178. THE RESPIRATION AND ANAEROBIC FERMENTATION OF TEA LEAF AND THEIR RELATIONSHIP TO TEA FERMENTATION

BY S. B. DEB AND E. A. HOUGHTON ROBERTS

*From the Tocklai Experimental Station, Indian Tea Association,
Cinnamara, Assam, India*

(Received 10 October 1940)

COMPARATIVELY little work has been carried out on the respiration of leaf tissue using manometric methods. Kempner [1936] measured rates of respiration of whole leaves from pine, tobacco, plum and oleander, in this manner, and Allen & Goddard [1938] recorded respiration rates of strips of leaf from wheat floated in water, using the Fenn micro-respirometer. Later, Marsh & Goddard [1939, 1], using this same apparatus, investigated the respiration of carrot leaf with 100 mg. samples floated on phosphate buffer. The success of these workers encouraged us to make similar observations on the rate of respiration of tea leaf.

These investigations into the respiration and anaerobic fermentation¹ of tea leaf, obtained both by the manometric and by the standard Pettenkofer methods, together with manometric measurements of the rate of tea fermentation and of simpler enzyme-substrate systems have enabled us to draw fairly definite conclusions as to the presence of the cytochrome system in tea leaf, and the almost complete inactivation of the H-carrying coenzymes (cozymase) on extensive mechanical damage to the leaf.

Methods

(1) *Manometric method of determination of respiration rate.* Disks, $\frac{1}{2}$ in. in diameter, are punched in the centres of the second leaf of shoots consisting of two leaves and a bud. 10–15 such disks, weighing approximately 250–400 mg., constitute a fairly representative sample; in fact in only one experiment out of 20 was there any serious variation between replicates.

The disks were suspended in 3 ml. water or $M/15$ phosphate buffer pH 5.8, contained in Warburg vessels. KOH, where necessary, was contained in the inner cups, rolls of filter paper being employed to ensure an adequate surface of the CO_2 absorbent. CO_2 outputs were obtained by difference between the mean uptakes of four vessels with KOH, and of another four without KOH, using the appropriate constants of the vessels for O_2 and CO_2 . For plant tissues in a medium of water this method appears to give results quite as accurate as those obtained by the method of Dickens & Šimer [Turner, 1938].

In these determinations the Warburg apparatus was covered by a black cloth, thus preventing any disturbance of the O_2 - CO_2 interchanges by photosynthetic processes.

¹ A careful distinction must be drawn between the two uses of the word fermentation. Tea fermentation is not an anaerobic process, and to avoid confusion is always referred to as tea fermentation.

(2) *Pettenkofer method*. 50–100 g. of leaf, fresh, withered or mechanically damaged, were placed in a respiration chamber. The gases (N_2 and air) were driven through this chamber from cylinders or aspirators at about 3 l./hr., first passing through two tall soda-lime absorption towers. A small $CaCl_2$ tube was placed between each respiration chamber and the Pettenkofer tubes, which were protected from the atmosphere by soda-lime tubes. At the end of each hour the excess $Ba(OH)_2$ was determined by addition of excess oxalic acid followed by back-titration with $Ba(OH)_2$. The results were expressed in ml. CO_2 absorbed.

In anaerobic experiments the respiration chamber containing the leaf was evacuated and filled with N_2 several times before absorption of CO_2 was started. This served to remove, to a large extent, CO_2 held by the tissue. No time could be allowed for the tissue to accommodate itself to fresh conditions as the withered and mechanically rolled samples of leaf are not in a condition where they are likely to survive for long. The values obtained are therefore probably not comparable with results obtained for leaf samples under more refined conditions but it is considered that this method, although not giving absolute values for anaerobic fermentation, will be sufficiently accurate to reveal differences in anaerobic fermentation due to mechanical damage to the leaf.

(3) *Tea fermentation*. Rates of tea fermentation were determined by the standard method described in earlier communications from this laboratory [Roberts, 1939; 1940].

Respiration of tea leaf

Results are most conveniently recorded as μ l. gas per 100 mg. tissue, for successive half-hour periods. Table 1 shows a typical result.

