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The effects of histamine in malaria

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Summary

1. Extracts of the blood of monkeys (*Macaca mulatta*) infected with *Plasmodium knowlesi* contain histamine. A mean total concentration of 0·15 μg/ml was present in the circulating blood.

2. No histamine was detected in the blood of healthy monkeys.

3. Vasodilatation and increased vascular permeability was observed at the site of injection of the extract into the skin and brain of guinea-pigs.

4. Infiltration of the dermal layer by leucocytes was observed after intradermal injection of the histamine extract. A similar response was obtained in the brain.

5. The extract of the blood from the infected animal produced hypotension in rabbits when administered intravenously.

6. The pathophysiological significance of histamine in malaria is discussed.

Introduction

Toxic effects of histamine were first described by Dale & Richards (1918). The amine has a profound direct effect on vascular smooth muscle (Lewis, 1927 ; Krogh, 1929) and induces changes in vascular reactivity as an endogenous chemical mediator in bacterial infections. Recently, the concept of histamine release by endotoxin has been advanced by Schayer (1960) and Hinshaw, Jordan & Vick (1961).

Excess of histamine has been demonstrated in venous blood returning from tissues subjected to reactive hyperaemia and from ischaemic tissues (Barsoum & Gaddum, 1935 ; Billings & Maegraith, 1938). This substance is responsible for the increased permeability observed in acute inflammation.

The present paper describes a study of this amine in the blood of monkeys (*Macaca mulatta*) infected with *Plasmodium knowlesi*, in relation to the pathogenesis of capillary damage.

Methods

Infection

Plasmodium knowlesi

The original Nuri strain of the parasite used was obtained from Dr. Davey (1957, Imperial Chemical Industries, Manchester) and has since been maintained in the Liverpool School of Tropical Medicine by blood passage through rhesus (*M.*

mulatta) monkeys. Healthy monkeys under phencyclidine (Sernylan) anaesthesia (1·5–2·0 mg/kg intramuscularly) were infected by intravenous injection of 2·0–5·0 ml of chronically infected (" strain ") heparinized blood containing 5–40 million parasites. The disease caused by this parasite usually terminates fatally in 6–9 days after inoculation.

Parasite count

Thin blood smears obtained from the infected animal by ear-prick without sedation were stained with Leishman's stain and examined under the microscope using the oil-immersion objective for parasite counting as routine ; thick films were used in the early stages. Parasite counts were made every morning and late in the afternoon throughout the infection. About 1,000 cells in a blood film were counted. Parasitaemia was defined as the ratio of parasitized to non-parasitized cells expressed as a percentage.

Collection of blood

The monkeys were anaesthetized with phencyclidine (1·5–2·0 mg/kg) and the femoral artery was cannulated for blood withdrawal.

Preparation of histamine extract

The method of extraction of histamine in the blood of control and infected animals was a modification of that of Barsoum & Gaddum (1935).

Arterial blood 5 ml (without anticoagulant) was collected from non-infected and from *P. knowlesi*-infected monkeys and added immediately to 10% HCl in a 50 ml (rotating) evaporating flask. The digest was heated to 95° C in a water bath for 1 h and then evaporated to dryness in a Rotavapour (Orme Scientific Ltd., Manchester) under reduced pressure. Two successive portions of 12·5 ml 95% ethanol were added separately to the residue and distilled off to remove the HCl. The residue was suspended in water and remaining traces of acid were neutralized with drops of N NaOH solution. The suspension was centrifuged at 6,500 rev/min for 20 min. The supernatant fluid was assayed on the isolated guinea-pig ileum preparation.

Isolated organ preparation

Guinea-pigs of either sex weighing 120–240 g were used. A 2–3 cm segment of the terminal ileum was cut off and suspended in a 5 ml bath of Tyrode solution at 35° C, and aerated with a constant flow of oxygen. Atropine (10^{-8} g/l.) was used to depress spontaneous contractions. Mepyramine was used in the bath as an antagonist, usually at a concentration of 10^{-7} g/litre. A dose cycle of 3 min and 20 min contact with the gut was used.

