STUDIES IN THE GENUS DIGITALIS

PART IV. THE INFLUENCE OF FERTILISERS AND OF LIME ON *D. purpurea*

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THE influence of fertilisers upon the growing plants of *Digitalis purpurea* has been investigated by a number of workers and a number of contradictory results have been reported. The larger number of these experiments have been field trials, directed to an increase in the weight of leaf produced by unit field area: fewer experiments have been concerned with the influence of fertilisers upon the therapeutic potency of the leaf produced, as indicated by biological assay or by glycosidal content. Sand culture or water culture investigations of this species are very few.

An unfavourable influence upon weight of crop by stable manure has been shown by Boshart[1], whereas Dauphinée[2] found cow manure to be the best fertiliser for increasing leaf yield. Ammonium sulphate was considered to be very useful by Boshart, but Dauphinée did not find it outstandingly valuable, although it was better than no fertiliser at all in its influence upon crop yields: Mascré[3] showed that an optimum level of treatment with ammonium sulphate existed, which should not be exceeded. Koch and Butler[4] found that, on a clay loam, the best results were obtained by using a mixed fertiliser containing sodium nitrate as the only source of nitrogen and they considered this salt to be the most essential single fertiliser. The same workers found calcium acid phosphate to be the most important single fertiliser for increased leaf yield when the plants were grown in sand. Mascré considered potassium to exert the most effect upon leaf yield, and phosphates also gave good increases; Boshart agreed that both potassium and phosphate exerted a favourable influence upon leaf yield but he considered that influence to be less marked.

Dauphinée, using the cat and guinea-pig methods of assay, concluded that plants treated with cow manure were of the highest quality; whilst Boshart found that stable manure had an unfavourable influence upon activity of the leaves. Parisis[5] showed that the most active galenicals were obtained from *D. purpurea* plants grown in soils treated with a mixed fertiliser containing nitrate, phosphate, calcium and magnesium ions but Dauphinée has reported that superphosphates appeared to depress leaf activity. It is unsafe to expect a parallelism in behaviour to fertilisers between co-generic species but in passing it may be noted that Court and Allemann[6] found a decrease in activity in leaves of *D. lanata* when plants were treated with stable manure, whereas artificial fertilisers, especially those containing nitrogen, produced an increased activity in leaves of this species.

Digitalis purpurea occurs naturally on acid soils[7], it is regarded as a marked calcifuge[8] and is seldom found in the wild state on limestone or

chalky soils. Siegfried[9] commented on this fact and found that the species could nevertheless be cultivated on calcareous soils. Boshart grew these plants near Munich in a soil naturally containing 0·5 to 1·0 per cent. of chalk, it was necessary to raise the plants in a seedbed and then transplant, after which they grew well. The complete fertiliser used by Koch and Butler for optimum crop yields on clay loam contained over 50 per cent. of chalk and was applied at a level of 1000 lb. chalk per acre ($3\frac{1}{3}$ oz. per square yard). James[10], reporting on drug cultivation in America, has stated that the lime-deficient soils of New England are dressed with lime before digitalis cultivation, which results in an increased crop yield. Sanna[11] found that this species would tolerate soils containing 5 to 6 per cent. of calcite but even such a small proportion of calcium resulted in a decrease in glycosidal content of the leaves. Court and Allemann state that *D. lanata* occurs naturally in Switzerland on lime-containing dolomitic soil but that when such plants were cultivated on light, humus-containing soils, poor in lime, the crop yield and activity were much higher. Duquénois[12] planted out seedlings of *D. purpurea* into infertile calcareous garden soil, *p*H 7·2 to 7·3, in Strasbourg and 2nd year leaves were collected for assay by the guinea-pig method. Control plants were taken from their natural sites on the eastern slopes of the Vosges and the potencies of both wild and experimental materials were found to be almost identical in 1948 and again in 1950, although the crop yield from the calcareous soil was low.

Such a conflict of views upon the influence of artificial fertilisers and of lime upon leaf yield and the limited number of results dealing with their influence upon leaf potency have led to the present investigation.

