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SKIN RESPONSE TO HISTAMINE OF THE PROTEIN-SENSITIZED GUINEA PIG

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AT A SYMPOSIUM in 1956, Perry[15] compared the skin reactivity of different species and noted that a high degree of response was not associated with a high histamine level. In an earlier report, however, Brocklehurst, Humphrey, and Perry[1] stated that a considerably reduced response to histamine was obtained in rats after administration of Compound 48/80, and this suggested that the species effect was the dominant feature. Guinea pigs were shown by Feldberg and Miles[4] to have a low skin content of histamine, whereas their degree of skin reactivity in allergic manifestations is acknowledged to be high. Brocklehurst and his associates[1] reported unpublished work by Perry which showed that the skin histamine of guinea pigs was resistant to histamine liberators. In the article by Feldberg and Miles,[4] mention is made of Miles' experiments demonstrating a rise in skin histamine after sensitization. In view of this, it was decided to carry out studies on the response to histamine of the hypersensitive guinea pig.

METHODS

Histamine sensitivity of the guinea pig skin was examined by the method of Miles and Miles[9] in which pontamine sky blue was injected intravenously, histamine being subsequently given intradermally into the depilated skin. Pontamine sky blue was made up as a 5 per cent solution and each guinea pig received 70 mg. per kilogram of body weight two hours before testing. The animals' backs were prepared and 0.1 ml. of histamine acid phosphate containing 1 μg, and 0.1 ml. of physiological saline as the control substance, were each injected into eight randomly determined sites. After thirty minutes the diameter of each blue area was measured and the total for histamine and for the control was obtained. The ratio of histamine to control responses was then calculated. The effect of a treatment was studied using batches of at least six nonpregnant female guinea pigs, weighing 500 to 700 grams, and the results were assessed by applying the analysis of variance.

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Sensitization.—Horse serum, 0.5 ml., was given intraperitoneally and the animals were examined twenty-one to twenty-four days later. The sensitivity was tested after the experiment, using terminal ileum in the isolated organ bath and, in the absence of a positive response, the results for that animal were discarded.

Tissue Analysis.—The guinea pig tissues were loaded with sodium by injection intraperitoneally of 0.2 ml. per 100 grams of body weight of a saturated sodium chloride solution. The experimental procedure was undertaken half an hour later.

Water and Electrolyte Depletion.—The method of Yannet and Darrow[20] was used to deplete the guinea pig tissues of water and electrolytes. Intraperitoneal injections of 10 ml. per 100 grams of body weight of a 5 per cent glucose solution were made and the studies were made three hours afterward.

Drug Administration.—Hydrocortisone,* 5 mg. in aqueous suspension, was injected intramuscularly twenty hours before the tests were made.

Ascorbic acid,† 2.5 mg. in aqueous solution, was given intramuscularly two hours before the examination.

Ro 3-0809,† a phenyl benzyl phosphate derivative with an anti-hyaluronidase action, was injected subcutaneously in doses of 2.5 mg. per 100 grams, of body weight as a 1 per cent solution half an hour before testing.

AHT,† which has the form of 8-chloro-2 methyl-1:3:4:5-tetrahydro-2 pyrid-[4, 3, b] indole hydrochloride, was given subcutaneously half an hour before the experiment in a 1 per cent aqueous solution using 2.5 mg. per 100 grams of body weight. This substance has a peripheral serotonin antagonistic effect and, in this dosage, appeared to produce no other relevant changes.

Assuming that 8 per cent of body weight of the guinea pig was blood, 0.01 mg. per milliliter of blood of d-lysergic acid diethylamide tartrate‡ was injected subcutaneously. The tests were performed half an hour later.

RESULTS

Effect of Sensitization.—The blue areas on the backs of the normal guinea pigs were about three times larger in response to the histamine than to the control solution. This is shown in the right column of Fig. 1. When the observations on eight normal and eight sensitized animals were compared, the histamine response was found to have increased in the latter (Fig. 1) by four times without an appreciable change in the control value. The ratio of histamine to control diameters was therefore raised to a significant extent ($P \cong 0.02$) by sensitization.