Table 1

		Hours											
		0-½	½-1	1-1½	1½-2	2-2½	2½-3	3-3½	3½-4	4-4½	4½-5	5-5½	5½-6
O_2 uptake (μ l.)	40	34	36	29	29	21	24	25	25	25	24	23	
CO_2 output (μ l.)	51	33	35	26	25	18	23	18	20	21	15	17	
R.Q.		1.27	0.97	0.97	0.90	0.86	0.86	0.96	0.72	0.80	0.84	0.63	0.74

The rapid fall in respiratory activity in the first 2 hr. is not considered to be of any physiological significance; the tissue is probably adjusting itself to its new environment. The fall in the rate is certainly not due to carbohydrate depletion since in 6 hr. the total output of CO_2 is 302 μ l. which is equivalent to 0.4 mg. glucose. 100 mg. of leaf contain more than this quantity of free glucose besides reserves of starch and other polysaccharides. The fall in the rate for the first 2 hr. therefore must be due to some other cause than substrate shortage.

The high initial R.Q., lasting as it does for only 30 min., is thought to be due to the expulsion of CO_2 from the intracellular spaces of the leaf as a result of the shaking at the higher temperature (36°). The preliminary equilibration seems to have been insufficient to have driven off this free CO_2 from the leaf.

There is a steady decrease in R.Q. throughout the whole period of shaking. Irregularities in this decrease are due to experimental error and taking all determinations into account no fluctuations such as occur in the above example are observed. It should be remembered that quite small errors in the original manometric reading may lead to an error of as much as 10% in the R.Q. This steady decrease in R.Q. cannot be put down to an increased share by protein and fat in respiration, as carbohydrate supplies in the leaf are ample for a considerably longer period of shaking than 6 hr. Some browning is observed at the

edges of the disks and we ascribe the decrease in R.Q. to a local "tea fermentation" brought about by disintegration of the tissue, near the cells damaged by the cut.

In view of these non-physiological factors, results on respiration obtained manometrically must be interpreted with caution; nevertheless the technique has proved quite useful for comparative purposes.

Effect of carbohydrate depletion on respiration. Leaf may be largely depleted of its carbohydrates by storage for several days in the dark, without appreciable change in turgor of the leaf if the atmosphere is saturated with water vapour. After 72 hr. a sour smell has usually developed, the leaf has a high bacterial infection, and a considerable proportion of the leaf surface will have browned. No enhancement in the rate of O_2 uptake as a result of this bacterial contamination has been observed.

The effect of this impoverishment of carbohydrates is to reduce both respiration rate and R.Q. Measurements of the respiration of leaf when fresh and after 1, 2 and 3 days' storage are recorded in Table 2. The figures represent $\mu\text{l./100 mg. tissue}$.

Table 2

	Days storage	Hours									
		0-½	½-1	1-1½	1½-2	2-2½	2½-3	3-3½	3½-4	4-4½	4½-5
O_2 uptake ($\mu\text{l.}$)	0	54	44	35	39	36	32	30	28	30	20
	1	50	44	34	30	31	29	32	24	22	20
	2	42	36	26	26	26	17	21	20	22	20
	3	44	32	24	28	24	24	24	20	23	23
CO_2 output ($\mu\text{l.}$)	0	60	47	37	39	36	33	28	27	27	18
	1	52	45	32	28	31	28	29	22	20	17
	2	40	33	24	23	23	13	17	18	18	18
	3	39	29	23	25	23	21	21	20	18	23
R.Q.	0	1.11	1.07	1.06	1.00	1.00	1.03	0.90	0.96	0.90	0.90
	1	1.04	1.02	0.94	0.93	1.00	0.96	0.90	0.92	0.91	0.85
	2	0.95	0.92	0.92	0.88	0.88	0.77	0.80	0.90	0.82	0.90
	3	0.98	0.91	0.95	0.89	0.95	0.87	0.87	1.00	0.78	1.00

The reductions in respiration rate and R.Q. during the first 2 days are significant. On the 3rd day there is no further fall in respiration rate, and a significant increase in the R.Q.

The results for the first 48 hr. suggest that as the carbohydrate content falls the rate of respiration falls, and that the proteins play an increasingly important part as respiratory material. The significant fall in the R.Q. is strong evidence in favour of the view that protein is being oxidized.

