THE ACTIVE CONSTITUENTS OF THE VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART I. GLYCOSIDES AND AGLYCONES

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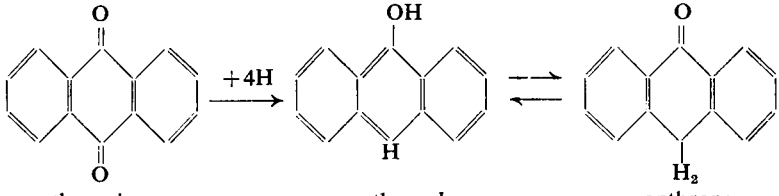
THE following are the common vegetable drugs containing anthracene derivatives ; Senna, leaf and pod (*Cassia acutifolia* and *C. angustifolia*) ; Cassia pulp (*Cassia fistula*) ; Cascara sagrada (*Rhamnus purshiana*) ; Frangula (*R. frangula*) ; Rhubarb (*Rheum* spp.) and Aloes (*Aloe* spp.). These drugs act as irritant purgatives and all respond to the Bornträger test[1] or suitable modifications[2,3] by means of which the anthracene derivatives are converted into free anthraquinone compounds which give pink to red colours in alkaline solution.

ANTHRACENE DERIVATIVES

The anthracene derivatives occur either free or in the form of glycosides, usually with glucose, though glucofrangulin also contains the sugar rhamnose[4]. The following aglycones have been reported :—

(1) *Anthraquinone compounds*, e.g. emodin, aloe-emodin, rhein. These compounds occur in all the drugs mentioned above, sometimes in very small amounts as in aloes[4] and in senna leaf[5] and sometimes in quite large amounts as in cascara[6] and rhubarb.[7]

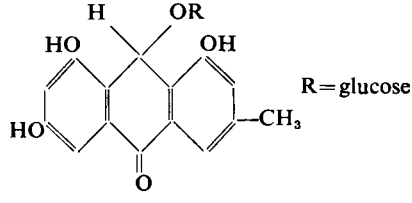
(2) *Anthranol compounds.* Anthranol (and its tautomeric isomer anthrone) is a reduced form of anthraquinone as shown



anthraquinone. anthranol. anthrone.

Hydroxy derivatives of anthranol and anthrone, corresponding to those of anthraquinone, occur frequently in these purgative drugs, e.g. aloe emodin anthranol in aloes.[8]

(3) *Oxanthrone compounds.* These are intermediate between anthraquinones and anthranols. Schindler[9] has shown that cascara bark contains a glycoside based on this structure :—



Glycoside from cascara.

On hydrolysis the aglycone is rapidly oxidised to emodin.

(4) *Dianthranol compounds.* Stoll, *et al.*[10] suggest that the aglycones in senna leaf may be dianthranol or dianthrone compounds which on oxidation in alkaline solution give a rhein-like compound.

For the purposes of this preliminary investigation these anthracene derivatives are classified as (*a*) glycosides, (*b*) free anthranol compounds, and (*c*) free anthraquinone compounds.

Active Constituents

Since all these anthracene purgative drugs can be made to give the Bornträger reaction under suitable conditions, it was natural that attempts should be made to determine the total content of anthracene derivatives as anthraquinones (calculated from the intensity of the red colour) and see if this amount corresponded to the biological activity of the pure anthraquinones prepared synthetically or otherwise. However, it soon became apparent that the "total anthraquinone content" could not account for the activity of these drugs. Thus Tutin and Clewer[11] found that 100 mg. of aloe emodin, or of emodin or of rhein were practically ineffective on human beings. This quantity of anthraquinones correspond to 4 g. of cascara bark, which is the maximum dose (B.P. 1932). Similarly, a recent attempt to correlate the colorimetric assay of frangula extract with its biological assay led the author to conclude that no parallelism exists between the two types of assay[12]. Similar conclusions were arrived at by Astruc and Giroux for cascara[31], and Ström and Kihlström for rhubarb[7].

An interesting series of experiments by Green, King, Beal *et al.* on cascara extract [14,15] seemed to offer an explanation of the superior activity of the crude drug and its preparation over the pure anthraquinones. They showed a definite synergistic action when the anthraquinones, aloe emodin, emodin and chrysophanol were given together to guinea-pigs. The response was much greater than with similar doses of these compounds separately, and as these compounds were stated to exist together in the drug extract it seemed reasonable to suppose that synergism of the anthraquinones accounted for the purgative action of cascara.

