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Cutaneous Reactions to Local Traumatization with Heat in Alloxan Diabetic Rabbits with and without Ketosis

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ABSTRACT

Short-term alloxan diabetic rabbits with and without ketosis were traumatized with local cutaneous application of heat. The degree of traumatization varied with regard to the temperature and duration used. The lowest degree of traumatization which caused intravenously injected Evans blue to be observed within the area of traumatization was determined. There was no difference between diabetic animals without ketosis and controls. A more pronounced traumatization of animals with ketosis was necessary if Evans blue was to be observed, but there was then a significant difference between these and controls. The traumatized skin areas were also studied histologically. The inflammatory reaction induced was judged as being weaker in animals with ketosis.

INTRODUCTION

The skin of the lower extremities in certain diabetics has previously been described as having an altered reaction to local thermal traumatization with heat and cold (7). This altered reaction is characteristic of patients with juvenile diabetes of long duration and in patients with maturity-onset diabetes. It is not connected with the diabetic metabolic derangement per se but to the occurrence of late diabetic lesions such as microangiopathy and polyneuropathy (7, 9, 11). The altered reaction was suggested to be the cause of gangrene and purpura localized to the lower extremities in patients with maturity-onset diabetes, especially in connection with cardiac decompensation (6. 7, 10, 11, 12).

It was also found that alloxan diabetic rats with long duration of the disease demonstrated a more pronounced reaction to local traumatization with heat than did controls and short-term alloxan diabetic rats (8). This more pronounced reaction consisted in an increased redness within the area of traumatization in alloxan diabetic rats with long duration of the disease, and was manifested one day after the traumatization and lasted for at least 3 weeks. In short-term alloxan diabetic rats no increased reaction was demonstrated until 2 weeks after the traumatization, compared with non-diabetic rats. The difference was slight. Alloxan diabetic rats seldom or never develop ketosis.

It is of interest to know if the diabetic metabolic derangement per se—especially a pronounced diabetic metabolic derangement with ketosis—influences the tissue reaction to different forms of traumatization. This question has been studied by several authors. The inflammatory reaction was reported to be decreased in alloxan diabetic animals after exposure to: implantation of cotton pellets (4, 22), experimental cutaneous mucormycosis (18), cutaneous injection of endotoxin prepared from *E. coli* (20) and intracutaneous injection of an antigen–antibody complex (21). An impaired wound healing in diabetics (14) and in alloxan diabetic rats (16) was also demonstrated.

Only Kiss et al. (3) studied the cutaneous reaction to local traumatization with heat. They investigated the leakage of intravenously injected trypan blue within the areas of traumatization on the shaved dorsal skin of alloxan diabetic rats. The leakage was studied 30 minutes after the cutaneous traumatization, which was obtained by applying a heated solid metallic cylinder to the skin for 30 seconds. There was a decreased leakage in short-term alloxan diabetic rats compared with controls. There is no information as to whether the temperature of the metallic cylinder was unaltered during the period of traumatization or whether the rats had ketosis or not; it is most likely that the diabetic rats did not have ketosis.

