PATHOLOGY OF SEA-SNAKE POISONING

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Sea-snake bite is an occupational hazard to fishermen in Asia (Reid and Lim, 1957), but the pathological lesions in man have not yet been properly described. In animals, Rogers (1903a, 1903b), Fraser and Elliot (1905), and Wright (1956) found a neurotoxic effect on the brain-stem and neuromuscular junctions. Kellaway, Cherry, and Williams (1932) considered that the venom of *Enhydrina schistosa* (Daudin) acted solely on the neuromuscular junction. Rogers (1903a), Nauck (1929), and Carey and Wright (1960) found little at necropsy. Lamb and Hunter (1907) injected *E. schistosa* venom subcutaneously into five monkeys and described degenerative changes in the central nervous system and peripheral nerves. In contrast, no histological abnormality was found in nerve tissue from two patients, although one did not die until three days after the bite (Reid, 1956a). Recent clinical observation has shown further discrepancies between the results of animal experiments and poisoning in human victims (Reid, 1961). Complementary to these clinical observations, we have found the chief pathological lesions to be in skeletal muscle, kidney, and liver. Our purpose is to describe these lesions and discuss some implications.

Material and Methods

This study is based on biopsy material from a patient who recovered from severe sea-snake poisoning and on the necropsy findings of seven patients. All patients, except Case 4, were personally observed during the toxaemia and at necropsy by one of us (H. A. R.). In Case 4 the clinical features and macroscopic findings at necropsy were personally confirmed with the doctor in charge. Pathological effects in Cases 7 and 8 have already been briefly described (Reid, 1956a), but, in view of the more recent findings, we have been able through the courtesy of Professor William Blackwood to review the histology in muscle and renal tissue. Case records of five subjects have been published elsewhere —Cases 1, 2, and 3 by Reid (1961, same case numbers); Case 7 by Reid (1956b); Case 8 by Reid (1956c, Case 3).

The constant high temperature (averaging 30° C.) and humidity of the Malayan climate is most unfavourable for the histological study of necropsy material, and particular efforts were made to combat this difficulty. The body was transferred to the mortuary refrigerator (temperature 9° C.) within 30 minutes of death in all cases except Case 4. The time between death and necropsy is shown in Table 1. Specimens for histology were immediately placed in formol-saline: in Cases 1 and 2 duplicate specimens were fixed in Bouin's fluid, and in Helly's fluid and alchohol in Case 3. Blocks were embedded in wax, and routine sections were cut and stained by haematoxylin and eosin. Sections of muscle were also stained by Masson's trichrome method and by phosphotungstic acid haematoxylin; other special stains were used as required.

Results

Findings are summarized in Tables I and II. Macroscopic changes are few, the most important being brown

TABLE I

Case No.	Hours Between Bite and Death	Hours Between Death and Necropsy	Macroscopic Findings (bladder contained brown or black urine in all cases)	Principal Histological Findings					
				Skeletal Muscles Examined with % Necrosis	Heart	Kidney	Liver	Lung	Other Organs
1	13	6	Few petechiae under liver capsules: liver pale	Deltoid 20%. Intercostal 30%. Diaphragm 33%	Normal	Congestion	Centrilobular degeneration. Portal round-cell infiltration	Patchy oedema	Brain-stem: normal
2	14½	2	Normal	See Table II	,,	Congested boundary zone	,, ,,	Small areas of collapse and emphysema	Parotid gland: normal. Lymph node: sinus catarrh. Thyroid: necrosis
3				Biopsy 3 days after bite: deltoid 30%. 6 months later: fine scarring					
4	25½	4½	Pale liver	Tongue 45%. Intercostal 65%. Biceps 33%. Quadriceps 35%	,,	Congested boundary zone	Hydropic degeneration parenchyma: Portal round-cell infiltration		Pancreas: normal. Bladder: chronic inflammation mucosa. Thyroid: normal
5	18½	7½	Normal	Eye. Lateral rectus 100%. Face 50%. Tongue 60%	Slight endocardial fibrosis	Congestion	Congestion. A few focal necroses. Portal polymorph infiltration	Small areas of collapse and emphysema	Oesophagus, stomach, bladder: chronic inflammation. Suprarenal: depletion lipoids cortex. Thyroid: normal
6	52	11	Right heart distended; purulent froth in main bronchi; kidneys bulged from capsule		Normal	Distal tubular necrosis	Centrilobular necrosis ½–2/3 lobules	Bronchitis	
7	77	15	Scanty petechiae in lung fissures and epicardium; pale liver; brain congested	Quadriceps 55%	(Coronary sclerosis)*	(Distal tubular necrosis)†	(Centrilobular degeneration. Portal polymorph infiltration)*	(Congested)*	(Brain-stem, basal ganglion, sciatic nerve: normal)*
8	12 days	31	Petechiae and ecchymoses throughout viscera	Quadriceps 55%	Slight fibrosis	Distal tubular necrosis			

