T. subterraneum would always correspond closely in composition with the amide and amino-acid values we have obtained in this one instance. In so far as it may be safe to generalize from the data at present available, the onset of senescence appears to have been accompanied by an increase in the cyst(e)ine content (and possibly in the tyrosine content) and a decrease in the methionine content of the whole protein in the leaf material. We do not know whether the compositions of individual proteins in the leaves have undergone change. It is possible that the only change has been in the proportions of the individual proteins. In this connexion it is pertinent to point out that change in haemoglobin composition with transition from foetal to adult mammalian life (as shown by Vickery (1944) for the histidine contents of foetal and adult bovine haemoglobins) may be due to a change in the ratio of two (or more) haemoglobin components (demonstrated for foetal and adult human material by Andersch, Wilson & Menten, 1944).

Effects of plant age and treatment (such as grazing) upon the cyst(e)ine and methionine contents of leaf proteins were judged by Smith & Wang (1941) to be fairly pronounced, but the validity of their conclusions was questioned by Lugg (1943) on the ground that the analytical methods they used would be highly unreliable with the impure leaf-protein preparations they analyzed. That changes in

the cyst(e)ine and methionine contents of the whole protein in the leaves of various plants may occur, particularly at the extremes of plant age, seems very likely, but our earlier work, covering considerable ranges of the intermediate ages of plants, has revealed no definite, progressive changes.

SUMMARY

1. The amide, tyrosine, tryptophan, cyst(e)ine, methionine, arginine, histidine and lysine contents of protein preparations from senescent leaves of *Trifolium subterraneum*, expressed as percentages of the protein N occurring in these forms, were found to be 5.39–5.71, 2.80–2.90, 1.65–1.85 (best 1.80), 1.42–1.47, 1.02–1.13, 13.5, 4.26 and 5.72, respectively.

2. From comparison of these values with those previously found for non-senescent material (arginine, histidine and lysine values were not available) and from other considerations, it has been concluded tentatively that the onset of senescence was accompanied by an increase in the cyst(e)ine and possibly in the tyrosine content, and a decrease in the methionine content of the whole protein in the tissues.

We wish to thank Mr D. S. Riceman for providing the leaf material used in this work.

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Nicotinic Acid in Cereals

1. THE EFFECT OF GERMINATION

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Nutrition surveys have shown that under present conditions dietary supplies of nicotinic acid are being obtained largely from cereals. These vary widely in their nicotinic acid content. Maize and oats are poor sources, rice and barley are better and whole wheat is probably the best of the staple cereals.

Kodicek (1940) found that chemical methods gave higher results for maize than biological methods, and suggested that better agreement might be obtained if extraction were carried out with hot water instead of acid or alkali. Melnick (1942), reporting the results of a collaborative study, stated that with acid extraction chemical methods gave higher results than

microbiological assays for low-extraction wheat flour. With aqueous extraction to exhaustion lower results were obtained by both methods, the chemical still being higher than the microbiological. James, Norris & Wokes (1947) confirmed Melnick's findings with acid extraction for low-extraction flour, and extended them to maize and yeast, using a modified chemical method which gave satisfactory agreement with microbiological assays for whole wheat, barley and other foods. Various American workers (Oser, Melnick & Siegel, 1941; Cheldelin & Williams, 1942; Krehl & Strong, 1944) have reported the presence in cereals of so-called precursors of nicotinic acid which, although water-soluble, are inactive microbiologically. Hydrolysis with acid or alkali, or enzyme treatment converts these 'precursors' into microbiologically active substances. Other American workers (Burkholder & McVeigh, 1942; Burkholder, 1943; Davis, Laufer & Saletan, 1943) have shown that the nicotinic acid content of wheat, barley, oats and maize increases after germination, thus indicating the occurrence of a precursor possibly related to the so-called precursor of the previous workers. We thought it desirable to study by chemical as well as by microbiological methods the day-to-day changes in nicotinic acid content during germination, and thus obtain a more complete picture.

METHODS

Chemical assays

These were made by the method described by James *et al.* (1947). The coefficient of variation of a single assay averaged 3.6 in 310 consecutive assays on 97 samples of cereals and cereal preparations.

