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REGIONAL VARIATIONS OF INCREASED PERMEABILITY OF SKIN CAPILLARIES INDUCED BY A HISTAMINE LIBERATOR AND THEIR RELATION TO THE HISTAMINE CONTENT OF THE SKIN

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Guinea-pigs with the vital dye Pontamine Sky Blue 6X in their circulation react to an *intradermal* injection of the histamine liberator 48/80 by an increase in capillary permeability, which results in a local intense staining of the skin tissue by the dye. This effect is apparently due to the local liberation of histamine (Miles, 1951; Miles & Miles, 1952); blueing of the skin can thus be used as an indicator of histamine liberation.

In the present experiments we extended this study to the effect on the skin of *intravenous* injection of 48/80 in guinea-pigs with circulating dye. Blueing of the skin occurred in these circumstances, but certain regions were regularly found to blue more rapidly and more intensely than others. It was thought possible that this uneven blueing was associated with differences in skin histamine and that the bluer regions contained the higher concentration. Accordingly, a survey was made of the histamine content of the various regions of the skin. A few experiments were also made on other species.

METHODS

Albino guinea-pigs, 300–500 g, were used. For the *dye test* the hair was removed as completely as possible with electrical clippers, taking care to avoid any damage to skin that might result in traumatic blueing (Miles & Miles, 1952) on injection of the dye. The animals were fixed in the extended position on their belly by four tapes tied immediately above the feet. The subcutaneous veins running diagonally towards the perineum on the posterior aspects of the thigh were exposed by incision of the skin and a 5% solution of Pontamine Sky Blue 6X ('pontamine blue') was injected into the vein of one leg, 1·3 ml./kg body weight, and a few minutes later 48/80 (2·5–5 mg/kg), in a solution of 0·85% (w/v) saline, into the vein of the other leg. The animal was immediately released for observation. After the full development of blueing in the skin, the animals were killed by chloroform or stunning, and the remaining stumps of hair removed by a depilating paste (composition: wheat flour, 350 g; talcum powder, 350 g; barium sulphide, 250 g; Castile soap powder, 50 g; water).

The dye test was also made in albino rabbits and mice and in one white female cat. In rabbits, the injections were made into the marginal vein of the ear; 12–15 mg/kg of 48/80 were given. In

mice, the 48/80 and dye were injected together into a tail vein; the dose of 48/80 was 1·8–2·2 mg/kg and that of pontamine blue 8 ml./kg of a 1% solution. The cat was anaesthetized with ether and the injections made into the femoral vein; otherwise the procedure was the same as for guinea-pigs.

The internal distribution of dye was examined in some animals. The blood was removed during anaesthesia by perfusion with warm saline solution from a cannula inserted through the right ventricle into the pulmonary artery, the right auricle being cut open.

For determining the *histamine content*, untreated guinea-pigs were killed with chloroform, depilated and carefully washed to remove all traces of the depilatory. Pieces of skin were excised and the loose subcutaneous tissues removed as far as possible. The cleaned specimen was weighed, ground with a little sand and extracted with acid saline. The histamine in the extract was assayed on the atropinized guinea-pig ileum preparation. The same procedure was used for cats and dogs. All the values cited for histamine refer to the weight of the base.