* Previously reported (Reid, 1956a). † Kindly confirmed by Professor J. H. Dible.

or black urine in the bladder. The liver may be pale. Colour and consistency of skeletal muscle are normal. Bite marks are inconspicuous and there are no visible changes in tissues immediately adjacent. Histological changes are as follows.

TABLE II.—*Widespread and Advanced Necroses in Case 2, Although the Patient Died Only 14½ Hours After the Bite*

Severity of Myonecrosis	Duration of Myonecrosis			
	Oldest. More than 70% Contracted	60% Contracted	40–50% Contracted	Most Recent. 25% or Less Contracted
Very severe; 70% or more fibres necrosed	Trapezius. Pharyngeal		Triceps. Biceps. Forearm flexor. Forearm extensor	
Severe; 50–70% fibres necrosed	Intercostal (upper)	Tongue. Intercostal (lower). Diaphragm. Gluteus maximus	Masseter	Quadriceps
Moderate; 45% necrosis			Pectoral	
Mild; 25% necrosis				Psoas. Gastrocnemius. Peroneus

Skeletal Muscle

There are widespread hyaline necroses in skeletal muscles. The toxin picks out individual muscle fibres, leaving a healthy fibre next to a necrosed one, and affects only one or more segments of varying length in a fibre, usually with an abrupt transition to normal muscle (see Fig. 1). The number of fibres with focal necroses varies from muscle to muscle: in the same patient there may be necroses in 20% or less of the fibres of one muscle while in another muscle every fibre shows necrosis (see Table II). The affected segment of a muscle fibre first becomes greatly swollen and the sarcoplasm undergoes coagulation necrosis. At this stage the nuclei and swollen myofibrils can still be distinguished, but the disks of the myofibrils soon lose their differential staining, and both nuclei and myofibrils disappear as they become fused into an amorphous hyaline mass. This necrotic material contracts, leaving an empty space beneath the sarcolemma, which remains intact although it may slightly collapse (see Fig. 2). Necrotic segments stain more intensely with acid dyes, but this is not conspicuous in routine sections and could easily be overlooked by casual or inexperienced observation. The myonecroses become very obvious with special stains. With Masson's trichome stain they appear bright red, while the sarcolemma is blue. The

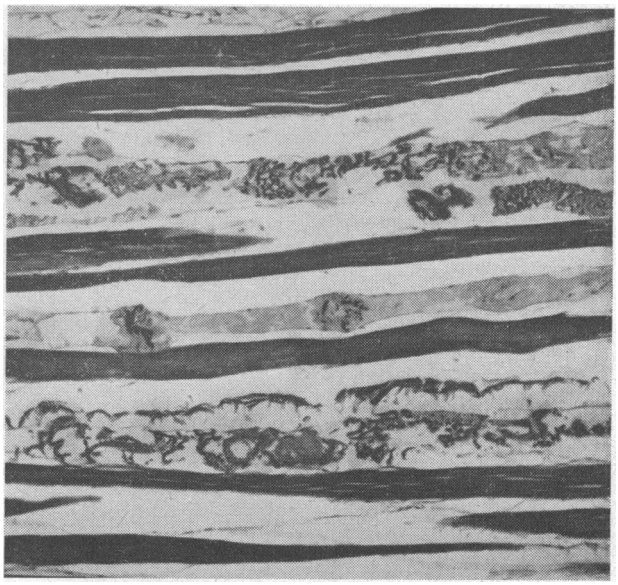

FIG. 2.—Later stage of myonecrosis with necrotic material contracted and tending to break up; the sarcolemma is still intact. (Phosphotungstic acid haematoxylin. ×110.)

picro-Mallory method stains them yellow; they are deep red with phosphotungstic acid haematoxylin and greenish with van Gieson's stain. Best's carmine stain shows a loss of glycogen from the necrosed segments.