On the other hand Casparis and Maeder[4], working on the similar drug frangula bark, concluded that the total activity of the bark was due to the glycoside gluco-frangulin. In fact they found (by experiments on man) that this glycoside was much more active than the corresponding amount of bark; the loss of activity, when in the crude drug, they attributed to the tannins present in the bark.

Straub and Gebhardt[16], working on senna leaf, discovered two active glycosides whose activity appeared to account for a large proportion of the activity of the leaf. Their work was continued by Stoll, *et al.*[10] who solated the glycosides in crystalline form and called them sennoside A and sennoside B.

Thus these recent series of experiments suggest that the activity of the anthracene purgatives may be accounted for by either (*a*) synergism of the free anthraquinone compounds as in cascara, or (*b*) highly active glycosides as in senna leaf and frangula bark. It is interesting to note that in both senna leaf[5] and frangula bark[17], more than nine-tenths of the anthracene

derivatives present occur as glycosides. Hence a determination of "total anthraquinones" would be virtually a determination of the glycosidal content, which (as stated above) is said to account for the total activity of these drugs. However, I have already quoted papers to show that such a correlation has not been found for every member of the group.

I decided therefore to investigate the whole series of anthracene drugs to see if any generalisation could be made as to what are the active constituents. The remainder of this paper describes the preliminary work towards this end, viz., the determination of the relative activity of (*a*) glycosides, (*b*) free anthranols and (*c*) free anthraquinones. The results of the experiments recorded show that for senna leaf, senna pod, sennosides A and B, rhubarb and cascara, the anthracene derivatives are highly active in the glycosidal form ; less active as free anthranols and much less active as free anthraquinones. A discussion of these findings is given at the end of the paper.

EXPERIMENTAL

Chemical and Biological Assays. Necessary requirements for this type of investigation, are reliable methods of chemical and biological assay. The *chemical assays* were based on the colorimetric methods of Kussmaul and Becker for senna[5], and Björling and Ehrlén for frangula[12]. Various modifications were necessary and it is hoped to publish details of chemical assays for each drug later. The glycosidal content of senna and preparations were estimated as sennosides A + B; that of cascara and of rhubarb as the oxidised aglycone, emodin. In order to make the figures comparable the glycoside content of senna is also given in terms of aglycone A + B. The *biological assays* were carried out by Mr. T. C. Lou[18].

1. *Preliminary experiments on Senna leaf.* Portions of powdered Tinnevelly leaf were subjected to increasing degrees of hydrolysis and the purgative activity of these fractions and that of the original leaf were compared by a bio-assay method based on that of Geiger[19]. The results, recorded in Table I, indicate that mild hydrolysis has little effect on the purgative activity but that more vigorous hydrolysis, and

TABLE I

PURGATIVE ACTIVITY OF SENNA LEAF FRACTIONS AFTER VARYING DEGREES OF HYDROLYSIS

Treatment	Temperature and Time	Bio-assay
1. Untreated leaf	——	100
2. Warmed with 0·16N hydrochloric acid in atmosphere of nitrogen	20° C. for 18 hours 70° C. for ½ hour	88
3. Warmed with 0·1N hydrochloric acid in atmosphere of carbon dioxide	90° C. for ½ hour 20° C. for 22 hours	77
4. Boiled in water under reflux	100° C. for 2 hours	36
5. Boiled in 1·5N hydrochloric acid under reflux...	approx. 120° C. for 2 hours	0

possibly oxidation (produced either by boiling in water or warming in strong acid solution in air) led to a marked loss in activity.

2. *Quantitative experiments using senna leaf glycosides.* The preliminary experiments suggest that hydrolysis of the leaf constituents, and possibly oxidation, leads to loss of activity. I obtained the leaf glycosides sennosides A and B in pure form and decided to repeat the previous experiments on a more quantitative basis.

A solution of sennoside A of suitable concentration was divided into three portions. One portion was hydrolysed and the liberated aglycones extracted and purified (as in the chemical assay process[5]); these were administered to mice in suitable doses. The second portion of the sennoside A solution was hydrolysed and oxidised (as in the chemical assay process[5]) and similar doses of the purified products were given to mice. The third portion of the original solution was used as a control.