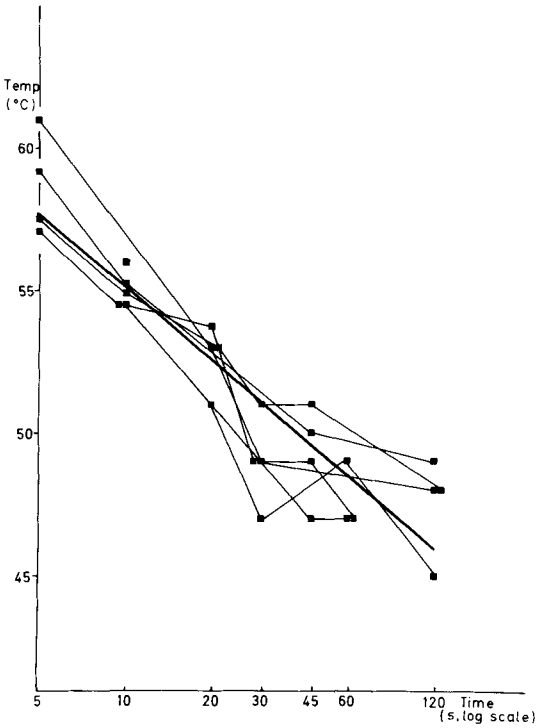

Fig. 1. Control animals. The effect of local cutaneous traumatization with heat. Minimal temperature and period of traumatization causing visible blue spots within the area of traumatization in connection with intravenously injected Evans blue. Numbers of animals=7. ■=observations. ——=calculated curve for the group.

As mentioned above, in our laboratory a slightly increased reaction was demonstrated 2 weeks after local traumatization with heat in short-term alloxan diabetic rats compared with controls (8). The diabetic rats had no ketosis. The results of the two cited studies are not directly comparable.

It was of interest to know if the diabetic metabolic derangement per se alters the cutaneous reaction to local thermal traumatization. We therefore studied this in alloxan diabetic rabbits which often develop, compared with alloxan diabetic rats, a more pronounced diabetic metabolic derangement with ketosis. The investigatory methods are, on the whole, in conformity with those of Kiss et al. (3).

MATERIAL AND METHODS

Twenty-eight rabbits were used, weighing from 2.7 to 4 kg. In 21 animals, 150–250 mg alloxan per kg body-weight was injected intravenously over a period of 5 min (5, 15). To counteract death from hypoglycemia, 9 g of glucose (30% solution) was given intravenously immediately after the injection of alloxan. At 4, 10 and, when necessary, at 24 hours after the injection of alloxan, 9–12 g of glucose was given by gastric tube. No insulin was given afterwards.

Blood glucose was determined by a modification of the glucose oxidase method (2). Regular estimations were made of urine volume and its concentration of glucose. Ketonuria refers in the following pages to a positive reaction (≥+ +) determined by Ketostix® (Ames Company).

After clipping away the hair of the abdomen and flanks of the animals anesthetized with ether, the skin was depilated with barium sulphide (13) 2–3 days after the injection. One day later local traumatization was performed on the anesthetized animals (pentobarbital 0.5 ml/kg intravenously).

The depilated areas were divided into smaller areas, about 3×3 cm in size, on an average 31 such areas on each animal. Local traumatization with heat was performed on these areas with different temperatures (range 45–63°C) and different periods of time (range 5–120 sec). The thermal injury was induced by placing the end surface of a straight cylindrical brass rod, 18 mm in diameter, in contact with the skin. Hot water ran through the brass rod and the indicated temperature was that of the part of the brass rod which rested against the skin. The pressure on the skin was the weight of the rod alone.

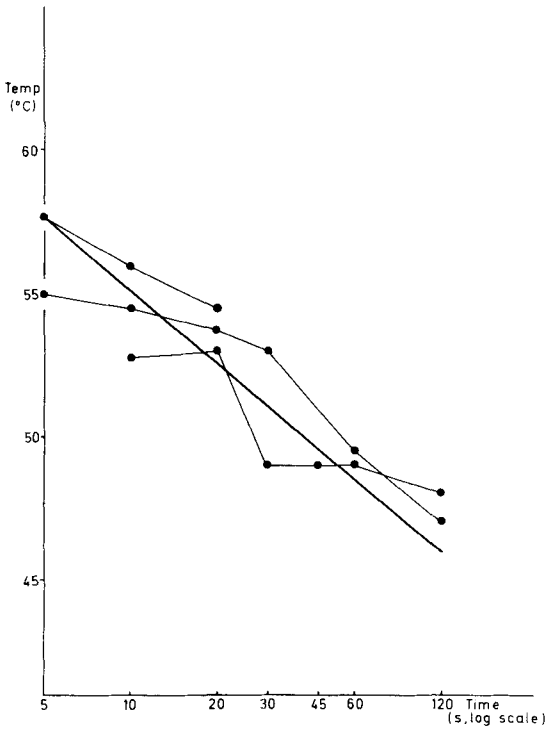

Fig. 2. Alloxan diabetic animals without ketosis. Numbers of animals=3. ●=observations. ——=calculated curve for the control group. For further information, see Fig. 1.

