The Effects of Partial Pressure of Oxygen upon Respiration and Nitrogen Fixation by Soybean Root Nodules

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SUMMARY

Increased oxygen tension (pO_2) caused increased respiration by excised soybean nodules of all ages. The increase took place in two steps, the first maximum occurring at about 50 % O_2 and the second at 90–100 % O_2 for actively nitrogen-fixing nodules. With increasing nodule age the first maximum occurred at decreasing pO_2 until, when fixation ceased at about 6 weeks, this maximum had disappeared. This effect was more marked at 30° than at 23°. The respiration of bacteroids increased with increasing pO_2 with a maximum at 2–3 % O_2 ; the curve indicated a simple saturation of the terminal respiratory pathway with O_2 . Increased pO_2 raised nitrogen fixation by excised nodules to a maximum which corresponded to the first maximum of the respiratory response to raised pO_2 ; higher pO_2 than this decreased nitrogen fixation. Sliced nodules showed the same effect but the stimulation of fixation at the lower pO_2 levels was not as great as with intact nodules. The Michaelis constant (K_m) for nitrogen fixation by intact excised nodules was relatively unaffected by pO_2 until this reached the pO_2 for maximum fixation when the K_m rose sharply. At external pO_2 of 80 %, oxygen was shown to be a competitive inhibitor of nitrogen fixation.

An explanation of these results is offered; it is suggested that the first part of the nodule O_2 consumption/ pO_2 curves is due to O_2 consumption by plant tissue and the second part to O_2 consumption by bacteroids. The two components are separated by an O_2 permeability barrier. When this barrier permits a rise in the pO_2 at the bacteroids, nitrogen fixation is inhibited as oxygen competes with nitrogen for the reducing power of the bacteroids.

INTRODUCTION

Allison, Ludwig, Hoover & Minor (1940) concluded from studies of the effects of increased oxygen tension upon nodule respiration that oxygen tension was low within the tissue of soybean root nodules. Ebertova (1959) measured low redox potentials within nodules during the period in which nitrogen was fixed. In contrast to these findings Ferguson & Bond (1954) found that increased oxygen tension in the root medium favoured nitrogen fixation by whole nodulated plants; and Burris, Magee & Bach (1955) found with sliced soybean nodules that oxygen tensions up to 50 % O_2 stimulated fixation but that higher tensions were inhibitory. Bond (1961) reported similar effects with root nodules of non-leguminous plants.

A unified hypothesis which relates the various phases of symbiotic nitrogen fixation in legume nodules has been proposed (Bergersen, 1960*b*) in which it is suggested that reducing power generated in the bacteroids brings about the ultimate production of NH_3 from N_2 . This hypothesis demands that oxygen tension at

the surface of the bacteroids be low (much less than the tension which saturates their terminal respiratory enzymes) if aerobic terminal respiration is not to compete with N_2 for their reducing power. The work to be reported here was undertaken to investigate the apparent paradox of oxygen both stimulating and inhibiting nitrogen fixation by legume nodules. In doing this the work also provided a test of one aspect of the hypothesis.

METHODS

Plant material. Lincoln variety soybeans were grown and nodule age recorded as previously described (Bergersen, 1958). Nodules were detached from the roots into beakers immersed in ice and experiments were begun within 1 hr. of picking the first nodule.

Bacteria. *Rhizobium japonicum* strain CC711 was used throughout and was grown on yeast-extract mannitol agar. Suspensions of bacteroids (the nodular form of the organisms) were obtained from crushed nodules as previously described (Bergersen, 1958).

Respirometry. Respiration was measured in double capillary Warburg manometers equipped with stopcocks between the fluid reservoirs and the capillaries to permit evacuation and flushing of the respirometers with various gas mixtures. In doing this the fluid was lowered to the hole of the stopcock and the respirometers evacuated and filled through both sides of the manometer. When filled, the fluid was raised and a positive pressure maintained until just before measurements began, when the excess pressure was released.

Respiration of whole and sliced nodules was measured in Warburg vessels (volume 30 ml.) containing 20 nodules and 0.25 ml. phosphate buffer (M/15, pH 7.0) with 0.2 ml. 40 % (w/v) KOH in the centre wells. Respiration of bacteroids was measured in the same vessels using 1.0 ml. bacteroid suspension and 1.0 ml. of 0.02 M-Na succinate or 1 ml. of phosphate buffer (M/15, pH 7.0).

