125. STUDIES ON THE 'FERMENTATION' OF CEYLON TEA

3. RESPIRATION AND TEA FERMENTATION

By H. B. SREERANGACHAR

From the Tea Research Institute of Ceylon, Talawakelle

(Received 29 September 1941)

THE production of CO_2 during fermentation in the manufacture of black tea has been observed by previous workers [Evans, 1928, 1, 2; Bosscha & Brzeskowsky, 1916; Roberts, 1939]. Evans (Ceylon) found that tea leaf like most other plant materials had a respiratory quotient of 1.0 which, however, did not appreciably change during withering. While crushing had no effect on the R.Q. of fresh leaf it caused a marked decrease in the R.Q. of withered leaf, due to a very large increase in the O_2 uptake associated with oxidation of polyphenols. This O_2 uptake, which is initially very rapid, gradually falls off until the ratio CO_2/O_2 again approximates to unity. The volume of CO_2 produced during fermentation decreases only slightly with time, e.g. 40 g. of rolled leaf at 18° , which produced 4.6 ml. in the 1st hr., still formed 3.8 ml. in the 7th. As a result of these observations Evans [1928, 2] concluded that 'the respiratory process is upset when the withered leaf is crushed, and during fermentation the normal process of respiration is not observed'.

Bosscha & Brzeskowsky [1916] showed that CO_2 is developed during the fermentation of completely sterilized leaf and concluded that it is not a product of growth of micro-organisms.

In north-east India, Roberts [1939; 1940] has studied in greater detail the output of CO_2 by fermenting tea leaf and has shown that it arises from oxidation of carbohydrates (glucose) present in tea. Roberts advanced an explanation in which this reaction is an intermediate step in his mechanism of fermentation. *o*-Quinones produced by oxidation of polyphenols by the cytochrome-cytochrome oxidase system either form condensation products or bring about the oxidation of non-tannin substances (glucose) through cozymase and dehydrogenases. The resulting reduced cozymase reacts with oxidized cytochrome giving reduced cytochrome which once again takes part in the cycle of reactions. Oxidation of carbohydrate is thus shown to influence continuous polyphenol oxidation.

In formulating this mechanism of fermentation Roberts has attempted to establish a relationship between respiration and fermentation of tea leaf. In fact, he is of the opinion that fermentation of tea is only an uncompensated respiration. Extensive mechanical injury resulting in the dispersion of coenzymes has been stated to be responsible for the switch over from respiration of tea leaf to its fermentation.

Parallel investigations on Ceylon leaf have led to interesting differences of conception of the relationship between fermentation and respiration. The leaf tissue resulting from the rolling process, and also, to a different extent, minced leaf, is regarded as a heterogeneous mixture of damaged and undamaged tissue. It seems improbable that even the finest mincing will result in the disruption of

every single cell. This means that undamaged tissue will continue more or less normal respiratory activity, while in the case of exposed cell contents enzymic oxidation with atmospheric O_2 will take place at a comparatively rapid rate. The latter activity accounts for the observed rapid increase of O_2 uptake by damaged tea leaf.

In the investigations described below, mincing was effected by means of a chromium-plated domestic mincing machine with the finest available cutter. In this case there is no question of complete disruption of the organic structure of the leaf although there is undoubtedly more damage than in factory 'rolled' leaf.

Evans showed that after an initial increase of O_2 uptake when leaf is damaged, with corresponding reduction of the R.Q. there was a steady reduction of O_2 uptake per hr. until the R.Q. again approached unity and remained at that level for several hours. The obvious explanation is that normal respiratory activity continues after the oxidation of exposed polyphenols has ceased.

It was shown by *in vitro* studies [Lamb & Sreerangachar, 1940] that the main reaction in fermentation, namely the oxidation of polyphenols, was not attended by any CO_2 production. Thus it was concluded tentatively that the CO_2 output observed in the fermenting leaf did not have its origin in the fermentation reaction itself. The apparatus employed for manometric studies was not, however, capable of high accuracy. The work has now been repeated and extended by the use of the more accurate Barcroft apparatus¹ and the results are reported in the present communication.

EXPERIMENTAL

Technique. The *in vitro* study of the O_2 uptake during the oxidation of tea tannin by our enzyme preparations was conducted by placing the enzyme powder in pliofilm boats left floating over the substrate solution in the reaction flasks. Shaking effects the admixture of the enzyme and substrate whenever desired. For absorption of CO_2 , KOH was added to the central tube in the flask, rolls of filter paper being employed to ensure an adequate surface for absorption. 2 ml. substrate solution (2.8%) and 50 mg. enzyme preparation, obtained by acetone treatment of fresh leaf as described in our previous communication [Lamb & Sreerangachar, 1940], were usually taken for each experiment.