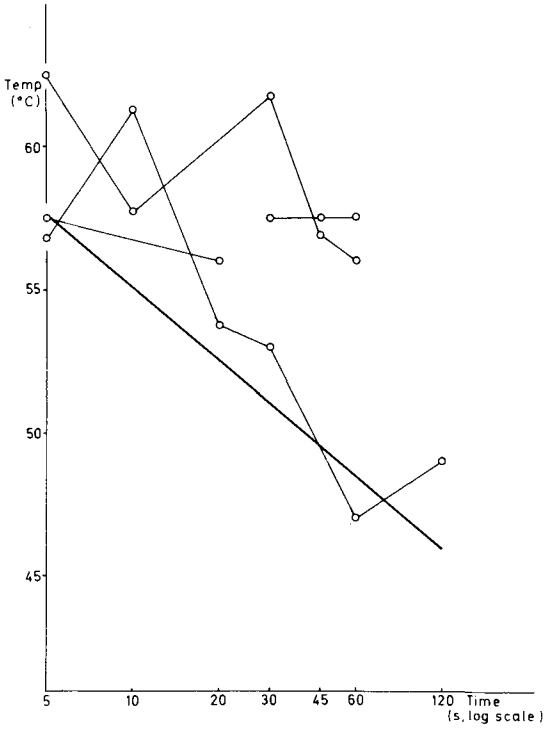

Fig. 3. Alloxan diabetic animals with ketosis. Numbers of animals=4. ○=observations. ——=calculated curve for the control group. For further information, see Fig. 1.

2.5 ml/kg Evans blue (2% solution) was injected intravenously immediately before the local traumatization with heat. This dye is bound by the plasma proteins in the blood stream (1, 17, 19), and when there is increased capillary permeability coloured patches will be seen. The traumatized areas were inspected ½ and 4 hours after the traumatization.

Furthermore, the water content of equivalent sites of traumatized and non-traumatized skin was determined in previously selected areas (23). The trauma consisted of heat applied for 10 sec at 60°C. The animals were killed 4 hours after the traumatization and the skin was cut exactly along a line made by a circular marking device 25 mm in diameter. The excision was performed through the panniculus carnosus down to the underlying fascia. Non-traumatized pieces of skin were removed in a corresponding manner from equivalent sites of the contralateral side of the same rabbit. The wet weight was then determined within 5 min after the excision. For dry weight determination, samples were dried in an oven at 110°C for 72 hours. The water content was determined from the wet weight (ww) and dry weight (dw) using the relationship (ww−dw)/dw. Determinations of, on an average, six pieces of skin from each animal were performed both before as well as after traumatization.

Traumatized skin surrounded by normal skin was excised for histological investigation, fixed in 10% neutral formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin–eosin.

The significance of the differences between means was calculated using Student's *t*-test. $P<0.05$ was chosen as the level for statistical significance.

RESULTS

Seven of the 21 rabbits injected with alloxan were excluded because the blood glucose and glucosuria values were only temporary. Another 7 of the rabbits died before the traumatization studies started, due to pneumonia, anuria or hypoglycemia. The remaining 7 rabbits were divided into two groups.

Group 1

Alloxan diabetic rabbits without ketosis, 3 animals. The blood glucose at the beginning of the traumatization was, on average, 405±65 mg/100 ml.

