# OBSERVATIONS ON THE AGEING OF DIGITALIS TINCTURES

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MANY workers have tried to explain the nature of the deteriorative changes occurring during storage of digitalis tinctures in which loss of potency is shown by the frog lymph sac method of assay, but not by the cat intravenous method[1,2,3,4,5,6,7,8,9,10]. This loss of potency could be explained[11] assuming the degradation of a solubilising agent and consequent change in rate of absorption from the frog lymph sac, but Brindle and Rigby[12] failed to show any potency change when saponin was removed from tinctures of digitalis. We have also failed, as shown in this paper, to restore the cardiac activity of aged tinctures by the addition of digitalis saponin and further, we have demonstrated a loss in potency using a frog intravenous assay in which variations due to absorption are avoided.

Haag[13] is of the opinion that deterioration might be due to hydrolysis of the cardiac glycosides to the less potent genins, in which case loss of potency would be more evident in the frog where the glycoside/genin potency ratio is much higher than in the cat[14,15,16,17]. The hydrolysis of such stable substances as digitoxin and gitoxin appeared to us improbable in such mild conditions, and although the hydrolytic product digitoxose has been reported[15] in some tinctures of digitalis we were unable, despite repeated attempts, to show the presence of this sugar in aged tinctures, although when a trace of pure digitoxose was added, its presence could be detected by chromatographic separation on paper. The potency change in frogs could be explained on the assumption that hydrolysis of primary glycosides to secondary glycosides occurs, without further degradation to genins. Each primary glycoside has a higher potency than its corresponding secondary glycoside when measured on the frog[15], but this is not so on the cat[18] and therefore the suggested change would be more evident in frog assays. It has been shown[19,20,21] that secondary glycosides give a greater colour density than equimolar amounts of primary glycosides with the Keller-Kiliani reagent and, since they are also more soluble in chloroform, spectrophotometric measurements were made to determine if the amount of chloroform-soluble glycosides had increased during storage of digitalis tinctures. This would provide evidence of the change from primary to secondary glycosides during storage.

## EXPERIMENTAL

To determine if loss was due to failure of the cardiac glycosides to be absorbed across the frog lymph sac membrane, digitonin was added to various aged tinctures and its effect on potency noted. The materials examined in this work included commercial tinctures, ethanolic (70 per cent.) solutions of digitoxin and tinctures prepared by us from leaf dried according to the pharmacopœial directions, and from leaf stabilised to

inactivate the enzymes. Each batch of leaf, collected from wild plants of *Digitalis purpurea*, was divided, one portion being dried at 55° C. while the other was first stabilised[11] by treating with ethanol vapour under pressure, prior to drying at 55° C. The leaf was powdered and stored in jars with ground-glass stoppers containing silica gel, to keep the moisture content to a minimum[22]. The tinctures were prepared by the 48-hour maceration process[23,24] and stored at room temperature for varying lengths of time. For this determination the materials used were 3 aged commercial tinctures

TABLE I

EFFECT OF ADDED SAPONIN ON AGED TINCTURES OF DIGITALIS IN THE FROG LYMPH SAC ASSAY

| Tincture | Age of tincture | Potency lost during storage, per cent. | Potency average of assay results calculated from frog mortality figures |
|---|---|---|---|
| A | 8 years | 39·3 | 112·6 |
| A + 0·1 per cent. of digitonin | | | 111·7 |
| B | 6 years | 16·2 | 118·0 |
| B + 0·1 per cent. of digitonin | | | 118·0 |
| C | 7 years | 29·6 | 98·3 |
| C + 0·2 per cent. of digitonin | | | 107·4 |
| T.S.1 | 7 months | 46·2 | 95·8 |
| T.S.1 + 0·2 per cent. of digitonin | | | 106·3 |
| D.1 | 1 year | 59·9 | 91·9 |
| D.1 + 0·8 per cent. of digitonin | | | 89·1 |

(A, B and C) and 2 tinctures (T.S. 1 and D1) prepared and stored by the authors. The potency of the first 3 tinctures, each of which contained 1 unit per ml. when fresh, was re-determined after storage by comparison with international standard digitalis powder and the potency of the last 2 tinctures compared with that of tinctures freshly prepared from the corresponding batches of dried leaf. The frog method as described in the British Pharmacopœia, 1948, using 48 frogs, was used and in each case loss of activity had occurred, the results (Table I) confirming those of Wokes[5].

