taining most of its activity during 16-hr incubation periods at 30°C.

Glucose-6-phosphate was measured using the Zwischenferment system (*3*). The Zwischenferment was obtained from *Leuconostoc mesenteroides* (*4, 5*). Fructose-6-phosphate was determined using the resorcinol method of Roe (*6*). Radioactivity was assayed at "infinite thinness" by drying 0.2 ml aliquots on planchets and counting with a standard end-window Geiger tube or, when necessary, with a continuous-flow gas counter. In the four experiments in which the specific activity of the hexose phosphate was the lowest, the counts per minute were 25 above background with a standard error of the counting rate of 10 percent. In all other studies the standard error was less than 5 percent. The glucose was separated from the hexose phosphates chromatographically using a small Dowex-1 formate column (5 mm high, 8 mm in diameter). The glucose was confined to the effluent. The glucose-6-phosphate and the fructose-6-phosphate were eluted, without significant separation, with 10 to 20 ml of buffer (sodium formate 0.05*M* and hydrochloric acid 0.002*M*, *p*H 5.0). ATP was assayed using hexokinase. ADP was measured by coupling hexokinase with myokinase.

The exchange reaction was followed by determining the specific activity of hexose phosphate (glucose-6-phosphate and fructose-6-phosphate) and comparing it with the theoretical activity of complete exchange. The latter value was obtained by dividing the total counts recovered from the column by the total micromoles of glucose and of hexose phosphate recovered. The specific activity of the two hexose phosphates was assumed to be equal. In four experiments, each with a 16-hr reaction period and in which no ADP or ATP was added, no radioactivity was detected in the eluates that contained the hexose phosphates. The results shown in Fig. 1 indicate faster exchange rates with increasing concentrations of ADP or ATP. The exchange appeared to be as well implemented by ADP as by ATP. In the ATP experiments the results were corrected quantitatively for the glucose phosphorylated directly by the "forward" reaction.

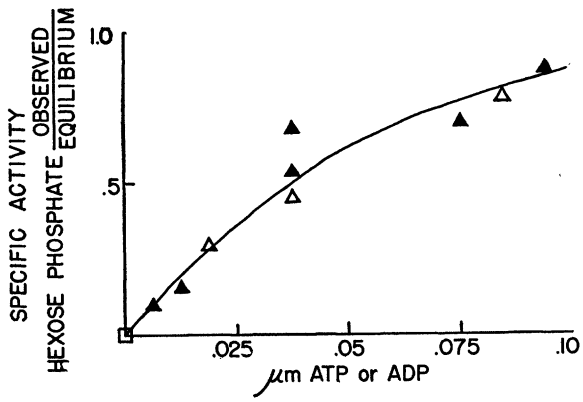

Fig. 1. Hexokinase exchange reaction. The reaction mixture consisted of 250 units of hexokinase, 2 μM of glucose, 2 μM of glucose-6-phosphate, and 0.05 μc (5000 counts/min) of C^{14}-labeled glucose (0.1 mg) in 0.01*M* $MgCl_2$ and 0.03*M* "Tris" buffer (*p*H 7.6). The total volume was 0.3 ml. The mixture was incubated for 16 hr at 30°C. □ represents four experiments, no added ATP or ADP; ▲, ATP; △, ADP.

In preliminary experiments, in which the amount of ADP added was increased to 5 μM, 25- to 50-fold increases in the exchange rate have been demonstrated. The exchange reaction has been observed with two other preparations of yeast hexokinase; the first was prepared using an extensive modification of the Berger procedure and the second was given to us by C. R. Park, who used a different purification method.

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* U.S. Public Health Service postdoctorate fellow.

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3 September 1954.

Effects of Light Intensity and Nitrogen Growth Source on Hydrogen Metabolism in *Rhodospirillum rubrum*

Harvey J. Stiffler and Howard Gest

Department of Microbiology, Western Reserve University School of Medicine, Cleveland, Ohio

The photosynthetic nitrogen-fixing bacterium *Rhodospirillum rubrum* produces molecular hydrogen photochemically during growth in media containing certain amino acids but does not do so when an ammonium salt is the nitrogen source (*1*). Resting cells derived from the former type of medium similarly catalyze light-dependent evolution of H_2 in the presence of various organic compounds (photo-evolution), and it has been shown that formation of this product by such cells is inhibited by addition of N_2 or ammonium salts (*1*). These observations indicate a close relationship between nitrogen metabolism and H_2 formation. The latter process in *R. rubrum* and physiologically similar organisms may be regarded as a manifestation of photochemical "reducing power." It is presumed that the terminal catalyst required for H_2 formation is the enzyme hydrogenase, which is ordinarily assayed by manometric measurement of H_2 utilization in the presence of a suitable electron acceptor (for example, ferricyanide in the present experiments) (*2*). Several types of evidence also suggest an important role for hydrogenase in the N_2 fixation process in aerobic nonphotosynthetic agents such as *Azotobacter*. In this connection, it is generally considered significant that cells of *Azotobacter* grown on N_2 usually show considerably higher hydrogenase activity than cells grown with ammonium salts as the nitrogen source (*3*).

