ETHANOL-INDUCED CHANGES IN THE INSULIN-CONTAINING RED CELL COUNT IN THE BLOOD OF ANIMALS AND MAN

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A cytochemical method of detection of insulin has recently been developed [3], and by its use to investigate blood cells it has been shown that the red cells are a unique depot and transport system of insulin [4]. Insulin, identical in its immunochemical, radioimmunologic, electrophoretic, and biological properties to standard crystalline human insulin has been isolated from human red blood cells [2]. Release of insulin from red cells is found under the influence of physiological factors such as glucose and lactic acid [5]. Release of insulin from red cells in vitro is also provoked by ethanol [1].

The aim of this investigation was to study changes in the level of insulin-containing red cells (ICRC) in the blood of laboratory animals and man under the influence of ethanol.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Wistar rats weighing 200-250 g, aged 3.5-4 months (10 rats formed the control group) and on 20 male chinchilla rabbits weighing 1-1.5 kg and aged 9-12 months (10 rabbits formed the control group). All the animals were kept on a standard diet under ordinary conditions in the animal house until required for the experiments.

Blood films were taken from all laboratory animals in the morning before feeding for cytochemical investigation of the initial ICRC level. The experimental animals were then given an intraperitoneal injection of 15% ethanol solution at the rate of 1 ml/100 g body weight, after which they exhibited external manifestations characteristic of acute alcohol intoxication: disturbance of movement coordination, tachycardia, increased respiration rate. The same volume of physiological saline was injected into the control animals.

Blood films for cytochemical investigation were taken 10, 45, and 120 min after the injection; all the experimental animals were then returned to a normal diet, and fasting blood films were again taken 24 h after injection of the test solutions.

To study the effect of ethanol on the ICRC level in man, 20 male volunteers aged 25-30 years and weighing 60-72 kg were chosen: They were physically and mentally healthy, and not addicted to alcohol (10 men formed the control group).

Blood films were taken from the finger of all subjects in the morning before breakfast for cytochemical investigation of ICRC, after which subjects of one group (10 persons) were given 100 ml of 40% ethanol to drink, whereas the other group (10 persons) were given the same volume of isotonic salt solution. In both cases, the subjects drank 100 ml of water after the test solution. Blood films were taken 10, 45, and 120 min later, after which all subjects were put on a diet which excluded any products that potentially might contain alcohol. Blood films were again taken from all subjects in the fasting state 24 h after taking ethanol.

To detect insulin in the red cells a cytochemical method including the following procedures was used. After preliminary fixation of the blood in an oxidizing mixture of solutions of potassium chromate and bichromate (6-8 h) and denaturation of the insulin molecule by treatment of the blood film with a solution of phosphotungstic acid (1-2 min), the film was treated with a solution of paraldehyde-fuchsine (20-30 min), which specifically stained bluish violet only those red cells which contain insulin. The film was then washed, taken through a series of alcohols, cleared in xylol, and mounted in Canada balsam.

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TABLE 1. Effect of Ethanol on Number of ICRC (in percent), in Blood of Animals and Man (M±m)

Experimental conditions	After injection of ethanol				
	normal	10 min	45 min	120 min	24 h
Rats (20)	83,6±7	—	—	—	—
Control (10)	—	83,6±4	82,5±5	83,9±3	84,7±2
Ethanol (10)	—	43,6±4	30,1±5	30,0±5	83,0±6
Rabbits (20)	72,8±4	—	—	—	—
Control (10)	—	72,5±3	72,3±4	71,6±1	77,3±7
Ethanol (10)	—	40,3±4	19,6±4	18,7±2	72,2±4
Man (20)	76,6±4	—	—	—	—
Control (10)	—	75,9±1	77,2±4	77,2±2	77,3±5
Ethanol (10)	—	53,5±5	37,0±5	37,1±4	77,1±3

Red cells containing and not containing insulin were counted in the blood film. Counting was done in several fields of vision (not less than 1000 cells), and the results, after determination of mean values, were expressed in percent.

EXPERIMENTAL RESULTS

Parenteral injection of average doses of ethanol into the fasting laboratory animals caused a sharp decrease in the percentage of ICRC in the blood (Table 1). The number of ICRC 10 min after injection of ethanol fell on average by 40% in rats and by 32% in rabbits. After 45 min, the number of ICRC in the animals' blood showed an even greater fall in each concrete case compared with the original values on average by 52% in rats and 53% in rabbits. This blood level of ICRC was maintained for 2 h. Toward the end of 24 h after the experiment the ICRC level was the same as initially.

Investigation of the volunteers showed that 10 min after they took ethanol their blood ICRC count was reduced by 22%, after 45 min it was reduced by 40%, and it remained for the next 2 h at this level. After 24 h the blood ICRC level was the same as initially. In the control groups no significant change in the numbers of ICRC in the peripheral blood was found.

Under the influence of ethanol the number of ICRC thus decreased in the experimental animals and human volunteers, but later, with elimination of ethanol from the body, this parameter returned to its initial value. The magnitude of the changes in ICRC in the animals of the different groups and in man can be explained by by differences in the blood alcohol concentration and also by physiological differences between mammals of different species.

Release of insulin from red cells is evidently utilized to intensify carbohydrate metabolism during alcohol intoxication. Later, with a return of the basal metabolic processes to normal, the initial level of ICRC is slowly restored, and this is accompanied by restoration of the metabolic systems responsible for meeting the energy requirements of the body to maintain an adequate response in extremal states.

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