129. MYOHAEMOGLOBIN IN THE URINE OF AIR RAID CASUALTIES WITH CRUSHING INJURY

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PATIENTS crushed beneath fallen masonry for several hours may develop 'shock' after release, with falling blood pressure, haemoconcentration and swelling of the affected part. With restoration of circulating blood volume by serum or plasma, the blood pressure returns to normal. Some of these patients, and some also who have shown no such stage of 'shock', pass smoky dark brown or red urine, often highly acid, and giving a positive benzidine reaction. The deposit, however, seldom shows red cells, but consists chiefly of brown pigmented casts. The patient may then either develop oliguria, with progressive azotaemia, dying within the week, or may recover with a diuresis. In either case the urine becomes progressively less pigmented and the casts less numerous.

Autopsy shows blanching and necrosis of muscle in the injured areas and, microscopically, loss of staining ability; in the kidneys there are seen tubular degeneration and pigmented casts similar to those seen in the urine.

The blanched appearance of the affected muscles at autopsy suggested the possibility that this urinary pigment might be myohaemoglobin [Bywaters & Beall, 1941]; Gilmour [1941] also emphasized this likelihood, pointing out the similarity to paralytic equine myohaemoglobinuria, as Minami [1923] had done previously.

Urine from such cases has therefore been examined. This has been possible through the kind co-operation of the following: Dr Gilmour (St Andrew's Hospital, Billericay), Dr Cruickshank, Dr Pochin and Dr Hynes (North-West Hospital and Hampstead General Hospital), Dr Joan Ross (Hill End Hospital, St Albans), Prof. Pickering (St Mary's Hospital, Paddington), Mr Patey, Mr Vaughan Hudson and Dr Hardwick (Middlesex Hospital), Mr Belsey and Dr Miles (St Thomas's Hospital), Dr Dow and Mr Riddell (St George's Hospital).

RESULTS

A. Examination with the Hartridge reversion spectroscope showed in seven cases a spectrum corresponding with that of oxymyohaemoglobin. The α band occurred at 582 mμ; after reduction with sodium hydrosulphite there was a broad band in the green. The carboxy compounds prepared from the several urines gave α bands between 578 and 579, and the 'met' compounds gave bands ranging between 633 and 636 mμ.

We prepared for comparison a solution of human myohaemoglobin from sartorius muscle (saline perfusion to wash out the erythrocytes and subsequent extraction of the minced muscle with phosphate buffer *p*H 6·5). Reference to

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Table 1 will show that the bands of the urinary pigment correspond both with the values given for myohaemoglobin in the literature and with those found for the muscle extract.

Table 1. *Position of α bands in mμ*

		Oxy compound	Carboxy compound	Met compound	References
Haemoglobin		578	572	630	
Myohaemoglobin: Ox		580	—	—	Watson [1935]
		580	577	—	Roche [1932]
		582	—	—	Shenk *et al.* [1934]
		582	—	—	Gunther [1921]
	Horse	580	577	—	Roche [1932]
		581	—	—	Watson [1935]
		581	—	—	Von Mócsy [1936]
		582	579	630	Theorell [1934]
		581·5	—	638	Mörner [1897]
	Dog	580	—	—	Kennedy & Whipple [1926]
		580	577	—	Ray & Paff [1930]
		580	577	—	Roche [1932]
		580	—	—	Watson [1935]
		581	—	—	Von Mócsy [1936]
	Other mammals excluding man	580	576–577	—	Roche [1932]
		579–580	—	—	Watson [1935]
		580	—	—	Keilin [1925]
	Pigeon	580	—	—	Keilin [1925]
	Ascaris	580	—	—	
	Man	581–582	579	636	This paper
Urinary pigment from crush injury cases		582	578–579	633–636	This paper

In three cases the supernatant urine, after centrifuging, showed the bands of met-myohaemoglobin as well as those of oxymyohaemoglobin. The deposit, dissolved in a few ml. of water with a few drops of *N* NaOH, showed haematin bands, rapidly passing into those of haemochromogen. In one of these three cases the amount of pigment was estimated quantitatively by a spectrophotometric method using the pyridine haemochromogen. The first specimen, obtained 12 hr. after admission, showed the spectrum of met-myohaemoglobin with a band at 633 mμ, and was reduced to the oxy-form with α band at 582. The haem pigment concentration of the sediment and supernatant combined was in this specimen 1·08 mg./ml. (expressed as oxyhaemoglobin). The second specimen, obtained 24 hr. after admission, contained much more deposit. The total pigment concentration was 4·91 mg./ml. A third specimen, passed 31 hr. after release, contained slightly less pigment; the remaining specimens contained only traces. Thus a total of about 3–3·5 g. of myohaemoglobin was excreted in this case.

B. *Ultra-filtration.* Since myohaemoglobin has a molecular weight of 17,500 [Svedberg & Pedersen, 1940] and haemoglobin one of 68,000, separation should be possible by filtration through suitable membranes. A 9 mμ 'Gradocol' collodion filter (Elford) was used. The first specimen from the case referred to above, after reduction with sodium hydrosulphite, afforded a brown filtrate in which the band of the ferrous pigment could again be detected at 582·8 mμ. The intensity was not so great as in the original unfiltered urine. A control urine containing oxyhaemoglobin was diluted to approximately the same pigment content as the crush injury specimen and filtered through a similar 9 mμ membrane. It afforded a colourless filtrate in which no trace of haemoglobin was detectable.

C. *Ultra-centrifuging.* Dr Kekwick (Lister Institute) kindly made ultra-centrifugal runs upon these urines. They were dialysed and concentrated by freezing and then afforded the following results.