RESULTS

Experiments on guinea-pigs

Induction of blueing by 48/80. The injection of pontamine blue alone produced during the first hour only a faint grey-blue coloration of the skin, providing a background on which the frank blue coloration produced by subsequent injection of 48/80 was readily detectable. In order to produce blueing with 48/80, doses of the order of 2·5–5 mg/kg were used. Some animals died within the first 2 min after the injection with acute respiratory failure; in others there was temporary respiratory spasm and the animal became prostrated, with a cold skin. In these animals blueing was feeble, presumably because the pressure in the skin capillaries was too low to permit extravasation of the dye (Miles & Miles, 1952). In most of the animals so treated, however, the signs of intoxication were slight or absent, and in these, development of blueing was rapid and extensive. Within 1 min of injecting 48/80, a faint blueing appeared in the eyelids at the inner or outer acanthus of the eyes, round the nostrils and lips; within 3 min there was intense blueing of the eyelids, the tip of the nose and round the mouth. During this period, blueing also started at the base of the ears, over the convolutions of the cartilage on their under surfaces, spreading outward until the inner and outer surfaces of the proximal half of the ear were a deep blue; over the brows, spreading ventrally and posteriorly until the whole head region was blued down to the neck and shoulders; round the areolar area of the nipples; round the anus, and round the scrotum or the vaginal orifice. The full extent of this blueing was complete within 10–15 min. The regions that were most deeply stained were those in which the blue first appeared. In the skin of the trunk and of the legs, blueing was fainter, often patchy and, in some areas, notably the ventral abdominal wall and the inner aspect of the thighs of the hind-legs, it was absent.

In animals fully blued with 48/80 there is a striking contrast between the intensely blue head region, the nipples and the perineum on the one hand, and the feebly and patchily stained skin of the trunk and hind-limbs on the other,

as illustrated in Pl. 1, animals A and B. Animal C was killed before blueing had fully developed and the intense early blueing round the nose, lips, eyes and the external meatus of the ear is evident. Pl. 1 also illustrates the traumatic blueing which always occurs in the skin of the legs where they are gripped by the tapes used in tying the animal during injection; and around the incisions made over the veins.

Internally there was a moderately intense blueing of the inner surface of the trachea, especially along the median posterior line, of the bronchi, of the medulla and inner aspect of the pelvis of the kidney and of the ureters. The whole digestive tract was also stained, but the colour was relatively feeble and had a greenish tinge. A similar staining, however, occurred in animals not treated with 48/80, but it was much less intense in the trachea, bronchi and medulla of the kidney. The medulla of the ribs and the cervical, axillary, mediastinal and inguinal lymph nodes were also blue. The colour in the ribs appears to be due to accumulation of the dye in a portion of the reticulo-endothelial system that happens to be visible through the semi-transparent bony wall of the ribs; it was roughly proportional to the lapse of time between intravenous injection of the dye and killing of the animal. The staining of the lymph nodes also became more intense with increasing lapse of time between injections of 48/80 and killing of the animal, and is presumably due, not to any direct action of 48/80 on lymph nodes, but to dye draining from the skin, bronchi and trachea.

Although the fact is not immediately relevant to the interpretation of the dye test, which is completed within 20 min of giving pontamine blue, it should be noted that in animals not treated with 48/80 the skin, trachea, bronchi, pelvis of kidney and testicles became moderately or intensely stained within 5–10 hr.

Mepyramine maleate in doses of 0·2–2·0 mg/kg given intravenously a few minutes before the 48/80 greatly diminished the extent and intensity of the blueing. At most there was a feeble blueing round the eyes, nose and lips which took 5 min or more to develop, and an even feebler blueing of the head and neck. Although the mepyramine markedly diminished the blueing induced by the dose of 48/80 usually employed, yet it did not reduce the proportion of animals in which this dose proved lethal or led to severe shock.

Histamine content of skin. The histamine content of the skin varied greatly in different regions, and the regional differences were substantially the same in all the animals tested. In Table 1 the histamine content of the skin is given in μg/g. The figures in the last column were obtained by first expressing the individual values in terms of the value for the abdominal skin taken as unity, and averaging the results for each region. These mean values are charted in the diagram of the body surface in Fig. 1.