The check in the rate of fall of respiratory activity and the significant rise in the R.Q. are not immediately explicable although it is interesting to point out that Yemm [1935] found similar decreases for starving barley leaves, and that after 3 days the R.Q. reached a minimum value, rising again later. Both for barley and for tea this change is associated with marked browning of the leaves and it is possible that, as with other senescent plant organs, the "climacteric" is reached [Kidd, 1935] which is associated with a temporary rise in respiratory activity.

Tea fermentation is not affected by depletion of carbohydrates to the same extent as is respiration. Rates of tea fermentation determined on successive days were as follows:

Days starvation	$-Q_{O_2}$
0	19.2
1	18.2
2	17.1
3	20.8

Two important conclusions can be drawn from this result: (1) Losses of carbohydrates during the withering process in tea manufacture are unlikely to affect the rate of fermentation. (2) The oxidase activity is but little affected by 3 days' starvation; the fall in respiratory activity is therefore probably due to substrate shortage.

Inhibition of respiration and tea fermentation by HCN and CO

In the determination of the inhibitory effect of cyanide on respiration and tea fermentation, certain modifications in the manometric technique are necessary. The disks or minces are suspended in *M*/15 phosphate buffer pH 5.8, and the KOH is replaced by the KOH-KCN mixtures prescribed by Krebs [1935]. If KOH be used for CO₂ absorption when cyanide is present, the HCN is slowly absorbed by the alkali and the original inhibition is reversed. The reversibility of the cyanide inhibition of tea respiration may be demonstrated in this way.

It is difficult to overcome the effect of HCN absorption completely in this way and partial reversals of cyanide inhibition after 2 hr. shaking are frequently observed. This is attributed to slight errors in making up the KOH-KCN mixtures, and to variations in the HCN partial pressure of such mixtures caused by CO₂ absorption.

Typical effects of cyanide on the respiration of the 1st and 5th leaves of the growing shoot, and on tea fermentation are shown in Tables 3-5.

Table 3. *Inhibition by cyanide of the respiration of the 1st leaf in the growing shoot*

Molar conc. of cyanide	Min.							
	10	20	30	45	60	90	120	150
	$\mu\text{l. O}_2/100 \text{ mg. tissue}$							
—	13	31	46	71	89	126	165	200
5×10^{-4}	9	19	28	41	44	55	60	70
2×10^{-3}	8	16	20	29	33	39	47	54
1×10^{-2}	7	14	20	26	29	35	43	45

Table 4. *Inhibition by cyanide of the respiration of the 5th leaf of the growing shoot*

Molar conc. of cyanide	Min.							
	10	20	30	45	60	90	120	150
	$\mu\text{l. O}_2/100 \text{ mg. tissue}$							
—	14	24	34	42	54	74	94	111
5×10^{-4}	11	22	28	36	48	62	70	82
2×10^{-3}	10	19	26	34	42	50	57	63
1×10^{-2}	7	13	18	22	26	33	36	40

Table 5. *Inhibition by cyanide of tea fermentation*

Molar conc. of cyanide	Min.					
	10	20	30	40	50	60
	$\mu\text{l. O}_2/100 \text{ mg. tissue}$					
—	75	128	170	184	193	199
5×10^{-4}	38	70	102	130	156	176
2×10^{-3}	22	36	50	62	76	90
1×10^{-2}	15	20	21	21	22	23

The cyanide apparently does not exert its full inhibitory effect on respiration for some 45 min. Table 6 shows the percentage inhibitions of the respiration as found in one experiment for the first 45 min. and subsequently.

Table 6. *Percentage inhibition of respiration by cyanide*

Molar conc. of cyanide	1st leaf of growing shoot		5th leaf of growing shoot	
	0-45 min.	45-150 min.	0-45 min.	45-150 min.
5×10^{-4}	43 %	78 %	14 %	33 %
2×10^{-3}	60	80	19	58
1×10^{-2}	64	86	48	74

For tea fermentation, initial rates show that 5×10^{-4} *M* HCN causes 49 % inhibition and 2×10^{-3} *M* HCN 71 % inhibition. The initial inhibition by 10^{-2} *M* HCN is still higher and after 20 min. this inhibition is almost complete.

The increased resistance of maturer leaf to cyanide poisoning is of interest, and recalls a similar finding by Marsh & Goddard [1939, 1] for carrot leaf. The consistent increase in the percentage inhibition with HCN concentration up to 10^{-2} *M* is also noteworthy. This is particularly marked in the case of tea fermentation.