Experimental

All cultivation experiments have been carried out in the Museum experimental grounds, Birdsgrove House, Mayfield. The soil was a light sandy loam in good heart which had been previously used for potato production. Strains of seeds of *Digitalis purpurea*, used in other selection and breeding experiments, have been employed; seedlings were raised in a heated greenhouse, were hardened off and were then planted out onto the prepared beds. In the fertiliser and the lime experiments 9 test plants formed a single block and adjacent blocks were separated by guard rows of plants which were not examined at any stage. Leaves were harvested from 1st year plants in late September in the late afternoon of a sunny day; for each plant any leaves on the outside of the rosette which were damaged or darkened in colour were rejected, also juvenile leaves and buds in the centre of each crown were not collected. The bulked leaves collected from plants in a block were immediately transferred to the drying shed and were rapidly dried in a forced draught at 55° C., after weighing they were powdered, returned to the drying shed for a further 12 hours and then sealed in screw-capped bottles containing silica gel[13]. Samples were estimated by means of the dinitrobenzoic acid method[14,15], which has been shown to yield results parallel to those given by the guinea-pig biological assay method when applied to leaf samples grown and

processed under similar conditions. Results have been expressed in terms of International Units of activity by comparison with the Standard Preparation of digitalis; moisture contents of samples varied only between 4 and 5 per cent. and these have not been considered in calculating results.

Fertiliser Trials

In the three years 1951, 1952, 1953, replicate factorial experiments using randomised blocks[16] were set up to investigate the influence of nitrogenous, phosphatic and potassium (NPK) fertiliser treatments. The fertilisers employed and their levels of treatment are set out in Table I. In each instance the appropriate amounts of fertiliser were worked into the blocks several days before planting out the seedlings.

TABLE I

ARTIFICIAL FERTILISERS (OZ. PER SQUARE YARD)

	1951	1952			1953	
		Low	Medium	High	High	Double High
N (sulphate of ammonia) ..	2	3	4	6	6	12
P (superphosphate)	1½	2	3½	5	12	24
K (sulphate of potassium) ..	¾	1	2	3	6	12

The 1951 experiment was carried out in triplicate at the one level of fertiliser treatment, which was that of normal horticultural practice. In 1952 duplicate experiments were carried out at three fertiliser levels, the "low" level being a little higher than that employed in 1951; the "high" level N content was double that of "low" level whilst P and K treatments were proportionately more increased. The levels of treatment selected

TABLE II

NITROGENOUS FERTILISERS 1954 CROP (OZ. PER SQUARE YARD)

Fertiliser	Base dressing	Top dressing		Total
		(*a*)	(*b*)	
Sulphate of ammonia ..	1	1	1	3
Nitrate of soda	1	2	1	4
Dried blood	2	2	1	5
Hoof and horn meal.. ..	3	2	0	5

for 1953 experiments were excessively high, with proportionately greater increases in the amounts of P and K; the randomised blocks were laid out in duplicate: also further duplicate blocks were prepared in which lime at the rate of 4 oz. per square yard had been previously worked into the soil to a depth of 9 inches. The design of experiment using 5 replications of the randomised block system was again employed in 1954 but was directed to an investigation of the behaviour of four different nitrogenous fertilisers. Amounts of fertilisers containing the same equivalent nitrogen content were taken and were applied partially as a base dressing before planting out and partially as top dressings during the growth of the plants. Treatments employed were those shown in Table II.

TABLE III

ARTIFICIAL FERTILISERS

Weight of dry leaf per plant (g.)

	1951	1952			1953			
		Low	Medium	High	High	High + lime	Double High	Double High + lime
Control	71	5	5	4	48	47	67	46
N	55	5	6	5	53	47	56	39
P	64	8	8	9	43	49	71	46
K	74	5	6	5	41	57	66	46
NP	75	6	4	6	60	71	77	53
NK	61	5	8	8	44	54	46	44
PK	87	5	7	9	43	41	59	47
NPK	62	7	7	9	44	51	37	48

TABLE IV

NITROGENOUS FERTILISERS 1954

Weight of dry leaf per plant (g.)

Fertiliser	Replicate blocks					Average
	1	2	3	4	5	
Control	29	27	19	22	18	23
Sulphate of ammonia	16	11	24	21	21	19
Nitrate of soda	10	11	15	14	10	12
Dried blood	53	23	31	26	15	30
Hoof and horn meal	29	27	29	28	24	27

TABLE V

ARTIFICIAL FERTILISERS

Activity of leaf (I.U./g.)