Histamine content is not associated with thickness of skin; thus in two

TABLE 1. Histamine content of various regions of skin of guinea-pigs weighing 400–600 g

Site	Histamine in μg/ g skin							Mean values (abdominal skin taken as unity)
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	
Ear margin	—	—	—	18·5	14·0	14·7	14·0	5·9
Ear base	—	—	—	12·5	10·3	13·0	12·0	4·6
Submental	15·5	—	17·2	12·0	11·2	13·3	10·5	4·5
Areola of nipples	15·5	—	14·2	—	—	—	11·3	3·7
Lids	11·0	—	8·9	11·5	10·0	—	—	3·7
Bristle area	13·0	—	11·5	7·3	10·5	—	—	3·6
Anal margin	—	—	—	6·0	—	8·7	11·7	3·1
Paws; forelegs	—	—	—	7·0	5·8	11·4	5·9	2·9
Paws; hind-legs	—	—	—	6·8	5·6	8·0	4·3	2·7
Vertex	10·0	9·2	8·9	8·0	5·8	—	—	2·6
Lips and nose	11·0	—	11·5	6·0	—	7·2	6·6	2·6
Cheek	—	—	—	5·0	—	7·6	8·5	2·4
Fore-leg	—	—	—	5·0	6·2	6·7	—	2·4
Scrotum	5·0	—	—	—	—	6·5	—	1·7
Abdomen	4·0	4·0	3·8	2·4	2·1	3·0	3·2	1·0
Calf	—	—	—	—	2·0	4·0	2·5	1·0
Back (middle lumbar)	3·0	3·8	4·2	2·4	1·5	2·6	2·2	0·9
Thigh	—	—	—	—	1·2	2·9	1·5	0·7

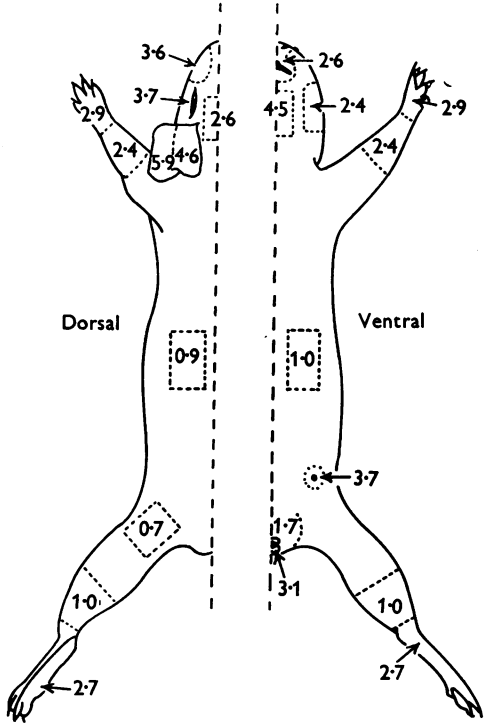


Fig. 2. Distribution of histamine in various regions of guinea-pig's skin. The figures are those given in the last column of Table 1 and represent mean values for each region, the abdominal skin being taken as unity.

thin-skinned areas the content may be high (ear) or low (abdomen); and similarly in two thick-skinned areas (vertex of skull and lumbar region). On the other hand, as may be seen by comparing Pl. 1 and Fig. 1, with the exception of the distal parts of the fore- and hind-limbs, the proportional distribution of histamine is directly related to the intensity of blueing under treatment with 48/80. The absence or paucity of blueing in certain regions does not appear to be due to insusceptibility of the contained histamine to liberation by 48/80, because when minimal effective doses of 48/80 are injected intradermally into guinea-pigs with circulating dye, the local blueing is of the same intensity whether the injection is made into 'histamine poor' regions, such as the skin of the face and limbs, abdomen or back, or into the skin of the neck or scalp.