Soluble products, notably myoglobin, enzymes, and electrolytes, are rapidly absorbed into the blood-stream. Insoluble necrotic fragments are phagocytosed by histiocytes and macrophages (see Fig. 3). Regeneration and repair begin one to two weeks after the bite with multiplication of surviving muscle nuclei (see Fig. 4). The muscle fibre is thus regenerated within its original sarcolemmal sheath and repair is remarkably complete, probably because very few muscle fibres are entirely necrosed. In Case 3, biopsy six months after the bite showed only a little fine scarring (see Fig. 5).

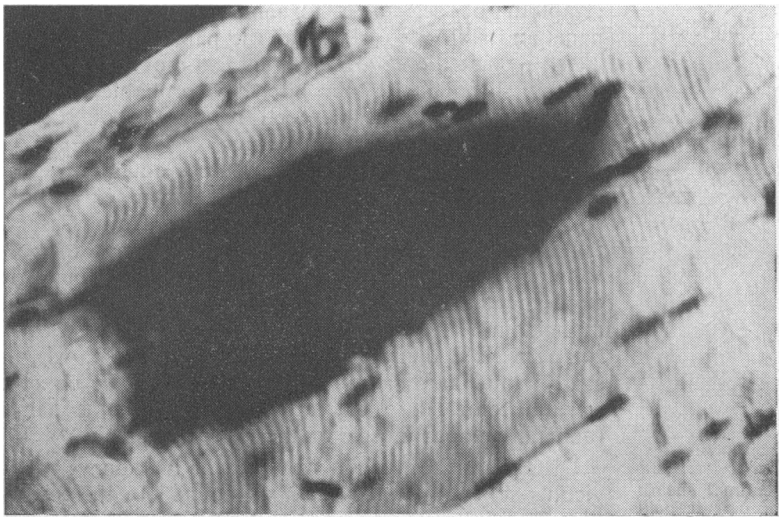

FIG. 1.—Early necrosis of a muscle fibre, showing swelling and abrupt transition to normal muscle. (van Gieson. ×400.)

Kidney

The earliest changes as shown by Cases 1, 2, 4, and 5, in which death occurred within 26 hours of the bite, are slight and entirely vascular. The whole kidney is engorged, but the boundary zone is intensely congested and stands out prominently. There are a few granular myoglobin casts in the tubules and there may be a little leakage of plasma and a few red cells from the congested glomeruli. Oedema, even of the boundary zone, is seldom marked and may be absent.

Distal tubular necrosis has been found in all three patients who survived for more than 48 hours after the bite. There is extensive necrosis of the epithelium of the loop of Henle, the second convoluted tubule, and the collecting tubules. Desquamated cells with granular and

amorphous debris fill the lumen and form casts in the distal and collecting tubules. Pigment giving a positive benzidine reaction is deposited in most of the casts, and even in some of the desquamated cells. The boundary zone is still intensely congested and the interstitial tissue is now oedematous, as well as showing both a diffuse and a focal cellular infiltration.

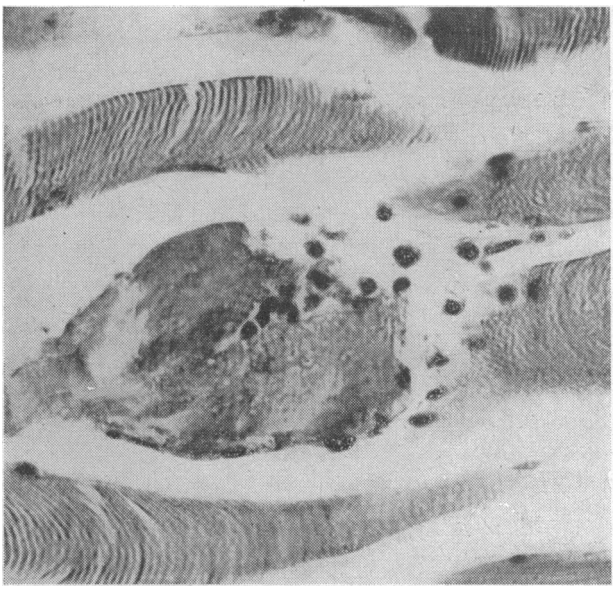

FIG. 3.—Phagocytosis of necrotic material (Case 8). (Haematoxylin and eosin. ×530.)