To a portion of each aged tincture, digitonin was added in proportions ranging from 0·1 to 0·8 per cent., and these solutions were compared directly with the corresponding tinctures to which no saponin had been added. The results in Table I show that addition of saponin to aged

tinctures produces no significant change in potency. Similar groups of frogs were injected with digitonin only, in the same proportions as given with the various tinctures, and the digitonin alone was shown to have no measurable effect.

To confirm further that loss of absorptive ability was not the reason for the loss of activity, an intravenous method initially similar to that used by Trevan and Boock[25] was employed, 0·002 ml. of a diluted tincture being injected intravenously every 10 seconds, by means of a hand-operated micrometer syringe. The injection was intermittent and tedious to perform, therefore an electrically-operated continuous injection apparatus was constructed by coupling the end of the micrometer to a synchronous motor (Fig. 1). When in use the micrometer head was rotated once every minute

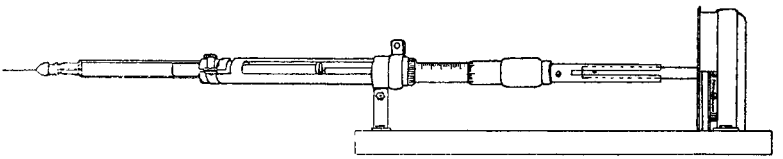

FIG. 1. Continuous injection apparatus.

and the plunger of the syringe pushed forward continuously to give a uniform injection at the rate of 0·01 ml. per minute.

Each test animal was partially submerged in 0·6 per cent. saline solution maintained at 27° C., in a large insulated tank, to avoid variations in susceptibility with changes in temperature[26,27,28]. The syringe passed through a hole in the side of the tank just above the surface of the saline solution. Because of the small size of the frog veins, special very fine needles, 0·011 in. diameter, were used.

*Method.* For each experiment the frog was weighed, then anæsthetised by injecting a 10 per cent. aqueous solution of urethane into the ventral lymph sac, at the rate of 0·04 ml. per g. of body weight. When completely anæsthetised, as evidenced by absence of reflex movements, the frog was pinned to a cork board and sufficient dissection performed to expose one of the prominent musculo-cutaneous veins[29] and also the ventricle of the heart. The frog was supported in the saline bath and the needle of the fully-charged syringe placed carefully into the exposed vein. A continuous injection of dilute tincture was given until the heart stopped in ventricular systole, the volume of solution injected being read from the micrometer.

By this method, employing comparable groups of healthy male frogs, aged tinctures of digitalis were compared with fresh tinctures made from the same raw materials. Some of the determinations were made on tinctures prepared from stabilised leaf and one was made on a simple ethanolic (70 per cent.) solution of digitoxin. To prepare the solutions for injection, 10 ml. of each tincture was evaporated to 5 ml. to remove most of the ethanol, the presence of which leads to irregular results in intravenous assays[7]. Further evaporation led to precipitation of colouring matter, etc. and an even suspension could not then be obtained on dilution. The

concentrate was diluted with 0·6 per cent. saline solution to give a 1 in 4, or in some cases a 1 in 6, dilution of the original tincture, the dilute solutions being prepared freshly each day to obviate errors due to any deterioration of digitalis principles in aqueous solution.[30] The injection of fresh and aged tinctures was alternated with successive frogs, animals of comparable weight being chosen.

The aged solution of digitoxin (sample B) and some of the aged tinctures which were assayed intravenously were also examined for loss of potency using the lymph-sac method and an aged solution of another sample of digitoxin (sample A) was also examined by the latter method. The results were taken by us to show that the loss of potency in tinctures of digitalis as shown by the frog is a real loss and is not due to failure of the absorption mechanism (see Tables II, III, IV).