Microbiological assays

The method of assay using *Lactobacillus arabinosus* 17/5 as test organism, first described by Snell & Wright (1941), was later modified by Krehl, Strong & Elvehjem (1943) and by Barton-Wright (1944). The latter method, or that described in a Report to the Society of Public Analysts (1946), was used in the present work. Results have been calculated by reference to the equation for the standard curve, calculated by the method of least squares, or by comparison of the slopes of the similarly calculated standard and sample curves. The latter method (Wood, 1945, 1946) is to be preferred, but valid assays will give closely agreeing results by either method. In some instances the limits of accuracy were calculated by the elegant, but somewhat lengthy, method of Finney (1945).

In 364 assays on 31 samples (see Table 1) the average coefficient of variation of a single assay lay between 6 and 7%.

Germination methods

Laboratory scale. Selected seeds (50–75 g.) were planted in sterile sand in Petri dishes, moistened with distilled water and grown in the dark in a warm room or at 27°. At

the end of the germination period (which was varied in different experiments) the seeds were washed free of sand with distilled water, and the degree of germination was determined by observing in each seed the ratio of the length of the shoot (acrosipire) to the length of the seed, as in the method of the Association of Official Agricultural Chemists (1945), but using an extended scale (i.e. 1–2, 2–4, above 4) to cover the greater amount of germination obtained. The seeds were sorted into groups according to this scale of acrosipire ratios and the percentage in each group was calculated. The seeds were then heated at 70–80° overnight and the average weight per seed was determined for different degrees of germination by weighing the whole of the seeds in each group. The seeds were then ground to no. 60 powder and the moisture content determined by heating at 105° for 3 hr. This was used to determine the average dry weight per seed.

Large scale 'stocking method'. The seeds were placed in calico bags containing 6–7 lb. each. These were first steeped at 10–12° for 1–4 days, according to the material, and then placed on the germinating floor under the same conditions as in the normal malting of barley. Malting was allowed to proceed as usual, records being kept of temperature and moisture additions. Each day one of the bags was removed to the kiln to check enzyme action. The kilned seeds were then sent to King's Langley. Here, weeds and other foreign matter, of which there was not more than 1%, were removed by hand. The given batch was sampled and the samples were examined in the same manner as the laboratory-germinated samples to determine the degree of germination. The average dry weight per seed in this batch was determined by weighing representative samples of 50–100 seeds. At least 9 such samples were taken. The coefficient of variation of the mean, calculated from the individual results on these 9 samples, lay between 1 and 2 (see Table 6). Before assay, a portion of the batch was ground to no. 60 powder, care being taken to ensure that the whole of the material passed through the sieve and was thoroughly mixed. This powdered material was stored in air-tight bottles and used for assay as soon as possible. Malted cereals were found to keep better in the unground condition.

Preparation of malt extracts

Since kilning had checked enzyme action without destroying the enzymes, the action of the latter could continue during the preparation of malt extract. In regard to barley, evidence had already been obtained (Klatzkin, Norris & Wokes, 1946) showing that in the brewing of malt extract more riboflavin might be produced. We therefore thought it of interest to examine this possibility for nicotinic acid. The malt extracts were prepared by methods ensuring the maximum yield of protein and aneurin (see Organ, Duncan, Westbrook & Wokes, 1943), since this might also be expected to ensure high yields of other B vitamins.

RESULTS

Barley

Large-scale experiments

Six typical batches of English barley were examined before and after large-scale malting, and the malt extracts made from the respective malted

barleys were also assayed. Mean results by the chemical method (see Table 2) showed no significant increases in nicotinic acid content at either stage.

Table 1. *Mean results by microbiological assay for the nicotinic acid content of samples*

B = Barley. M = Malted barley. ME = Malt extract.

Sample	Days germinated	No. of assays	Mean content ($\mu\text{g./g. dry wt.}$)
B 25	0	19	87 $\pm$ 2.2*
M 25	12	15	103 $\pm$ 1.9
ME 25	—	23	105 $\pm$ 2.6
B 32	0	6	102 $\pm$ 3.5
M 32	12	5	123 $\pm$ 2.8
ME 32	—	6	114 $\pm$ 2.2
B 35	0	19	109 $\pm$ 2.2
M 35	12	23	117 $\pm$ 2.5
ME 35	—	16	112 $\pm$ 2.4
B 36	0	12	125 $\pm$ 2.9
M 36	12	6	139 $\pm$ 2.9
ME 36	—	6	122 $\pm$ 1.4
B 38	0	15	115 $\pm$ 2.7
M 38	12	19	121 $\pm$ 3.0
ME 38	—	18	131 $\pm$ 3.8
B 40	0	16	86 $\pm$ 2.3
M 40	12	15	108 $\pm$ 2.1
ME 40	—	19	128 $\pm$ 2.7
Wheat	0	6	58.8 $\pm$ 0.9
Wheat	10	6	62.8 $\pm$ 0.8
Maize	0	19	31.4 $\pm$ 0.4
Maize	1	6	34.6 $\pm$ 0.7
Maize	2	4	36.8 $\pm$ 0.8
Maize	3	5	31.9 $\pm$ 1.1
Maize	6	4	27.3 $\pm$ 0.75
Maize	9	4	26.6 $\pm$ 0.45
Maize	12	16	33.8 $\pm$ 0.4
Oats	0	8	11.8 $\pm$ 0.27
Oats	4	6	17.0 $\pm$ 0.33
Oats	8	8	19.8 $\pm$ 0.56
Oats	12	14	21.0 $\pm$ 0.28