Observations on respiration were made by cutting leaf into a few fairly uniform pieces and suspending these pieces in distilled water. While working with leaf suspensions 200 mg. samples of leaf mince were usually taken. Mincing was done as rapidly and as finely as possible and the mince well mixed before sampling. The material was suspended in 2 ml. water and after closing the taps the apparatus was shaken at a speed of 120–140 oscillations per min. Comparatively very little oxidation occurs before commencement of readings and the differential principle of the Barcroft apparatus eliminates small temperature variations. Brodie's solution was employed as manometric fluid in order to obtain fairly large readings. Unless otherwise stated all experiments were carried out at 25° in a thermostat and O_2 absorption results recorded in $\mu l./200$ mg. fresh tissue weight, employing the modified formula of Dixon & Keilin [1933] for calculating the vessel constants. Agreement between replicates has been found to be very good and in the interpretation of results a consistent difference of 10% was considered to be significant.

¹ Kindly lent by Mr M. Sreenivasaya, Indian Institute of Science, Bangalore.

Oxidation of pure theotannin by tea enzyme preparation

Theotannin was prepared according to the method of Shaw [1935] and after removal of free gallic acid by dry ether a concentrated solution (2.8%) was obtained. 2 ml. of this solution were reacted upon by 50 mg. of enzyme powder preparation in one of the twin Barcroft vessels while the other contained suitable inactive control substances. The difference between the values of O_2 uptake with and without the use of KOH gave the amount of CO_2 , after correcting for the variation in the vessel constants for CO_2 and O_2 .

Table 1 records the results of two separate experiments which are typical of many others obtained in support of our previous conclusion that oxidation of pure tea tannin substrate is not accompanied by CO_2 output.

Table 1. O_2 uptake during enzymic oxidation of amorphous tea tannin

	min.	...	5	10	20	30	60	75	90	105
I: With KOH, μ l.	18		36	67	103	218	272	321	359	
Without KOH, μ l.	20		40	82	118	215	265	312	348	
	min.	...	10	20	40	65	80			
II: With KOH, μ l.	22		42	120	192	241				
Without KOH, μ l.	18		53	120	187	228				

The enzyme preparation contained both soluble and insoluble enzyme systems and when an attempt was made to estimate separately the activities of these, it was found that under the conditions of experiment the soluble enzyme was almost completely inactivated by the high tannin concentration. After the extraction of all soluble enzyme, the activity of the insoluble enzyme was only very slightly higher than the activity of the unextracted enzyme powder (Table 2).

Table 2

	min.	...	10	22	45	60	75	90	105	120
Extracted enzyme, μ l. O_2	51		87	136	158	187	219	241	261	
Unextracted enzyme, μ l. O_2	42		78	125	149	174	192	219	238	

The insoluble enzyme must be employed in the wet condition as otherwise its activity may suffer by drying. In a particular experiment the wet extracted enzyme was again treated with acetone and quantities (39.6 mg.) corresponding to the original weight of the unextracted enzyme (50 mg.) when employed on the same quantity of substrate showed about 40% decrease in the uptake of O_2 .

Reactions in tissue suspensions

As observed by Evans [1928, 1] and more recently by Deb & Roberts [1940], the R.Q. of respiring tea leaf is very nearly 1.0 and so very little volume change is recorded if no KOH is employed in the Barcroft vessels. But when the leaf is mechanically damaged, oxidation of polyphenol occurs in the damaged cells while in the undamaged cells the respiration continues: the R.Q. therefore falls enormously. Tables 3-6 show the values of R.Q. obtained for fresh and withered leaf before and after mincing, results being recorded in μ l. gas per 200 mg. tissue.

The change in the R.Q. on mechanical injury to the leaf can be explained as due to two principal causes, viz.: (1) high O_2 uptake required for polyphenol oxidation, and (2) reduction in the respiring leaf surface resulting in a lower CO_2 output. Of these causes the significance of the reduction in amount of respiratory tissue has not hitherto been recognized.