The results, recorded in Table II, show that the aglycone possesses about 1/3 of the activity of the parent glycoside, whereas the oxidised aglycone possesses no activity at all (when given in similar doses).

TABLE II

BIOLOGICAL AND CHEMICAL ASSAYS OF SENNOSIDE A AND FRACTIONS

Material	Bio-assay	Chemical assay
1. Sennoside A	100	100
2. Hydrolysed Sennoside A (3 N sulphuric acid at 95° C. for ½ hour)	32·5	96·4
3. Hydrolysed and oxidised sennoside A (above, oxidised in N sodium hydroxide and hydrogen peroxide)	0	85·3

Note: In theory, the results for the chemical assays should all be 100. The discrepancy in Expt. 2 is within experimental error; that in Expt. 3 can be accounted for by having to heat with hydrogen peroxide longer than usual, owing to the high concentration of the aglycones.

3. *Quantitative experiments using senna pod.* Similar experiments to those done on senna leaf glycosides were repeated on senna pod which is also said to contain sennosides A and B.[20] A large sample of Alexandrian senna pod was thoroughly mixed and stored in a cool place; a few hundred g. reduced to No. 60 powder was used as standard (= P_8). A potent extract of some of this powder was made by evaporating an infusion* under reduced pressure to a solid extract (E_1). This extract was assayed biologically and chemically against the standard powder. Some of it was then hydrolysed by heating in 5 per cent. hydrochloric acid at 90°C. for ½ hour, cooled and neutralised with sodium hydroxide. This hydrolysed product was re-assayed chemically and biologically against the Standard powder to which a calculated quantity of sodium chloride was added to balance that formed during the neutralising of the hydrolysate. The results of these experiments are shown in Table III.

These experiments on senna pod confirm the conclusions derived from the experiments on senna leaf and glycosides, in that hydrolysis leads to a marked loss in activity. They also show that a determination of the anthracene derivatives as "total anthraquinones" would be no guide

* Chemical assays showed that all the anthracene derivatives passed into an infusion made under suitable conditions.

to the biological activity. Thus in experiments 2 and 3:—biological activity is in ratio 6·8 : 1; glycosidal content is in ratio 8·3 : 1 ; " Total anthraquinones " is in ratio 1·1 : 1.

TABLE III

BIOLOGICAL AND CHEMICAL ASSAYS OF SENNA POD AND FRACTIONS

Material	Bio-assay	Chemical Assay (Results expressed as mg./g.) Glycosides		Free	Total
		Sennosides A+B	As Aglycones	As Aglycones A+B	As Aglycones A+B
1. Standard senna pod powder P_s	100	32·0	20·0	2·0	22·0
2. Potent extract of Pod E_1 ...	(i)301 (ii)376 | 339	132·4	82·8	8·2	91·1
3. Hydrolysed extract	Approx. 50	16·0	10·0	71·5	81·5

4. *Quantitative experiments using Rhubarb.* The experiments already described were made on senna and its preparations, where the aglycones were " artificially " produced by hydrolysis. In rhubarb and cascara, however, there is present " naturally " a large proportion of free aglycones, so that experiments carried out on these drugs will not only decide whether what is true of senna is applicable to other anthracene purgatives; but the proof of relative activity of the glycosides and aglycones can be obtained without resort to the drastic process of acid hydrolysis. Crude drugs and their preparations are complex mixtures and it is not always possible to forecast what effect hydrolysis of other constituents will have on the biological activity.

A weighed quantity of a Standard sample of powdered Chinese rhubarb (R_s) was exhausted with ether and acetone to remove the free emodins and the exhausted material was dried and re-weighed. There was a loss in weight of 15 per cent. The activity of this exhausted material (R_s) and of the Standard powder were compared by the biological and chemical methods already mentioned and the results are shown in Table IV. In calculating the potencies of R_{ex} allowance was made for the 15 per cent. loss in weight on exhaustion.

TABLE IV

BIOLOGICAL AND CHEMICAL ASSAYS OF RHUBARB AND FRACTIONS

Material	Bio-assay	Chemical Assay Total Anthracenes as Emodin : mg./g.
1. Standard rhubarb powder R_s	100	30·5
2. R_s exhausted with ether, etc. = R_{ex}	(i) 106, (ii) 84, (iii) 107 } = 99*	7·2*

* Allowance made for loss in weight of 15 per cent. on exhaustion.