* Standard error of mean.

Table 2. *Changes in nicotinic acid content of barley during malting and brewing, as shown by chemical assays*

Series no.	Nicotinic acid ($\mu\text{g./g. dry wt.}$)			Increase during	
	Barley	Malted barley	Malt extract	Malting (%)	Brewing (%)
25	99	104	106	5.1	2.0
32	111	116	116	4.5	0.0
35	123	112	125	-8.9	10.7
36	123	129	121	4.9	-6.5
38	127	126	122	-0.9	-3.2
40	112	120	122	7.1	0.9
Mean	116	118	119	1.9	0.4

Mean results by the microbiological method (see Table 1) showed an average increase of 14.7% during malting, but no significant increase during the brewing of the malt extract. Each of the six

samples showed, microbiologically, an increase during malting and in one sample this exceeded 25%. There was thus a divergence between the chemical and microbiological results. A comparison of these (Table 3) showed that, with malted barley and malt extract, the average results by the two methods were in good agreement, and the individual deviations were scarcely significant when compared with the respective coefficients of variation. On the other hand, with unmalted barley the mean chemical result was 12% higher than the mean microbiological result. In five out of six samples the chemical method gave appreciably higher results, and in at least one of these (series 40) the difference was highly significant ($p = 0.99$). Dr E. R. Dawson also kindly assayed this sample, barley 40, by the microbiological method and obtained a mean result of 90 $\mu\text{g./g. dry weight}$, confirming our microbiological result. With barley, therefore, the chemical method may give results appreciably higher than the microbiological method.

Table 3. *Comparison of chemical and microbiological results*

Series no.	Chemical results as % of microbiological results			Coefficient of variation of mean chemical result		
	Barley	Malted barley	Malt extract	Barley	Malted barley	Malt extract
25	114	101	101	4.5	0.03	1.2
32	109	94	102	7.4	7.8	4.9
35	113	91	112	4.6	3.6	4.9
36	98	93	99	2.0	4.6	4.1
38	110	104	93	3.9	3.5	5.1
40	130	111	97	1.6	5.9	5.3
Mean	112	99	101	—	—	—

These data summarize results obtained in 170 assays. At least 4 assays were made on each sample.

The results of microbiological assays indicate an apparent increase in nicotinic acid content when barley is malted. However, seeds lose weight on germination—the so-called malting loss—and this must be taken into account in assessing the true change in vitamin content. We therefore determined the average dry weight of a barley seed before and after malting. The results showed an average loss of 12–13% in weight, sufficient to account for almost the whole of the observed increase in nicotinic acid content. Hence, any nicotinic acid which had been formed from a precursor must have been used up in metabolic processes.

Small-scale experiments

In these, the barley was kept at 27° for 10 days, a sample from the material which was malted on the large scale being used. Acrospire counts showed that in some of the barley the degree of germination was considerably higher than in normal malting, the

shoot being longer than the seed in 33 % of the seeds, as compared with less than 1 % in normal malting. Nevertheless, no significant increases in nicotinic acid content were obtained.

Wheat

A large-scale experiment was carried out in which 1 cwt. of English wheat was germinated at 14–20° for 12 days. Perhaps owing to the warm weather, there was some development of mould after 10 days on the floor. The experiment was discontinued at that point, when the chemical results (Table 4)

nicotinic acid content when allowance was made for changes in dry weight. The chemical method showed a net loss of 6 % after 1 day on the germinating floor, and of 10 % after 12 days on the floor. The microbiological method showed no significant change. The chemical results were 107–116 % of the microbiological results.