Rabbit blood pressure

Animals weighing 3–4 kg were anaesthetized with urethane 1·25 g/kg body weight, made up in physiological saline and injected intravenously. The trachea was cannulated and artificial respiration applied when required. Blood pressure

was recorded from a cannula in the carotid artery through a mercury manometer (1 mmHg≡1·333 mbar), and injections of test substances were made into a cannulated femoral vein.

Capillary permeability test

Male albino guinea-pigs of 180–300 g were used. The trunk hair between shoulders and hips was closely clipped.

Pontamine sky blue dye 6BX (E. Gurr) in physiological saline was injected intravenously through the marginal ear vein of the test guinea-pig in a dose of 60 mg/kg body weight (Onabanjo & Maegraith, 1970c), after which the animal was referred to as " blued ". Intradermal injection of histamine extracts were made in 0·1–0·2 ml volumes 30 min after injecting the dye into the animals.

Technique of intracranial injection of histamine extracts

The technique used was as previously reported (Onabanjo & Maegraith, 1970a). Histamine extract was injected subdurally into " blued " guinea-pigs under pentobarbitone anaesthesia (4 mg/100 g body weight intraperitoneally) in 0·3–0·5 ml volumes.

Histopathological studies

Samples of excised guinea-pig skin used about the site of injection were fixed in Zenker's fluid ; thin slices (2–3 mm) of brain tissue were fixed in 10% formol saline. The tissues were stained with haematoxylin and eosin and examined for subsequent pathological effects.

Results

Histamine in blood

The blood of control animals contained no detectable histamine. Histamine was, however, present in extracts of blood from infected monkeys on about the eighth day of the disease, roughly 24 h before death. A contraction of the guinea-pig ileum caused by an extract from an infected animal is seen in Fig. 1. This response was abolished by mepyramine.

From the dose response relationship of the histamine extracts (matched with those of standard histamine phosphate (B.D.H.) as a base), the amount of histamine present in four infected animals was estimated (Table 1). A mean concentration of histamine of 0·15 μg/ml (Maegraith & Onabanjo, 1969) was found in the circulating blood in *P. knowlesi*-infected monkeys during the late stages of the disease (about 24 h before death). Controls were negative.

Skin reactions to histamine extracts

The technique used for intradermal injection of extracts into guinea-pigs was as described previously (Onabanjo & Maegraith, 1970c).

Extracts of the blood of *P. knowlesi*-infected monkeys were injected intradermally into " blued " guinea-pigs. A round area of blueing appeared in 3–5 min after injection and increased in diameter and intensity (depending on the concentration of the histamine extract injected) during the following 10–15 minutes. A total

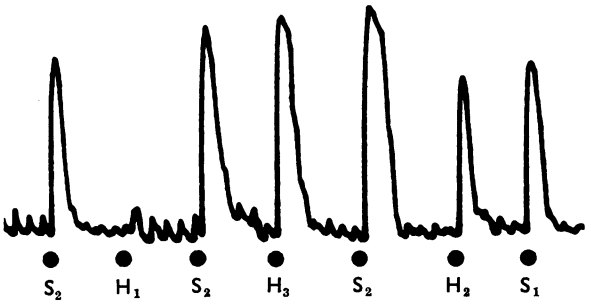

FIG. 1. Responses of the guinea-pig ileum suspended in 5 ml bath of atropinized (10^{-8} g/ml) Tyrode solution to samples of histamine extract and standard histamine acid phosphate are shown. S_1 and S_2=0·06 and 0·1 μg of histamine acid phosphate. H_1, H_2 and H_3=0·1, 0·15 and 0·2 ml of histamine extract from an infected monkey.