TABLE II

TYPICAL RESULT OF A FROG INTRAVENOUS ASSAY

| Tincture | Weight of frog, g. | Volume of 1–6 dilution injected | Equivalent volume of tincture per 100 g. of frog ($x$) | Mean of $x$ (M) | Standard error (E) |
|---|---|---|---|---|---|
| T.S.1, aged | 22·4 | 0·1806 | 0·1344 | 0·1447 | 0·0107 |
| | 27·8 | 0·3038 | 0·1821 | | |
| | 22·4 | 0·1526 | 0·1135 | | |
| | 26·9 | 0·2074 | 0·1285 | | |
| | 29·1 | 0·1902 | 0·1089 | | |
| | 23·0 | 0·2450 | 0·1775 | | |
| | 25·4 | 0·2020 | 0·1326 | | |
| | 21·8 | 0·2354 | 0·1800 | | |
| T.S.1(C), fresh | 16·0 | 0·1104 | 0·1150 | 0·1137 | 0·005825 |
| | 25·2 | 0·1836 | 0·1214 | | |
| | 22·4 | 0·1454 | 0·1084 | | |
| | 26·2 | 0·1858 | 0·1182 | | |
| | 28·3 | 0·1492 | 0·0879 | | |
| | 27·8 | 0·2376 | 0·1424 | | |
| | 29·5 | 0·2120 | 0·1198 | | |
| | 21·7 | 0·1256 | 0·0965 | | |

Significance test.
$t = 2{\cdot}54$. $p = 0{\cdot}05 - 0{\cdot}02$.

$$\text{Loss of potency} = 100 - \left[\frac{0{\cdot}1137}{0{\cdot}1447}\right] \times 100$$
$$= 21{\cdot}44 \text{ per cent.}$$

It is noticeable that the decrease in potency during storage of digitalis tinctures is gradual and eventually a state of no further loss occurs[5]. If the loss is due to hydrolysis of the secondary glycosides then free digitoxose should be present in aged tinctures and it has in fact been reported to occur in some tinctures of digitalis[15]. To demonstrate the presence of sugars by means of paper chromatography, tinctures must be decolourised and at first the lead subacetate process was used. Some of the sugars were lost by adsorption and the final concentrate was still coloured, therefore the following method was used to give a colourless concentrate. 50 ml. of tincture was evaporated to 10 ml. under reduced pressure, then shaken with 4 quantities, each of 10 ml., of chloroform, to remove the green colouring matter, genins and unchanged glycosides and sufficient kieselguhr was added to the dark brown aqueous liquid to form a paste, which was dried in a vacuum oven at 60° C. The product was powdered, sifted and extracted continuously for 3 hours with dry acetone. The pale yellow

TABLE III

INTRAVENOUS ASSAY OF A SERIES OF AGED DIGITALIS TINCTURES

| Tincture | Age of tincture, weeks | Number of frogs used | Mean volume of tincture per 100 g. of frog | Standard error | Probability *t*-test, $p =$ | Potency lost, per cent. |
|---|---|---|---|---|---|---|
| D.3 | 28 | 3 | 0·3301 | 0·006413 | 0·02 | 24·1 |
| D.3 (*a*) | 0 | 3 | 0·2504 | 0·020310 | | |
| D.3 | 36 | 12 | 0·1732 | 0·009473 | 0·05–0·02 | 19·2 |
| D.3 (b) | 0 | 12 | 0·1399 | 0·010810 | | |
| D.3 | 60 | 8 | 0·1624 | 0·006711 | 0·01–0·001 | 18·4 |
| D.3 (*c*) | 0 | 8 | 0·1326 | 0·005678 | | |
| D.1 | 38 | 12 | 0·1487 | 0·006290 | 0·01–0·001 | 21·3 |
| D.1 (*a*) | 0 | 12 | 0·1170 | 0·007698 | | |
| D.1 | 51 | 8 | 0·1564 | 0·012270 | 0·01–0·001 | 29·6 |
| D.1 (*b*) | 0 | 8 | 0·1101 | 0·003932 | | |
| T.D.1 | 18 | 8 | 0·1766 | 0·006301 | 0·001 | 21·5 |
| T.D.1 (*a*) | 0 | 8 | 0·1386 | 0·006231 | | |
| T.D.1 | 33 | 7 | 0·1515 | 0·011020 | 0·1–0·05 | 19·1 |
| T.D.1 (*b*) | 0 | 7 | 0·1225 | 0·009113 | | |
| T.S.1 | 21 | 8 | 0·1417 | 0·011730 | 0·7–0·6 | 4·7 |
| T.S.1 (*a*) | 0 | 8 | 0·1350 | 0·005123 | | |
| T.S.1 | 24 | 9 | 0·1897 | 0·015640 | 0·4–0·3 | 10·9 |
| T.S.1 (*b*) | 0 | 9 | 0·1690 | 0·013840 | | |
| T.S.1 | 36 | 8 | 0·1447 | 0·010730 | 0·05–0·02 | 21·4 |
| T.S.1 (*c*) | 0 | 8 | 0·1137 | 0·005825 | | |
| Digitoxin solution | 145 | 8 | 0·1517 | 0·018970 | 0·7–0·6 | 5·9 |
| " " | 0 | 8 | 0·1427 | 0·008769 | | |