Preliminary tests showed that little change in pO_2 due to uptake of oxygen resulted during measurement of the respiration rate during 30 min. with bacteroids or during 1 hr. with nodules; with bacteroids the pO_2 fell from 2.0 to 1.8 % and from 1 to 0.9 %. These small changes produced no detectable change of slope when the uptake of O_2 was plotted against time. With still lower pO_2 values however, a decline in rate could be detected after 20 min. In these cases only the initial rates were used.

In all cases respiration was expressed as qO_2 (μ l. uptake of O_2 /hr./mg. dry-weight tissue). In the first experiment a temperature of 30° was used. Later this was changed to 23°, the optimum for nitrogen fixation.

Gas mixtures. These were prepared from commercial gases through manifolds equipped with mercury manometers. The lines were evacuated by a rotary pump to 0.05 mm. Hg (McLeod gauge) and various scales graduated in percentage were used on the manometers according to the barometric pressure at the beginning of the experiment. Argon was used to bring the mixtures to one atmosphere. The following are mass spectrometric analyses of the gases used:

Nitrogen	N_2 (98.2 %), O_2 (0.1 %), A (1.7 %)
Argon	A (96.3 %), N_2 (3.2 %), O_2 (0.5 %)
Oxygen	O_2 (98.7 %), N_2 (1.3 %), A (trace)

For all purposes except the determination of the Michaelis constants, gas mixtures contained 10 % N_2 .

Isotopic nitrogen (^{15}N). $^{15}N_2$ gas was prepared from NH_3 (generated from NH_4^+ labelled NH_4NO_3) by oxidation over CuO at 600–700°. The gas was passed through the oxidant for 1 hr. after constant volume was reached and was then collected over Hg after being circulated through a liquid N_2 cooled trap where nitrogen-containing impurities condensed. Mass spectrometric analyses showed less than 1 % O_2 and no trace of mass 31 (^{15}NO), 45 or 46 ($^{14}N^{15}NO$ or $^{15}N_2O$) or 47 ($^{15}NO_2$).

Measurement of nitrogen fixation. Nodules were exposed at 23° to gas mixtures containing $^{15}N_2$ in 50 ml. Erlenmeyer flasks attached to a manifold. After exposure, the nodules were crushed in 3 N-HCl and the soluble material analysed for ^{15}N after Kjeldahl digestion, distillation of NH_3 and conversion of NH_3 to N_2 by alkaline $NaOBr$ containing 0.1 % KI (Francis, Mulligan & Wormall, 1959). The atoms % excess ^{15}N in the samples was a measure of the nitrogen fixation of the nodules during their exposure to the labelled gas mixture.

Mass-spectrometric determinations. All isotope measurements and gas analyses were done with an M86 (Atlas-Werke, Bremen) mass spectrometer. Atoms % ^{15}N were determined from the mass 29:28 ratios of the samples and the excess calculated from alternative readings of a sample of air N_2 contained in the other half of the double inlet system. All samples and standards were measured at the same inlet pressure (5 mm. Hg). Corrections were made for air leakage occurring during preparation of the samples by measuring the magnitude of the mass 28 and 32 peaks and applying corrections to the atoms % excess ^{15}N (Francis *et al.* 1959). In these experiments the maximum analytical error of repeated determinations on the same sample of gas was ± 2 % of the atoms % excess. The maximum analytical error of a complete determination, including digestion, distillation, conversion to N_2 , measurement and correction was ± 6.5 % of the atoms % excess. In any one experiment all values of ^{15}N excess of samples were adjusted by multiplying by c/x where c was the chosen atoms % excess of the incubation gas mixture and x was the actual value analysed. This step was necessary because contaminating N_2 in the other gases used in the mixture caused small variations in the percentage of ^{15}N in the mixtures of different composition which were used in an experiment; the step thus put all measurements on a common basis. Gas analyses were prepared from mass-spectra, the relative peak heights of which gave the partial pressures of the components of the mixture.

