The specificity of action of CSA from MSC in embryonic liver culture, however, remains questionable. The possibility likewise cannot be ruled out that MSC contains another factor (not colony-stimulating) which is responsible for the fall of CFU-S (and CFU-C?) level, and which may act indirectly through the stroma of the cultured liver. For example, cultures with MSC were found to differ appreciably in their external appearance from the controls, in their smaller zone of growth and a reduction in the total mass of the culture.

Examination of a series of increasingly complex hematopoietic systems from the agar culture to the living organism shows a decrease in the effect of the exogenous source of CSA: in a system of disconnected cells in a jelly-like medium dependence of CFU-C on CSA is complete, in embryonic liver organ culture CSA causes only a shift of differentiation toward the formation of mature cells, and the presence of CSA in bone marrow cultures on stroma no longer has any appreciable effect; finally, there is no firm evidence that granulopoietin is a colony-stimulating factor *in vivo* [6].

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EFFECT OF EXPERIMENTAL MODIFICATION OF THE GLUCOCORTICOID RHYTHM ON CIRCADIAN FLUCTUATIONS OF GLUCOSE TOLERANCE IN RATS

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When the insular function of the pancreas is normal it is rare to find significant circadian fluctuations in the blood sugar level [13] despite the discontinuous pattern of food intake and stress, and alternation of work and rest. This is largely due to the well-marked circadian rhythm of response of the β-cells to stimulating factors [10], which has also been demonstrated in experiments *in vitro* [8]. If the function or reliability of the β-cells is reduced, the role of the contrainsular hormones is enhanced, especially that of the glucorticoids, with their precise circadian rhythm, in the formation of daily fluctuations in the blood sugar. Stimulating glucose processing in reactions of gluconeogenesis, increasing the sensitivity of β-cells to glucose [12], acting in relation to many factors as insulin antagonists in target tissues, and inhibiting the binding of insulin with receptors [5], glucocor-

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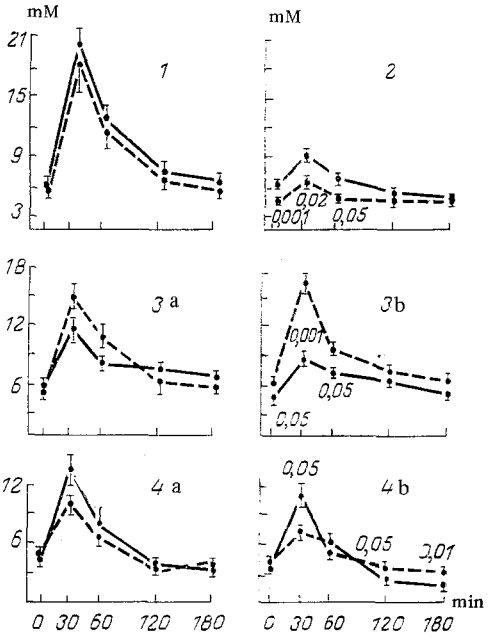

Fig. 1. Blood sugar levels in rats (in mM, M ± m) in GTT (0.5 g/100 g body weight) carried out at 10 a.m. (continuous line) and 7 p.m. (broken line). 1) Control; 2) adrenalectomy + physiological saline, glucose loading 0.25 g/100 g; 3a) cortisol at 8 a.m. (7-9 injections); 3b) cortisol at 5 p.m.; 4a) adrenalectomy + cortisol at 8 a.m.; 4b) adrenalectomy + cortisol at 5 p.m.

ticoids may directly or indirectly determine the rhythm of carbohydrate tolerance. Biorhythmologic aspects of insulin-glucocorticoid relations have not yet been adequately studied.

In the investigation described below the effect of exogenous modifications of the corticosteroid rhythm on circadian fluctuations in blood sugar after glucose loading was studied in rats.

EXPERIMENTAL METHOD

Male Wistar rats weighing 170 g were used. The animals were kept five in a cage at a temperature of 24 ± 1°C, with artificial lighting from 8 a.m. to 8 p.m. and with free access to food and water. The experiments were carried out in June.