	1951				1952			1953			
	1	2	3	Average	Low	Medium	High	High	High + lime	Double High	Double High + lime
Control	16·0	14·7	17·3	16·0	11·2	10·4	10·6	11·7	10·9	11·9	11·2
N	15·2	18·2	17·1	16·8	10·6	11·1	10·5	13·1	13·5	12·2	12·0
P	15·9	14·3	16·4	15·5	11·3	10·8	11·4	10·7	13·3	12·3	10·6
K	15·0	16·4	17·0	16·1	10·6	10·5	10·8	11·9	12·4	11·2	11·0
NP	19·3	15·5	15·9	16·9	11·4	9·7	10·1	11·7	13·5	13·2	11·6
NK	15·3	15·5	15·2	15·3	10·4	10·5	9·7	13·0	11·9	12·8	11·6
PK	15·6	18·3	16·8	16·9	10·7	10·2	11·2	12·7	11·5	12·4	12·1
NPK	18·0	16·4	15·2	16·5	11·7	10·5	12·2	12·3	11·1	11·8	11·4

TABLE VI

NITROGENOUS FERTILISERS 1954

Activity of leaf (I.U./g.)

Fertiliser	Replicate blocks					Average
	1	2	3	4	5	
Control	11·8	9·6	10·4	8·9	9·1	10·0
Sulphate of ammonia	9·8	9·1	10·0	7·9	8·6	9·1
Nitrate of soda	9·6	10·7	9·9	8·4	8·0	9·3
Dried blood	10·5	10·0	10·6	9·2	9·6	10·0
Hoof and horn meal	10·8	9·3	10·1	9·2	10·4	10·0

Digitalis purpurea strain B.26 was employed in 1951 and 1952, strain B.55 was used in 1953 and B.42 in 1954. The activities, in terms of International Units per g., of leaves taken from similar plants of these strains grown for other experiments in the same years were B.26, 1951, 15·6, 1952, 10·3; B.42, 1954, 10·4. Values for weight of dry leaf per plant, and for activity of leaf are given in Tables III to VI for all experiments and controls.

Liming Trials

Experiments were laid down in both 1951 and 1952 to show the effect of various quantities of lime on both leaf yield and activity, using randomised blocks. In 1951, lime levels from 2 oz. to 64 oz. per square yard were employed, the hydrated lime being lightly raked into the surface of the soil before the seedlings were transplanted. In 1952, levels of lime from ¼ lb. to 10 lb. per square yard were used, and these were worked into the soil by lightly forking, thus ensuring a somewhat deeper penetration of the lime. In 1953 similar levels of lime to those used in 1952 were employed but the lime was deeply dug into each plot and duplicate experiments were carried out. In 1954 three levels of lime at 3 lb., 6 lb. and 9 lb. to the square yard were used, and this was forked into the top six inches of soil, three replicate experiments being carried out.

The application of lime at different soil depths was investigated since the plants of *D. purpurea* possess a dense, shallow, fibrous root system mainly situated in the top 4 to 6 inches of soil, and seldom penetrating deeper.

At the time of harvesting the 1st year plants, *p*H readings of soil at 3, 6 and 9 inch depths were taken and these are recorded in Table VIII; the results for all experiments giving weight of dry leaf per plant and activity expressed in International Units per g., are given in Table VII. Strains of plants employed were B.155 in 1951, B.62 in 1952, B.55 in 1953 and B.22 in 1954.

TABLE VII

HYDRATED LIME TREATMENTS

Soil *p*H at a depth of 3 inches. Weight of dry leaf per plant (g.). Activity of leaf (I.U./g.)

Lime per sq. yd.	1951			1952			1953			1954		
	*p*H	Leaf	Activity	*p*H	Leaf	Activity	*p*H	Leaf	Activity	*p*H	Leaf	Activity
Control	6·5	40	12·0	6·7	19	11·1	6·2	33	10·0	6·2	6	8·7
2 oz.	6·5	51	11·4									
4 "	6·8	52	11·4	6·7	32	12·7						
8 "	6·5	40	11·4	7·5	35	14·5	6·5	35	9·8			
12 "	7·0	43	12·9									
1 lb.	7·0	53	12·9	7·5	30	14·4	6·5	39	9·9			
1½ "	6·8	44	12·0									
2 "				7·0	24	12·8	6·7	38	9·5			
2½ "	6·8	40	12·3									
3 "				7·5	24	14·2	6·5	18	9·7	7·2	4	9·3
4 "	7·5	38	12·6									
5 "				7·5	12	12·5	6·2	27	9·2			
6 "										6·7	3	10·2
7 "				7·5	25	15·9	7·2	34	10·6			
9 "										7·4	3	9·0
10 "				7·5	27	14·1	7·1	35	10·8			