RESULTS

The relationship between pO_2 and qO_2 for nodules and bacteroids

The effect of nodule size and slicing. The general form of the effects of pO_2 upon nodule respiration is seen in Fig. 1, which illustrates the results obtained with 23-day nodules. When qO_2 was plotted against pO_2 (the qO_2/pO_2 curve) the result was a distinct two-step curve with the first maximum at about 50 % O_2 and the second at about 90 % O_2 . This type of curve was repeatedly obtained throughout this work as long as nodules of a single age were used. The plants from which the nodules used in this experiment were taken fell into two well-defined groups: those with few large (3–4 mm. diam.) nodules and those with many small (1.5–3 mm. diam.) ones. These nodules were of the same age to within one day and each plant bore the same

Fig. 1. The effects of nodule size and slicing on qO_2/pO_2 curves for nodules. Respiration measured at 30° . $\times$, 23-day old nodules: large sliced; $\bullet$, 23-day-old nodules: small, whole; $\circ$, 23-day-old nodules: large, whole.

Fig. 2. The effects of nodule age on the qO_2/pO_2 curves for intact nodules, measured at 30° .

weight of nodules. The effects of this difference in size are seen in Fig. 1. The small nodules had a slightly higher qO_2 at each pO_2 value and the maximum occurred at slightly lower pO_2 than was the case for the large nodules. In all subsequent experiments only the large nodules were used because they were more easily handled.

When the large nodules were sliced (about 1 mm. thick) the general form of the qO_2/pO_2 curve was still apparent, but the first maximum was not as marked as with the intact nodules and the qO_2 at each pO_2 value was raised. The magnitude of the first step of the curve was increased by a little more than a third and that of the second step was slightly reduced.

The effects of nodule age on the qO_2/pO_2 curve. The qO_2/pO_2 curves were determined for nodules of different ages. The results are illustrated in Fig. 2. The two-step curve was found at all ages except 46 days. With young nodules (7–14 days) the two

Fig. 3. The qO_2/pO_2 curves for bacteroids isolated from nodules of various ages. Bacteroids from nodules aged: ○, 8 days, succinate; ●, 15 days, succinate; □, 22 days, succinate; ■, 47 days, succinate; ◻, 22 days, endogenous.

maxima occurred at lower pO_2 values than with actively nitrogen-fixing (21–23 days) nodules. As the nodules aged, both maxima occurred at progressively lower pO_2 . At 46 days the first maximum, when present at all, occurred at less than 5 % O_2 and nodule respiration was completely saturated with respect to O_2 at 10 %. From previous work (Bergersen, 1958, 1960*a*) it is known that fixation ceases when the nodules are about 42 days old. Concurrently with this experiment measurements were made of the respiration of isolated bacteroids and the relative proportions of the various nodule fractions were determined.

The effects of nodule age on the qO_2/pO_2 curves for bacteroids. Bacteroids were prepared from nodules aged 8, 15, 22 and 47 days and the respiration measured at a range of pO_2 (Fig. 3). The maximum qO_2 was attained at 2–3 % O_2 , and the curves are similar to those given by Burris & Wilson (1939). The qO_2/pO_2 curves are typical of the saturation of an enzyme with increasing concentrations of its substrate and there was no evidence of a two-step curve as seen with intact nodules. The main

effect of nodule age was that which has already been reported, as variations occurred in the maximum qO_2 which were in close agreement with the results of Bergersen (1958). At all ages the endogenous respiration was one-third of the respiration with succinate as substrate and was very stable, the qO_2 of 22-day-old bacteroids being unaltered after they had been standing in buffer at room temperature for 30 hr.

The effects of age on the composition of nodules. The composition of nodules is given in Table 1. For these determinations the nodules were crushed and the dry weights of the various fractions obtained after centrifugal separation. The nature of the fractions was checked microscopically. The composition became fairly stable from 15 days with the bacteroids making up about 25 % of the dry weight and the cell walls and large particles of the host accounting for about 40 %.

Table 1. *The composition of soybean root nodules of various ages determined on a dry-weight basis*

Fraction	Nodule age (days)				
	5	8	15	22	30
	Fraction (%)				
Large particles and cell walls (1)	80	56	43	39	38
Bacteroids (2)	3	19	28	25	25
Small particles (3)	1	2	2	3	3
Soluble (4)	15	23	26	26	26

(1) Sedimented from nodules crushed in buffer at 300 g for 4 min.

(2) Sedimented from the supernatant of (1) by 4000 g for 6–8 min.

(3) Sedimented from the supernatant of (2) by 6000 g for 20 min.

(4) Supernatant from (3).

