DIURNAL VARIATION IN THE CARDIAC ACTIVITY OF LEAVES OF *DIGITALIS PURPUREA* L.

By J. G. Dare and G. A. Nelson

From the Department of Pharmacology, University of Leeds

Received June 30, 1952

Variation in the activity of digitalis leaf was first observed by William Withering[1] who concluded that it was a seasonal effect. Subsequent observers[2,3,4,5,6,7,8,9,10] have sought to show that it may depend on such factors as season, soil, temperature, age of plant, and the like. The results of these investigations have been mainly inconclusive, but the suggestions put forward by Dafert[7] seemed of sufficient interest to merit further study, and it is a consideration of them which has led to the present work.

Dafert[7] was interested in the diurnal movement of plant reserves and had shown that they accumulate in the leaves of plants during the daytime. As he considered glycosides to be reserve substances he suggested that the maximum glycoside content would occur in digitalis leaves in the late afternoon. He concluded from some experiments that this was so. Little reliance can be placed on his results, partly because he collected his material only twice daily—at shortly before dawn and between 5 and 6 p.m.—but chiefly because of the inadequate method of biological assay which he used.

Whilst it is not generally accepted that glycosides are plant reserves, it nevertheless seemed possible that diurnal variation in glycoside content might occur in the leaf. Our experiments were designed to determine this point, for, if such a diurnal variation exists, it will clearly be necessary to take account of it in any investigation of such factors as season, soil, or climate, which might also affect the activity.

Material and Methods

16 seedlings were transplanted from the foot of a solitary wild parent to a cultivated plot, as uniform as possible in soil, shade, drainage, etc. Soil heavy clay, calcium deficient. The plants were arranged in a 4 × 4 square. Material was collected in July of the second year at the onset of flowering. Batches of leaf were gathered at 20.00 hours G.M.T. and thereafter at 3-hourly intervals until 20.00 hours on the following day. 9 gatherings were made, each consisting of 1 cauline and 1 radical leaf from each of 4 plants. At each gathering leaves were collected from one plant in each row and column of the square. The plants sampled varied from batch to batch, but, owing to the odd number of gatherings taken, 12 of the plants were sampled twice and 4 of them three times. Immediately after collection the leaves were set to dry in an oven at about 56° C. The drying took from 1½ to 2 hours. The batches of dried material—each weighing 2 to 3 g.—were separately reduced to No. 60 powder on the following day and stored in sealed glass ampoules.

Each batch was evaluated by one of us (J.G.D.) by the B.P., 1948

process, based on the method first described by Knaffle-Lenz,[11] in which the intravenous lethal dose for cavies is determined. The potency of each batch was estimated with reference to the Laboratory Standard Preparation.

Extracts of the batches were prepared by continuous extraction with absolute ethanol for 6 hours.[12] Excess of ethanol was then removed by distillation until 4 ml. of extract remained for each 1 g. of digitalis powder. These extracts were diluted with an equal volume of water and stored at 0° C. until required.

Burn[13] recommends that extracts be diluted before administration so as to contain 0·125 I.U./ml. Extracts of unknown potency were diluted arbitrarily and, if necessary, were adjusted after the first 2 observations to approximate to this value. The lethal dose for cavies is not directly proportional to body weight, small animals requiring a greater dose per 100 g. than large ones.[14] To obviate the difficulty arising from this, only animals weighing approximately 500 g. were used: the mean weight of all the animals was 504 g. with standard deviation ± 31 g. In each observation the first 5 ml. of diluted extract was administered at the rate of 1 ml./minute and the remainder at 0·3 ml./minute. The injection rates were maintained mechanically.

Experimental Data

From the volume of diluted extract used for each cavy, the lethal dose was calculated in terms of mg. of digitalis powder per 100 g. of body weight. Lethal doses of drugs are usually distributed log.-normally,[15] hence the log.-lethal doses are given in Tables I and III.

