tivity. Another is the system of reflex, motor, orienting reactions, enabling the quickest avoidance of a dangerous stimulus. Interaction between these systems is very worthwhile in the combined response of the animal to pain.

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LOW BLOOD HEPARIN LEVEL REDUCES SENSITIVITY TO THE HYPOGLYCEMIC ACTION

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Heparin is known to play an important role in physiological responses aimed at maintaining glucose homeostasis. For instance, if all the circulating reactive heparin in healthy rats is bound with protamine sulfate, a state of total resistance to the hypoglycemic action of both exogenous and endogenous insulin arises [7, 8]. Meanwhile, administration of heparin protects against the diabetogenic action of alloxan and reduces hyperglycemia in animals in the early stages of alloxan diabetes, and promotes restoration of μ-cell function [4, 6]. Insulin, in the form of a complex with heparin, has a stronger hypoglycemic action [5], and the diabetogenic factor loses its ability to induce hyperglycemia [9].

In the light of these data it is interesting to study the hypoglycemic effect of exogenous insulin in animals with a low blood heparin level. In this investigation animals with alloxan diabetes, whose blood heparin concentration is significantly below normal [10], and aging animals with reduced anticoagulating potential of the blood due to depression of the function of the anticlotting system and to atherosclerosis, aggravated by keeping the animal for a long time on an atherogenic diet [3], were used as the model.

EXPERIMENTAL METHOD

In the experiments of series I noninbred male rats weighing 160-200 g, kept on the ordinary laboratory diet, were used. Diabetes was induced by intravenous injection of alloxan (Spofa, Czechoslovakia) in a dose of 40 mg/kg after starvation for 24 h. Animals

*Deceased.

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TABLE 1. Blood Sugar Concentration in Animals with Alloxan Diabetes after Injection of Insulin and Insulin + Heparin (M ± m)

Experimental conditions	Sugar concentration, mg%		
	initially	30 min after injection	change in sugar concn., % of initial level
Alloxan diabetes: insulin + physiological saline (n = 12)	348,0±20,5	259,8±18,4	76,3±2,0
Insulin + heparin (n = 16)	327,0±12,8	156,0±9,4	49,1±1,5
Heparin (n = 7)	351,4±20,6	341,4±20,6	97,3±2,4
Healty animals: insulin + physiological saline (n = 16)	93,9±2,8	31,9±1,7	35,0±2,5
Insulin + heparin (n = 10)	86,4±1,2	33,6±3,1	38,4±2,8
Heparin (n = 17)	88,8±2,4	91,8±4,4	103,4±5,2

TABLE 2. Blood Sugar Concentration in Animals Receiving Atherogenic Diet, after Injection of Insulin and Insulin + Heparin (M ± m)

Experimental conditions	Sugar concentration, mg%		Change in sugar concentration, % of initial level
	initially	30 min after injection	
Atherogenic diet: insulin + physiological saline (n = 10)	144,0±3,6	108,0±2,7	76,0±2,4
Insulin + heparin (n = 10)	137,0±4,2	52,8±3,4	37,5±4,1
Heparin (n = 10)	138,0±3,4	130,9±5,3	93,4±3,5
Usual amount: insulin + physiological saline (n = 7)	93,3±4,0	51,7±4,0	55,4±4,3
Insulin + heparin (n = 14)	107,0±5,7	53,2±6,4	53,2±5,2
Heparin (n = 17)	88,8±2,4	91,8±4,4	103,4±5,2

Legend. Here and in Table 2: n denotes number of animals.

with stable hyperglycemia for not less than 30 days were used in the experiments. The experiments in series II were conducted on male rats aged 7-8 months, weighing 260-280 g, and receiving an atherogenic diet for a long time (5 months or more). The function of the anticlotting system of these animals was profoundly depressed, due both to their age and to the dietary factor, as shown by the following parameters: concentrations of fibrinogen and factor XIII, the R and K indices of the thromboelastogram, enzymic and nonenzymic fibrinolytic activity, and the heparin concentration. Male rats aged 2.5-3 months and weighing 160-200 g served as the control for them and also for animals with alloxan diabetes.

