STUDIES IN THE GENUS DIGITALIS

PART V. FERMENTATIVE DEGRADATION OF *D. purpurea* LEAF

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THE deterioration in therapeutic potency of the leaves of *Digitalis purpurea* when incorrectly dried or stored is well known. This is generally attributed to enzymic degradation of the glycosides in the presence of moisture but the nature of these changes is not fully understood. It is probable that the work of Cloetta[1] is responsible for the widely held view that digitoxin and gitoxin readily hydrolyse in the moist drug to liberate the much less potent aglycones digitoxigenin and gitoxigenin together with free digitoxose. The presence of free aglycones in digitalis leaf is supported by Kedde[2] who, using adsorption methods, found that up to 50 per cent. of the glycosidal mixture was of free aglycone. Svedsen and Jansen[3], using paper chromatographic methods, found that stabilised digitalis leaf contained much primary glycoside with smaller amounts of secondary glycosides and aglycones, whereas the dried leaves which had not been stabilised contained less primary glycosides but more secondary glycosides and aglycones.

Tattje and Van Os[4–6] found small amounts of aglycones present in the majority of the leaf samples examined. An average of figures which they record for 15 samples shows the glycosidal complex to contain 79 per cent. primary glycosides, 5 per cent. secondary glycosides and 16 per cent. aglycones in stabilised leaves whereas for non-stabilised leaves the corresponding figures are 32 per cent., 58 per cent. and 10 per cent. respectively. They used the picrate and modified Keller–Kiliani methods of estimation upon the chloroform extracts from cold water macerates of the powdered leaf prepared by either 1¼ hours shaking, or 3 days maceration at 30° C. The glycosidal complex in the 1¼ hour maceration was of primary and secondary glycosides also of aglycones. During the more prolonged maceration at 30° C. the primary glycosides were all converted to secondary glycosides, but these authors maintained that no degradation of secondary glycosides to aglycones occurred; thus the difference between the picrate and the Keller-Kiliani estimations for this extract was a measure of the aglycones present. The estimation of the primary glycosides depended on the difference in the Keller-Kiliani values for the two extracts, since in the former only two of the three digitoxose molecules present in these glycosides reacted, whereas all three molecules reacted after removal of the glucose by fermentation[7]. These authors have also pointed out that the recently isolated strospeside would be estimated as aglycone by their method since it contains gitoxigenin and digitalose[8].

Neuwald and colleagues[9–12] found good agreement between modified picrate-genin and modified Keller-Kiliani estimations of total glycosides and concluded that no free aglycones were present in the three leaf

samples examined. Wolfgramm and Weiss[13] used the picrate and Keller-Kiliani reagents to examine the chloroformic extracts from cold water macerates prepared by varying times of contact between water and drug from 1¼ hours to 48 hours. There was good agreement between the two assay values at each period of maceration, with only occasional exceptions, and the authors concluded that free aglycones were absent. Since the assay value increased with periods of maceration up to 24 hours, they concluded that secondary glycosides only were separated by chloroform, leaving the primary glycosides in the aqueous phase; on fermentation these were broken down to secondary glycosides and were then extracted; hence the increases in values as a result of prolonged soaking gave a measure of primary glycosides present. For this reason these authors preferred their methods of extraction to that used by Langejan and van Pinxteren[14] in which a mixture of chloroform and ethanol was employed to remove primary as well as secondary glycosides.

Wegner[15,16] used cold water extracts of digitalis leaf powder, macerating for varying periods up to 24 hours, also digitalis enzyme preparations were added to extracts prepared by hot infusion. Picrate and Keller-Kiliani assay values increased with increasing periods of fermentation, the increase being more marked for the sugar estimation until parity of values for the two assay processes was reached after 24 hours of maceration. Wegner thus concluded that there was no strong evidence for the presence of free aglycones; that part only of the primary glycosides were extracted from the aqueous phase with chloroform, of which only two digitoxose molecules reacted with the Keller-Kiliani reagent; fermentation converted all primary into secondary glycosides. He also found that, after such fermentation, 30 per cent. of the glycosides originally present in the aqueous phase were chloroform-insoluble. Fish and Todd[17] examined the stability of digitalis tinctures and calculated that the initial fall in activity was due to the conversion of primary to secondary glycosides, after which the tinctures were quite stable with no further degradation to aglycones.