The animals were divided into the following groups: 1) intact rats (control); 2) bilaterally adrenalectomized animals receiving daily intramuscular injections of physiological saline (0.1 ml/100 g) at 8 a.m. (subgroup 2a) or at 5 p.m. (subgroup 2b); 3) rats receiving a course of daily intramuscular injections of hydrocortisone acetate (0.5 mg/100 g) at 8 a.m. (subgroup 3a) or at 5 p.m. (subgroup 3b); 4) adrenalectomized animals receiving replacement injections of hydrocortisone in the same dose at 8 a.m. (subgroup 4a) or at 5 p.m. (subgroup 4b). Analysis of the results showed no correlation between the time of injection of physiological saline and glucose tolerance and for that reason data for subgroups 2a and 2b were pooled.

The course of injections of hydrocortisone began on the day after adrenalectomy and continued for 7-9 days. The injections were given at the times of minimum and maximum corticosterone secretion in rats kept under these conditions of lighting. Adrenalectomized animals received 0.9% sodium chloride solution instead of drinking water.

Sugar loading consisted of intraperitoneal injection of glucose solution in a dose of 0.5 g/100 g 12 h after removal of the food from the cages. To study circadian fluctuations in the glucose tolerance test (GTT) glucose was injected at 10 a.m. and 7 p.m., i.e., 2 h after the

TABLE 1. Diurnal Fluctuations of Blood Sugar and Its Mean Increase (mM, M ± m) with Modification of Glucocorticoid Rhythm

Group of animals	Blood sugar level after starvation for 12 h		P	Mean increase in blood sugar during GTT (0.5 g/100 g)		P
	10 a.m.	7 p.m.		10 a.m.	7 p.m.	
1) Control	5,95±0,15 (13)	5,45±0,21 (7)	<0,07	5,72±1,02 (12)	5,27±1,57 (7)	
2) Adrenalectomy	6,13±0,17 (5)	4,58±0,12 (9)	<0,001	0,83±0,15* (5)	0,44±0,14* (3**)	
3)						
Subgroup 3a) cortisol at 8 a.m.	5,91±0,26 (5)	5,42±0,29 (5)		2,31±0,78 (5)	3,92±0,34 (5)	
Subgroup 3b) cortisol at 5 p.m.	5,07±0,31 (5)	6,16±0,18 (5)	<0,05	2,19±0,33 (4)	3,92±0,44 (5)	<0,02
4)						
Subgroup 4a) adrenalectomy + cortisol at 8 a.m.	4,27±0,27 (9)	4,48±0,20 (8)		3,09±0,75 (9)	1,65±0,42 (8)	<0,02
Subgroup 4b) adrenalectomy + cortisol at 5 p.m.	3,47±0,29 (8)	3,83±0,25 (8)		3,03±0,45 (7)	1,40±0,31 (8)	<0,01

Legend. Number of animals shown in parentheses. *) Glucose load 0.25 g/100 g; **) six animals died from hypoglycemic coma during GTT with 0.5 g/100 g.

course of cortisol injections. The GTT was repeated on each animal not earlier than 36 h after the previous test. Sugar was determined by the orthotoluidine method in blood taken from the tail 30, 60, 120, and 180 min after glucose loading. The mean rise of blood sugar in the GTT was calculated by the equation in [3]. The significance of differences between values was estimated by Student's t test.

EXPERIMENTAL RESULTS

Analysis of background blood sugar concentrations in intact males showed a very small decrease in the evening. At the end of the day there was also a tendency for tolerance to increase, as shown by the smaller area beneath the blood sugar curve (Fig. 1: 1). These two facts can be explained by an increase in the basal secretion of insulin [7-11] and potentiation of its action on peripheral tissues [4, 9], and by increased sensitivity of the β-cells to glucose [8] at the beginning of the active period, both in nocturnal rodents and in man.