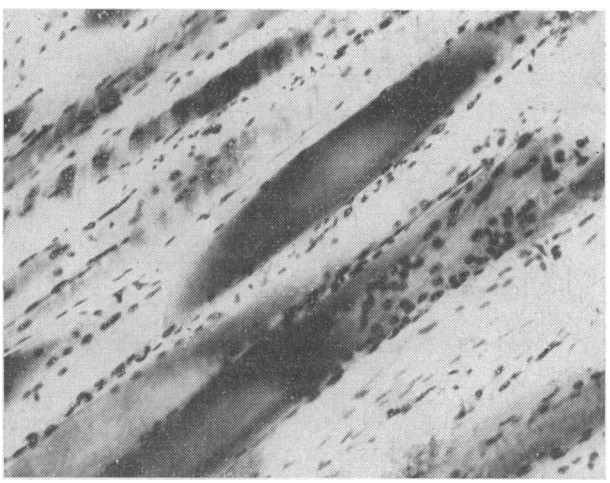

FIG. 4.—Multiplication of muscle nuclei (Case 8). (van Gieson. ×160.)

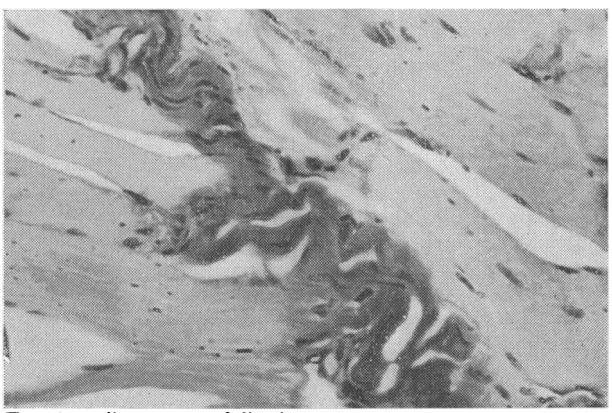

FIG. 5.—Fibrous scar following rupture of sarcolemmal sheath (Case 3: biopsy six months after bite). (Phosphotungstic acid haematoxylin. 140.)

The reactionary or healing stage is not often seen because victims usually die too quickly. Case 8 was our only example—the patient died 12 days after the bite. There may be considerable proliferation of the capsular cells of the glomeruli, presumably a reaction to the excreted myoglobin or its breakdown products. The first convoluted tubules appear normal, but, from the loop of Henle downwards, degenerating and regenerating epithelial cells crowd into the lumen, while some of the collecting tubules and excretory ducts are filled with a mass of proliferating cells. The interstitial tissue of the boundary zone and medulla is still oedematous and infiltrated with plasma cells, lymphocytes, and histiocytes, but now begins to appear more fibrous.

Other Areas

Liver.—There are no specific changes, but there is usually a centrilobular degeneration with a round- or mixed-cell infiltration of the portal areas.

Lungs.—Patchy oedema, small areas of collapse with compensatory emphysema, and early inflammatory changes in some bronchi were evident, but in no case did it appear likely that the lesions would play an important part in the clinical picture.

Cardiac and Smooth Muscle.—Apart from mild incidental fibrosis in two cases, no lesions have been found.

Other Organs.—The thyroid gland in Case 2 showed deficient colloid and many of the cells were necrotic, but the gland was normal in Cases 4 and 5. No significant lesions were seen in other organs. Professor William Blackwood has kindly confirmed the absence of histological changes in the nervous system.

Discussion

The pathology of sea-snake poisoning in man differs significantly from that reported in animal experiments. In human victims the primary histological lesion is necrosis of skeletal muscle. The myonecrosis occurs rapidly and is clinically apparent and widespread within half to one hour of the bite. Table II shows the extent and severity of the necrosis 14½ hours after the bite. These lesions are obviously sufficient to explain the chief symptom of sea-snake toxaemia—muscle movement pains and stiffness—as well as the paresis which ensues later. Since generalized muscle movement pains develop so soon after the bite, it is probable that sea-snake venom attacks the cell membrane of skeletal muscle directly in human victims. Myoglobinuria is proof of myonecrosis, although it does not indicate the cause. Professor J. H. Dible kindly examined sections of muscle from Case 3 and kidney from Case 7 and confirmed that the histological changes in both were indistinguishable from those he had found in experimental clostridial poisoning and in idiopathic myoglobinuria. Why does part of the muscle so often escape visible damage in such cases? Also, why is smooth and cardiac muscle so resistant? In no case were histological lesions attributable to the toxaemia found in smooth muscle or the myocardium.