TABLE 2. Histamine content of various regions of skin of three adult cats

Site	Histamine in μg/g skin			Mean values (abdominal skin taken as unity)
	Expt. 1	Expt. 2	Expt. 3	
Ear	120	95	100	4·9
Submental	—	44	42	2·0
Lids	39	36	50	1·9
Bristle area	34	32	40	1·6
Throat	32	31	34	1·5
Upper lip	—	—	42	1·5
Paws and toes	30	25	—	1·4
Areola of nipples	—	—	40	1·4
Vertex	—	30	28	1·3
Tail, distal	—	—	35	1·3
Thigh	—	—	33	1·2
Anal margin	—	25	27	1·1
Abdomen	20	18·2	28	1·0
Nipples	—	—	28	1·0
Tongue, ventral surface	—	18·0	—	1·0
Fore-leg	—	—	25	0·9
Tail, proximal	—	—	25	0·9
Back	19	11·5	10	0·7
Tongue, distal surface	—	9·5	—	0·5

Experiments on cats

In the one cat on which a dye test was made, blueing was most intense in the eyelids, particularly the lower lids, in the whisker-bearing skin of the upper jaw and the submental region of the lower jaw. It was moderate on the ridge of the nose; slight inside the outer ear; moderate immediately round the base of the nipples, particularly the two posterior pairs; and intense in the perianal and perivulval regions. There was only a faint staining of the remainder of the skin, with no particular distribution of intensity. Internally, the trachea, and especially the tissue between the cartilagenous rings, was strikingly stained.

Histamine estimations in three cats are given in Table 2. Mean values are recorded; but with the few tests made, they clearly have less weight than those in Table 1. In the cat the relation between histamine content and intensity of blueing is not so striking as it is in the guinea-pig, because for most areas tested

the range of proportional values for the histamine content is small. Nevertheless, the most intense blueing occurs in the areas with the greatest amounts of histamine, except that the blueing in the ear was not marked, though the histamine content was as high as 95–120 μg/g.

Experiments on dogs

Although no dye tests were made in this species, the following observations of Paton & Schachter (1951) strongly suggest that a subcutaneous injection of 48/80 (10 mg/kg) induces in the skin an increase in capillary permeability whose regional intensity resembles that in guinea-pigs as manifested by the dye test. They found that 'the facial swelling was particularly marked in the bristle area near the mouth, the eyelids and the pinna of the ear; these areas also showed considerable erythema. Oedema and erythema of other regions was not observed, except for the nipple area in one animal.'

TABLE 3. Histamine content of various regions of skin of two dogs (dog 1, 39 kg; dog 2, 26 kg)

Site	Histamine in μg/g skin	
	Expt. 1	Expt. 2
Bristle area	20·0	62
Ear	22·2	32
Lids	15·6	33*
Lips (hair bearing part)	—	20
Nipples	—	20
Areola round nipples	—	18
Submental	6·3	17
Front paw (dorsum)	—	16
Abdomen	8·6	15
Back	—	15
Lips (hairless part)	4·2	—

* Upper lids only.

Histamine estimations in two dogs are recorded in Table 3. It is noteworthy that the five skin regions with the highest histamine content, bristle area, eyelids, ears, lips and areola of nipples, became oedematous in Paton & Schachter's dogs.

Dye test in rabbits and mice

In rabbits the dye test revealed a picture in many respects similar to that in guinea-pigs; there was deep staining of the eyelids, of the internal aspect of the orifice of the ear, moderate staining of the muzzle and lips, areolar area of nipples and perianal regions, and slight staining at the base of the tail and the hind-limbs.

The response of mice to the combined injection of dye and 48/80 was variable. Within the range of doses of 48/80 used, the blueing in some animals did not differ from that in mice receiving pontamine blue alone; that is, a faint grey-blue over the whole body and slight staining of the muzzle, the root of the tail and the perianal region, and of the bony protuberances of all four feet

where they come in contact with the ground; in other mice the skin became blue all over, with a distinctly deeper staining over the head, shoulders and limbs.