If, however, such a hypothesis is correct then there should be some direct relation between the fall in R.Q. and the extent of crushing or mechanical

Table 3. *Respiration of fresh leaf*

min. ...	15	30	45	60	75	90	105	120
O ₂ uptake, μ l.	19	44	67	97	130	160	193	218
CO ₂ output, μ l.	22	49	71	98	125	152	178	201
R.Q.	1.20	1.11	1.06	1.00	0.93	0.95	0.92	0.92

Table 4. *Respiration of withered leaf*

min. ...	15	30	45	60	75	90	105	120
O ₂ uptake, μ l.	14	26	37	46	56	70	88	97
CO ₂ output, μ l.	13	27	38	49	58	69	86	89
R.Q.	0.96	1.05	1.02	1.06	1.04	0.99	0.91	0.91

Table 5. *R.Q. of fresh leaf mince*

min. ...	15	30	45	60	75	90
O ₂ uptake, μ l.	60	105	138	156	165	174
CO ₂ output, μ l.	19	28	46	53	53	56
R.Q.	0.31	0.27	0.34	0.34	0.32	0.32

Table 6. *R.Q. of withered leaf mince*

min. ...	15	30	45	60	75	90
O ₂ uptake, μ l.	40	76	112	147	185	225
CO ₂ output, μ l.	9	12	14	23	39	63
R.Q.	0.23	0.16	0.12	0.16	0.21	0.28

injury to the leaf. To put this to the test, two representative samples of leaf were taken: one part was minced and 200 mg. mince weighed out; of the other part 200 mg. leaf were first weighed and then ground with sand in a pestle and mortar. The O₂ uptake and CO₂ outputs of these samples when suspended in 2 ml. water were manometrically measured. Table 7 displays these results while Table 8 gives the changes in R.Q. for the 1st half hr. and the initial rate of fermentation ($-Q_{O_2}$) resulting from different degrees of tissue damage.

Table 7

min. ...	10	20	30	40	50	60	90
Total O ₂ uptake							
Minced leaf, μ l.	40	76	103	127	145	154	174
Ground leaf, μ l.	69	147	169	183	192	196	210
CO ₂ output							
Minced leaf, μ l.	14	26	35	46	49	53	60
Ground leaf, μ l.	10	19	30	37	44	46	51

Table 8

	R.Q.	$-Q_{O_2}$
Whole leaf	1.0	2.0
Minced leaf	0.34	6.0
Ground leaf	0.18	11.4

It may be concluded from the above results that a more intense mechanical damage is accompanied by a higher O₂ uptake and a lower CO₂ output. The ratio of damaged to undamaged cells would determine the actual extent of variations. Deb & Roberts [1940] have observed a similar effect of mechanical injury on the anaerobic fermentation¹ in which the greater the extent of mechanical damage the lower the rate of anaerobic fermentation.

¹ This is to be distinguished from 'tea fermentation', which is an entirely different, aerobic process.

The reactions in the damaged tissue have been explained by Roberts [1939] on the coenzyme dispersion theory. The decrease of carbohydrate oxidation or the production of CO_2 results from a dilution of coenzymes necessary for the process and more of the *o*-quinone produced by earlier reactions undergoes condensation. On suspension of the leaf mince in water, there will be a further dilution of coenzymes with similar effects as above on the carbohydrate oxidation and the accumulation of polyphenol oxidation products. It is possible, however, to explain this latter observation also by the respiration surface reduction theory. At higher dilutions the fine mince will be in a state of submersion under water and the leaf surface having access to air will be greatly diminished. Oxidation of polyphenol itself will be very little affected by this but the production of CO_2 will be markedly reduced.

In an attempt to correlate the results of manometric measurements of minced leaf suspensions with those of factory fermentation, Roberts has ignored the effect of different degrees of crushing on the rate of fermentation. Under the factory conditions, the rolling process does not bring about the same order of mechanical injury to the tissue as in fine mincing and it is highly probable that this difference may also be a factor in accounting for the much slower rate of fermentation in the factory.

Separation of fermentation and respiration reactions in the minced tea leaf

In order to investigate the origin of CO_2 production in the fermenting leaf it is necessary to separate the two coexisting processes namely fermentation and respiration, and the two methods employed for this purpose are described below.

(1) *Washing of minced leaf.* If the minced tissue is quickly washed free of the adhering juice and the contents of the damaged cells, fermentation is almost completely inhibited owing to lack of the polyphenolic substrate. This is shown by the fact that on omission of KOH in the Barcroft vessels, there is scarcely any registered O_2 uptake by the washed tissues which remain predominantly green even after 4 hr. The r.q. is 1.0 and respiration, which is confined to the undamaged cells, is unaffected by washing. In Table 9 are given results of two typical experiments which show that both the washed and unwashed tissues produce almost the same amount of CO_2 .