Similar results are obtained with tea oxidase preparations acting upon pyrogallol and *p*-phenylenediamine as substrates.

Table 7. *Inhibition by cyanide of the oxidation of pyrogallol by tea oxidase*

Molar conc. of cyanide	Min.					
	10	20	30	40	50	60
	$\mu\text{l. O}_2/100 \text{ mg. tissue}$					
—	108	190	252	312	366	404
5×10^{-4}	48	88	128	171	206	246
2×10^{-3}	23	33	49	62	72	98
1×10^{-2}	10	(8)	14	18	18	22

Table 8. *Inhibition by cyanide of the oxidation of p-phenylenediamine by tea oxidase*

Molar conc. of cyanide	Min.					
	10	20	30	40	50	60
	$\mu\text{l. O}_2/100 \text{ mg. tissue}$					
—	37	58	77	91	112	118
5×10^{-4}	28	49	67	86	93	106
2×10^{-3}	18	37	51	64	70	80
1×10^{-2}	8	15	17	17	17	17

The comparatively low sensitivity of these mixtures of enzyme and substrate to cyanide inhibition is apparent, 10^{-2} *M* HCN being required for complete inhibition. From these results it appears unlikely that the low sensitivity of tea fermentation to cyanide is due to combination of the HCN with tannin or other HCN-binding substances in the system. It would seem that the tea oxidase requires as high a concentration as 10^{-2} *M* before its activity is completely inhibited.

In the above experiments figures are quoted for the rates of oxidation of pyrogallol and *p*-phenylenediamine by a tea-oxidase preparation. Ascorbic acid and quinol were also found to act as substrates for this enzyme. 1 ml. of substrate solution (100 mg./50 ml. water) was added to 1 ml. of a suspension of 100 mg. tea oxidase in 10 ml. *p*H 5.8, *M*/5 phosphate and rates of uptake measured manometrically.

Table 9. *Oxidation of quinol and ascorbic acid by tea oxidase*

	Min.			
	15	30	45	60
	$\mu\text{l. O}_2/100 \text{ mg. tissue}$			
Ascorbic acid	26	44	59	76
Quinol	41	70	94	119

The relative rates of oxidation of various substrates by the tea oxidase are summarized below, expressed in terms of $\mu\text{l. O}_2/\text{hr.}/\text{mg.}$ of enzyme.

Table 10

Substrate	$-Q_{O_2}$
Ascorbic acid	10.4
Quinol	16.4
<i>p</i> -Phenylenediamine	22.2
Pyrogallol	65.1
Tea tannin	120*

* This value was obtained for a different sample of enzyme. Independent measurements confirm that tea tannin is oxidized about twice as rapidly as pyrogallol by the tea oxidase.

Inhibition by CO

An investigation into the reversal by light of the inhibition by CO of plant respiration is complicated by other effects of light on the interchange between O_2 and CO_2 in respiring plant tissue. Kempner [1936] overcame this by slight narcosis with $CHCl_3$ which he claimed inhibited CO_2 assimilation without decreasing the rate of respiration. With tea leaf such treatment results in a browning of the leaf due to tannin oxidation.

It was considered that the simplest way of investigating the effect of CO on the tea oxidase would be to use the method of Keilin [1929]. Tea oxidase preparations were incubated with the Nadi reagent in Thunberg tubes filled with various gas mixtures. At the end of 10 min. shaking the reaction was stopped by addition of 1 ml. 1 % KCN and the indophenol blue extracted with toluene and estimated colorimetrically. The effects of 75 % CO on the tea oxidase in the light and in complete darkness are summarized in Table 11.

Table 11

O_2 %	N_2 %	CO %	Illumination	Reaction
5	95	Nil	Light	++++
5	20	75	Light	+++(+)
5	20	75	Dark	++

There was about 50 % inhibition when the reaction was carried out in the dark, but in the light inhibition did not exceed 10 %.

Anaerobic fermentation of tea leaf

Recent work by Marsh & Goddard [1939, 2] on the relation between respiration and anaerobic fermentation in carrot root tissue has shown that inhibition of respiration, either by lowered O_2 tension or by poisons such as HCN, results in increased fermentation and hence in a rise in the R.Q. The R.Q. of respiring tea leaf is similarly enhanced by cyanide inhibition.