A small-scale experiment was carried out on the same sample in a warm room at 16–22° and also showed no increase in nicotinic acid content, although the acrospire counts indicated that a considerable degree of germination had occurred, the percentages of seeds with different acrospire ratios

Table 4. *Changes in dry weight and nicotinic acid content during germination of wheat*

Stage of malting		Nicotinic acid content by chemical method						
		Individual assays ($\mu\text{g./g. dry wt.}$)					($\mu\text{g./seed}$)	
							Actual	As % of initial
Days steeping	Days on floor					Mean		
0	0	64.5	56.6	65.7	65.6	63.8	2.46	100
2	6	68.2	67.5	—	—	67.9	2.46	100
2	9	85.3	84.9	83.5	82.9	84.2	2.82	115
2	10	92.3	91.6	—	—	92.0	3.24	132

showed a significant increase of 33 % in the nicotinic acid content, after allowance for the loss in weight of the seeds. Microbiological assays on the initial and final samples showed no increase, the average content of a single seed being 2.30 $\mu\text{g.}$ before germination and 2.27 $\mu\text{g.}$ after germination (both calculated on dry weight). On the ungerminated wheat the chemical result was 108 % of the microbiological. On the germinated wheat the chemical result was 147 % of the microbiological result, a significant difference.

Maize

A large-scale experiment was carried out with 1 cwt. of maize (White Horse Tooth variety) germinated at 15–18° for 12 days. Acrospire counts and weight changes showed that a fair degree of germination had occurred, but there was no increase in

being as follows: 0–0.5, 9 %; 0.5–1, 56 %; 1–2, 15 %; 2–4, 13 %; above 4, 7 %. After 8 days' germination there was a net loss of about 12 %, and after 10 days a net loss of about 15 % of nicotinic acid.

Rice

For this, we used paddy containing the husk. The small quantity available permitted of only small-scale experiments at 27°. In 9 days' germination the average net increase in nicotinic acid content was 17 % (Table 5). Of the seeds 93 % had germinated, but germination was rather uneven. Of the germinated seeds, 52 % had an acrospire ratio greater than 1.0. There was a much greater increase in nicotinic acid content in the seeds showing the greater degree of germination. In 7 days' germination 84 % of the seeds had germinated and the increase in

Table 5. *Changes in dry weight and in nicotinic acid content during germination of rice (paddy) as shown by chemical assays*

Period of germination (days)		Nicotinic acid content				
		Individual assays ($\mu\text{g./g. dry wt.}$)			($\mu\text{g./seed}$)	
					Actual	As % of initial
	Acrospire ratio			Mean		
0	—	74.1	73.0	73.6	1.88	100
7	1–4 (12 %)	95.2	100.0	97.6	2.41	128
9	Below 1 (48 %)	79.3	82.4	80.9	1.85	98
9	1–2 (33 %)	96.0	95.3	95.7	2.30	122
9	Above 2 (19 %)	124.0	116.4	120.2	2.85	152

Figures in parentheses represent percentage of total quantity of germinated rice which had reached the stated degree of germination.

seeds with acrospire ratios above 1.0 was 28% as compared with 33% for the corresponding degree of germination after 9 days. So far as we are aware, this is the first time that a significant increase in nicotinic acid content has been shown to occur in rice as the result of germination. It would seem worth while to carry out experiments on a larger scale when more material becomes available.

Oats

A large-scale experiment was carried out in which 1 cwt. of English oats was germinated at temperatures ranging from 15 to 18° for 12 days. The results (Table 6) showed by both chemical and microbiological methods almost the same percentage increase above the respective initial values. The average nicotinic acid content/seed by the microbiological method was, however, only about two-thirds of that determined chemically for the same sample. The differences between the chemical and microbiological figures were all highly significant ($p=0.99$). Each of the chemical figures in Table 6

content of an average seed in the latter part of the experiment had slowed down considerably since the rapid initial increase.

Acrospire counts on the samples in the large-scale experiment showed only a moderate degree of germination. We therefore carried out on the same batch of oats a small-scale experiment, aiming at a higher degree of germination by using a temperature of 27°. Acrospire counts showed that this had in fact occurred. After 10 days' germination, the chemical method showed a net increase of 108% compared with 43% in the large-scale experiment.