Table 9. *Effect of washing on CO_2 output*

	min.	...	10	20	30	40	50	60	70	80	90
I: Washed tissue, $\mu\text{l. CO}_2$	14		26	33	42	51	56	63	70	76	
Unwashed tissue, $\mu\text{l. CO}_2$	14		19	30	46	49	51	55	56	60	
II: Washed tissue, $\mu\text{l. CO}_2$	10		19	26	30	37	46	53			
Unwashed tissue, $\mu\text{l. CO}_2$	12		28	33	37	37	42	51			

There is no big difference between the CO_2 output of the washed leaf mince and the unwashed leaf mince. It seems reasonable, therefore, to conclude that in tea fermentation the CO_2 observed arises entirely from the respiratory activity of the undamaged portions of leaf.

(2) *Freezing.* Intact cellular structure seems to be necessary for proper functioning of those enzyme systems that are usually associated with respiration [Ashford, 1934; Yudkin, 1937; Geiger, 1939]. Cellular damage markedly affects respiratory activity. As the leaf cells undergo some rupture as a result of freezing and thawing, it was thought that this method could be utilized for

effecting a separation between fermentation and respiration. Lynen [1940] has found that the respiration of animal tissues is markedly inhibited after freezing in liquid air while certain insoluble enzyme systems remain unaffected. There is an insoluble enzyme system present in tea which is largely responsible for fermentation and the effect of freezing on this enzyme system cannot be appreciable as it was found that leaf frozen in dry ice fermented normally after thawing.

In the absence of liquid air which is not available here the effect of freezing in a bath of ice and salt and also by dry ice was studied. Although the leaf became brittle on freezing, the process was rather imperfect especially in the case of withered leaf. Nevertheless freezing was found to have a significant effect on the respiration and the manometric results obtained are given in Tables 10 and 11, both the frozen and unfrozen leaf being subjected as far as possible to the same degree of mincing.

Table 10. *Effect of freezing on CO₂ output*

min. ...	10	20	30	40	50	60	70	80	90
Freezing by ice and salt:									
Frozen tissue mince, μ l. CO ₂	5	7	12	14	19	23	35	33	37
Unfrozen tissue mince, μ l. CO ₂	14	23	30	40	51	63	88	91	97
Freezing by dry ice:									
Frozen tissue mince, μ l. CO ₂	5	7	12	19	23	28	33	37	42
Unfrozen tissue mince, μ l. CO ₂	12	23	33	44	53	65	74	84	93

Table 11. *Effect of freezing on O₂ uptake for polyphenol oxidation*

min. ...	10	20	30	40	50	60	70	90
Frozen, μ l. O ₂	16	36	54	71	87	103	116	142
Unfrozen, μ l. O ₂	9	27	58	74	89	107	127	138

The degree of freezing attained, although it injured the cells, did not by itself increase the O₂ uptake, because the polyphenols in the cells of the frozen material were not exposed to atmospheric O₂. Freezing, apparently, did not supplement the degree of exposure of polyphenols attained by mechanical crushing, but there was a marked inhibition of respiration on freezing, and in one experiment the respiration of frozen whole leaf was almost negligible. The general effect, however, of freezing was to suppress the respiration without affecting the fermentation process.

Effect of glucose addition on respiration and fermentation of tea leaf

If oxidation of carbohydrate is a part of the fermentation mechanism, changes in the concentration of carbohydrate, which is the normal respiratory substrate, may be expected to have an effect on fermentation. Deb & Roberts [1940] have shown that losses of carbohydrate during the withering process in tea manufacture are unlikely to affect the rate of fermentation. Starvation for 3 days results in an appreciable fall in the respiratory activity while oxidase activity is but little affected. Thus some sort of demarcation between the two processes is already apparent and further evidence is afforded here by a study of glucose addition to the fermenting and respiring leaf.

200 mg. of leaf (either whole leaf or minced leaf) were suspended in 2 ml. glucose solution containing 10 mg. glucose. The O₂ uptakes were measured manometrically both in the case of fresh leaf and the withered leaf and typical results are given in Tables 12 and 13. Table 12 contains data for total O₂ uptake

including that required for carbohydrate oxidation while Table 13 gives figures for O_2 absorption required for polyphenol oxidation only. These latter figures are obtained on the basis of the fact that on omission of KOH, changes due to respiration are barely registered in the apparatus and therefore the results obtained represent only the uptake of O_2 due to oxidation of polyphenols. The same observations are also applicable to reactions carried out in presence of glucose, because careful manometric measurements have shown that the R.Q. during the respiration of fresh or withered leaf in presence of glucose does not change appreciably from 1.0.

