value to 92 ± 16 nM. Compared to controls, the maximal frequency response was somewhat reduced but this effect was not statistically significant. A lack of effect of hypothyroidism on the efficacy of isoprenaline has also been reported for thyroidectomized rats[17]. Figure 2 also shows that the effects of thiamazole could be reversed when T_3 was injected daily for 3 days prior to the experiment.

The results of this study confirm that an increase in plasma T_3 level in previously normo- or hypothyroid animals enhances the potency of isoprenaline with respect to its chronotropic effect in isolated cardiac preparations. In addition, we have consistently observed a significantly higher efficacy. This observation is compatible with the view that the hormone treatment, at least in the rat, increases both the density of β-adrenergic bindings sites[2-4] and the number of functional receptors that are coupled to the physiological response. However, alternative explanations including the effects of T_3 at reaction steps beyond the receptor level cannot be excluded on the basis of our data. An inhibitory effect of T_3 on the extraneuronal uptake ($uptake_2$) of isoprenaline as a reason for the potency shift of this agonist is unlikely but cannot be excluded definitely. However, on the basis of known properties of $uptake_2$ in rat hearts[18] there is no way of explaining the increase in the chronotropic efficacy of isoprenaline by an inhibition of this uptake mechanism. Models of β-adrenoceptor function which assume that the number of functional receptors determines the rate at which the physiological response is approached rather than the absolute size of the response[19] are not applicable to our data without additional assumptions.

Clinical studies have suggested that the potency of isoprenaline is not consistently affected in hypo- or hyperthyroid patients[20]. If β-receptor density in humans is changed at all by thyroid hormone, then these findings could indicate that the discrepancy between the number of β-adrenergic binding sites and the density of functional receptors in humans is smaller than in the rat.

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2 S. Kempson, G.V. Marinetti and A. Shaw, Biochim. biophys. Acta *540*, 320 (1978).
3 L.T. Williams, R.J. Lefkowitz, A.M. Watanabe, D.R. Hathaway and H.R. Besch, Jr, J. biol. Chem. *252*, 2787 (1977).
4 J.S. Tsai and A. Chen, Nature *275*, 138 (1978).
5 S.P. Banerjee and L.S. Kung, Eur. J. Pharmac. *43*, 207 (1977).
6 G.S. Levey, Am. J. Med. *50*, 413 (1971).
7 K. Wildenthal, J. Pharmac. exp. Ther. *190*, 272 (1974).
8 A.J. Kaumann, Naunyn-Schmiedebergs Arch. Pharmak. *305*, 97 (1978).
9 J.C. Venter, Molec. Pharmac. *16*, 429 (1979).
10 R.P. Stephenson, Br. J. Pharmac. *11*, 379 (1956).
11 L.T. Williams and R.J. Lefkowitz, in: Receptor Binding Studies in Adrenergic Pharmacology, p. 127. Raven Press, New York 1978.
12 D.R. Waud, in: Advances in General and Cellular Pharmacology, vol. I, p. 145. Ed. T. Narahashi and C.P. Bianchi. Plenum Press, New York 1976.
13 G. Kunos, Br. J. Pharmac. *59*, 177 (1977).
14 H. Hashimoto and M. Nakashima, Eur. J. Pharmac. *50*, 337 (1978).
15 H.J. Schümann, E. Borowski and G. Gross, Eur. J. Pharmac. *56*, 145 (1979).
16 P.A. Longhurst and J.H. McNeill, Proc. west. Pharmac. Soc. *22*, 415 (1979).
17 J.H. McNeill and W.W. Simpson, Proc. west. Pharmac. Soc. *22*, 407 (1979).
18 U. Trendelenburg, Life Sci. *22*, 1217 (1978).
19 A.M. Tolkovsky and A. Levitzki, Biochemistry *17*, 3795 (1978).
20 D.G. McDevitt, J.G. Riddell, D.R. Hadden and D.A.D. Montgomery, Br. J. clin. Pharmac. *6*, 297 (1978).