TABLE 1. *Histamine levels in the blood of* P. knowlesi-*infected rhesus* (Macaca mulatta) *monkeys*

Monkey No.	% Parasitaemia	% Packed cell volume (P.C.V.)	Blood histamine content (μg/ml)	Mean ±S.D.
696	25·2	28·0	0·15	0·15±0·018
710	27·9	25·0	0·13	
730	45·9	35·0	0·18	
746	58·6	17·5	0·14	

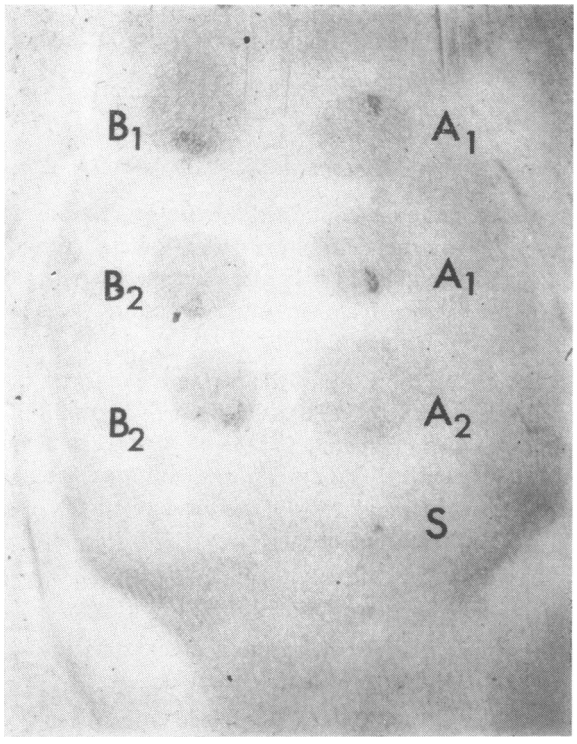

FIG. 2. Lesions produced by intradermal injection (0·1–0·2 ml) to a "blued" guinea-pig with histamine extracts from infected animals. A_1 and A_2, 0·01 and 0·03 μg/ml histamine extract from monkey 730; B_1 and B_2, 0·04 and 0·02 μg/ml extract from monkey 746; S, normal saline.

bleb area measuring from 17–25 mm diameter was obtained. A volume of 0·1–0·2 ml containing 0·01–0·04 μg of crude histamine extract produced this effect (Fig. 2); extracts of the blood from control monkeys gave uniformly weak reactions. The animals were killed 60 min after intradermal injections and portions of the skin were examined microscopically.

Inflammatory reactions

The sites of injection of the extracts of blood from infected monkeys showed pronounced inflammatory changes particularly in the dermal layer. The tissues were infiltrated with leucocytes (mostly lymphocytes and eosinophils, Fig. 3). No migration of leucocytes occurred at the site of injection of extracts of the blood of control animals.

Effects of histamine extracts in the brain

It was reported by Migasena & Maegraith (1965) that a breakdown in the blood–brain barrier occurred in the acute stages of *P. knowlesi* and *P. berghei* infections in animals. They suggested that this effect might be due to pharmacologically active substances released into the circulation and acting on the smooth muscles.

The large amount of histamine we found in the blood of infected animals suggests that this amine may be one of the agents responsible for the disturbance of the integrity of the blood vessels in the brain and elsewhere, since intradermal injection of extracts containing this substance increased permeability.

Extracts from infected animals were injected subdurally in 0·3–0·5 ml volumes in "blued" guinea-pigs (140–200 g weight); after some hours (Onabanjo & Maegraith,