TABLE VIII

SOIL *p*H BY DIFFERENT METHODS OF LIMING

	1951	1952	1953	1954
Weight of lime (sq. yd.)	2 oz.–4 lb.	¼–10 lb.	¼–10 lb.	3–9 lb.
Method of liming	light raking	light forking	deep digging	forking (6 in.)
*p*H Soil surface	6·7–7·5	7·0–7·5	—	—
3 inches below surface	6·5–7·5	6·7–7·5	6·5–7·2	6·7–7·4
6 " " "	6·3–7·0	7·2–7·5	6·5–6·7	6·6–7·6
9 " " "	6·5–6·8	6·8–7·4	6·0–6·7	6·0–6·6
*p*H Control	6·5	6·7	6·2	6·2

DISCUSSION

The level of artificial fertilisers for the 1951 trials was relatively low and the influence upon weight of leaf produced per plant was not marked; the only suggested stimulus to growth occurred when treatments of PK were applied (Table III). The plants harvested in 1952 were small, due to late planting out and to unfavourable weather conditions and it is probably unsafe to draw any firm conclusions as to the influence of artificial fertilisers upon weight of leaf per plant from these materials. There is however a suggestion that P at each of the three levels employed had stimulated growth and that NK, PK and NPK at "medium" and "high" levels of fertiliser had resulted in increases in plant weight.

The relatively poor increases in weight of leaf obtained in the first two years of these trials, despite increasingly heavy fertiliser treatments, suggested that the low *p*H of the soil in the experimental gardens or the lack of calcium were probably limiting factors, and hence added calcium was employed in 1953, a rapid release of ammonia being anticipated. The only significant increases in leaf weight per plant were found with NP treatment either with or without added lime. In general terms the first three years of factorial experiments with artificial fertilisers have not produced any striking increases in crop weight, even though uneconomically high levels of artificial fertilisers have been applied at times, whilst the application of ammonium sulphate alone has at times resulted in a small decrease in weight of leaf compared with the controls.

The 1954 trials reported in Table IV have shown that, for equal nitrogen content and applied at economic levels, the two organic fertilisers were significantly more effective than were the two inorganic nitrogenous fertilisers. Thus dried blood appeared, under the conditions of experiment, to produce a marked increase in weight of dry leaf, and hoof and horn meal produced a somewhat smaller but nevertheless definite increase: sulphate of ammonia failed to produce any increase in weight of dry leaf and in fact some marked decreases in weight occurred; nitrate of soda was still more marked in producing lower weights of dry leaf per plant than in the controls. These results are thus in direct contradiction to those of Koch and Butler[4] although the type of loam employed in our experiments differed markedly from the clays employed by those workers.

The influence of artificial fertilisers upon activity of leaf is set out in Tables V and VI. In 1951 the relatively low levels of fertilisers showed

no pronounced augmentation in activity although some slight increases were found using N, NP, PK and NPK. In the 1952 experiments N at "medium" level, P at "high" level, and NPK at both "low" and "high" levels produced some small increases in activity. In 1953 some increases in activity were found with N at "high" and "high + lime" levels, with P at "high + lime" level, with NP at "high + lime" and "double high" levels, with NK at "high" level and with PK at "high" and "double high + lime" levels.

None of the increases in activity were consistent and marked although "high + lime" and "high" fertiliser alone were the most marked. Of the eight different randomised block treatments recorded in Table V, four increases were found for N treatment, three for P in the presence of N, and three for P in the presence of K. It may thus be concluded that N, P and K have some influence upon activity of leaf in *Digitalis purpurea*, especially at medium to high levels of treatment. This augmentation is, however, not marked and does not appear to be of great economic value.

Although the organic nitrogenous fertilisers produced greater increases in leaf yield than the inorganic fertilisers employed in 1954, the difference in activities of such leaf groups was not so marked. Certainly the activities of leaf samples from both sulphate of ammonia and nitrate of soda treatments were somewhat lower than those of control or organic-fertiliser-treated samples but the differences shown in Table VI are too small to be significant.