Effect of seasonal variation on the acute toxicity of cyclophosphamide in the Chinese hamster *(Cricetulus griseus)* and the mouse under laboratory conditions

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Summary. The acute toxicity of cyclophosphamide, studied orally in chinese hamsters and intravenously in Tif: MAGf (SPF) mice, showed seasonal variation in Chinese hamsters but not in mice.

In an internal biological test in chinese hamsters and mice tolerance variations were observed in response to a similar dose of cyclophosphamide (Endoxan-Astra®) when given at different periods of the year. The dependence of some drug effects upon several biological rhythms has been reported in experimental animals[2-4]. It was decided therefore to test the occurrence of possible seasonal variation in the acute toxicity of cyclophosphamide in Chinese hamster and mouse under consistent laboratory conditions.

Methods. 14 parallel acute toxicity studies were done in Chinese hamsters *(Cricetulus griseus)* obtained from Chick-Line, Vineland, N.J., USA) and in Tif: MAGf (SPF) mice within a period of 3 years.

The tests were performed at day 17 of each of the following months: January, May, July, September and November. The Chinese hamsters, which had an average weight of 28 g and the mice (23 g) were caged in Macrolon® boxes (5 animals/box/sex/species) in a room maintained at a constant temperature of 22 ± 1 °C and a relative humidity of about. 55% with a 14-h light cycle/day. Water and pellet food (No. 890 for mice and No. 924 for Chinese hamster, Nafag, Gossau SG, CH) were given ad libitum. The animals were fasted overnight before treatment.

Freshly prepared solutions of cyclophosphamide (20 mg/ml aqua dest.) were administered to the Chinese hamsters by gavage and to the mice by i.v. injection (duration: 10 sec) into the tail vein. Groups of 5 males and 5 females were used for each dose level. All administrations were given in the morning, beginning at 08.00 h. The amount of cyclophosphamide administered was 600, 1000, 1300, 1600, 2000 mg/kg b.wt for Chinese hamsters and 100, 200, 300, 400, 500, 600, 700 mg/kg b.wt for mice.

Acute toxicity determinations were based on deaths occurring during a 15-day period. LD_{50} values and confidence limits were calculated by the probit analysis method[5]. The data were subjected to analysis of covariance which compared the monthly means and tested whether there was a linear trend over the $2\frac{1}{2}$ year period[6].

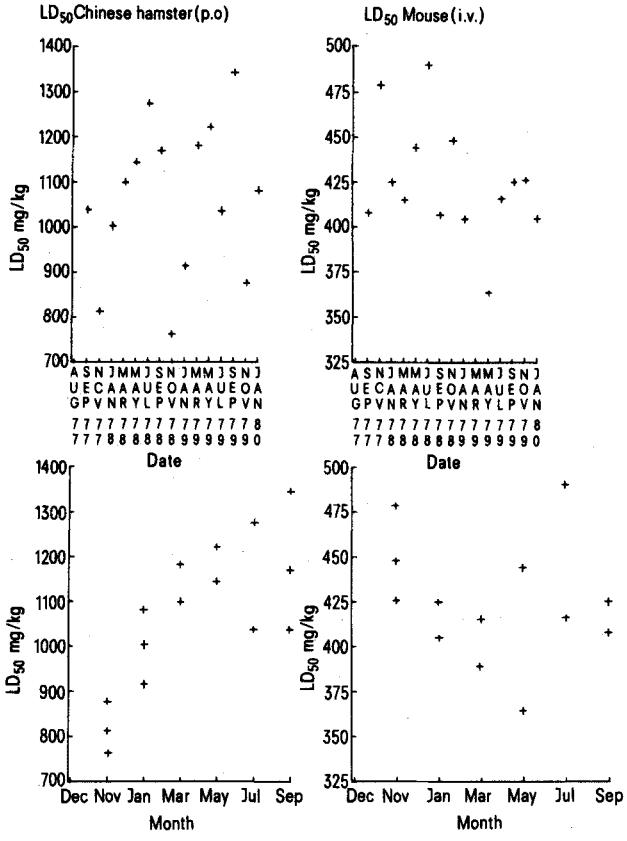
Acute toxicity of cyclophosphamide in Chinese hamster and mouse within a span of 3 years