TABLE I

LOG.-LETHAL DOSES TO CAVIES OF THE LABORATORY STANDARD PREPARATION

	Log.-lethal doses of Laboratory Standard Preparation in mg./100 g. of body-weight			
	Extract No. 1		Extract No. 2	
Cavy No.	Aug. 19, 1949	Aug. 22, 1949	Aug. 25, 1949	Sept. 16, 1949
1	1·33846	1·44576	1·29513	1·29711
2	1·25768	1·27439	1·21985	1·40993
3	1·40654	1·33102	1·34184	1·31239
4	1·34004	1·31408	1·33364	1·34339
5	1·27692	1·31069	1·33707	1·34125
6	1·34223	1·39076	1·40976	1·36154
7			1·32939	1·30362
8			1·30188	1·36136
9			1·28285	1·30620
10			1·26787	
11			1·37015	
Log-means	1·32698	1·34445	1·31722	1·33742

Grand mean log.-dose = 1·32984

Table I gives the data from which the grand mean log.-lethal dose of the Laboratory Standard Preparation was calculated. This mean was obtained from observations made on 4 different days, using 2 independently prepared extracts. The worth of the assays of the experimental

batches is dependent on (i) the consistency of the lethal dose, (ii) the consistency of the extraction process, and (iii) the stability of the extracts when stored for a short period. The analysis of variance presented in Table II shows that there is no significant difference between the 4 mean lethal doses: thus the mean lethal dose was constant from day to day; the 2 extracts were of equal potency; they were unaffected by storage.

TABLE II

ANALYSIS OF VARIANCE OF LOG.-LETHAL DOSES FOR THE STANDARD PREPARATION

Sources of variance	Degrees of freedom	Sums of squares	Mean squares
Between days ..	3	0·00360	0·00120
Within days	28	0·07116	0·00254
Total	31	0·07476	

Table III gives the data for all the observations on the experimental batches, the mean log. dose for the Laboratory Standard Preparation being the grand mean from Table I. The potency of each batch is expressed in I.U./g. with fiducial limits for P = 0·95.

TABLE III

LOG.-LETHAL DOSES TO CAVIES OF THE BATCHES OF DIGITALIS LEAF, AND THE ESTIMATED POTENCIES OF THE BATCH

	Log.-lethal doses of digitalis leaf in mg./100 g. of body-weight									
		July 4, 1949		July 5, 1949						
Cavy No.	Laboratory standard	20·00 hr. batch	23.00 hr. batch	02.00 hr. batch	05.00 hr. batch	08.00 hr. batch	11.00 hr. batch	14.00 hr. batch	17.00 hr. batch	20.00 hr. batch
1	See Table I for individual observations	1·56633	1·55630	1·47928	1·62066	1·49831	1·32553	1·40603	1·51161	1·55437
2		1·53059	1·66171	1·49122	1·57910	1·53606	1·45438	1·38131	1·57899	1·50638
3		1·54715	1·65562	1·48657	1·62582	1·60821	1·36380	1·44138	1·57531	1·57519
4		1·61045	1·55991	1·48898	1·54008	1·60402	1·38057	1·29292	1·58195	1·61888
5		1·60466	1·51134	1·47026	1·59174	1·44544	1·35025	1·50555	1·58838	1·53135
6		1·60217	1·68071	1·43917	1·53983	1·53007	1·38525	1·50624	1·56503	1·53995
7		1·57646	1·69285	1·46628	1·64885	1·60423	1·39076	1·53845	1·55763	1·50051
8		1·56217		1·41398	1·51001	1·46015	1·37291	1·54383	1·45803	1·59726
9		1·38632		1·49304	1·47783	1·55835	1·43520	1·53262	1·51228	1·58001
10				1·51627		1·55895				1·53262
11				1·51654						
12				1·62870						
13				1·52802						
14				1·44372						
Mean log. dose	1·32984	1·55403	1·61692	1·49015	1·57044	1·54038	1·38429	1·46093	1·54769	1·55365
Potency in I.U./g.	11·2	6·68	5·78	7·74	6·44	6·90	9·88	8·28	6·78	6·69
Fiducial limits in I.U./g. for P = 0·95		6·07–7·36	5·20–6·43	7·14–8·40	5·85–7·09	6·29–7·56	8·98–10·88	7·52–9·12	6·16–7·47	6·10–7·34

Results and Discussion

The results in Table III show the most active material to be that collected at 11.00 hours G.M.T., and the least active that collected at 23.00 hours G.M.T. That there is a highly significant difference between the activity of these 2 batches is evident from the graph, in which the potency in I.U./g. and the fiducial limits for each batch are shown.