The various preparations were injected and blood samples taken (3.8% sodium citrate was used as the anticoagulant) via the jugular vein. Blood for sugar estimation was taken 30 min after insulin was injected. To prevent immobilization stress the animals were not allowed to remain fixed for more than 5 min. The blood sugar concentration was determined by the method in [2], the plasma immunoreactive insulin level with the aid of an insulin RIA kit (Hungary), and the blood heparin concentration by the method in [12]. Insulin (insulin for injection, USSR) was injected into rats with alloxan diabetes in a dose of 0.3 UI/200 g, and into rats on an atherogenic diet in a dose of 0.2 IU/200 g. Animals of the corresponding control group received the same doses of insulin, but were given physiological saline instead of heparin. Heparin (Richter, Hungary) was injected simultaneously with insulin in doses of between 25 and 100 IU/200 g. The substances were injected in a volume of 0.5 ml.

EXPERIMENTAL RESULTS

Virtually no heparin could be detected (only traces were present) in the blood of the animals with alloxan diabetes and the concentration of immunoreactive insulin was 0.8 ± 0.3 μIU/ml, and in the healthy control rats the heparin concentration, in conventional units, was 7 IU/ml and the insulin concentration 21.3 ± 3.0 μIU/ml.

It will be clear from Table 1 that 30 min after injection of insulin in a dose of 0.3 IU/ml the blood sugar level in animals with alloxan diabetes was lowered by 23.7%, and in the control animals by 65%. If this weakened response to insulin by rats with diabetes was due to heparin insufficiently, administration of heparin should evidently restore it to normal. In fact, after injection of the same dose of insulin together with heparin (25-50 IU/200 g) the plasma sugar concentration of the animals with diabetes was reduced by 50.9%. Although this decrease was not so great as in the control rats, it nevertheless differed significantly ($p < 0.001$) from that produced in them by injection of insulin alone, evidence of at least partial, if not complete normalization of the reaction to insulin. Injection of insulin with heparin into the control animals did not potentiate the hypoglycemic action of insulin. Injection of heparin alone has no effect on the blood sugar level of either the control or the diabetic animals.

In series II experiments were carried out on animals with profound depression of their anticlotting system and with atherosclerosis and, consequently, with marked heparin deficiency the heparin concentration was 0.4 ± 0.5 IU/ml. Their blood sugar was about 140 mg% and their immunoreactive insulin 7.8 ± 1.6 μIU/ml (the corresponding values for the control animals were given above).

It will be clear from Table 2 that in rats with depressed function of their anticlotting system, just as in animals with alloxan diabetes, injection of insulin in a dose of 0.2 IU/200 g induced a hydroglycemic reaction that was much lower in degree than in the control (the fall of the sugar concentration was by 24 and 44.6%, respectively), whereas additional injection of heparin potentiated this reaction sharply, and the blood sugar level now fell by 62.5%. Injection of heparin alone, as in the previous series of experiments, had no effect on the blood sugar level under these experimental conditions (Table 2).

The results show that the decrease in sensitivity to the hypoglycemic action of insulin observed in animals with alloxan diabetes and in elderly animals, in which age changes were aggravated by atherosclerosis, induced by an atherogenic diet, and depression of the function of the anticlotting system, is linked with severe heparin deficiency in the animal. Compensation of this deficiency by means of oxygenous heparin goes a long way toward normalizing the reaction of these animals to insulin. This is in good agreement with previous observations showing that normal manifestation of the effect of insulin requires the presence of an adequate heparin concentration in the blood stream, and for that reason, after all the circulating heparin has been bound (with protamine sulfate or 2,5-ionene), total resistance to its hypoglycemic action arises [7, 8]. A number of suggestions may be put forward to explain this resistance. One is that insulin interactions with target tissue receptors only in the presence of heparin or in the form of an insulin–heparin complex.

Support for this view is given by data showing that the hypoglycemic activity of insulin is considerably increased in a complex of insulin formed with heparin *in vitro* [5].

Indirect evidence that heparin is involved in the realization of the action of peptide hormones is given by the fact that exogenous ACTH has no action in rats receiving protamine sulfate, and that the effect of adrenalin-stimulated release of endogenous ACTH is blocked [11].

The marked resistance of the animals used in a present investigation to the hypoglycemic action of insulin, which can be abolished by heparin, is thus evidence of the important role of the latter in the maintenance of glucose homeostasis in such states as diabetes and atherosclerosis with depression of function of the anticlotting system, and it indicates possible ways of correcting carbohydrate metabolism in these pathological states.

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