There is thus some real conflict in the published evidence on the occurrence or formation of free aglycones in dried digitalis leaf, the more recent work tending to suggest that little is found. All the foregoing comparative work had been based upon stabilised or normally processed drugs and we have no information about faulty processing, other than the behaviour of the leaf in the assay by 1–3 day fermentation itself, in which the secondary glycosides are said to be stable. A real conflict of evidence as to the extraction of the primary or secondary glycosides with chloroform also exists. To investigate these problems the following tests were made.

Extraction and Assay Methods

Cold water extracts were prepared by mixing powdered digitalis leaf 0·5 g. with water 50 ml. and shaking gently for 1¼ hours; decolourisation was effected by adding 5 ml. of 15 per cent. aqueous solution of lead acetate, shaking, allowing to stand, decanting and filtering. 37 ml. of filtrate (equivalent to ⅓ g. leaf) was shaken out with 4 × 20 ml. quantities

of either chloroform or a mixture of equal volumes of chloroform and ethanol 95 per cent. The mixed chloroformic extracts were dried over anhydrous sodium sulphate, filtered, the filter washed with chloroform, the filtrate adjusted to a suitable volume and divided into two halves each of which was evaporated to dryness. Using one dry chloroformic residue the glycosidal content was determined by the Keller-Kiliani reagent at

TABLE I

AQUEOUS MACERATES. DINITROBENZOIC ACID AND KELLER-KILIANI ASSAYS. EQUIVALENT DIGITOXIN CONTENTS (PER CENT.)

Leaf sample	A		B		C	
	Chloroform	Chloroform and ethanol	Chloroform	Chloroform and ethanol	Chloroform	Chloroform and ethanol
d_1	0·25	0·57	0·19	0·45	0·31	0·51
K_1	0·25	0·35	0·23	0·32	0·27	0·37
d_3	0·39	0·56	0·32	0·45	0·35	0·50
K_3	0·35	0·41	0·33	0·35	0·36	0·38

590 mμ as previously described[18], results being expressed as equivalent digitoxin (K_1). The second dry chloroformic residue was dissolved in 4 ml. of ethanol 17·5 per cent. and the glycosidal content determined by the dinitrobenzoic acid reagent at 535 mμ[18,19], results being expressed as equivalent digitoxin (d_1). The entire process was then repeated but macerating the leaf in water at 30° C. for 3 days: values K_3 and d_3 being determined.

TABLE II

DINITROBENZOIC REAGENT; ETHANOLIC AND COLD WATER EXTRACTS OF LEAVES. EQUIVALENT DIGITOXIN CONTENTS (PER CENT.)

Leaf sample	70 per cent. ethanolic extract of leaf	Aqueous maceration of leaf. Chloroform and ethanol extracts
A	0·57	0·56
B	0·50	0·45
C	0·53	0·50

Using three leaf samples A, B and C, the assay process was carried out in duplicate, employing chloroform and chloroform with ethanol in parallel experiments. The results are given in Table I. The assay values obtained by extraction of the leaf with ethanol 70 per cent., decolourisation and direct estimation with dinitrobenzoic acid[18,19] are compared with the 3-day maceration results in Table II.

It will be seen from this second Table that the results for each leaf by the two extraction methods are in good agreement and hence it is concluded that both methods are quantitative in extracting total glycosides which react with dinitrobenzoic acid reagent. The comparison of values in Table I shows that, from 3-day macerates, chloroform with ethanol extracts about 30 per cent. more material reacting with dinitrobenzoic acid than does chloroform alone; but this material appears to contain little or no digitoxose, since the K_3 values show almost insignificant increases. Hence this material is behaving as an aglycone in the estimation, it is not gitoxigenin or digitoxigenin which are chloroform-soluble and it is more probably digitalinum verum or strospeside which are free from digitoxose and are insoluble in chloroform.