Table 12. *Effect of cyanide on R.Q. of respiring tea leaf*

Molar conc. of HCN	$\mu\text{l. per } 100 \text{ mg. leaf per hr.}$		R.Q.
	O ₂	CO ₂	
Nil	120	144	1.20
2×10^{-3}	78	132	1.69
1×10^{-2}	42	84	2.00

The figures for O₂ uptake and CO₂ outputs are calculated from initial rates; hence the high R.Q. recorded for uninhibited respiration.

It was found possible to demonstrate a net evolution of CO₂ from tea leaf when disks were suspended in Warburg vessels in an atmosphere of N₂. The anaerobic fermentation however is better followed by the Pettenkofer method.

In one series of experiments, using 50 g. samples of fresh green leaf, the CO₂ evolved per hr. averaged 8.2 ml. (at N.T.P.) in a current of air, and 4.3 ml. in a current of N₂. As the quotient, CO₂ of fermentation/CO₂ of respiration, is greater than one-third, and in one experiment amounted to 0.69, the Pasteur effect is probably in operation. This cannot be established definitely unless it is certain that the ratio C₂H₅OH/CO₂ is close to unity in accordance with the equation for alcoholic fermentation,

and at the moment we have no evidence in this direction.

Effect of mechanical damage on anaerobic fermentation

In the previous section it was shown that inhibition of the respiration of tea leaf by cyanide caused an increase in the R.Q. No such increase could be found when the O₂ uptake of a finely minced tea-leaf suspension was inhibited by $10^{-2} M$ HCN. The total O₂ uptake of the poisoned system amounted only to 6 $\mu\text{l.}$ after 1 hr. and CO₂ evolution was too small to be measured. The net changes were too small to be accurately measured but the R.Q. was certainly less than 0.2. Thus, in contradistinction to undamaged tea leaf, no anaerobic glucose breakdown is evident in the finely minced tissue.

This is shown even more strikingly by results obtained with a suspension of finely minced tea leaf in water, shaken in an atmosphere of N₂. Naturally, the O₂ uptake under anaerobic conditions is nil; in addition, the CO₂ output does not differ significantly from zero after 90 min. shaking. This is in marked contrast to the findings for the whole tea leaf. The mincing of the tissue has apparently destroyed the power of the tissue to undergo anaerobic fermentation.

The manometric method is not well adapted to measuring very small rates of CO₂ production, and these findings do not necessarily prove that the destruction of the ability to undergo anaerobic fermentation is complete. In a fine mince however it may be concluded that the rate of anaerobic fermentation does not exceed 5% of the rate in undamaged tissue.

The Pettenkofer method, where CO₂ is determined directly, is a more accurate means of estimating small quantities of CO₂, and results obtained by this method show that for coarsely minced tea leaf, and for leaf that has undergone mechanical rolling in the factory, anaerobic fermentation is very largely inhibited. Complete inhibition is not observed but the damage during a coarse mincing or in the machines used for rolling tea leaf in the factory is not so complete as in a very fine mince.

Table 13. *Effect of mechanical injury on rate of anaerobic fermentation*

	ml. CO ₂ /100 g. fresh leaf/hr. in N ₂
Fresh green leaf	8.60 (7.02-9.46)
Fresh leaf coarsely minced in a meat chopper	3.26, 4.06
Withered leaf hand-rolled for 1 hr.	0.24
Withered leaf factory-rolled for 30 min.	0.50 (0.33-0.80)
Withered leaf factory-rolled for 70 min.	0.29 (0.16-0.40)

The above figures for fresh and for factory-rolled leaf are each average values for 5 separate determinations. Destruction of the power to undergo anaerobic fermentation by rolling the withered leaf, whether mechanically or by hand, is high (90-98 %). A coarse mincing in a meat chopper is not so effective and many intact pieces of tissue can be picked out from the chopped up leaf. Broadly speaking the greater the extent of mechanical damage the lower the rate of anaerobic fermentation.

DISCUSSION

The nature of the oxidase in tea leaf. As tea fermentation is considered to result from the disorganization of normal respiration, it is a matter of some importance to establish that the respiration of the tea leaf differs in no striking way from the respiration of other leaves. The analogy with barley, as far as the effects of carbohydrate impoverishment on respiration are concerned, is particularly striking as the two species are by no means closely related.

