol added.

In order to establish whether we have here to do with a reaction of true oxidation or of dehydrogenation, the influence upon the velocity of oxidation of the addition of methylene blue was investigated. The results (Table II) do not indicate any acceleration of reaction where this dye is added; on the contrary, a slight retardation is observed, possibly due to adsorption of methylene blue upon the surface of the catalyst in question.

The addition of potassium cyanide did not inhibit uricolysis (Table II).

Table II.

Substance added cc. per 100 cc. erythrocyte suspension			Original uric acid content mg./5 cc.	After 48 hours	
				Uric acid content mg./5 cc.	% loss of uric acid
			1.15	Traces	
Butyl alcohol	2		1.15	0.16	86.95
Butyl alcohol	4		1.15	0.605	47.3
Propyl alcohol	8		1.15	0.63	45.12
Propyl alcohol	16		1.15	0.785	31.73
<i>M</i> /500 methylene blue	10		0.372	0.145	61.02
			0.55	0.2	64.05
Mean	...	...	0.461	0.1725	62.76
<i>M</i> /1000 potassium cyanide	40		0.53	0.14	73.54
			0.64	0.16	75
Mean	...	...	0.58	0.15	74.27

C. The products of oxidation of uric acid in blood.

The following analyses were carried out with the object of identifying the products arising from the catalytic oxidation of uric acid under the influence of haemoglobin. Urea and ammonia were determined by the methods of Folin and Wu in fresh blood, and in blood to which uric acid had been added and which had remained for 48 hours at 37°. Allantoin was determined in other portions, using Wiechowski's method, and in separate portions purine-nitrogen was determined by the method of Krüger and Schittenhelm. The results, given in Table III, show that the major portion of the uric acid-nitrogen is found as urea, the remainder being ammonia-, allantoin- and purine-nitrogen.

Table III.

	Fresh erythrocytes mg. N in 100 cc.	After 48 hours mg. N in 100 cc.
Total uric acid content of blood	25.5	4.9
Ammonia	—	2.4
Urea	4.2	12.6
Allantoin	—	6.3
Totals	29.7	26.2

DISCUSSION.

Uric acid, on standing with blood, is quantitatively oxidised within 48 hours, yielding as products of oxidation, allantoin, urea and ammonia. The catalytic agent in question is absent from the blood-plasma and from the stromata of the erythrocytes, which are able only to adsorb uric acid in quantities not exceeding 10 % of that present in solution. Haemoglobin is probably the uricolytic catalyst in question.

It is well known that iron compounds play an important rôle as oxidation catalysts within the cell [see Warburg, 1914].

Robinson [1924], who used haemoglobin and haematin as catalysts for the oxidation of linseed oil, found that haematoporphyrin was without effect, and her experiments suggest that it is the iron present in haemoglobin that is responsible for oxidation of uric acid. This author showed further that cyanides do not inhibit the catalytic action of iron present in haemoglobin. This would point to the iron being present in some non-ionised form, as is the case with that of haemoglobin.

The results obtained with methylene blue indicate that the reaction of uricolysis under the influence of haemoglobin is rather one of oxidation than one of dehydrogenation.

SUMMARY.

1. Uric acid is quantitatively oxidised by blood within 48 hours at 37°.
2. Stromata and blood-plasma are inactive in this respect.
3. Haemoglobin is most probably the uricolytic agent present in blood.
4. The products of uricolysis in blood are allantoin, urea and ammonia.
5. The reaction of uricolysis is inhibited by propyl and butyl alcohols, but not by cyanides.
6. The reaction is one of true oxidation, and not of dehydrogenation.

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