TABLE IV

COMPARISON OF FROG LYMPH SAC AND INTRAVENOUS ASSAY RESULTS ON A SERIES OF AGED DIGITALIS PREPARATIONS

| Tincture | Age of tinctures, weeks | Loss of potency, per cent. | |
|---|---|---|---|
| | | Lymph sac method | Intravenous method |
| D.1 | 51 | 59·9 | 29·6 |
| T.D.1 | 33 | 54·0 | 19·1 |
| T.S.1 | 36 | 46·2 | 21·4 |
| Digitoxin A | 25 | +7·3 | — |
| Digitoxin B | 145 | 13·2 | 5·9 |

extract was concentrated, then passed through a 10 g. column of alumina, eluting with dry acetone, the eluate being collected in 10 ml. fractions. As shown by adding digitoxose to tinctures, any of this sugar present would be found in the first 2 fractions of eluate, and these were concentrated to a volume of 1 ml., several drops of each concentrate being applied to paper for chromatographic separation, whilst pure digitoxose, in acetone, was applied to each paper to act as a marker. Using Whatman No. 1 papers, both ascending and descending development methods were used with butanol, ethanol and water (4:1:5)[31] or phenol (80 per cent. in water)[32] as solvents. After development for 7 to 8 hours, the papers were dried, then sprayed with a suitable reagent to give coloured spots with the sugars present. The reagents used included xanthydrol, guaiacol, β-naphthylamine[33], napthoresorcinol, α-naphthol, orcinol, phloroglucinol, resorcinol[34],

aniline phthalate[35] and benzidine[36]. 0·2 per cent. ethanolic solutions of the above phenols with 10 per cent. of orthophosphoric acid[37] were used and the paper heated for 2 minutes at 100° to 105° C. The first 4 mentioned reagents gave the best results, especially xanthydrol, which gave a pink colour with digitoxose, the paper becoming yellow, and guaiacol which gave a dark grey colour with digitoxose against a colourless background.

In 5 tinctures examined no digitoxose was found even when 1 l. of starting material was used, but when about 2 mg. of digitoxose was added to 50 ml. of tincture and the above process carried out, the digitoxose could be readily detected. 10 aged tinctures examined by us as described by Brindle, Rigby and Sharma[15] showed no sign of digitoxose although here again when a trace of this sugar was added to the tinctures its presence

TABLE V

COLORIMETRIC ASSAY OF AGED AND FRESH TINCTURES OF DIGITALIS AND SOLUTION OF DIGITOXIN

CHLOROFORM EXTRACT FROM 5 ML. OF TINCTURE IN 10 ML. OF KELLER-KILIANI REAGENT

| Tincture | Colour density (590 mμ) | | Equivalent of digitoxin, mg. | |
|---|---|---|---|---|
| | Aged | Fresh | Aged | Fresh |
| G.D | 0·562 | 0·507 | 0·477 | 0·430 |
| M.D | 0·650 | 0·571 | 0·552 | 0·485 |
| T.D | 0·414 | 0·388 | 0·351 | 0·329 |
| D | 0·485 | 0·470 | 0·412 | 0·399 |
| G.S | 0·459 | 0·450 | 0·390 | 0·382 |
| M.S | 0·600 | 0·572 | 0·509 | 0·485 |
| T.S | 0·531 | 0·480 | 0·451 | 0·407 |
| Digitoxin B | 0·429 | 0·432 | 0·365 | 0·365 |

could be detected. Since the secondary glycosides of digitalis are much more soluble in chloroform than the primary glycosides, experiments were carried out to determine if the total chloroform-soluble glycosides increased during storage of tinctures.

The estimation of glycosides in digitalis tinctures by the Keller-Kiliani method has been criticised[38], but since we were primarily interested in the sugar part of the molecule, and further were comparing pairs of tinctures made from the same raw materials, the spectrophometric method as described by Rowson[38] was used on 5 ml. quantities of tincture. Lead subacetate at the 50 mg. level of Pb to each ml. of tincture was used for clarification and the shaking with 6 quantities, each of 10 ml. of chloroform was done in a mechanical shaker to ensure equal extraction from the solutions under comparison. To the residue left after evaporation of the chloroform, 10 ml. of the freshly prepared Keller-Kiliani reagent was added and measurement of colour intensity after half an hour was made at wavelength 590 mμ. The 1 cm. cells of a Unicam S.P. 600 Spectrophotometer were used in all determinations and the reagent served as a blank. Results are summarised in Table V.