Disparity of results is the more marked between the different solvent extractions from 1¼ hours aqueous macerates: the digitoxose-free glycoside such as digitalinum verum accounts for a portion of the difference in d_1 values, the extraction of primary glycosides by chloroform and ethanol accounting for the remainder. The good agreement between d_1 and d_3 values when based on chloroform with ethanol extracts should be noted. The K_3 values are also a maximum for these extracts due to the conversion of all primary to secondary glycosides in the 3-day fermentation with consequent availability of all three sugar molecules to react. Hence the 3-day aqueous maceration process followed by chloroform with ethanol extraction give results most nearly agreeing with those obtained by ethanolic extraction of this leaf. The guinea-pig biological assay of chloroform with ethanol and of chloroform extracts from decolourised tinctues of leaf sample E, as prepared for dinitrobenzoic acid assay[18,19], are reported in Table IV. It will be noted that about 30 per cent. of the glycosidal complex, as shown by potency, is insoluble in chloroform but soluble in chloroform with ethanol and this agrees well with the d_3 values recorded in Table I.

The presence of small amounts of chloroform-soluble free aglycones in leaf sample A is indicated by the difference $d_3 - K_3$ for the chloroform extract (Table I), this is approximately 10 per cent. of total glycoside present. Chloroform-soluble free aglycones are absent from leaf samples B and C.

Fermentation Studies

A preliminary test to investigate the behaviour of the glycoside content as shown by dinitrobenzoic acid assay in leaves subjected to different periods of humidification was set up. Weighed quantities of three leaf powders were each moistened with water, placed in covered petri dishes and incubated at 30° C. for periods of from 2 days to 6 weeks. Each

TABLE III

Fermented leaf. Dinitrobenzoic acid assay of 70 per cent. ethanol extracts

Leaf sample	D		E		F	
Period of fermentation	Loss in weight per cent.	Equivalent potency I.U./g.	Loss in weight per cent.	Equivalent potency I.U./g.	Loss in weight per cent.	Equivalent potency I.U./g.
0 days	—	11·9	—	11·8	—	12·5
3	26	9·7	—	—	—	—
7	29	7·2	35	7·1	—	—
14	—	—	41	2·4	—	—
21	—	—	44	2·1	—	—
28	—	—	—	—	49	3·2
35	—	—	—	—	47	3·4
42	—	—	—	—	46	3·0

sample was stirred daily and kept moist by adding water when necessary. When humidified for the requisite period of time, the dish lids were removed, the contents dried at 55° C. and re-weighed: losses in weight due to fermentation were calculated and weights of the materials necessary to make tinctures equivalent to 1 in 10 of the original leaf samples were taken.

Assays of these tinctures were made by the dinitrobenzoic acid process[19] and the equivalent potencies are given in Table III. Biological assays of sample E after 7 days and after 21 days' fermentation were also carried out and these are recorded in Table IV.

TABLE IV

BIOLOGICAL ASSAYS (GUINEA-PIG METHOD). LEAF SAMPLE E

Treatment of leaf	Equivalent leaf Potency I.U./g.
Decolourised 70 per cent. ethanolic tincture[18,19]:—	
extract with chloroform and ethanol	10·8
extract with chloroform	7·4
Leaf fermented 7 days	2·4
Leaf fermented 21 days	0·7

The fermentation process was repeated for periods of time up to 21 days, using two further leaf samples. Tinctures of the fermented materials were prepared and assayed as described above, the equivalent potencies being given in Table V. Further samples of the same fermented materials were also examined by the cold water extraction process for 1¼ hours or for 3 days at 30° C. as described above; allowance being made for the losses in weight due to fermentation. Glycosides were extracted from the 1¼ hour aqueous macerates with chloroform and ethanol; the 3 day aqueous

TABLE V

FERMENTED LEAF. DINITROBENZOIC ACID ASSAY OF 70 PER CENT. ETHANOL EXTRACTS

Leaf sample	G		H	
Period of fermentation	Loss in weight per cent.	Equivalent potency I.U./g.	Loss in weight per cent.	Equivalent potency I.U./g.
0 days	—	13·1	—	11·6
3	23	12·2	25	10·3
6	30	10·4	32	9·2
9	39	8·6	43	8·1
12	42	9·4	44	7·5
15	45	4·1	48	7·6
18	49	4·2	49	5·5
21	49	1·5	48	6·9

TABLE VI

FERMENTED LEAF SAMPLE G. AQUEOUS MACERATES. EQUIVALENT DIGITOXIN CONTENTS (PER CENT.)

