results of a group of experiments on any one sample fell within ±10% of the mean value. All determinations opposite a given experimental number were made on eggs from the same batch.

The lumiflavin reaction[1] was performed with the residues of 58W, 61W, and 67W, containing, in all, approximately 0.4 mg (5×10^{-7} moles) flavin-dinucleotide, as measured by manometric test. Approximately 4×10^{-7} moles lumiflavin were obtained.

Conclusions. These experiments appear to indicate that *Arbacia* eggs contain a flavin-dinucleotide similar to, or identical with, that found in other tissues.[1,4]

11813

Measures of Respiratory Activity With Resting Cells.*

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The "resting cell" technic has gained wide acceptance and has proved extremely useful in the study of bacterial respiration. Quastel and Whetham[1] who pioneered the application of the method, defined "resting bacteria" those which had been centrifuged, washed with saline, and finally well aerated. In the absence of a nitrogen source such cells do not proliferate, and hence observed responses are not believed to be complicated by growth phenomena. They state, "Such an emulsion is always employed under such conditions that growth does not occur. The reactions brought about under these conditions we consider to be reactions produced by the resting organism. . . . The term resting organism as we have defined it is useful for comparison with resting muscle and other tissues." It should be noted that there is nothing in this definition which precludes assimilation of a part of the substrate instead of its partial or complete oxidation. Working with *Esch. coli* and a few other species, Clifton[2] has demonstrated that assimilation usually occurs; Wilson[3] has provided evidence that resting cells of the rhizobia likewise assimilate part of the glucose furnished them.

4 Ochoa, S., and Rossiter, B. J., *Biochem. J.*, 1940, **33**, 2008.

* This work was aided by a grant from the Rockefeller Foundation.

1 Quastel, J. H., and Whetham, M. D., *Biochem. J.*, 1924, **18**, 519.

2 Clifton, C. E., *Enzymologia*, 1937, **4**, 246.

3 Wilson, P. W., *J. Bact.*, 1938, **35**, 601.

Allison and Hoover[4] have questioned the commonly recognized application of the term "resting cell"; they propose that a "resting cell should be defined not as merely a nonproliferating cell but as a nonproliferating and nonassimilating cell." They suggest, for example, that cells of *Rhizobium spp.* grown on a carbohydrate-free medium (Wilson[3]) are high in nitrogen and will, upon the addition of a substrate, assimilate this substrate and utilize their excess nitrogen in a "growth process."

The chief criterion for a resting cell suspension has always been the linearity of uptake on the addition of substrate. Any true growth response should be accompanied by an increased *rate* of respiration. We have observed in hundreds of runs with numerous strains of rhizobia and with a wide variety of substrates (excepting the polyhydric alcohols and acetate which are oxidized by adaptive enzymes as will be described in a later publication) that respiration of the rhizobia grown on a carbohydrate-free medium has been consistently linear.

The chief basis for the claim of Allison and Hoover[4] that suspensions of washed cells from a carbohydrate-free medium "grow" when carbohydrate is added is that such cells have a higher Q_{O_2} than those grown on a medium containing sugar. Although the traditional Q_{O_2} based on dry weight is satisfactory for tissue in which the ratio of respiring tissue to storage material (such as fat or complex carbohydrate) remains reasonably constant, it becomes unreliable when this does not obtain. This fact was recognized by Rubner in his classical studies on the energetics of different species and led him to compare values based on nitrogen content rather than dry weight. Wilson[3] emphasized that the $Q_{O_2}(N)$† is more reliable than the Q_{O_2} for measuring the respiration of the rhizobia since the quantity of gum produced by many species varies with their growth conditions. This gum, which is extremely difficult to separate from the cell, increases the apparent dry weight and thus lowers the observed Q_{O_2}. Similar considerations have been advanced by Berenblum, Chain and Heatley[5] in support of their belief that nucleic acid phosphorus is the most reliable measure of the true respiring tissue in liver, kidney and other animal cells.

[4] Allison, F. E., and Hoover, S. R., *Trans. Third Commission International Soc. of Soil Sci.*, 1939, Vol. A, 32.

† We have previously used q_{O_2} to designate O_2 uptake/hr/mg N, but the term $Q_{O_2}(N)$ used by Allison and his collaborators has more general application and is adopted here.

[5] Berenblum, I., Chain, E., and Heatley, N. B., *Biochem. J.*, 1939, **33**, 68.

TABLE I.
Influence of Composition of Media on Various Measures of Respiratory Activity of *Eh. trifolii* 209.