DISCUSSION

American workers using microbiological methods first showed that germination of cereals may, under certain conditions, increase their nicotinic acid content. Our own experience leads us to suggest that in further investigations it would be desirable to relate any observed increases to the degree of germination, as determined from acrospire counts and weight changes. This might explain some of the

Table 6. *Changes in nicotinic acid content and average weight/seed during germination of oats*

Sample no.	Treatment	Average dry weight of a seed (mg.)	Nicotinic acid content					
			Chemical method			Microbiological method		
			(μg./g. dry wt.)	(μg./seed)		(μg./seed)	(μg./seed)	
				Actual	As % of initial		Actual	As % of initial
1	Untreated	35.3±0.41*	19.5	0.69	100 ±0.3*	0.42	100	
2	Steeped 24 hr.	32.0±0.79	23.0	0.74	107 ±3.2	—	—	
3	Steeped +1 day on floor	32.8±0.54	26.9	0.88	127.5±0.6	—	—	
4	Steeped +2 days on floor	35.7±0.50	27.2	0.97	141 ±5.2	—	—	
5	Steeped +3 days on floor	32.9±0.31	30.7	1.01	146 ±0.4	—	—	
6	Steeped +4 days on floor	29.5±0.37	28.3	0.83	123 ±0.2	0.50	119	
8	Steeped +6 days on floor	32.1±0.28	27.8	0.89	129 ±1.7	—	—	
10	Steeped +8 days on floor	30.2±0.47	32.8	0.99	143 ±1.1	0.60	143	
12	Steeped +10 days on floor	30.7±0.54	34.9	1.07	155 ±3.1	—	—	
13	Steeped +11 days on floor	30.8±0.44	30.8	0.95	138 ±1.7	—	—	
14	Steeped +12 days on floor	31.5±0.64	34.4	1.08	157 ±13.4	0.66	157	

* Standard error of mean.

The nicotinic acid content for each sample is the mean of at least 4 assays, of which the extreme range in the initial sample was 98–104 and in the final sample 92–108% of the respective mean.

is the mean obtained from at least four assays. The coefficients of variation of the mean (as calculated from the respective standard deviations of the mean) for the figures for samples 1–13 ranged from 0.2 to 3.7 and averaged 1.4. For sample 14 there was a higher coefficient of variation of 8.5 obtained from seven assays. We cannot explain why the results for this sample showed this wider deviation. However, the nicotinic acid content/seed for sample 14 falls into line with the nicotinic acid content found in the other samples. The rate of increase in nicotinic acid

discrepancies between the findings of different workers and also help to build up a more complete picture of the germination process. For barley, e.g., Burkholder (1943) obtained with a single sample an increase of 70% in nicotinic acid content/g. dry weight. When allowance was made for loss in weight during germination about one third of this apparent increase disappeared. We did not obtain a significant net increase with any one of our six samples in assays either by chemical or by microbiological methods. Moreover, there was still no

significant increase when we prepared malt extracts from the malted barleys and thus gave still further scope to the germination enzymes. Our findings agree with those of Davis *et al.* (1943) who obtained an average increase of about 11 % in seven samples of barley as the result of germination, but made no allowance for loss in weight during germination, which would probably explain the whole of their observed increase.

With wheat, Davis *et al.* (1943) obtained an average increase of about 15 % in five samples. Our results indicate that the loss in weight during germination of wheat seeds (Fig. 1) might easily reach 15 %. If allowance were made for this loss in weight, the results of Davis *et al.* would probably not show any significant increase. Burkholder (1943) obtained a net increase of 24 % with a single sample. We obtained a net increase of 33 % by the chemical method, but no increase microbiologically.

Maize is of particular interest because of its low initial content of nicotinic acid, which has been held responsible for the prevalence of pellagra in parts of the world where it is a staple food. Burkholder & McVeigh (1942) obtained an increase of 141 %, uncorrected for loss in weight during germination. Burkholder (1943), after making such correction, obtained a net increase of 258 % on another sample. We did not obtain, either by chemical or microbiological methods, any increase in a sample with an initial content of 35.9 $\mu\text{g./g.}$ dry matter. This may have been due to the high initial content as compared with 17 and 9.5 $\mu\text{g./g.}$ dry matter respectively in the samples of the American workers. American findings indicate that greater increases may be expected with cereals of lower initial nicotinic acid content. Our results, on the whole, support this suggestion, but rice was an exception, since, using a high degree of germination, we secured an increase of 52 % in a sample with an initial content of 74 $\mu\text{g./g.}$ The mean increase in the whole sample after 9 days' germination was only 17 %. In the work above our chemical assays had given the same general picture as our microbiological assays, and thus provided additional evidence of the reliability of the chemical method (cf. James *et al.* 1947).