Effect of Electrolyte Change.—An earlier paper (Buxton and McDowall[2]) reported that, after protein sensitization, the skin of the guinea pig had a

*From Roussel Laboratories, Harrow Road, London.
†From Roche Products Ltd., Welwyn Garden City, Herts.
‡From Sandoz Products, Great Castle St., London.

lower sodium content than that of the normal animal. But when the normal animal was subjected to water and electrolyte depletion by means of hypertonic glucose solution given intraperitoneally, the response to histamine was little changed, and the increase in the histamine to control ratio did not reach a significant level. In addition to this, the procedure used to raise the skin sodium content was accompanied by a greater spread of dye after injections of both histamine and saline, with a wide range of response. The ratio of the histamine to saline diameters was not altered to a significant extent.

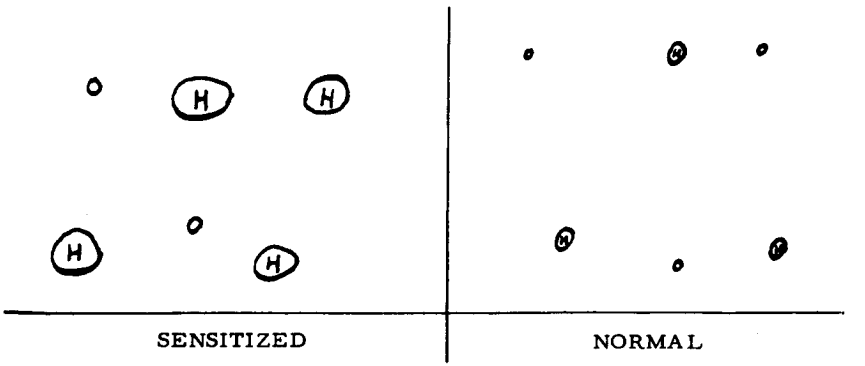

Fig. 1.—Part of the injected fields of a sensitized and normal guinea pig for comparison of the histamine response *(H)*. The control injections are unmarked.

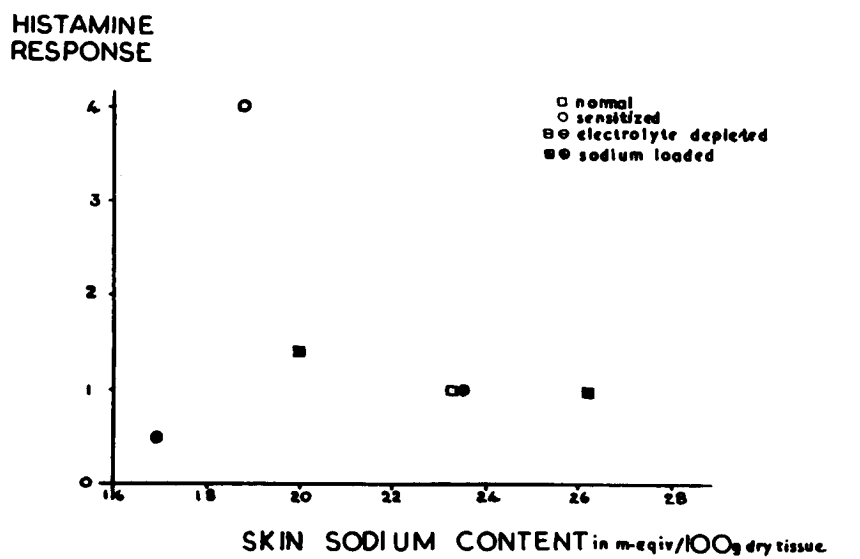

Fig. 2.—The relationship between the histamine response using an arbitrary scale and the guinea pig skin sodium content.