The effects of pO_2 upon nitrogen fixation ($^{15}N_2$ uptake)

It has already been shown by Burris *et al.* (1955) that pO_2 affects nitrogen fixation by excised soybean nodules, and it was necessary to ascertain that these effects were not due to an increase or a decrease in the time during which N-fixing capacity was retained by the excised nodules. Figure 4 shows that this was not the case, since at 20, 37 and 81 % O_2 , fixation was linear with time for at least 90 min. No fixation at all was detected at 5 % O_2 ; this is in agreement with all the present experiments, in which N_2 fixation could be extrapolated to zero at about 5 % O_2 for intact nodules. In this experiment, exposure to $^{15}N_2$ commenced 55 min. after the first nodule was detached.

Nitrogen fixation increased with increasing pO_2 up to 30–50 %, above which it was inhibited. With intact nodules, the rise in fixation with increasing pO_2 from 10 to 50 % was greater than the decrease which occurred from 50 to 80 %, but with sliced nodules the inhibition was as great as the stimulation over a similar increment of pO_2 (Table 2; Fig. 5).

The effects of nodule age upon ^{15}N uptake with different pO_2 values. The amount of nitrogen fixed at the optimum pO_2 varied with nodule age, being greatest at 28–32 days (Table 2), and the optimum pO_2 decreased from 50 % at 32 days to 30 % at 40 days.

The relationship between the qO_2/pO_2 curve and $^{15}N_2$ uptake with varying pO_2 . The

data in Table 2 clearly show that the pO_2 range of the first maximum of the qO_2/pO_2 curve corresponded with the pO_2 range for maximum nitrogen fixation. Figure 5 illustrates the form of the curves for nodules aged 32 days. This relationship was not found in older nodules (36 and 40 days) when respiration was measured at 30° . In these cases the first maximum of the qO_2/pO_2 curve occurred at a lower pO_2 than at 23° (compare Fig. 2 and Table 2).

Fig. 4

Fig. 4. The time course of ^{15}N uptake by 26 day old intact nodules. ●, 37% O_2 , ○, 81% O_2 , x, 20% O_2 , 10%; +, 5% O_2 , all gas mixtures contain 10% $^{15}N_2$ (46 atoms %).

Fig. 5

Fig. 5. The relationship between the qO_2/pO_2 curve and the nitrogen fixation/ pO_2 curve for intact 32 day-old nodules. Both fixation and respiration measured concurrently at 23° .

The effects of pO_2 on the kinetics of nitrogen fixation. The kinetics of nitrogen fixation by intact nodules were examined by measuring the effect of N_2 concentration upon the nitrogen fixed in 1 hr. with different pO_2 values. The ^{15}N excess of the nodules after 1 hr. was a valid measurement of the velocity (v) at any particular N_2 concentration (s) since fixation was linear with time for at least 90 min. The Dixon (1953) modification of the Lineweaver & Burk (1934) graphical method was then used to determine the Michaelis constant (K_m) and the maximum velocity (V_{max}) for nitrogen fixation in the Michaelis-Menten equation

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{pN_2}$$

by plotting $1/v$ against $1/s$ at each pO_2 , for nodules aged from 25 to 27 days. The oxygen tension in this work as in the respiratory work was taken as the initial value. The N_2 concentration was taken as the final pN_2 measured in a gas sample taken at the end of the incubation. At each pN_2 for any pO_2 , duplicate determinations of ^{15}N excess were made on each of two separate samples of nodules.

Statistical methods were necessary in order to exploit the data fully. The following is an outline of the methods of analysis used as a result of examination of the form of the data. The variance of $1/v$ increased with $1/v$ so that a weighted regression procedure was necessary. To determine the weight function the regression of the

difference between (or range of) the sample means of two duplicates on the average of the means was determined in a relationship of the form: $\text{range} = b_1(1/v) + b_2(1/v)^2$. This relationship itself was fitted using weights inversely proportional to $(1/\text{range})^2$. The coefficient of the linear term, b_1 , was significant while b_2 was not, although there is little doubt of the reality of the curvature. However, these data do not conform to a relationship: $\text{range} = b(1/v)^2$, as suggested by Wilkinson (1961). The weights were taken as the inverse of the squares of the range estimated from the regression relation of any average $1/v$. The weighted regression of $1/v$ on $1/pN_2$ was fitted using standard least squares procedure. The confidence limits of the intercepts on the x axis ($-1/K_m$) and on the y axis ($1/V_{\max}$) were calculated using standard procedures.

