# THE BIOLOGICAL ASSAY OF VEGETABLE PURGATIVES

## PART III.—CASCARA AND ITS PREPARATIONS

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BIOLOGICAL methods of assay for senna, rhubarb, and their preparations have been reviewed and described in the previous papers of this series[1,2]. Green, King and Beal[3] used guinea-pigs and white rats to measure the purgative activity of cascara (*Rhamnus Purshianus* D.C.). The activity was measured by determining the rate of fæcal output (g. per hour), the fæces being collected and weighed every 3 hours. The cascara was administered by pipette in the form of fluid extracts. They stated that the response of the white rat was not so uniform as that of the guinea-pig which also gave occasional erratic results, but repeated tests with animals comparable in size and age were in reasonably good agreement. Hazleton and Talbert[4] used white mice as the test animal, and also found that results were not so satisfactory as with senna. This was because the " wet fæces " formed were not so distinctive as those formed when senna was used.

In the present series of investigations, mice were chosen as the test animals, and the advantages of using mice have been discussed in Part I[1]. The indefinite response obtained by Hazleton and Talbert may be due to insufficient dosage, as we obtained a very distinct response after administering suitable doses of cascara and its preparations.

### RELATION BETWEEN DOSE AND RESPONSE

To see whether the same method as that described for senna and rhubarb can equally well be applied to cascara, a series of graded doses

TABLE I

NUMBER OF WET FÆCES PRODUCED BY PAIRS OF MICE AFTER ADMINISTERING DRY EXTRACT OF CASCARA B.P.

| Pair of mice (No.) | Dose in mg. per pair of mice | | | | | Totals |
|---|---|---|---|---|---|---|
| | 80 mg. | 120 mg. | 180 mg. | 270 mg. | 405 mg. | |
| 1 ... ... ... ... ... | 1 | 4 | 19 | 25 | 27 | 76 |
| 2 ... ... ... ... ... | 5 | 1 | 15 | 25 | 26 | 72 |
| 3 ... ... ... ... ... | 1 | 5 | 12 | 24 | 20 | 62 |
| 4 ... ... ... ... ... | 1 | 10 | 17 | 17 | 37 | 82 |
| 5 ... ... ... ... ... | 2 | 8 | 19 | 24 | 29 | 82 |
| Totals ... ... ... ... | 10 | 28 | 82 | 115 | 139 | 374 |
| Averages ... ... ... ... | 2 | 5·6 | 16·4 | 23·0 | 27·8 | — |

of powdered cascara bark suspended in distilled water was given to mice using the same technique as that described for senna. The results showed that the activity of cascara bark was so low that a very large dose had to be given in order to induce a positive response. This large dose when suspended in 0·5 ml. of water (the usual volume of a single dose) produced a suspension which was too thick for convenient administration. Therefore the experiment was repeated using a sample of dry extract of cascara B.P. instead of the powdered bark. It was found that suitable doses could be administered in water without difficulty. The results of the experiment are recorded in Table I.

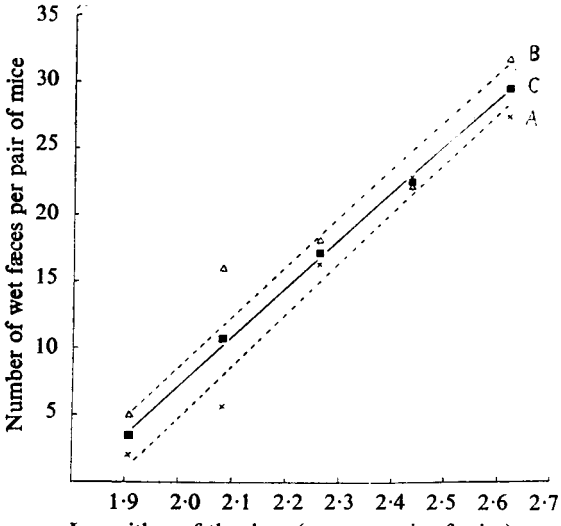


FIG. 1.—Relation of response to the logarithm of the dose.

Table I clearly shows that an increase in the dose of cascara extract produced an increase in the number of wet fæces as with senna and rhubarb. A graph was then constructed by plotting response against the log dose (Fig. 1 A). The graph shows that all the points fall reasonably well on a straight line except the response to the second dose, which was rather low. The experiment was then repeated and similar results were obtained, except the response to the second dose was rather high (Fig. 1 B). However, when the average responses of the two experiments are taken, all the points fit very well into a straight line (Fig. 1 C).

*Analysis of Variance.* To determine how well the supposed linear relationship between log dose and response fits the data, and also to examine the nature of discrepancies, if any, an analysis of the variance was carried out for both experiments. The results of the analysis for the first experiment are recorded in Table II. The results for the second experiment have been omitted here because they are in close agreement with that of the first experiment. The calculations were carried out according to the usual procedure (Finney[5], Emmens[6]), which is similar to that adopted in Part II[2].

TABLE II

ANALYSIS OF VARIANCE FOR THE DATA OF TABLE I

| Source of variation | Degrees of Freedom | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|
| Between doses ... ... ... | 4 | 2435·76 | — | — | — |
| Regression ... ... ... | 1 | 2376·86 | 1237·86 | 155·8 | <0·001 |
| Deviation from regression... | 3 | 58·90 | 17·30 | 1·2 | >0·2 |
| Within doses ... ... ... | 20 | 293·20 | 14·66 | — | — |
| Total ... ... ... ... | 24 | 2728·96 | — | — | — |

From the above table, we may conclude that the regression of the log dose upon the number of wet fæces is highly significant, and there is no significant deviation from this regression; therefore, a linear relationship between log dose and response can be assumed.

## APPLICATION OF THE METHOD

The above analysis of variance has proved that the method of biological assay described for senna and rhubarb can also be used for the bioassay of cascara provided the test preparation is sufficiently strong in purgative activity so that the purgative dose can be conveniently administered to mice. A number of assays were carried out using a sample of dry extract of cascara B.P. as laboratory standard and the same procedures as that described for senna and rhubarb. Generally a "4 point" assay such as that described for senna is sufficient, but for higher accuracy a "6 point" assay like that described for rhubarb is advisable. The results of these assays will be reported later.

## SUMMARY

1. An attempt has been made to apply to cascara bark (*Rhamnus Purshianus* D.C.) and its preparations the biological method of assay devised for senna[1] and rhubarb[2] in which white mice are used as the test animals.

2. The powdered bark cannot be used as a standard as the doses required to produce distinctive responses are too inconveniently large to be administered in suspension.

3. By using potent extracts of the bark it has been possible to apply the method successfully to cascara and its preparations.

## REFERENCES

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5. Finney, in Burn, *Biological Standardization*, Oxford University Press, London, 2nd Ed., 1950, 54.
6. Emmens, *Principles of Biological Assay*, Chapman and Hall, London, 1948, 40.