The colour development with time at 470 mμ and 590 mμ was determined on the tinctures and showed fair agreement with previous workers[21,38], who employed pure digitoxin. The absorption spectra of tinctures (Fig. 2)

showed that the highest peak was at 470 mμ and not at 590 mμ, thus differing from the spectra obtained by Rowson[38] and confirmed by us with digitoxin or pure digitoxose where peaks occur at the same wavelengths the highest being at 590 mμ. However, at all wavelengths in the range shown, the aged tinctures gave a higher colour density than the fresh tinctures, but the most stable colour and most reproducible results were obtained at 590 mμ; therefore readings were taken at this wavelength.

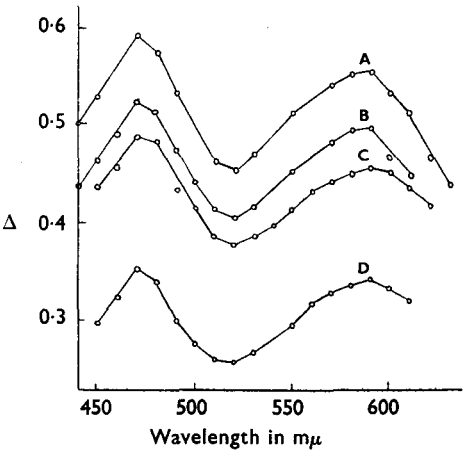


FIG. 2. Keller-Kiliani reagent and chloroform soluble glycosides from tinctures of digitalis.

A — Aged tincture prepared from dried leaf.
B — Fresh tincture prepared from dried leaf.
C — Aged tincture prepared from stabilised leaf.
D — Fresh tincture prepared from stabilised leaf.

(No comparison of colour density is intended between tinctures A, B, and tinctures C, D, since they were prepared from batches of leaf collected at different times.)

An ethanolic (70 per cent.) solution of digitoxin (B) which had been stored for 9 months at room temperature and a portion of the same solution which had been stored at room temperature for 8 months and at 55° C. for 1 month, were compared spectrophotometrically with fresh solution of digitoxin (B). 0·5 ml. of solution, corresponding to 0·365 mg. of glycoside, was diluted with 4·5 ml. of water and shaken with 6 quantities each of 5 ml. of chloroform. The mixed chloroform extracts were evaporated to dryness, the residue mixed with 10 ml. of Keller-Kiliani reagent and the colour density measured at 590 mμ after 30 minutes. The results in Table V show that no change had occurred in the glycoside solutions during storage.

Two samples of digitoxin, one supplied as containing 1369 units per g., the other 900 units per g., were tested biologically (frog lymph sac) and chemically (spectrophotometer) and while the samples were found equipotent by the chemical method, the biological assay showed them to be of quite different strengths, the potencies being of the order stated above.

## DISCUSSION OF RESULTS

On storage of several months, loss of potency occurs in digitalis tinctures but not in ethanolic (70 per cent.) solutions of digitoxin. The loss appears to be much greater when estimated by the lymph sac method than by an intravenous assay on frogs. However, a definite loss is indicated by the intravenous method, thus conflicting with the published results obtained

using cats. Also the addition of saponin to aged tinctures does not restore any of the lost potency determined by the lymph sac method and thus it is shown that disagreement between frog and cat assay results is not due to differences in absorption. The discrepancy can be accounted for only by assuming the presence in aged tinctures of substances with different relative potencies in frog and cat. Since no measurable quantity of free digitoxose could be found in aged tinctures and since the proportion of chloroform-soluble glycosides increased slightly on ageing, the change in potency did not appear to be due to hydrolysis of the secondary glycosides to genins. Biological and chemical assay results show that digitoxin in ethanolic (70 per cent.) solution is stable on storage, but the methods used gave quite different estimates of potency of the two samples examined. Conversion of primary into secondary glycosides would account for the higher chemical values of aged tinctures obtained by us and also by Goldstein[39], who used the alkaline picrate method of assay. It would also account for potency changes of tinctures as measured on frogs in which digitoxin and gitoxin are much less potent than purpurea glycosides A and B, respectively[15]. With cats, digitoxin is actually more potent than purpurea glycoside A[18] and therefore an increase, rather than a decrease, in potency might be expected on ageing.