As a first approach to closer study of the function

of hydrogenase in nitrogen metabolism in *R. rubrum* (strain SI), we have investigated (*4*) the relative activities of hydrogenase and the photoevolution system in resting cells obtained from glutamate plus malate and NH_4Cl plus malate media. The former medium was prepared as described in an earlier publication (ref. *5*, G3X medium), with the exception that the yeast extract concentration was reduced from 600 to 400 mg/lit; the NH_4Cl plus malate medium was identical in all respects, except that 1 g of NH_4Cl per liter was substituted for the glutamate. In all instances the experimental cultures were sown with a 1-percent inoculum from a 24-hr culture grown in a complex nitrogen-rich medium (see G5 medium, ref. *5*). The bottle cultures (125 ml) were maintained at 30°C either 18.5 or 33 in. from a 150-w General Electric reflector flood lamp and were consequently exposed to average intensities of approximately 400 or 130 ft-ca, respectively.

Typical data illustrating cell yields obtained in the two media, as well as the hydrogenase and photoevolution activities of the harvested cells, are listed in Table 1. The results of two separate experiments at each age and light intensity (during growth) are given in terms of milligrams dry weight of cells per liter (cell yield) or $QH_2(N)$—that is, microliters of H_2 evolved or utilized per hour per milligram of cell nitrogen at 30°C. There was no appreciable dilution effect on the hydrogenase or photoevolution activities over the range of cell concentrations used in the assays.

It is evident that growth in both media is greatly enhanced by increase of light intensity from 130 to 400 ft-ca. The cell yield data also suggest that glutamate nitrogen is assimilated more readily than ammonia nitrogen during the first 24 hr. In accord with the results previously noted in growing culture experiments, resting cells derived from the ammonia medium do not produce H_2 photochemically. The ability of glutamate-grown cells to catalyze photoevolution (from pyruvate) is considerably greater in organisms grown at 400 ft-ca than in those grown at the lower light intensity. In contrast with "400 ft-ca cells," organisms cultivated at 130 ft-ca produce H_2 at the rates indicated only after an appreciable induction period. The $QH_2(N)$ values for photoevolution show a definite rise with increasing cell age at both light intensities.

Although incapable of photoevolution, ammonia-grown cells contain hydrogenase and the $QH_2(N)$ of this activity is seen to increase markedly with age. A light-intensity effect, comparable to the one already discussed, has been consistently noted in 48-hr and older cells. Pyruvate is rapidly decomposed in the light by ammonia-grown cells with the liberation of CO_2 (for example, $QCO_2(N)$ of 200 observed with 72-hr cells), and it appears that failure to produce H_2 is not the result of a deficiency of hydrogenase. It also seems unlikely that lack of H_2 formation could be entirely due to utilization of metabolic hydrogen for reductive amination of pyruvate with intracellular ammonia. The present results suggest the possibility that a specific electron transport system is required in addition to the hydrogenase enzyme (as measured here) for evolution of H_2 and that the former is not produced in cells growing with ammonia as the sole source of nitrogen.

The hydrogenase activity level in cells grown on glutamate does not increase as greatly with age as is the case when ammonia is the nitrogen source, and a light-intensity effect, although frequently noted, has not been uniformly found. It is evident that the relative hydrogenase activities of the two nutritional types is greatly influenced by age and thus there is no apparent *unique* relationship between nitrogen growth source, comparing ammonia and glutamate, and capacity to activate H_2 for reduction of ferricyanide.

As is indicated by the data in Table 1, all the cell types examined thus far that were capable of photoevolution of H_2 displayed hydrogenase activity, whereas the converse does not necessarily hold. Since the requirements for *rapid* growth (24 to 72 hr) of

Table 1. Hydrogen metabolism and cell yields of *R. rubrum* grown under different conditions.

Age (hr)	Activity	Cells from glutamate medium				Cells from ammonia medium			
		130 ft-ca		400 ft-ca		130 ft-ca		400 ft-ca	
24	Hydrogenase*	136	200	127	146	48	98	36	
	Photoevolution†	0	36	63	100	0	0	0	
	Cell yield‡	220	220	440	490	90	120	120	
36	Hydrogenase	155	146	209	318	77	32	74	24
	Photoevolution	56	57	182	200	0	0	0	0
	Cell yield	490	480	890	990	420	370	920	920
69	Hydrogenase	307	300	364	307	492	492	764	800
	Photoevolution	138	100	264	209	0	0	0	0
	Cell yield	940	800	1350	1400	810	1070	1280	1460

* *Hydrogenase assay*: Results expressed as microliters of H_2 utilized per hour per milligram of cell nitrogen at 30°C. Water-washed cells were suspended in 0.125*M* phosphate buffer *p*H 6.8; final fluid volume, 2 ml. At zero time, 166 μM of $K_3Fe(CN)_6$ was added. KOH present in center well of the Warburg vessel. Gas phase, hydrogen. Dark.