Table 12. *Total O_2 uptake*

	min.	...	10	20	30	40	60	80	100
Fresh whole leaf, μ l.:									
Leaf + glucose			13	31	45	58	94	120	147
Leaf + water			9	22	36	45	54	85	120
Fresh leaf mince, μ l.:									
Leaf + glucose			9	36	58	89	127	152	170
Leaf + water			4	27	45	80	112	127	134
Withered whole leaf, μ l.:									
Leaf + glucose			13	31	40	51	80	107	138
Leaf + water			4	13	18	18	27	36	47
Withered leaf mince, μ l.:									
Leaf + glucose			22	45	67	80	94	103	
Leaf + water			13	31	42	54	67	71	

Table 13. *O_2 uptake for polyphenol oxidation*

	min.	...	10	20	30	40	60	90
Fresh leaf mince, μ l.:								
With glucose			31	58	83	98	120	132
Without glucose			34	65	89	103	114	136
Withered leaf mince, μ l.:								
With glucose			11	29	47	58	85	107
Without glucose			13	29	47	62	78	100

Addition of glucose stimulates respiration as shown by the increase in the O_2 uptake of the whole leaf. This stimulation is very marked in the case of withered leaf which may possibly be due to one or both of the two causes, namely, (1) the substrate shortage occurring during withering is made up by the addition of glucose and (2) the carbohydrate is better imbibed into the leaf as a result of an increased permeability of withered leaf [Evans, 1927].

An increase in the total O_2 uptake of the minced leaf as a result of adding glucose is indicated in Table 12. This may be due to a stimulation of either fermentation as denoted by polyphenol oxidation or of respiration. But the figures in Table 13 reveal that on omission of KOH the O_2 uptake due to fermentation remains unaffected by glucose. It must therefore be concluded that glucose, when added to minced leaf, stimulates respiration but not fermentation.

Now it has already been shown that the O_2 uptake by washed tissue mince can be completely attributed to the respiratory activity and that CO_2 output is not affected by washing the minced tissues. Stimulation of respiration must therefore be demonstrable by adding glucose to the washed tissues. Table 14

Table 14. *CO_2 production by washed leaf mince*

	min.	...	10	20	30	40	60	90
With glucose, μ l. CO_2			16	30	39	46	56	67
Without glucose, μ l. CO_2			13	23	23	28	30	35

shows the figures for the CO_2 production with and without the addition of glucose, by 200 mg. fresh leaf mince after washing the tissues.

The increase in the respiratory CO_2 of the washed tissues on addition of glucose as shown above is of the same order as the increase in the total O_2 uptake of the unwashed tissues. It may therefore be stated that whether fermentation is taking place or not respiration is equally stimulated by glucose.

When glucose (10 mg. in 2 ml.) is added to an *in vitro* mixture of purified amorphous theotannin (2 ml. of 4.1 %) and the acetone enzyme (50 mg.) there is no increase in the O_2 uptake and also there is no CO_2 evolution. These results, which are given in Table 15, thus confirm the fact that glucose has no effect on the fermentation reaction.

Table 15. *Effect of addition of glucose to an in vitro enzyme-substrate mixture*

	min.	...	10	20	30	60	90	120
	$\mu\text{l. O}_2$ uptake							
Reaction with glucose, KOH present	11		47	78	136	236	292	
Reaction with glucose, KOH absent	13		47	74	149	232	281	
Reaction without glucose, KOH present	25		40	71	147	232	285	

The general conclusion from all these results can only be in support of the view that respiration and fermentation, although occurring side by side in the fermenting leaf, are really quite independent of each other.

DISCUSSION

In the light of the present conclusions Evans's results can now be satisfactorily explained, his statement that during fermentation the normal process of respiration is not observed being strictly confined only to the damaged tissues. His observation that the volume of CO_2 produced during fermentation decreases only very slightly from the 1st hr. to the 7th shows that respiration, being a slow process, continues at almost the initial rate even long after the fermentation has come to a standstill. This obviously cannot happen if CO_2 production is due to the fermentation reaction itself. The constancy of CO_2 production and the rapid decrease in polyphenol oxidation with time together result in the fact that the ratio CO_2/O_2 approximates once again to unity, when it is only the respiratory activity that is being measured.

