Brit. J. Pharmacol. (1957), **12**, 100.

SEASONAL VARIATIONS IN THE RESPONSE OF RATS TO THE ANTIDIURETIC HORMONE

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(RECEIVED NOVEMBER 5, 1956)

The antidiuretic response of unanaesthetized rats to intravenous doses of vasopressin has been compared in the different seasons of the year. Significant seasonal differences in sensitivity to the hormone were found; thus the rats responded to smaller doses in spring and summer. Discrimination between doses appeared to be best in summer. Relative variability in response seemed to be least in winter. There appeared from season to season to be a concomitant variation in the same or opposite direction between sensitivity in terms of the average minimum effective dose and environmental factors such as temperature, barometric pressure and relative humidity.

One of the difficulties in antidiuretic assays on rats lies in the variation in sensitivity of test animals to intravenous injections of posterior pituitary extracts. For instance, one rat may respond with a marked inhibition of water diuresis to 5 microunits (μU.) of vasopressin per 100 g. of body weight, while another may need 100 μU./100 g. to produce a similar response using the same method of assay. The present work is an attempt to analyse factors which may have some influence on this variability in response. Our material consisted of 281 rats which received two or more doses of the standard pituitary preparation (Pitressin). All the animals were males of the same strain (Wistar); they were under the care of the same animal attendant; the number of rats per cage and the diet remained the same throughout the period of experiment. All the rats were of approximately the same age and weight. They were prepared for the assay in the same manner. Their water load was kept between 6 and 8% of their body weight, as Ginsburg and Heller (1953) have shown that changes in response attributable to differences in water load within this range are insignificant. All injections were made by the same worker and the volume of fluid injected was kept constant. The time of day at which the injections were made was varied at random.

METHODS

The procedure of Ginsburg and Heller (1953) was used for the antidiuretic assay with the modification that the bladder as well as a jugular vein was cannulated. Urine volumes were measured at 5 min. intervals. Antidiuretic responses were calculated as % antidiuresis, that is, as the difference between the rate of urine secretion in the collecting period preceding injection (V_1) and that during the two collecting periods after injection (V_2) expressed as a percentage of the pre-injection rate thus:

$$\% \text{ antidiuresis} = [V_1 - V_2)/V_1] \times 100$$

A dose producing between 30 and 40% antidiuresis was regarded as the minimum effective dose.

Pitressin (Parke, Davis and Co.) was used throughout. The pressor activity of different batches was assayed by the method of Dekanski (1952) in comparison with international standard powder. The results were in good agreement with the estimates of the manufacturers (see Ginsburg, 1956).

RESULTS

The experiments were made during a period of about 1½ years. The results in Table I are arranged according to the sensitivity of animals. In the second column, the frequency distribution of animals (numbers and %) is shown according

TABLE I

VARIATION IN SEASONAL FREQUENCY DISTRIBUTION OF MINIMUM EFFECTIVE DOSES OF PITRESSIN INJECTED INTRAVENOUSLY INTO UNANAESTHETIZED RATS

Pitressin (μU./100 g.)	Whole Yr.		Winter		Spring		Summer		Autumn	
	No.	%	No.	%	No.	%	No.	%	No.	%
3·125	16	5·7	1	1·8	6	10·5	9	8·3	0	0
6·25	22	7·8	1	1·8	5	8·8	15	13·8	1	1·7
10	39	13·9	2	3·5	5	8·8	30	27·5	2	3·5
12·5	50	17·7	7	12·3	14	24·6	14	12·8	15	25·9
25	98	34·9	21	36·8	24	42·1	33	30·3	20	34·5
50	47	16·7	25	43·9	3	5·3	4	3·7	15	25·9
100	9	3·2	0	0	0	0	4	3·7	5	8·6

to the minimum effective dose during the whole calendar year. The columns following give the frequency distributions in each of the four seasons. Fig. 1 expresses these results graphically. The two S-shaped curves for summer and spring fall close to one another, while those for autumn and winter are both displaced to the right by about the same amount. In the summer, 62% of the rats responded to 12.5 μU./100 g. or less, while in the winter only 19% did.