Table 2. *The influence of the partial pressure of O₂ upon respiration and nitrogen fixation of excised soybean nodules of various ages*

Nodule age (days)	Measurement	Partial pressure of O ₂ (%)									
		5	10	20	30	40	50	60	70	80	90
23	qO ₂ *	—	1.66	2.48	—	3.41	3.60	4.38	5.07	—	5.10
	¹⁵ N excess†	—	0.064	0.202	—	0.626	0.653	0.550	0.451	0.428	—
	(atoms %)	—	0.060	0.156	—	0.766	—	0.583	0.467	0.420	—
23 (sliced)	qO ₂ *	—	2.45	3.50	—	4.36	4.52	4.87	—	5.89	6.23
	¹⁵ N excess†	—	0.050	0.195	—	0.410	0.423	—	0.311	0.143	0.056
	(atoms %)	—	—	—	—	—	—	—	—	—	—
28	qO ₂	—	0.40	0.72	—	1.10	1.20	1.41	1.83	—	1.97
	¹⁵ N excess†	—	0.188	0.649	—	0.949	1.071	1.113	0.882	—	0.769
	(atoms %)	—	0.199	0.661	—	0.975	1.127	—	0.851	—	0.760
32	qO ₂	—	0.62	0.81	—	1.04	1.18	1.64	1.94	—	2.31
	¹⁵ N excess	—	0.067	0.434	0.919	1.105	—	0.926	—	0.613	—
	(atoms %)	—	0.071	0.428	0.921	1.116	1.111	0.930	—	0.609	—
36	qO ₂	—	0.75	1.23	1.46	1.70	1.68	2.04	—	2.90	—
	¹⁵ N excess	—	0.110	0.479	0.920	1.003	—	0.901	—	0.840	0.780
	(atoms %)	—	0.110	0.482	0.926	1.012	—	0.900	—	0.832	0.712
40	qO ₂	0.25	0.68	1.37	1.52	1.80	—	2.77	—	2.90	—
	¹⁵ N excess	—	0.019	0.195	0.598	0.598	0.531	0.403	—	0.399	—
	(atoms %)	—	0.019	0.205	0.629	0.559	0.497	0.494	—	0.380	—

qO₂ = $\mu\text{l./hr./mg. dry wt. nodule}$. Respiration was measured on 20 nodules in each Warburg vessel; 0.5 ml. $M/15$ phosphate buffer (pH 7.0), was added to prevent drying out of the tissue; centre wells contained 0.2 ml 20% KOH.

¹⁵N uptake was measured by analysing the portion of the nodules which was soluble in 3N-HCl after 1½ hr. exposure at 23° to gas mixtures enriched in ¹⁵N. Figures for duplicate nodule samples are shown.

* The respiration of the 23-day-old nodules was measured at 30°. All other qO₂ values shown were measured at 23°.

† Corrected ¹⁵N excess figures; all results are expressed as if the incubation gas contained 55.8 atoms % excess ¹⁵N.

The results are given in Table 3. It will be seen that $V_{\max}$ increased with pO₂. K_m was only slightly affected by pO₂ at the lower levels but above 40% increased very sharply. The intercepts on the y axis for 60 and 80% O₂ were not significantly different but the slope was greater for 80% O₂. These results meet the criteria for competitive inhibition of nitrogen fixation by oxygen when comparing 60 and 80% O₂. The intercepts on the y axis for 50 and 60% O₂ were just significantly different, but the slope was greater for 60% O₂. Thus, comparing these two partial pressures

of O_2 , the results approach competitive inhibition. The Dixon-Lineweaver-Burk plots for these three partial pressures of O_2 are presented in Fig. 6.

Figure 7 shows the change in K_m with pO_2 and the change in nitrogen fixation at a constant pN_2 (10 %). These latter figures were determined from the regression diagrams of $1/v$ on $1/pN_2$ at each pO_2 . Figure 7 thus shows the change in the kinetics of the nitrogen-fixation reaction in relation to the earlier experiments, in which the effects of pO_2 upon fixation were determined with pN_2 equal to 10 % (Table 2 and Fig. 5).

Fig. 6

Fig. 7

Fig. 6. The Dixon-Lineweaver-Burk plot for nitrogen fixation by intact 26 day-old nodules at 50, 60 and 80 % O_2 . $\circ-\circ$, 80 % O_2 ; $\bullet-\bullet$, 60 % O_2 ; $\times-\times$, 50 % O_2 .