The effects of poisons on tea leaf respiration are also very similar to those found for other leaves. Thus James & Hora [1940] have shown that $M/500$ HCN causes 64 % inhibition of the respiration of barley leaves, and that $M/50$ HCN is required for complete inhibition. Further resemblances to tea were shown by the lag period before the HCN exerted its full effect, and the rise in the R.Q. to a value greater than unity as a result of HCN poisoning.

According to Ross [1938] the respiration of *Nitella* is only 37 % inhibited by $M/1000$ HCN. For carrot leaf [Marsh & Goddard, 1939, 1] inhibition by $M/1000$ HCN of the respiration amounts only to 61-72 % and even with $M/100$ HCN inhibition is by no means complete.

For such leaf tissues as have been investigated, in which the cytochrome system is probably operating, inhibition by cyanide is not always complete and often does not reach its maximum effect until the concentration of poison is $M/100$. This is in marked contradistinction to yeast where $M/2000$ HCN brings about complete inhibition.

For tea this relatively low sensitivity to cyanide persists in simple enzyme-substrate systems, and it appears that the oxidase system is less sensitive to cyanide than the cytochrome system from yeast. Alternatively there may be more than one oxidase system in the tissue, one of which is cyanide-stable.

This latter alternative deserves careful consideration. Boswell & Whiting [1938] have already concluded that the catechol oxidase system accounts for only two-thirds of the respiratory activity in potato tubers. The presence of a cyanide-stable oxidase system in carrot leaf, which increases with maturity, has also been deduced by Marsh & Goddard [1939, 1] who found that in the fully mature leaf, cyanide may sometimes stimulate respiration. For tea the maturer leaves are less sensitive to cyanide inhibition, so we have here some slight evidence in favour of a cyanide-stable oxidase, the relative activity of which increases with the age of the tissue.

There is another possible explanation of the low sensitivity of respiring plant tissues to the inhibitory effect of cyanides which, we believe, has not as yet been put forward. Jones [1920] stated that plant tissues generally give a more intense reaction for Fe than do animal tissues, and Ingalls & Shive [1931] demonstrated that only a small fraction of the Fe content is water-soluble. Miller [1938] states that the greater part of the Fe in plants is immobile, so it appears that a considerable portion of the Fe may be in complex organic form. The amount of this Fe is considerably in excess of that which can be accounted for by the cytochrome system. We suggest that this "fixed" Fe is able to form complexes with cyanide, so that with competition for cyanides between this fixed Fe and the respiratory system, considerably more cyanide is required for complete suppression of respiratory activity. No definite evidence can as yet be advanced in favour of this hypothesis but it is interesting to note that the less cyanide-sensitive 5th leaves of the growing tea shoot have a significantly higher Fe content. Mr P. B. Sen Gupta of this laboratory has determined the Fe contents of the 1st and 5th leaves of the growing shoot and found them to be 0.088 and 0.112% of dry matter respectively.

The CO inhibition of tea oxidase and its reversal by light is a characteristic of the cytochrome system. Similar behaviour has also been recorded for carrot respiration (leaf and root) by Marsh & Goddard [1939, 1] and for several plant tissues including the leaves of tobacco, plum and oleander [Kempner, 1936]. A relatively low inhibition by cyanide of leaf respiration may therefore be associated with a light-reversible inhibition by CO of the same order as that found for yeast respiration.

Further evidence for the presence of a cytochrome-like system in tea leaf is provided by the catholicity of substrates oxidized by a tea oxidase preparation. The range of substances oxidized is too varied to be in accordance with current views on the specificity of an enzyme towards its substrates. On the other hand, this behaviour is what would be expected were the cytochrome system operative, as the method of preparation of the tea oxidase is such that cytochrome would be found in the final product. By the same token catechol oxidase is unlikely to be present, as the method of alcohol precipitation used in the preparation of the crude enzyme would yield a product free, or nearly free, from such alcohol-soluble carriers as catechol or its derivatives.

It may therefore be concluded that either the cytochrome system or one very closely resembling it is responsible both for respiration of the tea leaf and for tea fermentation.