These results fall into line with those already obtained. The removal of a large amount of free aglycones did not result in any loss of activity, thus indicating that the main activity lies in the (ether-insoluble) glycosides.

The free aglycones consisted almost entirely of anthraquinone compounds, which would account for the entire absence of activity in this fraction.

5. *Quantitative Experiments using Cascara Sagrada bark.* Cascara bark, like rhubarb, contains a large proportion of free aglycones as well as glycosides. Biological experiments indicated there was insufficient activity per g. of crude drug for bio-assay work. Accordingly, a potent extract (EC) was made in a similar manner to that used with senna pod. It was found that this dried extract could not conveniently be exhausted with ether, acetone or methylal; accordingly, a suitable solution in water was prepared and half of this was shaken with ether till the bulk of the free compounds was removed. The exhausted solution was warmed to remove ether and adjusted to volume. These two solutions were assayed biologically and chemically and the results are shown in Table V.

TABLE V

BIOLOGICAL AND CHEMICAL ASSAYS ON CASCARA AND FRACTIONS

Material	Bio-assay	Chemical assay mg./g.		
		Glycosides (as emodin)	Free Aglycones (as emodin)	Total Anthraquinones (as emodin)
1. Cascara extract (EC) ...	(i) 100	20·2	19·0	39·2
2. Exhausted cascara extract (EC*ex*)	(i) 110·5 | 100 (ii) 89·3 |	20·2	4·0	24·2

The results are exactly similar to those for rhubarb; chemical tests also showed that the free compounds were in the anthraquinone form, which once more accounts for the lack of activity in this fraction.

6. *Experiments with pure anthracene derivatives.* In order to confirm previous findings and also to determine the relative activities of anthranols and anthraquinones, pure anthracene derivatives were prepared, as below, and tested by the bio-assay method.

1. *Aloe Emodin.* Prepared from aloin by the method of Cahn and Simonsen[21]. Obtained orange needles. M.pt. 223°C. (uncorrected); 231°C. (corr.) (Cahn and Simonsen[24] give 218°C.; Liddell, *et al.*[14] give 222 to 223°C.).

2. *Aloe emodin Anthranol.* Prepared from aloin by the method of Hauser[8]. Obtained yellow needles. M.pt. 200°C. (uncorr.) (Hauser gives 194° to 195°C.).

3. *Emodin (frangula emodin).* Prepared from chrysarobin by the method of Gardner[22]. Obtained silky orange needles. M.pt. 256·5°C. (corr.) (Gardner gives 254° to 256°C.).

4. *Chrysophanol.* Prepared from chrysarobin by the method of Gardner[23]. Obtained golden yellow crystals, M.pt. 197° to 198°C. (corr.). (Gardner gives 193° to 194°C. (corr.). Naylor and Gardner[24] give 195·6° to 196·2°C.).

5. *Rhein.* Prepared from rhubarb and purified by sublimation *in vacuo*. Obtained orange yellow needles. M.pt. 319° to 321°C. (uncorr.). (Oesterle and Tisza[25] give 321° to 321·5°C.)

(*a*) *Qualitative experiment*

5·0 mg. per mouse of each of these compounds was given to groups of 5 mice with the following results :—

1. Aloe emodin ... ... 9 wet fæces produced
2. Aloe emodin anthranol ... ... 23 wet fæces ,,
3. Emodin ... ... 0 ,, ,, i.e. no response
4. Chrysophanol ... ... 0 ,, ,, ,, ,,
5. Rhein ... ... 0 ,, ,, ,, ,,

These results are purely qualitative and indicate that aloe emodin and its anthranol are more active than the other compounds. It was decided to compare the activity of the two former compounds on a quantitative basis.

(*b*) *Comparison of Aloe emodin and aloe emodin anthranol with senna pod* (P_s)

The two compounds were assayed biologically against the Standard Senna Pod (P_s) with the following results :—

Aloe emodin

(i) 1 g. has same activity as 0·561 g. of P_s.
(ii) 1 g. ,, ,, ,, 0·494 ,, ,,
Mean = 0·527 ,, ,, (=10·5 mg. of aglycone A + B)

Aloe emodin anthranol

(i) 1 g. has same activity as 4·49 g. of P_s.
(ii) 1 g. ,, ,, ,, 4·68 ,, ,,
Mean = 4·58 ,, ,, (=91·6 mg. of aglycone A + B)

NOTE.—1 g. of senna pod (P_s) contains 20 mg. of aglycone A+B.