Distal tubular necrosis was present in kidneys of all victims surviving longer than 48 hours. In these cases acute renal failure appeared to be the immediate factor

causing death. Experimentally, Bywaters and Stead (1944) showed that myoglobin injected into healthy rabbits caused no renal damage unless the urine was acidified. It is therefore unlikely that the myoglobin released in sea-snake poisoning is solely responsible for the renal lesions. Is the venom nephrotoxic? Reid and Jenkins (1948) showed that cobra venom acted directly on the kidney of eviscerated adrenalectomized cats, causing liberation of rennin. Hypertension was observed in Case 1 and 3; progressive hyperkalaemia was the immediate cause of death in Cases 1 and 5. Renal damage may be mainly responsible for these two features—a renal pressor factor leading to hypertension while progressive hyperkalaemia arose because diminished renal excretion failed to cope with the extra load from cellular release of potassium. Against this concept of sea-snake venom being directly nephrotoxic is the paucity of visible damage in patients dying within 24 hours of the bite. By analogy with the skeletal-muscle lesions so evident in sea-snake poisoning one would certainly expect to find renal necrosis even when death occurs shortly after the bite. We have been unable to demonstrate the fine fatty globules which Graber and Sevitt (1959) found in the glomerular tufts and which they thought might be a histological sign of structural changes affecting glomerular filtration in burned patients.

The liver may appear distinctly pale at necropsy. Feldberg and Kellaway (1938a) described depigmentation and release of adenyl compounds from the liver experimentally perfused with cobra venom. Similar depigmentation occurred with staphylococcal toxin, mercuric chloride (Feldberg and Kellaway, 1938b), and clostridial toxin (Kellaway, Reid, and Trethewie, 1941). The centrilobular degeneration in sea-snake poisoning may therefore be due to direct venom damage. In Case 2 there was no obvious clinical explanation why death occurred when it did (Reid, 1961): in such cases liver damage may be an important factor.

Clearly, there are important differences in pathology between sea-snake poisoning in man and in the animals so far reported. Myonecrosis was not found at necropsy on a goat which died 30 hours after injection of *E. schistosa* venom; rabbits dying acute deaths showed no lesion in muscle, liver, or kidney (Carey and Wright, 1960). Possible explanations for these discrepancies include: (1) species may differ in their reactions; (2) some change occurs in sea-snake venom during desiccation; and (3) myonecrosis has been overlooked. To elucidate the problem we have recently injected dogs with *E. schistosa* venom, both freshly obtained and reconstituted from dried venom. In each case typical widespread necrosis of skeletal muscle was histologically evident. This suggests that myonecrosis has previously been overlooked.

Summary

The principal pathological lesion of sea-snake poisoning in man is necrosis of skeletal muscle.

As with other types of myonecrosis, renal damage often results.

Histological changes in the nervous system and in smooth and cardiac muscle are notably absent.

We thank Professor William Blackwood and Professor J. H. Dible for their help and interest.

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HYPOVITAMINOSIS-A IN A FAMILY WITH TYLOSIS AND CLINODACTYLY

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Tylosis palmaris et plantaris constitutes one of the group of polykeratoses (Touraine, 1954) and is a rare familial ectodermal anomaly of the palms and soles characterized by marked hyperkeratosis. The most comprehensive review of the subject is that of Cockayne (1933).

The association between various hyperkeratotic and dyskeratotic skin diseases and hypovitaminosis-A has been noted (Rapaport *et al.*, 1942; Peck *et al.*, 1943a, 1943b; Leitner and Moore, 1946; Leitner and Ford, 1947). Low blood levels of vitamin A were found in tylosis (Porter, 1951) and some improvement in the skin lesions occurred on giving high doses of the vitamin.

The present report is a study of a tylostic patient who was admitted to hospital with a myocardial infarction and a good description of tylosis in the family. The histopathological and clinical features of tylosis in this family have been more extensively dealt with in an earlier publication (Klintworth and Anderson, 1961).

Materials and Methods

A family of 59 members through five generations was investigated. Wherever possible they were personally interviewed. Where this was not practicable a first-hand collaborative description was taken from several other members of the family. Particular attention was paid to the presence of tylosis, cardiovascular disorders, manifestations of hypovitaminosis-A, and dysphagia. In those deceased the cause of death was ascertained. Specimens of blood were taken from willing members for estimation of the serum vitamin A, carotene, and lipid.

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