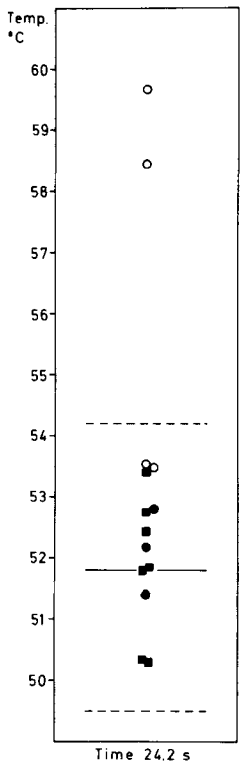

Fig. 4. Comparison between controls (■), alloxan diabetic animals without ketosis (●)and alloxan diabetic animals with ketosis (○) concerning the cutaneous effect of local traumatization with heat. Minimal temperature which at 24.2 sec (see text) causes visible blue spots within the area of traumatization after intravenously injected Evans blue. ——=calculated value for the control group and ---=±2 sigma for the control group.

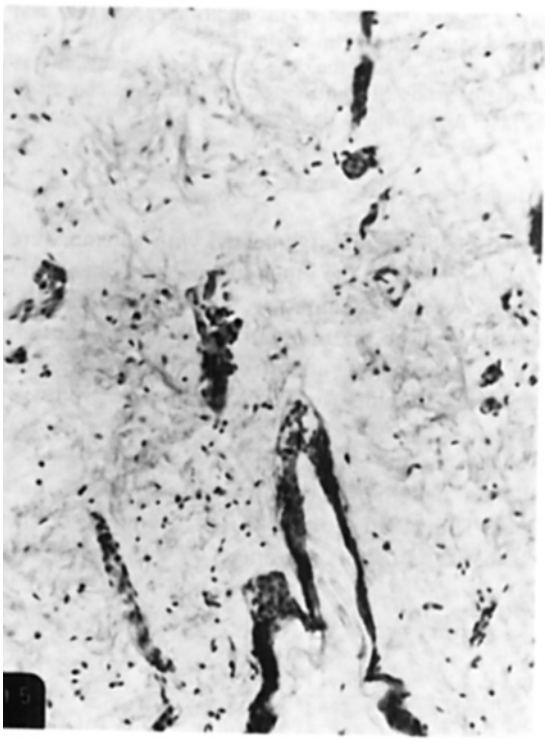

Fig. 5. Section of traumatized skin from diabetic rabbit with ketosis. Traumatization 61°C for 10 sec. Marked edema with a few inflammatory cells in the tissue beneath the epithelium. Hematoxylin–eosin, ×160.

Group 2

Alloxan diabetic rabbits with ketosis, 4 animals. The blood glucose at the beginning of the traumatization was, on average, 462±116 mg/100 ml. Ketostix® ++ or +++.

There were 7 control animals.

The lowest temperatures which just produced visible blue spots within the areas of traumatization at different periods of traumatization are indicated in Figs. 1–3. The cutaneous reactions were the same after ½ and 4 hours. If the results of all the observations of the controls (Fig. 1) are calculated as a right line the equation will be: $y=63.64-8.53\times\log x$, where y is the lowest temperature and x is the period of traumatization. A corresponding curve was also calculated for each of the 7 controls, for each of the 3 diabetic animals without ketosis and for each of the 4 diabetic animals with ketosis. It is to be noted concerning the latter 14 equations that the constant mentioned above, −8.53, which was determined from the group of controls, was used to calculate the second constant of these 14 equations. In Fig. 4 controls, diabetic animals without ketosis and diabetic animals with ketosis were compared with regard to the effect of local cutaneous traumatization with heat. 24.2 seconds was the mean of the periods of traumatization of all observations made. The lowest temperature of traumatization, that which at 24.2 sec causes visible blue spots within the area of traumatization after intravenous injection of Evans blue, was calculated according to the equations mentioned above for each individual of the three groups of animals. It is evident from Fig. 4 that there was good agreement between controls and alloxan diabetic animals without ketosis. In 2 of the 4 alloxan diabetic animals with ketosis the values were three sigma above the mean of the 7 controls. When the 7 controls were compared with the 4 alloxan diabetic rabbits with ketosis the mean difference was 4.45±1.94. The difference was statistically significant ($p<0.05$).