DISCUSSION

If we consider the blueing of the skin induced by intravenous injections of 48/80 in guinea-pigs with circulating dye as evidence of increased permeability of the capillaries, our results show clearly that there is a definite regional pattern in the manifestation of this increase. There is good evidence that the increased permeability is an effect of released histamine, because: (1) 48/80 is a potent histamine liberator; (2) histamine itself, like 48/80, will induce on intradermal injection a local blueing in animals with circulating dye; and (3) the general blueing produced by intravenous injections of 48/80, like the local blueing produced by intradermal injections of either 48/80 or of histamine (Miles & Miles, 1952), is antagonized by mepyramine. The demonstration of regional differences in histamine content of the skin that are broadly correlated with the intensity of blueing is consistent with this interpretation and provides an explanation for the regional pattern of increased permeability we have observed.

So far, a systematic survey of the relation between histamine content of the skin and the areas prone to manifestations of increased capillary permeability by 48/80 has been made only in the guinea-pig. There are, nevertheless, several indications of a similar regional pattern in other species. Thus, in rats, mice, rabbits, cats and dogs the eyelids, ears, nose, mouth, to some extent the head, the paws, the areola of the nipples and the anal and perineal regions are sites where increased capillary permeability, as manifested by frank oedema or by exudation of circulating dye, most readily occurs under the influence of 48/80 and other histamine releasing agents. As far as information is available, in each species these regions are those with the relatively higher content of histamine.

It is noteworthy that in the various species proneness to increased permeability does not occur in all the aforementioned regions. For instance, in guinea-pigs the skin of the feet did not blue readily, in spite of its relatively high histamine content. However, one of us (A. A. M.) has recently observed that in guinea-pigs sensitized with various antibodies the simultaneous injection of antigen and dye produces blueing which extends to the feet as well. The difference in histamine release by 48/80 and antigen may depend on the rapidity and firmness with which 48/80 is fixed to guinea-pig tissue (Miles & Miles, 1952), as compared with the antigen; after intravenous injection, the 48/80 may be removed rapidly from the blood and so tend to be selectively concentrated in the areas of skin with the most abundant blood supply. In rats and mice intraperitoneally injected egg-white as well as 48/80 certainly reaches the limbs in effective quantities, because oedema formation is par-

ticularly pronounced in the region of the feet (for references see Feldberg & Talesnik, 1953). Dogs and cats behave in this respect apparently more like guinea-pigs; in dogs the histamine content of the skin of the feet is also not particularly high. Further, the ears of guinea-pigs, cats and dogs have a high histamine content of the skin at the base but an even higher one of the more distal parts; nevertheless, signs of increased capillary permeability are confined to or predominate at the base.

There is another kind of discrepancy between proneness to increased capillary permeability and histamine content of the skin: the range of intensity of blueing or of oedema formation in the different regions of the body is greater than the range of histamine concentration. For example, in guinea-pigs the difference in the histamine content of the skin of the abdomen and eyelids is only about fourfold, but when injections of varying concentration of dye dissolved in normal serum are made into the skin of an untreated guinea-pig, the concentration of dye required to stain the skin to match the staining of the eyelids and nose in animals with circulating dye treated with 48/80 is of the order of a hundredfold greater than the concentration staining the skin to match the abdomen of the 48/80-treated animal. In fact, on the evidence of blueing alone, one would say that no histamine was released in the abdominal skin of the guinea-pig by 48/80, although the histamine it contains, between 2·4 and 4 μg/g, is sufficient to produce intense local blueing when released by 48/80 injected intradermally.