Treatments with hydrated lime were most effective when lightly dug into the top 6 inches of soil and under such conditions the soil *p*H values at depths of 3 and 6 inches were changed from 6·2 to 6·7 to as high as 7·5 (Table VIII). Deep digging-in of the lime was much less useful in influencing the *p*H of the upper levels of the soil in which the digitalis roots are located. It will be noted that this latter method of lime application was used in 1953 and the results for leaf yield and leaf activity, as recorded in Table VII for that year, do not parallel those obtained in the experiments carried out in other years. No evidence can be produced in this work to distinguish between the influence of calcium and of hydroxyl ions in the lime treatments, and both may well be significant.

The present investigation confirms the conclusions of other workers that seedling plants of *D. purpurea* will grow satisfactorily when planted out into a soil made distinctly alkaline by the presence of lime. Such plants grow as vigorously as the controls when lime treatments up to 10 lb. per square yard have been applied, with no loss in leaf yield; rather, with lime treatments up to 1 lb. per square yard, a definite increase in leaf yield per plant has been recorded and is shown in Table VII. The activity of these lime-treated plants has never been depressed as compared with the controls and thus the work of Duquénois[12] is confirmed. Conversely, there is distinct evidence of increases in glycosidal content in these plants and, in 1952, plants grown in soil treated with 7 lb. of lime per square yard were found to contain 15·9 International Units of activity whilst the controls showed 11·1 Units of activity.

Summary and Conclusions

The influence of fertilisers upon the 1st year leaves of *Digitalis purpurea* plants grown in light sandy garden loam, *p*H 6·2 to 6·7, are as follows:

1. Sulphate of ammonia in low, medium or high levels of treatment does not increase leaf yield: at medium and high levels it may fractionally increase glycosidal content of the leaves. This is most marked in the presence of lime.

2. Nitrate of soda decreases leaf yield but exerts no influence upon glycosidal content of the leaves.

3. Dried blood and, to a less marked extent, hoof and horn meal increase leaf yield but do not influence glycosidal content of the leaves.

4. Phosphatic fertiliser alone and in association with potassium sulphate or with sulphate of ammonia may produce a small increase in leaf yield and in glycosidal content of the leaves.

5. Hydrated lime in quantities up to 1 lb. per square yard, with change of soil *p*H up to 7·5, increases leaf yield, and quantities up to 10 lb. per square yard are tolerated by the plants without decrease in leaf yield. Under similar conditions glycosidal content of leaves is never depressed and generally an increase has been recorded, a maximum augmentation to 143 per cent. being obtained with a treatment of 7 lb. of lime per square yard.

6. The remarkable constancy of glycosidal content of leaves under all treatments excepting that with lime is most significant.

7. Normal horticultural NPK fertilisers containing organic nitrogen together with hydrated lime are recommended for optimum plant growth.

These investigations are being continued.

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References

1. Boshart, *Pharm. Monatsch.*, 1931, **12**, 203.
2. Dauphinée, *Amer. J. Pharm.*, 1924, **96**, 796.
3. Mascré, *Pharm. Monatsh.*, 1931, **12**, 204.
4. Koch and Butler, *Amer. J. Pharm.*, 1919, **91**, 211.
5. Parisis, *Atti soc. ital. progresso sci.*, 1935, **23**, 136.
6. Court and Allemann, *Pharm. Acta Helvet.*, 1943, **18**, 369.
7. Clapham, Tutin and Warburg, *Flora of the British Isles*, Cambridge, 1952, p. 874.
8. Tansley, *The British Islands and their Vegetation*, Cambridge, 1939, p. 285.
9. Siegfried, *Pharm. Acta Helvet.*, 1943, **18**, 535.
10. James, Oxford Medicinal Plants Scheme, Drug Plant Research in the U.S.A., p. 9.
11. Sanna, *Atti V congr. nazl. chim. pura applicata Rome*, 1935, **1**, 208.
12. Duquénois, *Ann. Pharm. franç.*, 1952, **10**, 177.
13. Rowson, *Pharm. J.*, 1954, **172**, 472.
14. Rowson, *J. Pharm. Pharmacol.*, 1952, **4**, 814.
15. Rowson, *ibid.*, 924.
16. Hayes and Immer, *Methods of Plant Breeding*, McGraw Hill, 1942, p. 307.