These frequency distributions were transformed into logarithmic distributions. Such distributions if normal or approximately so would, in their cumulative form, plot as straight lines on a log-probability grid, that is on a system of co-ordinates whose abscissa is the logarithmic variable and whose ordinate is the Gaussian integral. The distribution for the whole year, plotted on such a probability grid (Fig. 2), is linear apart from the extremes and justifies the conclusion of a log normal distribution ; those for the seasonal distributions give less satisfactory lines, probably due to the small number of animals. The position of the lines suggests that the mean dose required for response differs from season to season, whereas the slopes of the lines which indicate

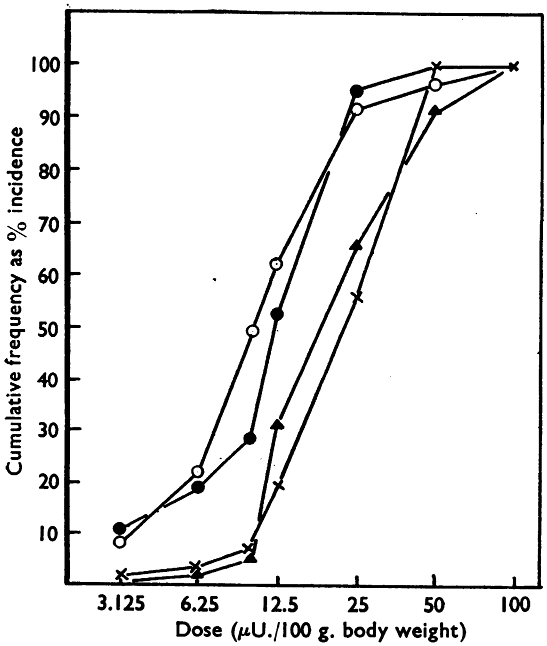

FIG. 1.—Cumulative frequency distribution of the minimum effective doses of Pitressin. Antidiuretic assays on rats. Intravenous injections. ×——× winter; ●——● spring; ○——○ summer; ▲——▲ autumn. The dose scale is logarithmic, but is graduated in true doses.

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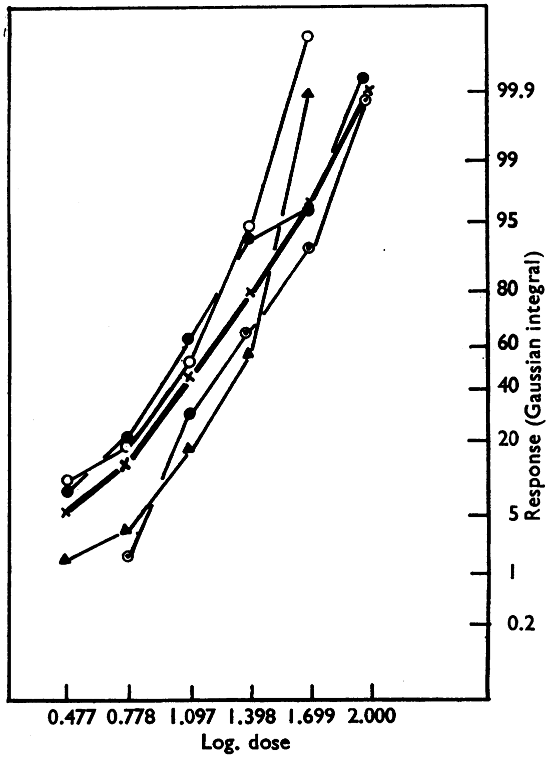

FIG. 2.—Probability graph of the seasonal frequency distributions in sensitivity. X——X whole year; ▲——▲ winter; O——O spring; ●——● summer; ⊙——⊙ autumn. The results of the experiments with 10 μU./100 g. (see Table I) are omitted.

the standard deviations are not very different from one another.

The arithmetic means and standard deviations of the log distributions are given in Table II. As is well known, the arithmetic mean and standard deviation of the logarithmic distribution stand in functional relationship to the corresponding statistics of the geometric distribution, the anti-log of M_{log} being the geometric mean G, and the anti-log of σ_{log} being the geometric standard deviation σ_g (Galton and MacAlister, 1879; Gaddum, 1945). These constants for the geometric distribution are shown in col. 5 and 6 of

TABLE II

LOGARITHMIC DISTRIBUTIONS (MEAN AND STANDARD DEVIATION) OF THE RESULTS SHOWN IN TABLE I

Seasons	M_{log}	σ_{log}
Winter ..	1·452	0·274
Spring ..	1·154	0·330
Summer	1·122	0·345
Autumn	1·426	0·329
Whole year	1·258	0·351