No. of experiment	Month of the year	Season	LD_{50} confidence limits mg/kg Chinese hamster (p.o.)	Mouse (i.v.)
		Summer		
1	September		1038 (878-1228)	408 (374-444)
2	November	Autumn	814 (670-989)	479 (434-529)
3	January	Winter	1004 (892-1130)	425 (384-470)
4	March		1100 (989-1223)	415 (376-458)
		Spring		
5	May		1145 (1014-1293)	444 (407-485)
6	July		1275 (1190-1365)	490 (465-517)
		Summer		
7	September		1171 (1045-1312)	407 (358-463)
8	November	Autumn	763 (607-959)	448 (396-508)
9	January	Winter	916 (768-1091)	405 (363-451)
10	March		1182 (1047-1334)	389 (352-429)
		Spring		
11	May		1222 (1146-1303)	364 (317-419)
12	July		1037 (931-1154)	416 (367-473)
		Summer		
13	September		1345 (1227-1476)	425 (376-481)
14	November	Autumn	877 (740-1038)	426 (395-459)

Results. The results of this studies are summarized in the table. In the Chinese hamster cyclophosphamide administered orally at sublethal and lethal dose caused reduced spontaneous activity, muscular hypotonia, hyperreflexia, ataxia, irregular respiration, roughening of the coat and prostration. Death occurred between 1 and 13 days (in the majority 5-7 days) after administration, with signs of emaciation and depression.

In the mouse, cyclophosphamide given i.v. produced, in addition to the above mentioned symptoms, tremor, tonic-clonic convulsions and cyanosis. In the majority of the cases death occurred with convulsions and respiratory failure within 30 min after injection. No gross differences were observed between male and female Chinese hamsters or mice with regard to symptomatology and mortality.

For the mouse the covariance analysis of the LD_{50} showed that the monthly means were not statistically significantly different; they ranged from 402 to 453 mg/kg. There was an indication of a downward trend during the period, but this was not statistically significant. For the hamster the linear trend was also not significant; however, there was a significant difference between the monthly means. Subsequent analysis showed that the November mean (818 mg/kg) was significantly different from that for the remaining months, whilst amongst these 5 there were no significant differences (means ranging from 1001 to 1185 mg/kg). See figure below.



Seasonal variations in the acute toxicity of cyclophosphamide in Chinese hamster and mouse measured within 3 years.

Discussion. The data obtained for the Chinese hamster are in contrast to those obtained for the mouse, which showed less seasonal variation in the acute toxicity of cyclophosphamide. However, the different routes of administration in the animals used allowed no close comparison. The fact that in the month of November all calculated LD_{50} values in the Chinese hamster declined, might indicate a possible seasonal variation in the acute toxicity of cyclophosphamide in this species. This variation is presumably due to a lower rate of metabolism with occurs in hibernating animals. Therefore it appears that the various physiological function of the Chinese hamsters under consistent laboratory conditions are controlled by an endogenous rhythmicity[8,9]. These observations suggest that investigators using Chinese hamsters should consider the possible seasonal fluctuations which occur in this species.

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2 E. M. Boyd, Can. med. Ass. J. *98*, 278 (1968).
3 J. F. von Ledebur and K. Wachholder, Pflügers Arch. *231*, 114 (1932).
4 H. von Mayersbach, Drug Res. *28*, 1824 (1978).
5 A. Goulden, in: Methods of Statistical Analysis, p. 404. John Wiley, New York/London 1960.
6 SAS User's Guide, in: Statistical Analysis System. Raleigh, North Carolina 1979.
7 R. Bloch and B. Canguilhelm, C.r. Soc. Biol. *160*, 1500 (1966).
8 P. Berthold, in: Endogene Jahresperiodik. Universitätsverlag GmbH, Konstanz 1974.
9 K. Hoffmann, Drug Res. *28*, 1836 (1978).