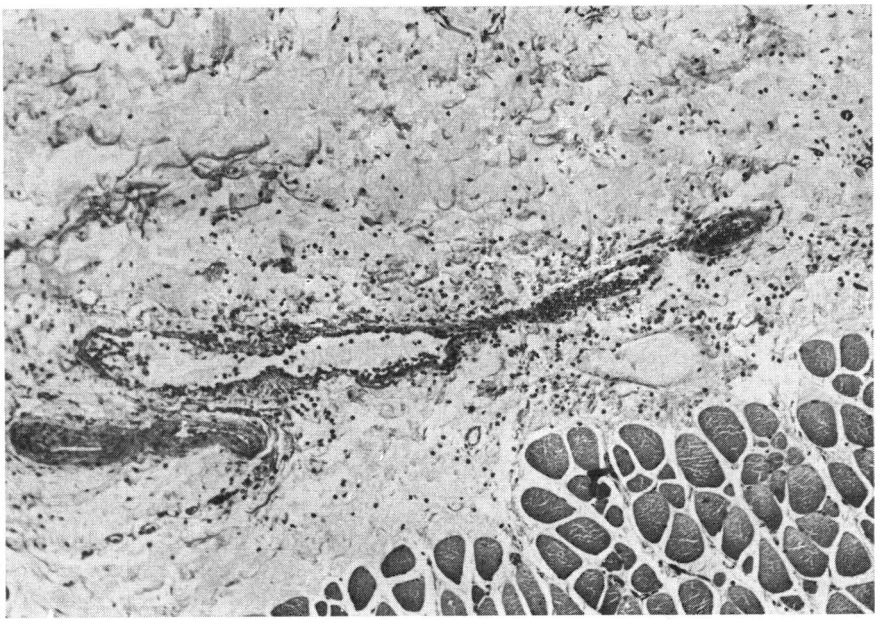

FIG. 3. Massive infiltration of the dermal layer by leucocytes of "blued" guinea-pig skin section, after intradermal injection of the histamine extract from an infected monkey.

1970a) blueing occurred throughout the brain. This indicated the passage of plasma proteins and water into the perivascular and cerebrospinal fluid (Maegraith & Onabanjo, 1969 ; Onabanjo & Maegraith, 1970b). The result was the development of " stasis ". Extracts from the blood of control monkeys produced no such effect under similar conditions.

Pathological changes in the brain

The overall appearance of the lesions produced after injection of extracts from infected monkeys into guinea-pig brain was indicative of the physiological activity which had taken place.

Inflammatory reactions were observed in the lateral ventricles (Onabanjo & Maegraith, 1970a). Marked infiltration by polymorphs and lymphocytes of the meninges (Fig. 4), the choroid plexus and the ependymal (Fig. 5) areas was observed.

Effect of inhibitors

Antihistamine drugs (Anthisan, Benadryl and Phenergan) in doses of 20 mg/kg body weight injected intraperitoneally into guinea-pigs 1 h before " blueing " almost completely blocked the effect of the histamine extracts after intradermal and subdural injections.

The permeability increasing activity of the extract was not affected when histamine-protease inhibitor (such as soy-bean trypsin inhibitor) digest was injected intradermally into " blued " animals.

Effect of extract on cardiovascular system

The dilatation of the small blood vessels is the most characteristic action of histamine on the vascular tree. This results from direct action on the musculature of the vessels, and is independent of innervation (Dale & Laidlaw, 1910, 1911).

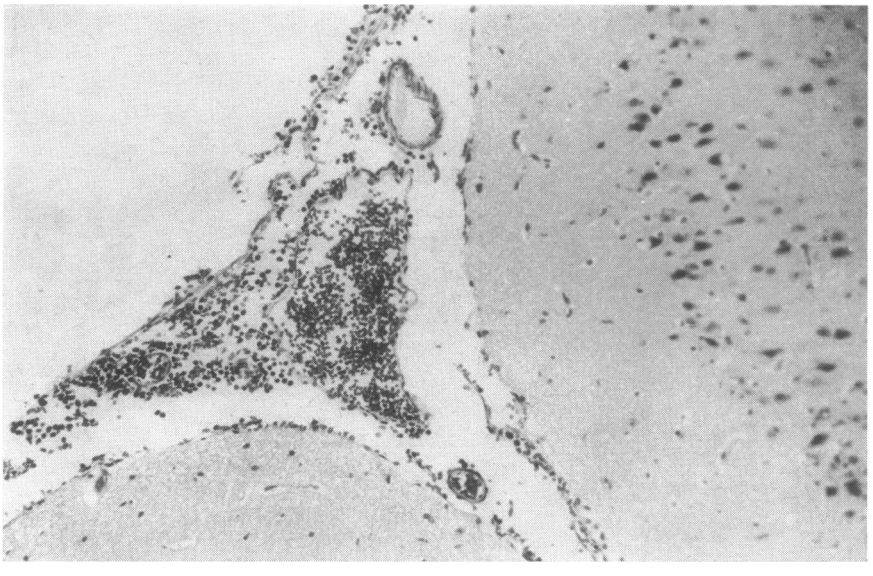

FIG. 4. Infiltration by leucocytes of the meninges of the guinea-pig brain after intracerebral injection of the histamine extract from an infected monkey.