Conversely, the sensitized animals receiving a sodium load showed a reduction in histamine response without appreciably changing the control areas. In this way, the ratio of the responses fell to a quarter of the former value, which was a significant difference ($P < 0.01$). If, however, the electrolyte content of the skin was lowered by a hypertonic glucose injection, the dye spread was increased after injections of histamine and saline, but, as the latter effect was the greater, the ratio of histamine to saline areas was reduced to a significant extent ($P < 0.01$).

Fig. 2 shows these results in graphic form. The histamine-saline ratio of the normal animal is assumed to be unity and the experimental observations are presented with reference to this. The skin sodium content is given in milliequivalents per 100 grams of dry tissue. No obvious relationship between the sodium level in the skin and the histamine response is apparent. In the normal animals the effect of histamine was little changed by the experimental procedures but the reaction in sensitized guinea pigs was reduced.

Effect of Drugs.—

Anti-hyaluronidase: Changes in tissue permeability can account for an increased spread of a dye in a tissue. Duran-Reynals' work on Spreading Factor, which led to the identification of hyaluronidase, described such a factor in skin (Claude and Duran-Reynals[3]). Studies were therefore undertaken using a drug to inhibit hyaluronidase and so obtain information as to whether the increase of dye spread in sensitized guinea pigs in response to histamine could be attributed to this enzyme.

After injections of Ro 3-0809 in twenty-four guinea pigs, the twelve sensitized animals showed a reduced response to histamine, the histamine-control ratio falling to the level of the untreated normal animals, which represents a highly significant effect ($P < 0.01$). In the twelve normal guinea pigs no significant change was found after this treatment.

Hydrocortisone: Kellaway and Cowell[8] first reported the enhanced sensitivity to histamine of the adrenalectomized cat. It seemed logical therefore to determine whether the increased response of the sensitized guinea pig was reduced by the appropriate adrenal hormone. Hydrocortisone did, in fact, diminish the histamine-saline ratio by a significant extent ($P < 0.02$), but mainly by increasing the effect of the control solution. The ratio was not, however, brought to the normal level. The normal guinea pigs treated with hydrocortisone presented small changes after histamine and saline injections so that the ratio was increased but not to a significant extent.

Ascorbic acid: The relationship between ascorbic acid and the adrenocortical hormones is still a matter for debate. Perla found that histamine resistance was not increased in the adrenalectomized rat by giving ascorbic acid.[12] But he found that a scorbutic diet increased the sensitivity and a single massive injection of ascorbic acid conferred some protection against protein shock (Perla and Marmorston[13]). The results in our experiments of a single administration of ascorbic acid to normal and sensitized guinea pigs

were similar to those obtained by giving hydrocortisone, namely a reduced response in the sensitized and an increased one in the normal group; but, since the observations were less scattered, these were significant at the 1 and 5 per cent levels, respectively.

Antiserotonin: The anaphylactoid reaction in rats was shown by Parratt and West[11] to be due to the release of 5-hydroxytryptamine. As a state of hypersensitivity precedes anaphylaxis, the effect of an antagonist to serotonin on the sensitized animal was considered worthy of examination. AHT reduced the histamine response dramatically in both normal and sensitized guinea pigs, the ratio of histamine to saline being significantly lowered ($P < 0.01$).

This finding was further examined in the sensitized animals by giving lysergic acid diethylamide which antagonizes 5-hydroxytryptamine. The results confirmed the observations with AHT, the histamine response being decreased by a significant amount ($P < 0.01$).

The ratios of histamine to saline measurement under the different treatments are summarized in Table I. The value for the normal animals was taken as unity as in compiling Fig. 2, and the remaining observations were calculated in relation to this.

TABLE I. HISTAMINE: SALINE RATIOS OF BLUED AREAS WITH DIFFERENT TREATMENTS

	UN-TREATED	SODIUM-LOADED	SODIUM-DEPLETED	HYDRO-CORTI-SONE	ASCORBIC ACID	ANTI-HYALURONIDASE (RO 3-0809)	ANTI-SERO-TONIN (AHT)	LSD
Normal	1.0	2.5	1.4	2.2	1.5*	0.8	0.5*	—
Sensitized	4.0*	1.0*	0.5*	1.8*	2.2*	2.7*	0.3*	2.2*

*These findings represent a significant change.