Oats, however, told a different story. Our chemical results for oats ungerminated and at different stages of germination were consistently 60–70 % higher than the microbiological values, though both methods showed the same net increase (57 % of the respective initial values) as the result of germination. This increase was considerably lower than the net increase of 352 % obtained by Burkholder (1943) microbiologically with one sample of oats. He, however, used oats with a much lower initial value and also obtained a much higher degree of germination. Fig. 1 shows the loss in weight at different stages of germination in our various samples, the data being

used to calculate the net changes in nicotinic acid content recorded in our tables. The discrepancy between the chemical and the microbiological results was clearly established by the results of numerous assays, and will require further investigation.

Fig. 1. Changes in dry weight of seeds during germination. All seeds in our experiments were steeped in cold water for 24 hr. before germination. Results obtained on wheat by Burkholder (1943) x—x, Klatzkin, Norris & Wokes (present study) x—x; on oats by Burkholder (1943) *—*—*, McVeigh (1944) ▲····▲, Klatzkin, Norris & Wokes (present study) ⊗—⊗.

SUMMARY

1. Samples of barley, maize, rice (paddy) and oats have been germinated in the laboratory under known conditions. Large-scale germination experiments have been carried out on samples of barley, wheat, maize and oats. The degree of germination was followed by acrospire counts and by changes in dry weight. Nicotinic acid assays have been made on the dried powdered material, both by a chemical and a microbiological method, at different stages of germination.

2. In oats a net increase was obtained of 57–108 %. Rice gave net increases of 0–52 % with different degrees of germination. With wheat, barley and maize no significant increase was obtained.

3. It is suggested that the considerably greater increases reported by American workers may be due to their obtaining a higher degree of germination, and to some extent also to their omitting to allow for changes in weight during germination.

4. The chemical method gave satisfactory agreement with the microbiological method with all samples, except the wheat and oats and one sample of unmalted barley, where the chemical method gave higher results.

We are indebted to the Curator of the Imperial Institute, South Kensington, for a sample of Burma paddy, to Samuel Thompson and Sons Ltd. for carrying out large-scale malting of our samples of barley, wheat, oats and maize,

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A Polarographic Study of some Stilbene Derivatives of Biological Interest

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4-Aminostilbene and some related compounds have been shown to have an inhibitory action on the growth of young rats and on malignant tumours (Haddow, Kon & Harris, 1945). Certain of these substances are also potent carcinogens capable of eliciting tumours at sites remote from that of administration (Haddow, Kon & Harris, 1946). The ethylenic bond present in stilbene has been found to be electroreducible at the dropping mercury electrode (Laitinen & Wawzonek, 1942) and the present investigation was undertaken for the purpose of comparing the polarographic behaviour of compounds of this series showing differing biological activities. It was also considered that the data obtained in such experiments would be of value in assessing the suitability of the polarographic method of analysis in metabolic studies. Since stilboestrol is also a stilbene derivative (4:4'-dihydroxy- $\alpha\beta$ -diethylstilbene), and similar problems arise in connexion with investigations of its metabolism in the human subject (Warren, Goulden & Robinson, 1948), it was decided to include synthetic oestrogens of this type in the investigation.

EXPERIMENTAL

Materials

All the substances used in these experiments were pure compounds. A number of the stilbene derivatives containing basic substituents was kindly put at our disposal by Prof. G. A. R. Kon and Dr R. J. C. Harris. We are also greatly indebted to Prof. E. C. Dodds and Dr W. Lawson for generous gifts of stilboestrol derivatives.

Solvent and electrolyte

The solvent employed was a dioxan-water mixture (3 : 1 by vol.) (Laitinen & Wawzonek, 1942). Commercial dioxan (British Drug Houses Ltd. 'purified') was refluxed for 12 hr. with sodium and then fractionated. Repetition of the sodium treatment was sometimes necessary in order to achieve very negative decomposition potentials. Only recently purified dioxan was used since there was some deterioration on storage.

Laitinen & Wawzonek recommend the use of tetrabutylammonium iodide as a supporting electrolyte for the attainment of very negative discharge potentials. In the present experiments tetraethylammonium iodide was found to be quite suitable, and it was easily prepared by heating