Period of maceration	1¼ hours		3 days			
Extraction solvent	Chloroform and ethanol		Chloroform		Chloroform and ethanol after chloroform	
Period of fermentation	d_{1T}	K_{1T}	d_3	K_3	d_{3CA}	K_{3CA}
0 days	0·52	0·32	0·36	0·32	0·13	0·04
3	0·35	0·33	0·30	0·28	0·05	0·03
6	0·35	0·28	0·32	0·25	0·05	0·04
9	0·30	0·27	0·30	0·23	0·04	0·03
12	0·33	0·23	0·29	0·20	0·04	0·03
15	0·17	0·19	0·16	0·15	0·01	0·04
18	0·17	0·20	0·16	0·16	0·02	0·05
21	0·05	0·13	0·04	0·07	0·01	0·04

macerates were extracted first with chloroform and then with a mixture of chloroform and ethanol. Dinitrobenzoic acid and Keller-Kiliani assays were then applied to each fraction and these are recorded in Tables VI and VII.

TABLE VII

FERMENTED LEAF SAMPLE H. AQUEOUS MACERATES. EQUIVALENT DIGITOXIN CONTENTS (PER CENT.)

Period of maceration	1¼ hours		3 days			
Extraction solvent	Chloroform and ethanol		Chloroform		Chloroform and ethanol after chloroform	
Period of fermentation	d_{1T}	K_{1T}	d_3	K_3	d_{3CA}	K_{3CA}
0 days	0·50	0·32	0·34	0·31	0·12	0·04
3	0·36	0·26	0·31	0·26	0·05	0·03
6	0·31	0·25	0·28	0·22	0·04	0·03
9	0·29	0·24	0·26	0·21	0·03	0·03
12	0·26	0·23	0·27	0·20	0·02	0·03
15	0·18	0·20	0·13	0·14	0·01	0·04
18	0·18	0·19	0·16	0·14	0·03	0·04
21	0·03	0·08	0·03	0·03	0·02	0·04

TABLE VIII

FERMENTED LEAF SAMPLES G AND H. APPARENT CONTENTS OF PRIMARY AND SECONDARY GLYCOSIDES AND OF AGLYCONES (PER CENT.)

Leaf sample	G			H		
Period of fermentation	Primary glycosides	Secondary glycosides	Aglycones etc.	Primary glycosides	Secondary glycosides	Algycones etc.
0 day	0·15	0·25	0·08	0·11	0·26	0·07
3	Nil	0·33	0·02	0·11	0·20	0·03
6	"	0·28	0·03	Nil	0·25	0·03
9	"	0·27	0·02	"	0·24	0·02
12	"	0·23	0·05	"	0·23	0·01
15	"	0·19	Nil	"	0·20	Nil
18	"	0·20	"	"	0·19	"
21	"	0·13	"	"	0·08	"

DISCUSSION

The presence of about 30 per cent. of the total glycoside complex in the form of chloroform-insoluble but chloroform with ethanol-soluble material has already been noted in leaf samples A, B, C and E as recorded in Tables I and IV. Similar results were obtained for leaf samples G and H when controls were examined by means of the 3-day aqueous macerate method, the d_{3CA} value being about 26 per cent. of the total glycosidal material estimated, as shown in the first line of Tables VI and VII. The almost complete absence of digitoxose sugar in this chloroform-insoluble material was also confirmed by the very low assay figures with the Keller-Kiliani reagent (K_{3CA}). This material is neither digitoxigenin nor gitoxigenin, but may be a chloroform-insoluble glycoside free from digitoxose such as digitalinum verum.