Fig. 7. The relationship between the change in K_m with pO_2 and the change in fixation (v) with pO_2 when the gas phase contained 10 % $^{15}N_2$.

Table 3. Data from the Lineweaver-Burk plots of nitrogen fixation at different pO_2 values. The intercepts on the x axis gave $-1/K_m$ and on the y axis $1/V_{max}$.

Initial pO_2 (%)	$1/V_{max}$		V_{max}		$-1/K_m$		K_m	
	Esti- mate	95 % limits	Esti- mate	95 % limits	Esti- mate	95 % limits	Esti- mate	95 % limits
20	3.4157	3.9365 2.8949	0.2927	0.2540 0.3454	13.9361	18.8963 10.3181	0.0718	0.0529 0.0969
30	2.5058	3.1368 1.8748	0.3991	0.3188 0.5384	11.8712	18.2242 7.5261	0.0842	0.0549 0.1329
40	1.1769	1.5145 0.8393	0.8497	0.6603 1.1915	7.8766	12.7561 4.6755	0.1269	0.0784 0.2139
50	0.7245	0.9440 0.5050	1.3802	1.0593 1.9802	3.7775	5.4518 2.4055	0.2647	0.1834 0.4157
60	0.5660	0.7168 0.4152	1.7667	1.3951 2.4085	2.1954	3.0111 1.4992	0.4554	0.3321 0.6670
80	0.4036	0.6247 0.1825	2.4777	1.6008 5.4795	0.9402	1.5798 0.3954	1.0636	0.6330 2.5291

DISCUSSION

There are intriguing effects of oxygen tension both on nodule respiration and on nitrogen fixation. In discussing these results it is convenient to divide this part of the paper into sections, but it should be realized that the results are composed of inter-related observations.

The effects of pO_2 on nodule respiration. In the widest sense the results presented are in agreement with the work of others (e.g. Allison *et al.* 1940; Ebertova, 1959) who have concluded that the oxygen tension in legume root nodules in air is low. This is shown by the 2–3-fold increase in qO_2 when the external pO_2 is increased from air tension (20 %) to 90 %. The additional information which has come from the present work is that this increase in respiration is not simple. The failure of others to observe the two-step nature of the qO_2/pO_2 curve can be attributed largely to the fact that no precautions were taken about nodule age. Nodules of a range of ages would have the first maximum at different pO_2 values and hence the whole curve would tend to be smoothed out. An explanation of the qO_2/pO_2 curve is offered.

Interpretation of the nodule respiration data. From studies of the anatomy and cytology of soybean nodules the bacteroids are seen to be the innermost component since in the central tissue in which they occur they are enclosed within membrane envelopes in the cytoplasm of the host cells (Bergersen & Briggs, 1958). It may also be noted again that the tissues of soybean nodules are uniform in cell age since these nodules have no growing point (Bergersen, 1958). From these considerations and assuming (i) that bacteroids within the nodules have a qO_2 similar to their endogenous qO_2 as measured in the Warburg; and (ii) that root tissue has a similar qO_2 to the plant tissue component of nodules, it is possible to offer an explanation of the unusual pattern of nodule respiration with increasing pO_2 . Because the bacteroids are the innermost respiring component it is logical to suggest that their respiration is the last to be saturated with respect to O_2 as the external pO_2 increases; that is to say, the second step of the qO_2/pO_2 curve for the nodules represents the bacteroid respiration increasing to a maximum with increased penetration of O_2 . Table 1 shows that for nodules aged 22 days the bacteroids composed 25 % of their dry weight. Figure 3 shows that the maximum respiration of bacteroids isolated from these nodules occurs at 2–3 % O_2 . If we now consider the data for nodules aged 21 days (Fig. 2), the bacteroids (22-day sample, Fig. 3) respiring at the maximum endogenous rate would account for 2.5 $\mu l./hr.$ of O_2 uptake. This is the magnitude of the second step of the qO_2/pO_2 curve (Fig. 2, 21 days).