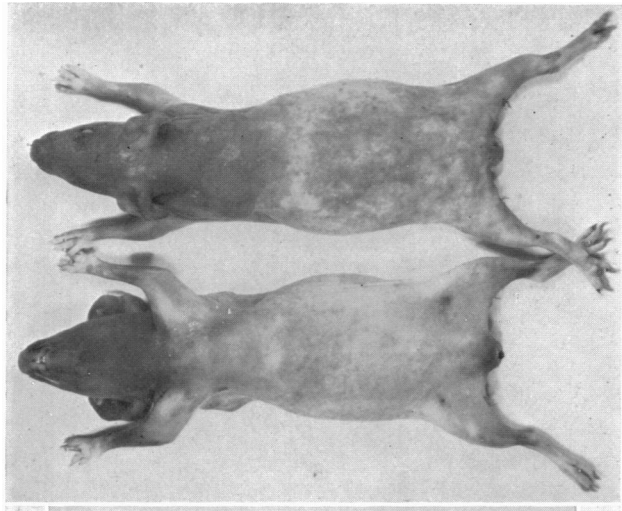
In addition to the differences in histamine content of the skin, there may therefore be other contributory factors determining the regional pattern of increased permeability of the skin capillaries seen after 48/80. The vascularity of the skin or the amount of blood supplied to it may vary; some regions may contain arteriovenous anastomoses; the sensitivity of the skin capillaries to endogenous histamine may not be the same over the whole body surface; and the readiness with which the extravascular tissue of the skin is able to accumulate dye or oedema fluid may vary according to the looseness of the tissue, or the ability of the tissue constituents to take up dye or oedema fluid.

SUMMARY

1. When the histamine liberator 48/80 is injected intravenously into guinea-pigs with the vital dye Pontamine Sky Blue in their circulation, the skin rapidly becomes blue. This blueing, which is antagonized by mepyramine, is presumably due to increased capillary permeability following the release of histamine.

2. The blueing varies in intensity in different regions of the body, being most intense in the head region, the nipples and perineum.

3. This characteristic regional pattern of blueing is directly related to the histamine content of the skin.



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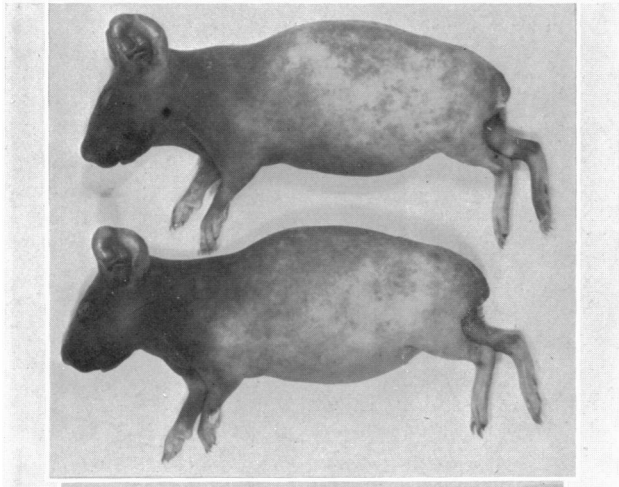

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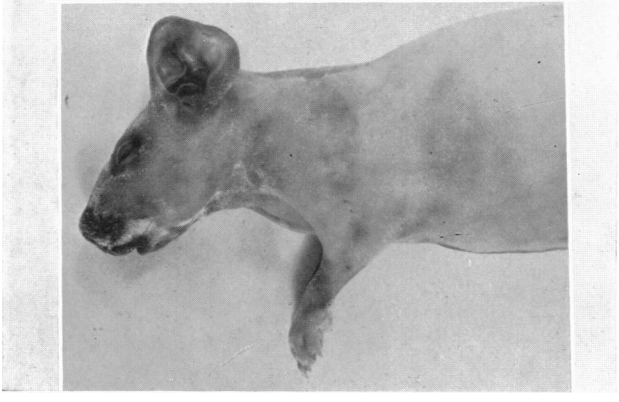

C

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4. The manifestations of increased permeability of the skin capillaries induced by 48/80 or by egg-white in other laboratory animals have, in general, a similar regional pattern, and the available evidence suggests that this also is determined primarily by regional variations of histamine content of the skin.

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EXPLANATION OF PLATE

Blueing of the skin after intravenous injection of 48/80 into three guinea-pigs with pontamine blue in their circulation. Animals A and B were killed after full blueing had developed and depilated. Guinea-pig C was killed before the blueing was complete to show the early blueing round the lips, nose, eyes and ears. The depilation of this animal is incomplete; the white areas round the mouth and along the base of the jaw are remnants of hair.