Using the results for leaf sample A, Table I, as an example it is seen that 0·15 per cent. ($d_{3CA} - K_{3CA}$) of this chloroform-insoluble glycoside is present. Thus the total amount of other glycosides and aglycones originally present in the leaf is 0·41 per cent. ($d_{1CA} - 0·15$). It thus follows

that chloroform alone fails to extract 0·16 per cent. ($0·41 - d_1$) of this group, and this will be primary glycoside which is very feebly soluble in chloroform. Now in the chloroform with ethanol extract the difference between the two Keller-Kiliani values is one-third of the amount of primary glycoside present since only two digitoxose molecules react in the primary glycoside molecule (K_{1CA}) whereas all three digitoxose molecules react after degradation to the secondary glycoside (K_{3CA}). Hence the leaf contains 0·18 per cent. of total primary glycosides ($3 \times (K_{3CA} - K_{1CA})$) and so the 0·16 per cent. of primary glycoside, not extracted by chloroform alone after 1¼ hour aqueous maceration, comprises practically the whole of the primary glycosides present in the leaf. Similar calculations may be made for leaf samples B and C (Table I). Undue reliance should not be placed on such absolute calculations for it seems probable that the solubilities of the components in the glycosidal complex will differ from those determined on pure glycosides. Nevertheless, these results support the findings of Wolfgramm and Weiss[13] that the primary glycosides are not extracted by chloroform from aqueous leaf macerates, the results also support the work of Langejan and van Pinxteren[14] that primary glycosides are extracted from such aqueous macerates by means of chloroform and ethanol.

The good agreement between the dinitrobenzoic acid assay results for ethanolic extractions of leaves and for aqueous macerates followed by chloroform with ethanol extractions is of importance (Table II). Since aglycones appear to be almost completely absent, even in deliberately fermented samples, and since digitalinum verum is of high potency, it follows that the former method of extraction may be used with confidence, for it extracts maximum glycosidal material.

The fall in total glycosidal material, as estimated by dinitrobenzoic acid, in ethanol extracts of leaf samples which have been subjected to progressive humidification at 30° C., is marked as is seen in Tables III to V and very low values are reached after about 14 days. This decrease, associated with a fall in dry weight of the leaf tissues, is most readily explained by fermentative degradation of cell contents. It is reasonable to hypothesise that the aglycone portions of the glycoside molecules are progressively degraded by these fermentative changes, at least in so far as the butenolide group, which reacts with the dinitrobenzoic acid reagent, is concerned.

The most outstanding fact which arises from these investigations is that it has proved impossible to produce free aglycones in leaf samples submitted to extremely bad conditions of storage. These moist, warm conditions rapidly affected the samples adversely as judged by odour, colour and general appearance also by loss in dry weight of up to 49 per cent. The rapid fall in dinitrobenzoic acid assays of 70 per cent. ethanolic extracts, associated with these changes, has already been pointed out and similar decreases were found with this assay on aqueous macerates prepared from these fermented materials as shown in Table VI and VII. The decreases in Keller-Kiliani assay values with increased periods of leaf fermentation are of the same order, but not quite so great as the decreases in dinitrobenzoic acid values. Hence the difference $d_3 - K_3$, which is a measure

of aglycone, becomes smaller and eventually reaches a zero value. Thus in these degradation changes the aglycone portion of the glycoside molecule, or at least the butenolide linkage thereof, disappears, as rapidly or slightly more rapidly than the digitoxose sugar moiety. There is never any evidence that the digitoxose is split off from the aglycone, and, if this does occur, the subsequent degradative changes are more rapid, for free aglycones do not accumulate.

The amount of primary glycoside originally present in the leaf sample G, as indicated by the difference in Keller-Kiliani values on chloroform with ethanol extracts, had completely disappeared after 3 days of fermentation of the material; in leaf sample H it had disappeared after 6 days' fermentation. This was to be anticipated and confirms the findings of other workers which were discussed above. The chloroform-insoluble glycosides free from digitoxose, such as digitalinum verum, which are associated with the primary glycosides (d_{3CA}, Tables VI and VII) also disappear relatively rapidly as fermentation proceeds. Hence the relative stability of the secondary glycosides is demonstrated. The more rapid fall in biological assay values after 7 and 21 days' fermentation (Table IV) should however be noted.