		Q_{O_2}	$Q_{O_2}(N)$	$Q_{O_2}(P)$	$Q_{O_2}(C)$	Q_{O_2}(cell)	Endogenous: % of glucose respiration	% N
Experiment I: Constant nitrogen, varying carbohydrate.								
1. 100 ml yeast extract, no sucrose	E*	12.5	100	1030	26.9	17.9	21	12.48
	G*	60.2	482	4900	130.0	86.4		
2. 100 " " " plus .25% sucrose	E	17.0	176	1890	34.8	23.2	32	9.65
	G	52.9	548	5870	108.2	72.0		
3. 100 " " " " .5% "	E	20.2	316	3600	42.2	44.5	62	6.4
	G	32.4	507	5810	67.7	71.5		
4. 100† " " " " 1% "	E	19.8	331	3760	42.8	43.6	64	5.98
	G	30.9	517	5880	66.8	68.2		
Experiment II: Constant carbohydrate, varying nitrogen.								
5. 1% sucrose, 10 ml yeast extract	E	19.7	365	3330	51.3	81.7	64.5	5.4
	G	30.5	565	5760	79.5	126.5		
6. 1% " 50 " " "	E	22.0	363	3860	49.4	49.5	66	6.06
	G	33.1	550	5840	74.7	75.0		
7. 1%† " 100 " " "	E	18.9	288	3080	41.9	38.4	56	6.55
	G	33.6	512	5500	74.6	68.4		
Experiment III: Synthetic media.								
8. Allison's medium plus .25% sucrose	E	11.7	151	1990	28.5	31.1	30	6.46
	G	38.7	600	6600	94.5	103.1		
9. " " " 1% "	E	14.5	246	2700	34.0	37.6	36	5.91
	G	40.0	676	7420	93.5	103.5		

*E—endogenous. G—glucose.
†Note that these two media are the same; a comparison of values indicates how well results can be duplicated.
$Q_{O_2}(N)$ mm³ O_2 uptake/hr/mg N content.
$Q_{O_2}(P)$ " " " nucleic acid P content.
$Q_{O_2}(C)$ " " " total carbon content.
Q_{O_2}(celi) mm³ O_2 uptake/hr/cell $\times 10^{10}$.
All measurements at 34°C, pH 6.5, M/45 phosphate buffer, M/150 glucose.

These points are illustrated by the data in Table I which summarizes the respiration rates of washed cells of *Rh. trifolii* (Wisconsin strain 209) grown for three days at 28°C on media containing variable amounts of yeast water and sucrose.‡ Comparisons have been made on the basis of dry weight, nitrogen content, nucleic acid phosphorus content, total carbon, and cell numbers. Dry weights were obtained by drying suspensions on tared watch glasses at 100°C. Nitrogen was determined by the semi-micro Kjeldahl analysis of Umbreit and Bond.[7] The method of Berenblum and Chain,[8] modified for use with bacterial cells, was employed for nucleic acid phosphorus. Total carbon was found by a semi-micro modification of the method of Friedemann and Kendall.[9] Cell counts were made with the Petroff-Hauser counter.

Respiration rates on a sodium succinate substrate (not included in Table I) were higher than when glucose was supplied to organisms grown on the carbohydrate-free medium and on the synthetic medium, but the rates on succinate were less than the glucose oxidation with all other suspensions.

As would be anticipated (Wilson[3]), the Q_{O_2} was dependent upon the gum production of the cells, and high endogenous respiration characterized the gummy cells from media containing carbohydrate. Organisms grown on the base medium without carbohydrate had a nitrogen content of 12.5% and a Q_{O_2} on glucose of 60.2, whereas organisms grown with 1% sucrose had a nitrogen content of 6% and a Q_{O_2} of 30.9; the spread in values is 95%. An identical range of 95% was obtained in the values based on carbon content of cells (66.8-130.0). In contrast the $Q_{O_2}(N)$ values ranged only from 482 to 548, a spread of 13%. The range in the $Q_{O_2}(P)$ of 4900 to 5880 represents a difference of 20%. The maximum variation in respiration per cell was from 68.2 to 86.4 or 26%. The endogenous respiration, as percentage of that in the presence of glucose, rose from 21%

‡ The media were as follows: Media 1-7 contained the salt mixture of Medium 79 (Fred and Waksman[6]); 100 ml of double strength yeast water per liter plus variable quantities of sucrose were used in media 1-4. Variable amounts of yeast water plus 1% sucrose were used in media 5-7. Media 8 and 9 contained the salt mixture of Allison and Hoover[4] plus 0.4 g NH_4Cl and 0.1 μg biotin concentrate (0.4%) per liter. All media contained 2.5% agar and were adjusted to pH 7.0.