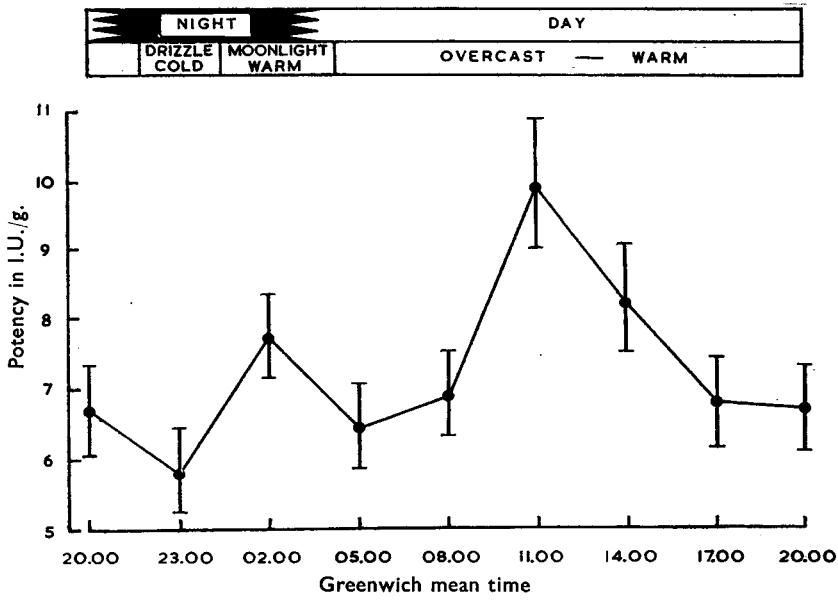

Fig. 1. Showing the estimated cardiac activities of 9 batches of digitalis leaf collected at 3-hourly intervals during 24 hours. The vertical line through each point indicates the fiducial limits (P = 0·95) of that estimate.

With the exception of the batch collected at 02.00 hours, there is a continuous increase in activity from about midnight to a maximum about noon, after which the activity steadily declines. Although there is a large variation in activity throughout the 24 hours, this follows a regular cycle, in which the first and last batches, collected at the same time of day, are equi-active. Furthermore, the Laboratory Standard Preparation, which was prepared from the remaining leaves of the plants collected at 11.00 hours on the following day, had an activity of the same order as that of the 11.00 hours experimental batch although it was somewhat more active. This greater activity may depend on the fact that the Standard material was collected on a day of continuous bright sunshine, whereas the corresponding experimental material was collected on a sunless day.

It is evident from these results that consideration of other factors which may affect the cardiac activity of digitalis must take account of the hour at which the material is collected. Even in so comprehensive an investigation as that of Watson and James[9] this factor was ignored, and it may

be that much of the variation they observed, and for which they could not account, was due to it.

The anomalous activity of the 02.00-hour material cannot be explained, but may be caused by a nocturnal alteration in metabolism, by the considerable improvement in the weather between 23.00 hours and 02.00 hours on the night in question, or by sampling variation. The last however does not seem likely, because there is no noticeable variation between batches collected from different plants at the same time of day, nor is there any evidence of irregularity in the rise and fall in activity observed in the material collected after 02.00 hours.

SUMMARY

1. Samples of *Digitalis purpurea* leaf were collected from the same group of plants at 3-hourly intervals throughout 24 hours and dried under uniform conditions. The cardiac activity of the samples was estimated biologically.

2. The activity was found to vary significantly throughout the day, being maximal about noon and minimal about midnight.

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