## Summary and Conclusions

1. It has been confirmed that saponin has no effect on the results of the "overnight" frog method of assay for digitalis.

2. The loss of potency during storage of digitalis tinctures is a real loss of cardiac activity in frogs, since it is shown by an intravenous method of assay. This method detects 20 to 30 per cent. loss of potency during 12 months storage as compared with up to 60 per cent. loss as shown by the lymph sac method of assay.

3. A chemical assay based on the Keller-Kiliani colour reaction does not indicate loss of strength in digitalis tinctures during storage.

4. Tinctures prepared from stabilised digitalis leaf are not stable on storage.

5. Digitoxin in ethanolic (70 per cent.) solution is stable on storage.

6. In aged tinctures of digitalis loss of potency to frogs appears to be due to hydrolysis of primary glycosides to secondary glycosides without further breakdown to genins.

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## DISCUSSION

The two papers on digitalis were discussed together. The first paper was presented by MR. G. RIGBY and the second by MR. F. FISH.

MR. F. FISH asked Mr. Rigby whether they had looked for digitoxose in the tinctures. At the previous year's Conference they had reported finding it in two samples, but perhaps the reagent which they used this year was not capable of detecting digitoxose. He himself could not find it. It would have been useful had the authors given more experimental detail.

DR. J. M. ROWSON (London), dealing with the first paper, suggested that the dinitrobenzoate reagent and lead subacetate decolourisation, with a reasonably fresh tincture, gave a similar colour to the naked eye to that given by digitoxin or other glycosides. It was only when the tincture was old that the brown colour seemed to occur. He had re-examined a commercial tincture which he had examined 3 years previously, and had confirmed that it gave a much more brown colour than the many hundreds of tinctures, prepared freshly from leaves dried in his own laboratory. The dinitrobenzoate reagent gave two absorption peaks. Decolourised old tinctures gave a first peak which he had always found to be below 400 m$\mu$ rather than at 420 m$\mu$ quoted by Rigby and his colleagues. The method for the dinitrobenzoate estimation should have been given. The second peak employed in the assay process is clearly

defined at 535 mμ. No doubt the authors used the method reported in their own paper last year, but one could be led to believe that they were using the Kedde method. He asked for more details of the columns used. He had tried 2 methods—alumina, with and without charcoal, but only with charcoal had his filtrate been water-white. He wanted to know the column sizes in order to get the details correct for use. Whether the alumina method or the lead subacetate method of decolourisation was used, the results obtained by dinitrobenzoate seemed to be the same. The estimate of loss given in the paper—6 to 8 per cent.—was about the same as that which he had given in his paper two years ago, using lead subacetate. Last year he had expressed doubt as to whether the simple dinitrobenzoate estimation was adequate to determine the potency of ageing tinctures, but he thought that the chemical method might be employed to give further help. Could Mr. Rigby give the ages of his tinctures? Using the guinea-pig he had found a 25 per cent. decomposition after storage on the shelf for about 3 months. Did the authors suggest that there had been a formation of aglycone in the tinctures?

Turning to the second paper, he said that until the relationship between chemical and biological estimations was settled they would be faced with still further problems. The loss in ageing tinctures in the intravenous frog method was lower than in the lymph sac method. Possibly the extra factor of absorption across the lymph sac membrane had been eliminated. He agreed with Mr. Fish's thesis that the primary glycosides readily decomposed, losing glucose molecules, and that aglycones were not produced even in an ageing tincture. This was in agreement with work he had carried out on fermented leaf. If fermentation were allowed to proceed, there was complete breakdown of the secondary glycosides but at no stage did aglycone increase to any extent. This was supported by Wegner and other Continental workers. He had condemned the Keller-Kiliani reagent 2 years ago because extraction was not complete with chloroform, though he had shown that this was not so with chloroform-ethanol mixture. A second difficulty was that only two digitoxose molecules of the primary glycoside reacted with the reagent. With the primary glycoside broken down to secondary, all three digitoxose molecules would react. Thus Mr. Fish found his apparent increase in glycoside by ageing simply because glucose had been eliminated and 3 digitoxose molecules had reacted instead of 2. Mr. Fish had said that this apparent increase in glycoside might also explain Goldstein's results, but in fact Goldstein had been using the picrate method. The fact that Mr. Fish had found the peak at 470 mμ to be the higher was due to interaction of the aglycones of both gitoxin and digitoxin with the reagent and to the time elapsing before taking the reading. Had the author any comments on Table I, which showed that 3 tinctures 6 to 8 years old showed less loss of potency than others which were up to 12 months old?