† *Photoevolution assay*: Results expressed as microliters of H_2 produced per hour per milligram of cell nitrogen at 30°C. Water-washed cells were suspended in 0.05*M* phosphate buffer *p*H 6.7; final fluid volume, 2 ml. At zero time, 50 μM of sodium pyruvate was added and illumination begun (approx. 1000 ft-ca). KOH present in center well. Gas phase, helium.

‡ Milligrams dry weight per liter.

R. rubrum with N_2 as the sole or primary nitrogen source are still unknown, comparable experiments with such cells have not yet been possible. These nutritional requirements are now under investigation.

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On the Anti-inflammatory Mechanism of Hydrocortisone (Compound F)

Valy Menkin

Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia

My earlier studies (1940, 1942) have indicated that adrenocortical extract or cortisone suppresses the increased permeability of small blood vessels caused by an alkaline exudate or its contained leukotaxine. Subsequently, it was shown that cellular activity, as gaged by the incidence of cell division, was considerably reduced in the eggs of the sea urchin, *Arbacia punctulata* by the presence in sea water of corticosteroids (*1–6*). Severely injured cells, such as those encountered in acute inflammation, liberate, as a result of their activity, numerous chemical factors capable of reasonably explaining the various manifestations of inflammation (*7*). These factors include, among others, leukotaxine and the leukocytosis-promoting factor (LPF). Leukotaxine explains the initial increased small blood vessel permeability and the migration of polymorphonuclear leukocytes into an inflamed area (*7, 8*). The LPF induces a discharge of leukocytes from the bone marrow and in part explains the mechanism of leukocytosis often accompanying an acute inflammation (*7*). Observations were undertaken (*9*) to determine whether the presence of compound F in an inflamed area would suppress the activity of injured cells, so that they no longer would be able to produce adequate amounts of active leukotaxine or of the LPF (*10*).

Acute inflammation was induced in dogs by the intrapleural injection under pentobarbital anesthesia of 1.5 ml of turpentine. The experimental animals were then injected at the same site with a suspension in saline of 10 to 20 mg hydrocortisone (compound F, free alcohol) (*11*), repeated at daily intervals for 3 to 4 days. The control dogs, following the intrapleural injection of turpentine, received daily injections of saline into the inflamed area. The increased permeability of the small blood vessels was determined, as was previously described, by the extent of accumulation of intravenously injected trypan blue into the treated cutaneous areas on the abdomen of rabbits (*1–4, 6*).

Leukotaxine was extracted, as is described elsewhere, from a given sample of exudate withdrawn from the chest cavity of experimental and control dogs (*12, 13*). Following repeated injections of hydrocortisone (compound F) into the inflamed area, the activity of leukotaxine in regard to its capacity of increasing small vessel permeability was considerably reduced (Table 1, Fig. 1). The outward migration of leukocytes

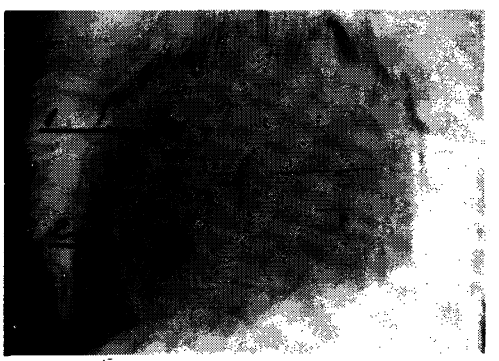

Fig. 1. Inability of leukotaxine derived from compound F-treated exudate to induce increased permeability of small blood vessels. Rabbit 21–19: *Area 1*, 0.4 ml of leukotaxine extracted from exudate of dog 254-T following two successive daily injections of compound F into inflamed area. Total of about 42 mg of hydrocortisone in 6 ml of saline injected into inflamed pleural cavity of the dog from which leukotaxine had been extracted from a given quantity of exudative material. *Area 2*, 0.4 ml of leukotaxine extracted from exudate of dog 255-T following two successive injections of a total of 6 ml of saline into inflamed area. Leukotaxine extracted from an identical quantity of exudative material as in the case of dog 254-T. At end of experiment 6 ml of 1-percent trypan blue in saline was injected intravenously. The injections of areas 1 and 2 were made in the dermis of the abdomen.

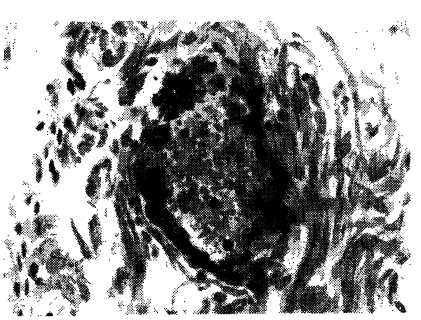

Fig. 2. Effect of leukotaxine in inducing margination of polymorphonuclear leukocytes in skin of rabbit 21–22. Taken about 1 hr after intracutaneous injection of leukotaxine extracted in turn from a sample of canine exudate. The exudate was derived from the pleural cavity of a dog previously injected with turpentine into that region. The animal had received two successive daily injections of saline, each 3 ml. The section is that of a small blood vessel, possibly a venule. (×200)