TABLE III
CHANGES IN RESPONSE AND DISCRIMINATION RELATED TO SEASONAL ENVIRONMENTAL FACTORS

1	2	3	4	5	6	7		8
	Mean Temperature (° F.)	Mean Barometric Pressure (Millibars)	Mean Relative Humidity (%)	Dose (Geometric Distribution)		Response (% Antidiuresis)		
				G	σ_g	Means $\bar{S}_1$	$\bar{S}_2$	Regression $\bar{S}_2/\bar{S}_1$
Winter ..	37·3	1,009·5	84·2	28·3	1·88	41·3	64·5	1·56
Spring ..	52·1	1,017·2	78·0	14·3	2·14	39·4	63·8	1·62
Summer	60·1	1,019·2	77·2	13·3	2·21	32·3	57·9	1·79
Autumn	46·6	1,013·5	88·1	26·7	2·13	40·5	62·3	1·54
Whole year ..				18·1	2·24			

Table III. Col. 5 shows the changes in average sensitivity with the season. It was greater in the spring and summer and smaller in the autumn and winter. The differences between the means are significant between winter and spring ($t=4.87$, $P<0.001$), and summer and autumn ($t=5.62$, $P<0.001$), but not significant between spring and summer ($t=0.56$, $P>0.5$) and autumn and winter ($t=0.48$, $P>0.6$).

The geometric standard deviations represent a much more homogeneous series than the geometric means, suggesting that the variation relative to the mean dose within a group of test animals remained similar from season to season, in spite of the different dose level required for response. This inference is drawn from the following relation between the standard deviation of the logarithmic and of the geometric distribution.

$$\sigma_{\log} = \log\sigma_g - \log G = \log (\sigma_g/G)$$

The logarithmic standard deviation is thus seen to be the logarithm of the relative standard deviation of the geometric distribution. Since the ratio of the standard deviation to the mean is known as the coefficient of variation, which is used for the purpose of comparing variability relative to the mean, similarity of the logarithmic standard deviations implies similarity of the relative variability.

However, it cannot be excluded that the series of geometric standard deviations exhibits a trend in the direction opposite to that of the means. If this could be established, it would afford a clue to a change in variability between the animals from season to season. However, only the logarithmic standard deviation for the winter appears significantly different from the other three values by Fisher's F test at the 0.05 level.

So far, the responses have not been considered quantitatively. Col. 7 and 8 of Table III show the magnitude of the response to a smaller dose (S_1) and a larger dose (S_2) in 163 animals in which S_2 was twice S_1—i.e., twice the "minimum effective dose." Col. 7 gives the mean response to each of the two doses and col. 8 the regression. The trend of the regression admits the conclusion that the variation from season to season in the responses to the larger dose was not parallel to that of the smaller dose. That is to say, discrimination between doses seemed greater in the summer and, perhaps, in the spring than in autumn and winter.

As the antidiuretic response could be related to the seasons of the year, it was felt worth while to inquire whether the response was associated with any meteorological condition. In Table III three physical characteristics of the seasons, recorded in Bristol, are given in col. 2, 3, and 4. It can be seen that the higher the mean temperature and barometric pressure the greater apparently the sensitivity to vasopressin. However, there seemed to be an inverse relationship between sensitivity and relative humidity.

DISCUSSION

A seasonal variation in the quantitative response to posterior pituitary extracts has been demonstrated in frogs (Heller, 1930; Oldham, 1936; Boyd, Mack, and Smith, 1939) and toads (Jørgensen, 1950). In both species, the water-balance-effect of a given dose is greater in summer than in winter. It would now seem that an increase in the sensitivity to vasopressin during spring and summer can also be shown in the rat, a mammalian species in which seasonal variations in sexual activity are not pronounced and in which adaptability to climatic changes is excellent. It could not be established in this investigation which of the physical characteristics of seasonal changes account for the fluctuations in sensitivity and in discrimination between doses. The results suggest that there is a relation between the degree of responsiveness to the antidiuretic hormone and factors such as environmental temperature, barometric pressure, and humidity. These associations may not be causal, but they may be taken to indicate that the influence of these factors on mammalian water metabolism and neurohypophysial function deserves further study. Such work is in progress.

Our sincere thanks are due to Mr. G. E. Clothier, of the Long Ashton Horticultural Research Station, who supplied the meteorological information.

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