

LETTERS TO THE EDITOR

for the initial injection gave 40,515 counts/minute. The experiment was repeated after an interval of 4 weeks except that the rats which had received the gentisate injections in the previous experiment now received the saline, and vice versa.

The results are given in Table I and show that the administration of the gentisate produced no significant effects on either the rate or the total cumulative excretion of ^{14}C in the urine. The rats receiving gentisate showed an initial diuresis during the first 15 hours of the experiment but this did not alter the urinary excretion of radiocarbon. Thus, it must be concluded that despite the close chemical similarity of salicylate and gentisate it is unlikely that the latter substance displaces salicylate from its intracellular binding sites.

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New Possibilities for the Biological Assay of Digitalis

SIR,—In the research of cardiotonic-glycoside plants it is essential to determine the biological value of preparations containing glycoside mixtures or glycosides in the pure state, and it often happens that only a minimal quantity is available. Thus, biological titrations on larger animals or animals of a relatively high resistance to cardiotonic glycoside activity may be impossible.

Presuming that birds other than pigeons could be used to evaluate cardiotonic glycosides, attempts were made to use turtle-doves (*Streptopelia roseogrisea*) and sparrows (*Passer domesticus*). Since both the turtle-doves and especially the sparrows weigh less than pigeons, smaller quantities of the active materials have been thought to be needed.

The titrations on turtle-doves and sparrows were made by the method prescribed in the B.P. 1953 for pigeons, ignoring the sex of the birds. The solution to be examined was injected into sparrows by means of a micro-syringe connected to a plastic tube and intracutaneous canula. The weight of the turtle-doves varied between 133 and 205 g., whereas that of sparrows varied between 12.5 and 18.5 g.

Experiments were carried out with *Digitalis purpurea* from two different sources (Preparations A and B) on pigeons, turtle-doves and sparrows comparing the results with the Yugoslav Digitalis standard preparation. Results of these experiments are given in Table I. As can be seen, these were not significantly different for the three species used.

Titrations of the Yugoslav Digitalis standard preparation were repeated at intervals of 3–6 months to check the reproducibility of the results. These results are given in Table II.

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As can be seen from Table II, it seems that the average lethal dose undergoes practically no changes during the stated period of time, the variations between the three results obtained on the same animal being not significantly different.

It is seen that the biological titration on turtle-doves can be made with smaller quantities of material than with pigeons, and the range of the results

TABLE I
TITRATION OF TWO COMMERCIAL PREPARATIONS

Preparation	Int. unit	Limits of error per cent (P = 0.05)	Number of animals used	
			Standard preparation	Sample
Pigeons A	10.8	91.9-108.8	12	6
B	12.9	90.9-110.1	12	6
Turtle-doves A	10.6	97.7-102.4	8	6
B	12.7	98.2-101.8	8	6
Sparrows A	10.3	86.0-116.2	8	6
B	10.6	85.5-117.9	8	6

TABLE II
DETERMINATION OF THE LD OF THE YUGOSLAV DIGITALIS STANDARD PREPARATION

	Number of animals	Mean of weights of animals in g.	Mean of log's of lethal doses	Σd^2	Coefficient of variation
Pigeons	12	273.5	1.22246	0.01609	3.13
	12	299.7	1.24510	0.21603	3.56
	12	284.7	1.20897	0.02011	3.54
Turtle-doves ..	6	150.8	1.23890	0.00727	3.08
	5	161.2	1.21965	0.00563	3.08
	7	183.1	1.21045	0.00933	3.26
Sparrows	9	17.2	1.12448	0.03188	5.61
	8	17.4	1.14281	0.15470	13.01
	8	16.9	1.11992	0.09001	10.11

was found to be even smaller than for pigeons. Turtle-doves are as easy to breed as pigeons.

Sparrows are relatively resistant to digitalis, and the concentration of the solution to be injected should be 3-4 times higher than for pigeons or turtle-doves. On the other hand, the weight of the sparrows being nearly 10 times smaller than that of the pigeons, the determination can be made with quantities of material so small that they can be eluted from a paper chromatogram.

Although the range of the results obtained is larger than that obtained with turtle-doves and pigeons, they can be considered satisfactory. Sparrows are not so easily bred as pigeons and turtle-doves, special conditions having to be established.

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