The independence of carbohydrate oxidation and polyphenol oxidation casts doubt on the fermentation mechanism put forward by Roberts which is already open to the criticism that most of the participating components have never been directly demonstrated in tea. The parallelism obtained by Roberts between the polyphenol oxidation and the carbohydrate oxidation was not generally found here. These appear to depend upon the nature of the leaf in the first place and then on the degree of mechanical damage. Furthermore, the inhibitory effect of the addition of ascorbic acid [Roberts, 1939] and HCN [Deb & Roberts, 1940] is different for polyphenol oxidation and carbohydrate oxidation.

The reaction in certain tissues has been explained by Szent-Györgyi & Vietorisz [1931] on the basis of the assumption that an enzyme and its substrate, an *o*-dihydroxy compound, are kept separate from one another in the uninjured cells but come into contact with each other when the cells are mechanically injured. Sutter [1936] considers that the increased O_2 uptake of the minced

tissues depends upon the facilitated diffusion of O_2 . These may serve as possible alternatives to the theory of coenzyme dispersion in the damaged tissues as put forward by Roberts in his mechanism of tea fermentation. The lower carbohydrate oxidation on mincing and on dilution of the minced tea leaf tissue can be accounted for by the reduction of respiring cell surface by these operations. The extent of crushing has been shown to be a factor in explaining the disparity between the manometric results with tissue suspensions and the factory conditions.

Evidence for the occurrence of polyphenol oxidase in tea was adduced in a previous communication [Lamb & Sreerangachar, 1940], where the objections to the cytochrome and peroxidase theories were also presented. Our studies are mostly confined to fermentation and it is probable that cytochrome oxidase and peroxidase may both be functioning in the respiratory process of the tea leaf. Roberts [1940] has suggested a revision of classification of plant tissues based on the respiratory enzyme contained in the tissue and he thinks that in plants where oxidase activity is weak the respiratory enzyme may be cytochrome oxidase. This may be possible but, so far, the relation of these respiratory enzymes to tea fermentation cannot be regarded as established. It may be of interest here to mention that our acetone-treated enzyme preparation failed to show any dehydrogenase (succinic) activity. The dehydrogenase or its coenzyme may have been inactivated during preparation; yet the enzyme preparation brought about polyphenol oxidation with the greatest ease.

Reference must also be made again to our finding [Lamb & Sreerangachar, 1940] of a particular tea bush that fails to ferment. The respiratory mechanism is intact in this leaf because a plant must respire. The tannin content is also quite normal. Now, if the respiratory mechanism is indeed responsible for the fermentation of the leaf on mechanical injury, it becomes difficult to understand the failure of this leaf to ferment when minced. The requirement of an additional factor is thus clearly indicated and it has been experimentally shown that the deficient factor is indeed the polyphenol oxidase which is directly responsible for fermentation.

SUMMARY

The CO_2 produced by the fermenting leaf of the tea plant is shown to arise from the respiratory activity of the undamaged portions of leaf. *In vitro* enzymic oxidation of tea polyphenols is not accompanied by any CO_2 production in the presence of carbohydrates. Respiration and tea fermentation are thus found to be two separate processes occurring side by side in the damaged tea leaf but quite independent of each other.

The author's thanks are due to Dr R. V. Norris, the Director, for permission to publish these results and to Mr J. Lamb, Biochemist, for helpful criticism throughout this work.

REFERENCES

- Ashford (1934). *Biochem. J.* **28**, 2229.
Bosscha & Brzeskowsky (1916). Med. v. proefstation v. thee, **47**.
Deb & Roberts (1940). *Biochem. J.* **34**, 1507.
Dixon & Keilin (1933). *Biochem. J.* **27**, 86.
Evans (1927). *Bull. Tea Res. Inst. Ceylon*, **2**, 20.
—— (1928, 1). *Bull. Tea Res. Inst. Ceylon*, **3**, 34.
—— (1928, 2). *Tea Quart.* **3**, 28.
Geiger (1939). *Biochem. J.* **33**, 877.
Lamb & Sreerangachar (1940). *Biochem. J.* **34**, 1472.
Lynen (1940). *Hoppe-Seyl. Z.* **264**, 146.
Roberts (1939). *Biochem. J.* **33**, 836.
—— (1940). *Biochem. J.* **34**, 500.
Shaw (1935). *Bull. Uni. Plant. Ass. South India*, **4a**.
Sutter (1936). *Ergebn. Enzymforsch.* **5**.
Szent-Györgyi & Vietorisz (1931). *Biochem. Z.* **233**, 236.
Yudkin (1937). *Biochem. J.* **31**, 1065.