The water content of traumatized and non-

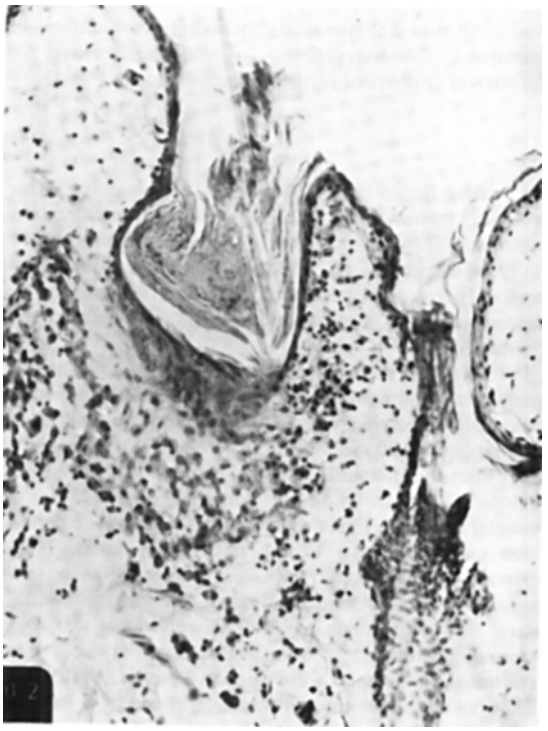

Fig. 6. Section of traumatized skin from control animal. Traumatization 59.5°C for 10 sec. A moderate inflammatory reaction is seen beneath the epithelium. Most of the cells are polymorphonuclear granulocytes. Hematoxylin–eosin, ×160.

traumatized skin was determined in the alloxan diabetic animals with ketosis and in 2 controls. The increase in water content was, for 2 of the diabetic animals with ketosis 10 and 20%, and for 2 of the controls 63 and 101%. Histological studies were made of the traumatized skin of controls and of diabetic animals with ketosis. After a pronounced traumatization (61°C for 10 sec to 63°C for 30 sec) dilatation of subepidermal vessels and signs of oedema formation were seen in the skin of the diabetic animals. Very few inflammatory cells were seen, located mainly subepidermally (Fig. 5). In the controls a very intense inflammatory reaction was seen even at lower temperatures (57°C for 5 sec to 59°C for 10 sec). The blood vessels were dilated and sometimes marked diapedesis was observed, mainly of polymorphonuclear granulocytes (Fig. 6). Degenerative changes were seen in the epidermis, hair follicles and sebaceous glands.

DISCUSSION

The purpose of the present investigation was to study whether the diabetic metabolic derangement per se (especially pronounced diabetic metabolic derangement with ketosis) has any effect on the cutaneous reaction to local traumatization with heat.

With regard to the appearance of Evans blue within the traumatized areas, there were no differences between controls and alloxan diabetic animals without ketosis. Diabetics with ketosis had, however, a smaller leakage than controls, the difference being statistically significant. There was a tendency to a smaller increase in the water content of the traumatized skin of diabetic animals with ketosis than in controls. There was also a histologically weaker inflammatory response of traumatized skin in diabetic animals with ketosis than in controls. A decreased inflammatory reaction in connection with diabetic metabolic derangement is in agreement with the literature, as referred to in the introduction.

Kiss et al. (3) described a reduced leakage of intravenously injected trypan blue within the skin areas traumatized with heat in alloxan diabetic rats. Information concerning ketosis is lacking. The difference between the results of Kiss et al. (3) and of those of the present study may be ascribable to the different species and methods used. Trypan blue, used by Kiss et al., and Evans blue, used by us, differ concerning the rate of elimination from the blood steam after intravenous injection as well as in the manner of binding to plasma proteins (17).

It is not clear, however, what causes the demonstrated reduction in the leakage of intravenously injected Evans blue within the skin area traumatized with heat in alloxan diabetic rabbits with ketosis.

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