These results show that—

(*a*) Aloe emodin anthranol possesses about 9 times the activity of the corresponding anthraquinone, aloe emodin.

(*b*) The glycosides in senna pod (calculated as aglycones) possess about 11 times the activity of aloe emodin anthranol and about 100 times the activity of the anthraquinone.

(*c*) The (reduced) aglycone of sennoside A is about 1/3 as active as the glycoside (see Table II). Hence this aglycone is much more active than the simple anthranol of aloe emodin.

DISCUSSION

The experiments recorded in this paper show that the main purgative activity of the anthracene derivatives is shown when they are in the glycoside form; and that of the free aglycones the reduced (anthranol) form, though less active than the corresponding glycoside, is much more active than the oxidised (anthraquinone) form. A question raised by these results is what part the aglycones play in purgation. According to the experiments of Okada[26] and Straub and Triendl[27], on senna leaf, the free emodins (anthraquinones) are the active compounds provided they reach the large intestine. Apparently the bulk of the free emodins disappear during metabolism[28], and Straub and Triendl[27] suggest that the sugar moiety of the glycoside acts as a " transporter " for the active

aglycone. The glycoside is not hydrolysed in the stomach and so reaches the large intestine where it is hydrolysed and the liberated (and presumably oxidised) aglycone then exerts its action. The work described in this paper has not only confirmed this theory for senna leaf and shown that the same is true of senna pod, rhubarb and cascara, but also indicates that the sugar moiety plays the further role of "protector," preventing the orally active anthranol from oxidation during storage to orally inactive anthraquinone. In all the drugs studied it was found that nearly all the free aglycones are present in the anthraquinone form (though the glycosides contained "anthranol" aglycones), indicating that after hydrolysis the liberated anthranols are fairly rapidly oxidised during storage. Furthermore, if the pure senna glycosides are heated in N sodium hydroxide with hydrogen peroxide, no oxidation takes place after hydrolysis of the glycosides (in acid), however, the aglycones are rapidly oxidised under similar conditions. This "protector" theory is in line with what is known of the constitution of these glycosides (e.g. in cascara[9], aloin[29]). The sugar is attached to the meso group and so would protect the anthranol structure.

These theories appear to conflict with that of Liddell, King and Beal[14], who claim synergism of the anthraquinones as the explanation of the activity of cascara. While their experiments do show that synergism occurs, they fail to show that this synergistic effect accounts for the total activity of the crude drug. They used 1·5 ml. of a commercial sample of fluid extract of cascara (U.S.P.) as standard in the bio-assay, but unfortunately did not determine the amount of anthraquinones in this standard. Gibson and Schwarting[6] have shown that this amount varies considerably; their highest figure is 2·9 mg. per ml. Thus, at the most 1·5 ml. of standard may contain about 4·5 mg. of anthraquinones, whereas Liddell *et al* found it required 12·5 to 25 mg. of the synergistic mixture to produce a similar effect. However, if sufficiently large doses of free anthraquinones are given by the mouth, purgation results; indicating that a proportion has reached the large intestine. In such circumstances, synergism may be an important factor. To be effective, comparatively large quantities must be given, e.g. 100 to 300 mg. for man[11,27]. This quantity corresponds with the dose of the synthetic anthraquinone, 1 : 8 dihydroxyanthraquinone* (Istin) of which 150 to 450 mg. is necessary as a purgative.

The results also show that a chemical assay which merely determines the total content of anthracene derivatives, irrespective of their form of occurrence, will not correspond to the purgative activity. An interesting example of this occurs in a paper recently published from Finland on the chemical and biological assays of Chinese and rhapontic rhubarbs[7]. The former contains less "total anthraquinones" yet is twice as active as the rhapontic and the authors conclude that the chemical assay does not give a true picture of the laxative effect. However, their figures for "combined anthraquinones" (glycosides) are in the ratio of 9·25 to 5·08 respectively, which corresponds very closely to the biological assays.