Intravenous injections of extracts from infected monkeys into rabbits in doses 0·08–0·14 μg produced a substantial but temporary fall in systemic blood pressure (Fig. 6); extracts from non-infected animals had no effect.

Discussion

The pathological processes involved in malignant malaria create an ever-widening chain reaction which builds up to the clinical and pathological picture of the disease by the interaction of reversible processes, many of them non-specific (Maegraith, 1966), which may eventually lead to irreversible tissue damage and the death of the host.

The work described here shows that the peripheral blood of infected monkeys in the acute stages of the disease contains histamine, which is active specifically on

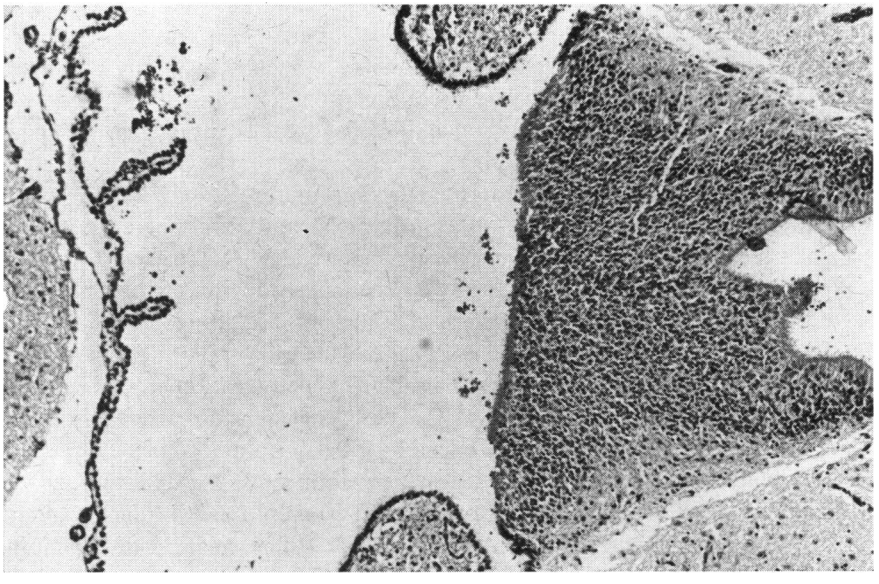

FIG. 5. Marked leucocyte migration into the ependyma of the guinea-pig brain after subdural injection of the histamine extract from an infected animal.

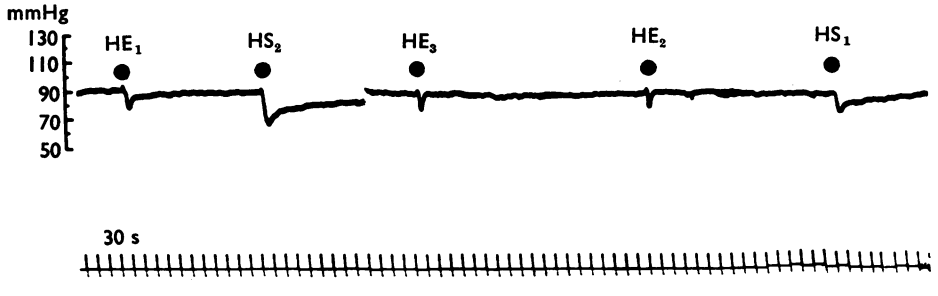

FIG. 6. Arterial pressure changes in a rabbit (4·0 kg) following intravenous doses of extracts of histamine from infected animals. Dose intervals, 5 min. 0·2 and 0·5 μg of standard histamine (HS_1 and HS_2). 0·08, 0·134 and 0·14 μg of histamine extracts from infected animals (HE_1, HE_2 and HE_3).

smooth muscles, particularly in the small vessels and which acts on the vascular endothelium, increasing its permeability.