Turning to the plant tissue component of the nodule respiration, it is seen that this is made up of the respiration of the cortical tissue and the central (bacteroid-containing) cells, the peripheries of which contain mitochondria (unpublished electron microscope observations). It was found that 23-day soybean root segments had a qO_2 of 2.52 at 20 % O_2 and 3.36 in 100 % O_2 . These values are close to those of Allison *et al.* (1940), namely, 2.12 and 2.88, respectively. They support the suggestion that the first part of the qO_2/pO_2 curve represents the saturation with O_2 of the plant tissue respiration of the nodule, because 75 % of the nodule dry weight is plant tissue and 75 % of 3.36 (the maximum qO_2 of root tissue) is close to the magnitude of the first maximum of the curve for these nodules (Fig. 2). The suggested explanation is summarized in Fig. 8, which shows how the nodule qO_2/pO_2 curve

could be the sum of the respiration of the plant tissue and of the bacteroids as the respirations of these components are successively saturated with O_2 .

Assuming that this interpretation is correct, it is also possible to deduce the internal pO_2 at various external oxygen tensions. These deduced values are shown on the lower scale of Fig. 8.

Fig. 8. The explanation of the qO_2/pO_2 curve for nodules. The observed curve is considered to be the sum of the curves for the plant tissue and the bacteroids as these are successively saturated with respect to O_2 . —, Observed respiration 1 mg. nodules aged 21 days; ---, observed respiration 0.75 mg. root aged 21 days;, calculated respiration 0.25 mg. bacteroids.

This explanation of the nodule respiration data implies that there is an O_2 diffusion barrier between the host respiration and that of the bacteroids. The latter, with their high respiratory rate (about five times higher than that of the root tissue cells in terms of the dry weight) would respire any O_2 passing the outer respiring regions of the nodule as, with increasing external pO_2 , these regions approached saturation with respect to O_2 . There would be no break in the curve unless there was a further barrier which did not permit the passage of appreciable amounts of O_2 until a certain partial pressure had been exceeded. This diffusion barrier may be the membrane envelopes within which the bacteroids are enclosed (Bergersen & Briggs, 1958).

The effects of pO_2 upon nitrogen fixation. The data of Table 2 fully agree with those of Burris *et al.* (1955) who found similar responses to increased pO_2 with sliced nodules. However, in my work there was considerably less variation in $^{15}N_2$ uptake than was the experience of these authors or of Aprison, Magee & Burris (1954), who found it necessary to use sliced nodules to decrease variation. This decreased variability of nitrogen fixation in the present work is attributable once again to the use

of nodules of a single age in any one experiment. The use of nodules of mixed ages and of successive nodule samples from ageing plants will give rise to errors because the shape of the fixation/ pO_2 curve changes with age.

The nature of the stimulation of nitrogen fixation with increased pO_2 up to about 50 % in any one nodule sample remains obscure. The increased respiration of the host tissues in this pO_2 range may provide more substrates to the bacteroids or more acceptors for the fixed nitrogen. There is a significant correspondence between the first maximum of the qO_2/pO_2 curve and the optimum pO_2 for nitrogen fixation (Fig. 5). If the interpretation of the respiratory data which has been offered is correct, it would therefore seem that the penetration of O_2 into the vicinity of the bacteroids results in inhibition of nitrogen fixation. This has been confirmed by the kinetic data.

Parker & Scutt (1960) showed that, within certain limits, O_2 and N_2 competed as terminal hydrogen acceptors in nitrogen fixation by *Azotobacter vinelandii* and thus fixation may be regarded as a form of respiration. That a similar phenomenon might account for the inhibition of nodule nitrogen fixation at high pO_2 , described by Burris *et al.* (1955), has been suggested by Bergersen (1960*b*). The kinetic studies reported here show that there was in fact a competitive inhibition of nitrogen fixation by O_2 at high external pO_2 . The value for K_m of 0.07 atmosphere N_2 for 20 % O_2 is higher than the 0.025 obtained by Burris *et al.* (1955), probably because they used sliced rather than intact nodules. The Michaelis constant (K_m) also rose sharply in a range at which an internal O_2 diffusion barrier became permeable. The competitive nature of the inhibition is not necessarily due to a direct competition for the active site of the nitrogen-reducing enzyme. Any diversion of reducing power from any part of an electron transport chain could have a competitive effect. Thus, diversion of the bacteroid-reducing power to aerobic respiration could have a competitive effect even if the actual reduction of nitrogen or nitrogen containing intermediate compounds took place at a site remote from the bacteroids but linked with them by an electron transport chain. The results reported in the present work therefore support the hypothesis proposed elsewhere (Bergersen 1960*b*), that one of the main functions of the bacteroids in nodule nitrogen fixation is that they are a source of reducing power which is used for the ultimate reduction of N_2 to NH_3 .

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