Further Work

Having established that the glycosidal fraction of these crude drugs possesses the main activity, I propose to study this fraction in greater detail, as it is obvious that the aglycones of the various glycosides vary considerably in their activity, e.g. the aglycones of sennosides A and B are much more active than the simple anthranol of aloe emodin. It is further hoped to publish details of chemical assays and to examine galenicals made from these drugs to see whether all the glycosides are extracted and whether they are preserved during storage. Hazleton and Talbert [30] state that the U.S.P. fluid extract contains only 35 per cent. of the activity of senna leaf. Preliminary experiments which we have performed on a commercial sample of dry extract of cascara showed it was slightly less active than the crude drug; whereas it should be about four times as active. Similar experiments on a dried extract of senna pod made by the B.P. method showed that it had only 1/6 of the activity of the pod, instead of 3 to 4 times its activity.

Summary

1. A review of the published theories which seek to account for the activity of purgative drugs containing anthracene derivatives is given.

2. The relative activity of anthracene derivatives occurring as (*a*) glycosides, (*b*) anthranols and (*c*) anthraquinones, in certain crude drugs has been determined by biological means.

3. A similar investigation of isolated glycosides, anthranols and anthraquinones has been carried out.

4. The results show that the anthracene derivatives are highly active as anthranol glycosides; less active as free anthranols and much less active as free anthraquinones.

5. The more complex aglycone (dianthranol) of sennoside A is more active than the simple anthranol of aloe emodin.

6. The theory is advanced that the sugar moiety of the glycoside not only acts as a " transporter " of the active aglycone, enabling it to reach the large intestine, but is also a " protector " which prevents oxidation of the aglycone to the relatively (orally) inactive anthraquinone.

7. The experiments also show that a determination of " total anthraquinones " irrespective of their form of occurrence will be no guide to biological activity.

8. Further work on the glycoside fraction of these crude drugs is being carried out.

I would like especially to thank Mr. T. C. Lou for performing all the bio-assays included in this paper, for preparing the pure anthracene compounds and for much valuable assistance and many useful suggestions during the course of the work. I am also grateful to the Pharmacology Department of this School for facilities for carrying out the bio-assays; and to the Department of Physical Chemistry for the loan of certain spectrophotometric equipment necessary for the colorimetric assays. Acknowledgments are made to Sandoz Products Ltd. for the gift of sennosides A and B, and to Mr. A. H. Fenton for the preliminary biological work.

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DISCUSSION

The Papers on Vegetable Purgatives by Mr. Lou and Dr. Fairbairn were discussed together

THE CHAIRMAN said that until recently there had not been a really satisfactory assay of these drugs. Mr. Lou's method was a development of that described last year by Collier and Harris.

DR. I. MICHAELS (London) said that senna pods were said to be more certain than the leaves in their laxative action, and to cause less griping. It appeared that sennosides A and B were present in both, and it would be logical to conclude that the pods contained a higher proportion of active principles than the leaves. It would be interesting to know the comparative figures for the active principles of Alexandrian and Tinnevelly pods so as to be able to assess the reason for the market price of the Alexandrian pods being three times that of the Tinnevelly pods. Griping was said to be associated with the anthracene compounds. The seeds were said to be the cause of griping, and yet it was recorded that the seeds did not contain anthracene derivatives. Modern theory stated that the senna glycosides passed unchanged through the stomach to the intestines, where they were absorbed into the blood stream: here they were hydrolysed. There was a latent period of 10 to 12 hours before the active emodin reached the large intestine, where it stimulated the

peristaltic movements of purgation. The emodins in a badly prepared extract were responsible for the griping pains by stimulating peristalsis in the small intestine. Mr. Lou's method complied with the accepted principles of biological assay. There could be little disagreement with the choice of powdered senna as a standard for the assay of senna and its preparations, although there was room for controversy as to the method of administration. It should be regarded, however, as an intermediate stage in the establishment of a standard based on a compound of known composition.

DR. E. F. HERSANT (Dagenham) said if the glycoside were not hydrolysed in the stomach, was it not possible that after hydrolysis in the large intestine the unoxidised aglycone would partly account for the greater activity of the glycosides.

DR. F. SAID (Egypt) said that glycosides were usually hydrolysed by acids, and if they were not hydrolysed in the stomach they were unlikely to be hydrolysed by the alkali in the duodenum. If the glycosides were absorbed in the stomach and were excreted in the intestine, would it be possible to inject a solution of an anthraquinone and see if it was so excreted.