If the methods of calculation, proposed by Tattje[5] for the estimation of primary and secondary glycosides and of aglycones, are applied to the chloroform and ethanol values in Tables VI and VII, the figures of Table VIII are obtained for leaf samples G and H.

Digitalinum verum and other non-digitoxose glycosides are here shown as aglycones. Because of differences between solubilities of individual glycosides and of their mixtures, these calculated values cannot be regarded as absolute but the results do support the general conclusions already made.

Summary and Conclusions

1. Leaf powders of *Digitalis purpurea*, when moistened and allowed to ferment at 30° C. for periods of time up to 42 days showed: (*a*) a rapid conversion of primary to secondary glycosides; (*b*) a progressive degradation of secondary glycosides, involving both the aglycone and sugar parts of the molecule; (*c*) free aglycones did not accumulate at any stage.

2. The glycoside complex of *Digitalis purpurea*, extracted with either water or 70 per cent. ethanol may contain about 50 per cent. of material insoluble in chloroform, but soluble in chloroform with ethanol and possessing high pharmacological activity; this comprises: (*a*) practically the whole of the primary glycosides, and (*b*) up to 30 per cent. of glycosidal material free from digitoxose.

3. The dinitrobenzoic assay process, applied to decolourised preparations of 70 per cent. ethanol extracts of leaf powders, estimates the whole of this material.

Our thanks are due to the Laboratory Animals Bureau for the biological assays of Table IV.

REFERENCES

1. Cloetta, *Arch. exp. Path. Pharmak.*, 1926, **112**, 261.
2. Kedde, *Pharm. Weekbl.*, 1947, **82**, 741.
3. Svedsen and Jansen, *Pharm. Act. Helvet.*, 1950, **25**, 241.
4. Van Os and Tattje, *Pharm. Weekbl.*, 1951, **86**, 104.
5. Tattje, *Stabilisatie en Samenstelling van het glycosidenmengsel van Folia Digitalis*, Thesis. Groningen, 1952.
6. Tattje and van Os, *Pharm. Weekbl.*, 1953, **88**, 237.
7. Stoll and Kreis, *Helv. chim. Acta*, 1935, **18**, 120.
8. Van Os and Tattje, *Pharm. Weekbl.*, 1955, **90**, 901.
9. Neuwald, *Arch. Pharm.*, 1950, **283**, 93.
10. Zollner, Diekmann and Neuwald, *ibid.*, 1950, **283**, 265.
11. Neuwald and Diekmann, *ibid.*, 1952, **285**, 19.
12. Neuwald, *ibid.*, 1952, **285**, 22.
13. Wolfgramm and Weiss, *Pharmazie*, 1955, **10**, 292.
14. Langejan and van Pinxteren, *Pharm. Weekbl.*, 1953, **88**, 529.
15. Wegner, *Arzneimit.-Forsch.*, 1952, **2**, 382.
16. Wegner, *Pharmazie*, 1952, **7**, 373.
17. Fish and Todd, *J. Pharm. Pharmacol.*, 1954, **6**, 894.
18. Rowson, *ibid.*, 1952, **4**, 825.
19. Rowson, *ibid.*, 1955, **7**, 924.

DISCUSSION

The paper was presented by DR. J. M. ROWSON.

The CHAIRMAN said that workers on digitalis frequently referred to fermentation. Was this fermentation hydrolysis caused by micro-organisms which might be a definite species associated with the drug or by enzymes? Had the authors any evidence that the surface of the leaves was sterile?

PROFESSOR H. BRINDLE (Manchester) said the evidence for the presence of aglycones in digitalis was conflicting. The authors used the extraction method but the value of certain parts of their work would have been enhanced if they had also used chromatography. For example, they speculated that one substance might be digitalinum verum, whereas had chromatography been applied they would have been sure. Speaking from memory, he had found in unfermented leaf a higher ratio of primary glycosides to secondary than the authors had. In Table VIII the primary glycosides present in Sample H had not changed after three days' fermentation—was this a misprint?