DISCUSSION

The papers were presented together by THE AUTHOR.

DR. F. FISH (Glasgow) said that the author seemed to be recommending the use of extemporaneously made tinctures. He disagreed with the use of the tincture, although if a doctor prescribed it, it must be dispensed. A patient could not be trusted to measure out 15 drops of a tincture and dilute it with water as required, though he could be trusted to take tablets as directed. The work on fertilisers needed to be continued much longer before a clear picture would be obtained. It appeared that in one year the fertiliser gave a better leaf yield than lime, while in another year the same fertiliser gave a poor yield. He thought that the number of plants used—nine—was too small.

MR. G. R. A. SHORT (London) agreed with Dr. Fish that nine plants were rather few, and he wondered whether they could be supplemented by undertaking some experiments in sand culture.

DR. G. RIGBY (Manchester), referring to the variable factors encountered in the biological assay, said that in Manchester they had performed similar tests and found similar effects. The reason might be, as Dr. Rowson found, that alteration of ethanol concentration produced no alteration in its extinction. They, on the other hand, found that as the ethanol concentration was increased from 35 per cent. to 75 per cent. by volume, the extinction of a reaction mixture containing a constant weight of e.g., digitoxin, progressively fell. This, they felt, was related to the essentially ionic nature of the reaction itself, which might be retarded in the mixture containing the lower proportion of water. He suggested that the amount of water in the mixtures assayed varied considerably.

MR. K. L. SMITH (Nottingham) said that he was surprised the biological assays agreed so well. He had intended to recommend Dr. Rowson to forget about chemical assays but he would postpone making that recommendation until the chemists could agree among themselves.

PROFESSOR H. BRINDLE (Manchester) said that Dr. Fish had dismissed the use of tincture of digitalis too readily. Had he evidence to show that, in the human subject, powder of digitalis was equivalent in action to the tincture? When the powder was administered, the patient's alimentary tract was used as the extraction apparatus, and this varied considerably. A more constant result would be obtained from the tincture, of which the potency was known.

DR. F. J. ELLIOTT (Edinburgh) referred to the importance of root development.

MR. A. D. POWELL (Nottingham) suggested that the lime in Dr. Rowson's experiments might have acted as a soil corrective and improved the tilth of the soil, without itself being a growth substance.

DR. F. FISH (Glasgow) said that Professor Brindle, in observing that there was a considerable variation between patients, had put his finger on the main point. It was for the clinician to decide what kind of preparation was to be given to a patient. Most patients were left to dose themselves with digitalis. Nurses could be trusted to measure doses fairly

accurately, and the use of fresh tincture was therefore practicable in hospitals. But, if the tincture were diluted with water, as was often required, its potency would be lost in about a day.

DR. ROWSON, replying, said that he should apologise for having held up publication of the first paper for about two years, but he had published a summary in *The Pharmaceutical Journal* last year. He was sorry that the tincture was under a cloud, especially as tincture was by no means as liable to final breakdown of primary glycosides to secondary as they had thought two years ago. After an initial breakdown it was probably that the tincture remained constant for a long period. He thought that Dr. Fish went too far in his comments on the variation in results from one year to another. In 1952 they had had bad weather, and at Birdsgrove House the plants had not grown so well. In 1951 there was a good yield of leaf, and 1952 was poor. In 1953 the weights were lower because they had done their planting later. Concerning the number of plants, they tried to run three replicate blocks. Last year there were five, so they were getting a reasonable number of plants. The cultivation would be extended, and at a later stage they would have to go to the drug growers for help in cultivation. They were attempting a little sand culture this year, and were also trying to grow the plants in limestone country. He had tried planting straight on to lime soil, but without success. He did not think that changes in the tilth of the soil was the explanation for the effect of lime. He said that he would not commit himself as to the ionic nature of the dinitrobenzoic acid colour reaction. He did not believe that colour intensity was greatly influenced by change in the ethanol concentration of the reaction mixture, but all reaction mixtures quoted in the first paper had about the same ethanol content. He thought that under controlled conditions of plant cultivation and processing the chemical estimation gave results that were parallel with those of the biological estimation. He was pleased to note that Mr. Smith considered the results satisfactory and Dr. Rowson agreed that the chemical method might well replace the biological assay of digitalis leaf. The limits of error of the chemical method were lower than of the biological method.