DISCUSSION

Perry[14] found that the skin histamine of the normal guinea pig was resistant to histamine liberators. The effect of protein sensitization was to raise the skin histamine content (Miles[10]), but whether the increase in histamine response, which is reported above, was due to this or to other changes provoked by the sensitization process is not yet clear. The effects might either take the form of an exaggerated response or of a response which differed qualitatively. Since Brocklehurst and his co-workers,[1] using rats given Compound 48/80 which liberated 90 per cent of the total skin histamine which is all that is able to be released, were still able to elicit the Arthus phenomenon and passive cutaneous anaphylaxis, a substance other than histamine must have been involved. Fink[5] demonstrated the complete inhibition of the anaphylactic contraction of the uterus of the sensitized mouse by LSD and reserpine, and Fischer and Lecomte[6] found that after anaphylactic shock in rabbits the urinary excretion of 5-HIAA was raised. These experiments among other reports suggest that 5-hydroxytryptamine was involved in anaphylactic responses in the rat, the mouse, and the rabbit. The studies reported, although on the hypersensitive guinea pig, showed an effect on the histamine response

after both the 511-T antagonists. These results would suggest that not only is 511-T concerned in the increased response to histamine after sensitization but that it also plays a part in the response in normal guinea pigs. The skin in normal guinea pigs has few mast cells over the area studied and there is at present no evidence that serotonin is found in these cells in the guinea pig. Riley and West,[17] in fact, showed that intravenous administration of histamine in rats did not produce mast cell damage in normal dosage. It would seem, therefore, that mast cells are not involved in this type of response in the rat. If the same is true in the guinea pig, there is no reason to comment on the relationship of serotonin and the mast cell, or the absence of mast cells in the skin, when considering the histamine response in the guinea pig skin. The location of serotonin in the skin and the mechanism of its release after histamine injection remain obscure.

The change in histamine response which was brought about by hydrocortisone was not a significant one in the normal guinea pigs. This finding is in agreement with that of Miles and Miles[9] who used cortisone. In the sensitized animals, however, a significant reduction in effect was demonstrated. Sudak, Wyman, and Fulton[19] found that cortisone gave some protection against large doses of histamine and it may be that an increased sensitivity to histamine in the sensitized animals evoked a similar type of response.

The state of sensitization in the guinea pig was shown to be associated with alteration in some tissue constituents (Buxton and McDowall[2]). This paper reported only on the water, sodium, and potassium content of certain tissues, including the skin. Procedures expected to change these constituents did not effect the histamine response in such a way as to lend suport to the suggestion that the electrolyte content of the skin and the histamine response were related, but it may be that other tissue changes are more imporant in this respect. A reduction in the hyaluronic acid or an increase in the hyaluronidase, for example, would be an interesting finding in view of the fact that the hyaluronidase antagonist was effective in reducing the histamine response in the sensitized guinea pigs but not in the normal animals. Certain of our other observations would fit in with such a theory. Seifter and associates[18] observed that cortisone opposed the action of hyaluronidase when the permeability of synovial membrane was used to examine this, and Hayes and Baker[7] reported the reduction of dye spread induced by hyaluronidase as an acute response to adrenocortical extract. Similarly, Reppert, Donegan, and Hines[16] noted the inhibitory effect of ascorbic acid on dye spread in rabbits and suggested that the hyaluronidase-hyaluronic acid system was influenced by this vitamin. Further work is required on this.

SUMMARY

1. Female guinea pigs were sensitized with horse serum. Histamine response was measured using pontamine sky blue.

2. Sensitization increased the histamine response.

3. Sodium loading and depletion reduced the response after sensitization but did not alter the findings in normal animals.

4. The histamine response in sensitized animals was reduced by hydrocortisone, ascorbic acid, and anti-hyaluronidase, and antiserotonin and d-lysergic acid diethylamide tartrate. These findings are discussed.

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