In urine from a case of crush injury, two components were present in about equal proportions, with sedimentation constants of approximately 2·3 and 4·5 respectively, suggesting that both myohaemoglobin and haemoglobin or albumin were present. The control urine containing haemoglobin showed a single component only, with a sedimentation content of approximately 4·5. (Haemoglobin and serum albumin have sedimentation constants of 4·5, whilst Svedberg & Pedersen [1940] give the value 2·0 for myohaemoglobin.)

D. *Plasma* from such patients shows no spectroscopic bands. This is to be expected since the molecule of myohaemoglobin is a quarter of the size of that of haemoglobin and the renal threshold correspondingly lower [Camus & Pagniez, 1902; Hektoen *et al.* 1928; Carlström, 1931]. It is of particular interest that no methaemalbumin was detected spectroscopically in the plasma.

E. *Renal casts.* The pigmented tubular deposit gives a positive reaction in paraffin sections by a modified benzidine reaction. Its identification as a haem pigment has been established by spectroscopic means. The technique evolved was as follows: unstained, frozen sections were laid on the microscope slide and drained. A drop of freshly prepared mixture of pyridine containing about 2 % of hydrazine hydrate was added. The granules in the tubular lumen were seen to turn pink. When viewed through the spectroscopic eyepiece, the characteristic absorption bands of pyridine haemochromogen were now visible. This proves that the pigment is a haem pigment and its general behaviour suggests that it is haematin. It is still visible in kidney material perfused before fixation. (The reaction cannot be used, however, to differentiate between myohaemoglobin and haemoglobin.)

Discussion

After these results had been obtained, references were found to cases of crushing injury seen by German observers during the last war. Minami [1923], examining post mortem material, suggested that the pigment might be myohaemoglobin and asserted his intention, if he had the opportunity, of proving it spectroscopically, but there is no record of this having being done.

Our findings prove conclusively that myohaemoglobin is excreted in the urine of such patients. It is thus direct evidence of muscle damage, and may be present even when little clinical injury is apparent. Spectroscopic examination of urine may therefore be useful in cases where the alternative possibility of haemoglobinuria due to renal contusion or mismatched transfusion exists. There have been recorded cases, however [Husfeldt & Bjering, 1937], and we have seen one such personally [Bywaters & Graham, 1941], in which a syndrome similar to that following crushing injury developed following automobile accidents, with damage to muscle blood supply but without prolonged crush. In all such cases, where 'haemoglobinuria' is associated with muscle disease or damage, spectroscopic examination should be made.

Whether muscle pigment of itself plays any part in the pathogenesis of renal failure is doubtful. It may play (*a*) a leading role, (*b*) an adjuvant role or (*c*) no role at all.

(*a*) The problem is similar to that of haemoglobinuria following mismatched transfusion. It has been shown in man that amounts of haemoglobin up to 30 ml. of corpuscles [Sellards & Minot, 1916] or 350 mg./kg. [Fairley, 1940] may be given intravenously and excreted without ill effect. Preliminary observations of

our own lead us to the opinion that small quantities of myohaemoglobin prepared from human muscle are excreted rapidly and harmlessly through the human and rabbit kidneys after intravenous injection. The few cases recorded of spontaneous 'paralytic' myohaemoglobinuria in man are important in this respect; two of the five [Paul, 1924; Gunther, 1924] died. The other three cases recovered after multiple attacks [Meyer-Betz, 1910; Hittmair, 1925; Millikan, 1939]. It seems probable that, in these cases, factors other than myohaemoglobin are concerned in bringing about the fatal outcome, since in the mildest of the three (Millikan) there was little abnormality except pigmented urine and increased blood potassium after exercise.

(*b*) As in the similar problem of haemoglobinuria after intravascular haemolysis, it may be that associated conditions such as the rate of urinary secretion, the *p*H of the urine and the amount of tubular resorption can modify the response of the kidney to the pigment. It has been claimed, for instance, on the basis of animal experiments, that the acidity of the urine is important in changing haemoglobin filtered through the glomeruli into the less soluble acid haematin or methaemoglobin [Baker & Dodds, 1925; cf. Navasquez, 1940] which may then precipitate in the tubules. Whether or not this is so in man, with an amount of excreted haemoglobin small in comparison with that used in experimental animals, it still remains to be shown that such blockage is adequate in man to produce renal failure. Experiments in this direction are in progress.

(*c*) The third possibility is that myohaemoglobin in the urine is no more than an indicator of muscle damage and that other substances released from muscle at the same time are responsible for the renal damage. The high acidity of the urine and the lowered blood alkali reserve [Bywaters & Beall, 1941] witness a considerable uptake of acid substances; it has been shown in paralytic equine myohaemoglobinuria [Grzycki, 1934] that muscle contains less phosphagen, and blood more lactic acid and phosphate, than normal. MacKay & Oliver [1935], Duguid [1936] and McFarlane [1941] have shown that phosphate in large dosage produces renal lesions in animals somewhat similar in distribution to those seen in the crush syndrome.

The study of these other substances released from muscle at the same time as pigment should not be overlooked and investigations are in progress along these lines.

Summary

Spectroscopic examination of the urine from seven patients crushed under debris showed the presence of myohaemoglobin. This was confirmed by ultrafiltration and by ultracentrifuging.

Kidney sections examined by a histochemical technique showed the presence of haem pigment within the tubules. In one case the amount of pigment excreted was measured quantitatively. The factors involved in this type of renal failure are discussed.

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