MR. G. J. RIGBY (in a written contribution read by Professor Brindle) said it was surprising the authors had not employed chromatography in their investigations. The authors had calculated that one leaf sample contained 0·18 per cent. of total primary glycosides, and said that 0·16 per cent. of primary glycosides not extracted by chloroform alone approximated closely to that figure. The paper would be less difficult to understand if the symbols used for this calculation had been clearly defined. The value 0·18 per cent. had been calculated from the expression $3(K_{3CA}-K_{1CA})$ which took no account of the different molecular weights of the primary and secondary glycosides. The authors had assumed that 2 mg. of a secondary glycoside would react with the Keller-Kiliani reagent to the same degree as 3 mg. of the corresponding primary glycoside. In fact,

one molecule of each were reported to react to the same degree. That required a correction in the paper. The percentage of total primary glycoside present should be calculated as 3·63 ($K_{3CA}-K_{1CA}$). The figure 0·18 per cent. would then become 0·22 per cent., showing that chloroform alone extracted about 30 per cent. of the total primary glycosides and not 11 per cent. as suggested by the authors. The term "equivalent digitoxin" was ambiguous. Did the authors mean "producing the same extinction as x mg. of digitoxin", or did they mean that two g. molecules of desacetyl-digilanid A was equivalent to two molecules of digitoxin?

DR. G. E. FOSTER (Dartford) said he understood the authors to state that the activity of moist digitalis leaf was completely destroyed on storage. That had not been his experience. He had found that it fell fairly rapidly for a time but then remained constant over a long period.

DR. S. E. WRIGHT (Sydney) said the authors had tended to reject the idea that genins were present in digitalis. That was difficult to establish as the genins were not easy to identify. It was interesting to note that Kaiser and co-workers had isolated some compounds recently which were formyl derivatives.

MR. E. H. B. SELLWOOD (London) referred to the relative amounts of the biological activity extracted in chloroform, with and without alcohol. He had extracted the chloroform-soluble glycosides from a number of samples of leaf and isolated them in solid form, obtaining a yield of approximately 1 g. assaying biologically at about 400 units/g. from a pound of leaf. It was obvious that a considerable amount of biological potency was unaccounted for because this was equivalent to about 1 unit per g. of leaf, whereas the leaf itself assayed at 10–12 units per g. The missing activity may be in the water soluble fraction.

DR. J. W. FAIRBAIRN (London) said that with anthraquinone drugs he had found that during storage or extraction the glycosides broke down, but one could not recover a corresponding amount of the aglycones. At the beginning of the paper the reader was offered two statements of fact. The first was that there were aglycones present, and the second that they were not present. At the end of the paper the authors stated aglycones did not accumulate. It would be interesting to hear how they explained the first statement.

DR. J. M. ROWSON, in reply, said that no attempt had been made to control or to follow the nature of the fermentation. The leaves were kept moist in dishes, and after several days' fermentation there was a good growth of mould on the surface. He had not examined the leaves for micro-organisms, but he felt sure that they would not be sterile. The naturally occurring enzymes were present since these are only destroyed by hot water extraction. He had resisted the use of chromatography up to the present time because he knew that others had undertaken chromatographic studies, and it was undesirable that the work should overlap too much. With regard to the high proportion of secondary to primary glycosides, his recoveries were in good agreement with those of Van Os and Tattje which were quoted. The presence of digitalinum verum in

Digitalis purpurea leaves of different geographical sources had been proved by other workers; he accepted their findings and made use of the new evidence. There was no misprint in Table VIII. The symbols d_{3CA} and K_{3CA} etc. were adequately defined in the paper. The calculations in the paper were correct, since all results were expressed as equivalent to digitoxin. Mr. Rigby has wrongly interpreted this fact. As to the activity of the leaf being destroyed on storage, the leaf he was examining was very moist and it fermented. It was not comparable with the slow drying or leaf storage. The formyl compounds of Kaiser were interesting. Early references to the presence or absence of aglycones in the dried leaf were not clear, and Cloetta in 1927 contributed much to the idea of their presence.