MR. V. REED (London) asked if the author had any explanation of why the public taste for senna preparations had altered so much in the last 30 or 40 years. At first there had been a big demand for senna leaves, then senna pods had come in. Now there were senna leaves, Tinnevelly senna pods and Alexandrian senna pods, all taken for the same purpose. Was there any real difference in the actual active principle of the three things?

DR. T. E. WALLIS (London) said he was glad to see a Department of Pharmacognosy taking an interest in the biological side, as was the case in similar departments in other countries. He asked for Mr. Lou's opinion on the use of *Daphnia* in this type of work.

DR. D. C. GARRATT (Nottingham) said that it was necessary to get an accurate chemical assay before they could fully appreciate the really good work that had obviously been done.

DR. WALKER (London) said that in the last twelve months he had had to make comparisons clinically of liquid extracts of senna and frangula. The experience had been that many of the senna extracts examined had shown very poor activity compared with senna pods themselves, whereas the difference in the activity in frangula extracts had not been so marked. It would appear that the conclusions reached had been on single samples of powdered senna and cascara. Had the author gone far enough yet to offer any information as to variations between different samples of the powdered drugs.

DR. F. HARTLEY (London) asked whether, in arriving at the conditions for the hydrolysis of glycosides in Table 1 the times and conditions had been arbitrarily chosen, or were they known to be the most favourable for hydrolysis? If Dr. Fairbairn could give supplementary data on the rate of hydrolysis of the glycosides, it would throw light on the

conditions. It was said that by boiling at 110° under a reflux with water for two hours, 60 per cent. of the senna glycosides had been hydrolysed. Could Dr. Fairbairn say whether substantially all that decomposition had occurred during a shorter period such as 15 or 20 minutes.

MR. H. DEANE (Long Melford) said that the method appeared to be accurate for testing purgative drugs. Human volunteers had so far seemed the only way of testing, but the staff of laboratories considered that it was not part of their duties. Senna was more sensitive to heat than rhubarb or cascara; a freshly-made infusion of senna pods was much more active than a liquid extract made from the same pods.

MR. H. B. MACKIE (Brighton) said that it had long been known that with cascara partial hydrolysis decreased therapeutic activity, but increased palatability. With a solid preparation which could be protected by coating palatability was unimportant. In liquid preparations, the therapeutic action had to be balanced with flavour and other factors, if the product was bitter and nauseating it could not be swallowed. In the preparation of decoction of aloes, the aloes was boiled with potassium carbonate and the dose of decoction was equivalent to twice the dose of aloes to get the same therapeutic result, but it was then pleasant to take; 50 per cent. of the activity of the aloes had been lost in boiling.

DR. FAIRBAIRN, replying, said that the glycosides were the active principles, but the particular glycosides present in different drugs might vary, thus giving different activities. Regarding anthranols, presumably as soon as the glycosides were hydrolysed the free anthranol enhanced the activity. It was true that the stomach contained acid, but Table I showed that it needed fairly strong acids at high temperature to produce hydrolysis. Dr. Collier had reported last year that he had injected liquid extract of senna into the veins of mice, but there had been no purgative action.

MR. LOU said it was, generally speaking, much better if a definite compound was used as a standard, but unfortunately these purgative drugs contained various glycosides which might be different in structure. If sennoside A or B was adopted as a standard, and the product assayed on either compound, the result might be in conflict with a chemical test. It was obvious that workers in this field ought to be careful about the element of biological variation. Statistical considerations had been applied to all the tests they had made and the errors were within 15 per cent. They had no experience with the *Daphnia* method. There was a wide zoological gap between *Daphnia* and human beings, and they had thought it preferable to use animals where the gap was much smaller. He had tested a few preparations on himself. Those preparations which were inactive in himself, or had very low potency, had no activity in mice. In Munch's book on bio-assays, there were data to show that the relation of dose between a mouse and a human being differed with different purgatives. For senna the ratio was about 1 to 300. For instance, if the minimum effective dose for mice was 6 mg. the human dose would be 1·8 g. which was within the dose range of the B.P. for senna. He had carried out laboratory experiments on extracting both pods and leaves, but the latter gave difficulty because of the large quantity of mucilage they contained.