[6] Fred, E. B., and Waksman, S. A., *Laboratory Manual of General Microbiology*, New York, 1928.

[7] Umbreit, W. W., and Bond, V. S., *Ind. Eng. Chem. Anal. Ed.*, 1936, **8**, 276

[8] Berenblum, I., and Chain, E., *Biochem. J.*, 1938, **32**, 295.

[9] Friedemann, T. E., and Kendall, A. E., *J. Biol. Chem.*, 1929, **82**, 45.

for cells grown in absence of added carbohydrate (and hence little gum) to 64% for cells grown with 1% sucrose.

Examination of the data of Experiment II indicates that in the media in which the carbohydrate level was constant but with varying concentrations of yeast extract, the nitrogen content, and presumably gum content, differed little; hence all measures of respiratory activity were equally constant. All cells were high in gum, and the endogenous respiration was about 60% of that on glucose. In Experiment III the organisms were grown on synthetic media which were essentially those used by Allison and Hoover. The Q_{O_2} of these cells on glucose was approximately 40 at 34°C (29.6 at 28°C). In other experiments the following average values have been obtained with cells grown on Allison's medium containing varying levels of sucrose (glucose as substrate at 34°C): 0.25% sucrose—Q_{O_2}, 45, $Q_{O_2}(N)$, 553, % N, 8.0; 0.5% sucrose—Q_{O_2} 31.2, $Q_{O_2}(N)$, 510, % N, 6.1; 1.0% sucrose—Q_{O_2}, 35, $Q_{O_2}(N)$, 532, % N, 6.2. Allison and Hoover[4] report that their organism (*Rh. meliloti* 131) grown in such media had a Q_{O_2} on glucose at 28°C of 6 to 9. Aside from strain variation, we are unable to explain this discrepancy.

A noteworthy feature of the data in Table I is that cells grown on the modified Allison's synthetic media in general showed higher activity than those grown on the yeast extract media plus added carbohydrate. A synthetic medium is ordinarily to be preferred since it is more reproducible than are media using yeast extract, and though endogenous respiration of rhizobia grown on this particular one is greater than when grown in the absence of carbohydrate, it is not excessive. The chief objection to its use is the extreme difficulty encountered in separating cells from extraneous gum, when the carbohydrate content of the medium is greater than 0.25%.

In summary, it is recognized that assimilation occurs with suspensions of resting cells, but there is no definite evidence that the ordinary measurements made with these cells (O_2 uptake and CO_2 production) are concerned with any cell function other than respiration. The factor of assimilation should be considered in interpreting results, but as yet no convincing evidence has been brought forth that the occurrence of assimilation is a serious objection to the use of resting suspensions.

Conclusions. The $Q_{O_2}(N)$ has much to recommend it as the most suitable measure of respiration activity. With the rhizobia, for example, not only does it show the least variation of the measures employed, independent of the composition of the medium used for

growth of the cells, but also it rests upon a Kjeldahl analysis which is probably the easiest and most accurate of the determinations made.

As long as respiration of washed cells remains linear with time, assimilation of a portion of the substrate should not seriously interfere with interpretation of the results.

11814

Production of Anti-Bacterial Agglutinins by Carp and Trout at 10° C.

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In recent years costly losses to the salmon industries and trout hatcheries of middle Europe, Great Britain, and North America have been caused by a generalized bacteremia, the etiological agent of which is *Bacterium salmonicida.* These losses have raised the question as to the possibility of inducing immunity in brood stocks of these species by the use of bacterial vaccines.

There is no physiological characteristic of fish, amphibians, or reptiles known to the author that indicates that the reaction of these animals to the injection of a bacterial vaccine should differ from the response of the warm blooded vertebrates. Nevertheless, a search of the literature has failed to reveal a single attempt to immunize fish with vaccines. Indeed, there are very few studies reporting the production of any antibodies by cold-blooded animals upon the injection of foreign proteins, and most of these reports concern animals held at 20°C or above.

Five studies report the production of antibodies against red blood cells. Lazar[1] injected frogs with washed bovine erythrocytes and incited the development of agglutinin titers of 1:10 to 1:80 in some individuals. Many animals, however, were negative. Schwarzmann[2] also found that frogs developed hemoagglutinins on the injection of erythrocytes. Allen and McDaniel[3] held 2 groups of frogs at room temperature (22°C to 27°C) and 2 similar groups

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1 Lazar, Erwin, *Wien. Klin. Wchnschr.*, 1904, 1057.

2 Schwarzmann, L., *Z. f. Immunitatsf.*, 1927, **51**, 138.

3 Allen, F. W., and McDaniel, C., *J. Immunol.*, 1937, **32**, 143.