The biorhythmologic data show that the insulin-producing cells were functioning under stress at the end of the period of daylight and beginning of darkness, when corticosterone secretion and the food intake of rats are maximal [2]. Under normal conditions at this time the effects of insulin evidently are very slightly stronger than those of the contrainsular hormones, and this leads to a very indistinct rhythm of the blood sugar concentration and tolerance in intact rats.

The fall in concentration of the two most important contrainsular hormones (corticosterone and adrenalin) after adrenalectomy (group 2) caused the blood sugar to fall during the evening (time of maximal secretion and reactivity of β-cells). Replacement injections of cortisol at the end of the period of daylight (subgroup 4b) led to a further considerable fall in the background sugar level (Table 1), evidence of the role of adrenalin in the hyperglycemic or β-cytotrophic effect of glucocorticoids. After the morning injection of cortisol (subgroup 4a) a smaller decrease was observed in the background sugar level than after evening injection.

In rats with intact adrenals injections of the hormone at the beginning of the period of daylight (subgroup 3a) did not change the background sugar concentrations compared with the control. This is in agreement with the observed absence of a hyperglycemic effect of "stressor" doses of cortisol when injected without glucagon and adrenalin [14]. Conversely, injections of the hormone on the approach of darkness caused an increase in the background blood sugar level in the evening and a decrease in the morning, significant in both cases compared with the corresponding values in intact rats. The data thus show that the animal is relatively refractory to the hyperglycemic action of cortisol when injected in the negative phase of the natural glucocorticoid rhythm, i.e., during the morning.

Analysis of the blood sugar curves after the loading test gave the following results. Adrenalectomy led to a significant decrease in the area below the curve in all groups, especially during the evening, so that the rhythm of tolerance became more marked than in the control (Table 1; Fig. 1: 2, 4a, 4b). Removal of the contrainsular factors (group 2) led to marked hypoglycemia in the late stages of the evening GTT, so that the dose of glucose injected could be halved. Replacement injections of cortisol under both conditions (subgroups 4a and 4b) did not return tolerance to the control level: the mean increase in blood sugar during the GTT remained significantly lower (Table 1).

Hydrocortisone loading of rats with intact adrenals (subgroups 3a and 3b) gave the following results. Injection of the hormone under both conditions reduced the mean rise of blood sugar caused by injection of glucose compared with the control (Table 1). On the other hand, this can be explained by some increase in sensitivity of the β-cells to glucose under the influence of glucocorticoids and, consequently, the more intensive insulin release [12]. On the other hand, lowering the reactivity of the pituitary-adrenal system during exogenous hyperglucocorticoidemia determines the release of corticosterone, which raises the sugar level, in response to hypoglycemia [15].

The rhythm of tolerance was reversed (Fig. 1: 3a, 3b): the area below the morning blood sugar curve and the mean rise in the sugar level became less than after the evening GTT, the values of which did not differ significantly from those in the control rats in the evening. The morning–evening difference in profile of the blood sugar curves was most marked at the first points of the GTT (30 and 60 min) and was statistically significant only for cortisol injections at the end of the day, but nothing more than a tendency was exhibited in subgroup 3a (Table 1).

Reversal of the rhythm of tolerance independently of the time of injection of the hormone can be regarded as the result of the hypercortisolemia observed in both cases. In the evening (the phase of increased secretion of adrenalin, which depresses β-cell function [1]) the hyperglycemic effect of the glucocorticoids was exhibited more strongly, whereas at the beginning of the resting period the facilitating action on insulin mobilization was manifested.

Some adrenalectomized rats (group 2) died 2-3 h after injection of glucose in a dose of 0.5 g/100 g from hypoglycemic coma. They began to die when their mean blood sugar level was 3.6 mM, whereas adrenalectomized rats receiving cortisol remained alive even at lower concentrations – down to 2.5 mM. This is evidence of a marked increase in resistance of the CNS to hypoglycemia under the influence of cortisol.

It can be concluded that the action of glucorticoids on the blood sugar level depends on the time of injection and it is determined not only by concentration ratios with insulin and adrenalin, but also by phase angles between the circadian rhythms of the hormones.

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