The comparative study of the reaction of many insect species on the JH-active IGRs makes the limits for their practical application apparent. However, selectivity, low activity against parasitoids in eggs and larvae[6,45], low toxicity and general environmental safety[46] increase their real pesticide value.

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Circadian variation of the streptozotocin-diabetogenic effect in mice[1]

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Summary. Streptozotocin was injected in normal mice every 4 h, during the day. Greatest number of diabetic animals were obtained at 16.00 h (95%) and lowest at 08.00 h (50%). Magnitude of hyperglycemia also showed similar distribution. This effect might be considered when planning its use for both experimental and clinical purposes.

The induction of experimental diabetes by the use of β-cytotoxic agents[3] is an useful tool for people working in the field of diabetes, both for research and clinical purposes in the treatment of severe cases of hyperinsulinism[4,5]. Although alloxan was previously extensively employed in animals, its use is no longer encouraged, mainly on account of its high toxicity[6]. For this reason, in the last few years streptozotocin (SZ)[7], a less toxic compound, has been almost the only drug used to induce chemical pancreatectomy.

Recently, it has been demonstrated that normal rats modify during the day their susceptibility to alloxan diabetogenic effect, despite the prior fasting period[8]. On the other hand, the doses of SZ employed to induce diabetes in mice seems to be considerably higher than the one used in other species[9]. This fact might represent a larger risk of secondary lesions due to the toxic effects of the drug. Since the final purpose of using β-cytotoxic drugs is to have the largest number of diabetic animals with the smallest doses and consequently the lowest incidence of extrapancreatic alterations, we assume that it would be of interest to search for a circadian rhythm of susceptibility of SZ. With this idea in mind, normal mice were injected at different times of the day with different doses of SZ.

Materials and methods. Normal female mice from the C3Hs strain, caged in groups of 8, were used. They were maintained in a room at a constant temperature of 23 °C and with free access to food and water. Lights were automatically switched on and off at 06.00 and 18.00 h, respectively. Under this light regime, animals engage in exercise and foold intake only during the darkness period[10].

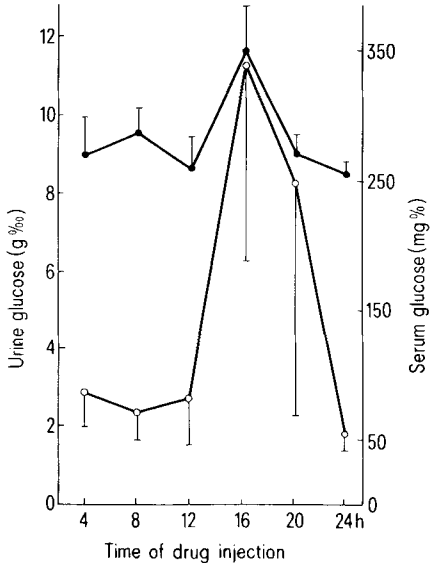
Streptozotocin (Upjohn U-9889, lot No.60140) was diluted in cold citrate buffer pH 4.5 and immediately injected, solutions being discarded 5 min after its preparation. Several doses (0, 50, 100, 175, 200 and 250 mg/kg b.wt) were injected i.v. using one of the tail veins, at 04.00, 08.00, 12.00, 16.00, 20.00 and 24.00 h on different days. After the injection, urine glucose was controlled daily (Gluco-Cinta, Lilly) and b.wt once a week. 21 days after the injection, all the animals were sacrificed at 12.00 h and blood samples were obtained from each animal for serum glucose[11] determination. Statistical analysis of the data was done accord-

Daily percentage of animals with glucosuria

Time of injection	5	6	7	8	9	10	11	12	13 ..... 21 days
00.00 h	50	50	50	50	50	50	50	50	50 50
04.00 h	63	63	63	63	63	63	63	63	63 63
08.00 h	0	0	34	34	34	34	50	50	50 50
12.00 h	0	0	28	72	72	72	72	72	72 72
16.00 h	32	62	62	62	62	95	95	95	95 95
20.00 h	0	0	31	31	31	31	31	31	62 62

ing to t-test for independent samples. Differences resulting in P-values higher than 0.05 were considered not significant.

Results. The table shows the daily percentage of animals with glycosuria when 250 mg/kg SZ was injected i.v. at different times of day. It can be seen that both the time at which mice start with glycosuria, as well as the final total number of animals with this pathological finding, exhibits a highly significant time-dependence. Glucose in urine appeared earlier in the group of animals injected at 16.00, 24.00 and 04.00 h. On the other hand, the largest final percentage of animals with glycosuria was recorded in the group injected at 16.00 h (95%), while the group injected at 04.00 and 08.00 h showed the lowest ones (50%). Conversely, though the values of the glucose concentration in urine were different in each group, this difference was not statistically significant (figure), mainly due to the high degree of dispersion of the data. No urine glucose was detected all through the experiment with the SZ doses of 50, 100 and 175 mg/kg. Otherwise, only a few animals injected at 16.00 h showed a moderate glycosuria with SZ dosage of 200 mg/kg. The figure also shows the serum glucose levels in the controls as well as in diabetic animals at the end of the experiment. For this purpose, animals were recorded as 'diabetic' only when the serum glucose levels were above 180 mg% and exhibited a constant glycosuria for at least 5 days. The highest values were found in the group injected at 16.00 h, and the lowest ones in the group injected at 24.00 h.



Urine glucose (○——○) and serum glucose (●——●) levels 21 days after SZ injection (250 mg/kg/b.wt). Each point represents average ±SEM.

Discussion. Our results show that in the C3Hs strain of mice, SZ produces its well-known diabetogenic effect only when injected in a dose of 250 mg/kg b.wt. They confirm the large drug requirement in mice[9]. On the other hand, the percentage of diabetic animals obtained following its injection was different, according to the day-time administration. This circadian rhythm of diabetogenic effect of SZ is similar to the one obtained with alloxan by Hernandez et al.[8]. These authors also demonstrate that rhythm was still present in animals submitted to identical prior fasting period. As we have previously shown, in these animals a 24-h fasting period was unable to modify the circadian rhythm of gastric conetent, serum glucose and IRI levels[12]. Thus, it was assumed that the present results are the consequence of some unidentified factors rather than of the uneven fasting period of each group.

Greater diabetogenic effect of the drug was obtained at 12.00 and 16.00 h, around the middle of the animal resting period. According to our previous results, these 2 points are within the span of greatest β-cell capacity to release insulin[13] and the lowest islet content of a metabolic substrate like glycogen[14]. Pyridine nucleotide metabolism has been regarded as the key step for the mechanism of the diabetogenic effect of SZ. Accordingly, a different degree of either activity or affinity of the NAD-$NADH_2$ system to the SZ action during the day, might be responsible for the present results[15].

Within the diabetic animals, the serum glucose levels as well as the glucose concentration in urine, exhibited a similar circadian variation, i.e. peak and trough values located at 16.00 and 24.00 h, respectively. These findings would suggest that not only the percentage of diabetic animals at 16.00 h was higher, but the magnitude of the hyperglycemic state was greater as well. Therefore, with the injection of SZ at different times of 1 day, it is possible to find a period of maximal effectiveness of its diabetogenic effect.

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