The effect of mechanical injury on anaerobic fermentation. Anaerobic fermentation is quite easily demonstrable in the tea leaf either by depressing the rate of aerobic respiration by inhibitors or by measuring the CO₂ output in an atmosphere of N₂. The behaviour of tea leaf under such conditions is very similar to that of carrot tissue [Marsh & Goddard, 1939, 2] and barley [James & Hora, 1940].

James & Arney [1939] have recently demonstrated that phosphate esters play essentially similar parts in the respiration of barley to those established in the case of yeast; hence it does not seem unreasonable to conclude that the role of cozymase is the same in anaerobic fermentation in both yeast and plant tissues.

Now the experimental results recorded in this communication show without doubt that extensive mechanical damage to the tissue may almost completely destroy the ability of the tissue to undergo anaerobic fermentation under otherwise suitable conditions. This cannot be attributed to extensive inactivation of the dehydrogenase, for under aerobic conditions, where oxidation products of

tea tannin may function as H-acceptors [Roberts & Sarma, 1940], carbohydrate oxidation is quite evident, and such oxidation requires the co-operation of several dehydrogenases. The failure of the mechanically damaged tissue to undergo anaerobic fermentation must therefore be attributed to an inhibition of one of the H-acceptors.

On *a priori* grounds it would seem that the H-acceptor inactivated by extensive damage to the tissue must be cozymase (or possibly coenzyme II, or both) as it is difficult to visualize the inactivation of any other H-acceptor which would have such different effects on the rate of carbohydrate breakdown under aerobic and anaerobic conditions. It is hoped when supplies of cozymase become available, to subject these conclusions to experimental verification.

SUMMARY

The respiration of tea leaf has been studied. The R.Q. for fresh leaf is close to 1.0 and decreases as a result of carbohydrate impoverishment. Rate of respiration, but not oxidase activity, is significantly depressed as a result of starvation in the absence of light.

Respiration and tea fermentation require $M/100$ HCN for maximum inhibition. Tea oxidase-substrate systems have the same cyanide sensitivity. It is suggested that the low cyanide sensitivity of plant oxidases in general may be due to the presence of other organic Fe compounds in the tissue which compete with the oxidase system for the cyanide.

The CO inhibition of the tea oxidase is reversed by light. This, and the wide range of substrates oxidized, suggests that the tea oxidase is a cytochrome oxidase.

Anaerobic fermentation is demonstrated in tea leaf after cyanide inhibition or in an atmosphere of N_2 . Extensive damage to the tissue almost completely destroys its ability to undergo anaerobic fermentation. As dehydrogenase activity is not affected to the same extent it is concluded that the effect of injury to the tissues is to inactivate the coenzymes concerned in carbohydrate oxidation.

The authors wish to thank Mr P. H. Carpenter for his interest in this work, and the Indian Tea Association for permission to publish these results.

REFERENCES

- Allen & Goddard (1938). *Amer. J. Bot.* **25**, 613.
- Boswell & Whiting (1938). *Ann. Bot., Lond.*, N.S. **2**, 847.
- Ingalls & Shive (1931). *Plant Physiol.* **6**, 103.
- James & Arney (1939). *New Phytol.* **38**, 340.
- & Hora (1940). *Ann. Bot., Lond.*, N.S. **4**, 107.
- Jones (1920). *Biochem. J.* **14**, 654.
- Keilin (1929). *Proc. roy. Soc. B*, **104**, 206.
- Kempner (1936). *Plant Physiol.* **11**, 605.
- Kidd (1935). *Nature, Lond.*, **135**, 327.
- Krebs (1935). *Biochem. J.* **29**, 1632.
- Marsh & Goddard (1939, 1). *Amer. J. Bot.* **26**, 724.
- (1939, 2). *Amer. J. Bot.* **26**, 767.
- Miller (1938). *Plant Physiol.*, p. 334. New York: McGraw Hill.
- Roberts (1939). *Biochem. J.* **33**, 842.
- (1940). *Biochem. J.* **34**, 500.
- & Sarma (1940). *Biochem. J.* **34**, 1517.
- Ross (1938). *Amer. J. Bot.* **25**, 458.
- Turner (1938). *New Phytol.* **37**, 232.
- Yemm (1935). *Proc. roy. Soc. B*, **117**, 483, 504.