Histamine plays a significant part in the development of the phenomena of acute inflammation, and it is suggested that it is acting similarly in malarial infection, producing vasodilatation and slowing the local circulation and increasing endothelial permeability, the effect of which depends on the anatomical site. In the brain, where the small vessels are relatively impermeable, histamine causes loss of protein and water through the walls and consequent stasis with impedance and eventual cessation of local blood flow.

The absolute amount of histamine in the blood of uninfected monkeys has not been investigated ; no histamine could be detected in the blood of healthy monkeys. A substantial amount was, however, found in the blood of infected animals in the late stages of the disease.

It is of interest to speculate on the origin and release of the histamine that appears in the blood of *P. knowlesi*-infected animals. Malaria parasites destroy the erythrocytes in which they develop. It is possible that at schizogony the ruptured erythrocytes may release into the plasma certain protein residues of peptide nature (Goodwin & Richards, 1960), including kallikrein, which can act on the enzyme phospholipase A to produce histamine. The release of the histamine from rat mast cells suspension was recently reported by Amundsen, Ofstad & Hagen (1969) using pancreatic exudate (containing phospholipase A and kallikrein activities) from dogs with experimental acute haemorrhagic pancreatitis. Release of the amine could also occur in malaria in which kallikrein activity in the circulating blood is increased (Onabanjo & Maegraith, 1970c). On the other hand, histamine is released during tissue injury (Lewis, 1929) and Billings & Maegraith (1938) demonstrated an increase in blood draining an ischaemic limb. This suggests that the amine may be a product rather than an initiator of the pathological chain reaction, although, once present, it can be presumed to exert its pharmacological effects which will contribute to the vasodilatation, increase in permeability of the endothelium of the small vessels and to vasomotor effects observed in *P. knowlesi*-infected monkeys (Skirrow, Chongsuphajaisiddhi & Maegraith, 1964). The relation of histamine to the production of shock (Dale & Richards, 1918) is also relevant to our findings in the terminal stages of the infection.

The results of the present investigation show that histamine is present in increased amounts in *P. knowlesi* infection.

Diapedesis of cells from local vessels was observed at the site of injection of histamine extracts into the skin and brain of guinea-pigs, accompanied by vasodilatation and increased vascular permeability. The tissues of both skin and brain in the sites injected were infiltrated with polymorphs, lymphocytes and eosinophils. The dilated small vessels of the injected area were filled with tightly packed erythrocytes. The increase in permeability of the vessel walls was demonstrated in both skin and brain (Maegraith & Onabanjo, 1969), by the escape of dyed protein following the injection of the extract. The effect of this escape of protein is probably the same as in acute inflammation, leading to local plasma concentration, accumulation of erythrocytes and ultimately to stasis.

The effects of histamine on the local circulation may contribute to the production of generalized vascular failure. Increased capillary permeability may in some cases

affect the circulating plasma volume and blood may be pooled in certain peripheral areas where there is vasodilatation and distal vasoconstriction. This results in reduction of venous return to the heart associated with increasing reduction of cardiac output and finally shock.

The effect of histamine on the haemodynamics of the sick monkey must be taken into account, but this is not easy to assess. Injection of extracts of the blood of malaria-infected monkeys produced a fall in arterial blood pressure in the rabbit. Since histamine circulating in high concentration produces shock, which is preceded by circulatory stasis (Best & Solandt, 1940), it may act in this way in malaria.

The extract from the blood of the *P. knowlesi*-infected monkeys contained a substance we identified pharmacologically as histamine. This caused increase in capillary permeability, escape of protein and fluid and the development of local stasis with consequent slowing of the circulation. This process is probably non-specific (Maegraith, 1948); it can be reversed by anti-inflammatory compounds (Migasena & Maegraith, 1967). It is reasonable to assume, therefore, that these phenomena in malaria closely simulate the equivalent effects in acute inflammation, in which histamine plays an important role.

The identification of histamine and other substances in the blood of infected monkeys (Onabanjo & Maegraith, 1970c; Onabanjo, Bhabani & Maegraith, 1970) and their pharmacological capabilities makes more intelligible the concept of malaria as a pathological process (Maegraith, 1951).

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