DR. W. MITCHELL (London) remarked that Mr. Rigby had not given the ages of the commercial tinctures used. Was it not likely that even under more closely defined experimental conditions two possible variables

were the type of alumina used and adsorption on the precipitate caused by lead subacetate?

MR. H. DEANE (Long Melford) asked for the source of the commercial tinctures. Were they obtained from retail shops or from manufacturers?

MR. G. A. STEWART (Dartford) said that he had studied the B.P. methods with commercial preparations obtained soon after manufacture, but different results were obtained from the same tincture. It was probable that in clinical use patients standardised themselves to the tinctures used.

DR. G. FOSTER (Dartford) said that the majority of digitalis was used in tablet form, and he did not think they would progress by trying to develop a chemical assay on the total glycosides. He felt that chromatographic methods might be more helpful.

MR. RIGBY, in reply, agreed that the presence or absence of saponin did not appreciably affect the potency of the tincture when tested in frogs by the lymph sac method, but he thought it was an assumption to say that for this reason the effects of absorption from the lymph sac could be discounted in that type of assay. Many other factors were involved in the absorption of tinctures from the sac.

He had been placing quantities of digitoxose up to 0·4 mg. on top of the column and eluating with acetone and, with 70 per cent. ethanol, he had not found digitoxose in the eluates. The dimensions of the column were not mentioned in the second paper, but he and his colleagues had described the chromatographic alumina they had used, and had given the length, which was 10 cm. He considered this more valuable than giving the weight. It was better to make an estimation using dinitrobenzoate reagent in such a way that the colour developed was the true colour of the glycoside, but the lead subacetate precipitation method did not give that; a brown colour was obtained. Dr. Rowson's method estimated the components of the brown colour but it was untidy. He denied that Dr. Rowson's figures for loss of colour-active constituents were the same as the 6 to 8 per cent. given in the paper. In Dr. Rowson's work published in 1952 the losses were given as 15 per cent. He hoped that Dr. Rowson did not imply that aglycones were never present in the tincture, for fundamental consideration suggested that they were almost certainly present. He agreed with Dr. Foster that tablets were to be preferred to tinctures and that chromatography was probably one of the few hopes for an investigation of this sort. Replying to Mr. Deane, he said that tinctures were obtained from manufacturers or local wholesalers and he could not remember seeing any date of assay on them.

MR. FISH, in reply, said he was glad of the support of Dr. Rowson, who had been unable to find genins in the tinctures and who had suggested that the hydrolysis stopped at that point. Dr. Rowson's figures of 25 per cent. loss, using the intravenous guinea-pig method, agreed with the results by the intravenous frog method. In referring to Goldstein's paper, he had not meant to imply that the secondary glycosides themselves gave a higher reading than the total glycosides. Speaking of the time

of measuring colour densities, he said that at 470 mμ the colour developed much more quickly and reached a peak in 5 to 10 minutes, while at 590 mμ it took 30 to 40 minutes. In his experience, the colour density at 470 mμ remained higher than at 590 mμ for about 2 hours. Commercial tinctures showed a loss of from 16 to 39 per cent., whereas a tincture which he had prepared himself showed a 60 per cent. loss. The assays of the commercial tinctures had probably been done on the cat or guinea-pig and not on the frog, whereas he had tested his tincture, both in the first place and after several months storage, against frogs. He agreed with Dr. Mitchell that even if a chemical assay determined precisely all the glycosides they would not be much further forward unless they had a precise measure of the pharmacological activity of the various constituents. The tinctures he had used had been obtained from various commercial houses and the date of standardisation had been given on them. The authors hoped to publish a method using what they regarded as clinical end-points, to measure the cardio-tonic rather than cardio-toxic effects of digitalis, and employing the electro-cardiograph method on frogs. He said that the assay method for digitoxin had been the 18-hour method, and he still could not find digitoxose unless he added it first.

MR. RIGBY, further in reply, pointed out that last year they had been decolourising by the lead subacetate method, but when they used an alumina column they found no digitoxose.