as a tool for the critical examination of Feulgen microspectrophotometry is the counting of chromosomes. It is evident that there is a good accordance between the DNA results of Feulgen microspectrophotometry and chromosome numbers, a comparison that is of special significance in tissues showing multiple DNA values.

The third method used for comparison concerns DNA determinations by *ultraviolet* microspectrophotometry. Although it may be argued that another microspectrophotometric method is not too suitable for comparison, nevertheless, the different principles of the two methods and accordance of the ultraviolet data with the Feulgen data are of sufficient interest to justify its inclusion in Table 1.

Coming now to the second point—namely, the sensitivity and accuracy of the microspectrophotometric DNA determinations—the excellent agreement of the *multiple* DNA values obtained by Feulgen microspectrophotometry with chromosome numbers, as well as with biochemical analysis, illustrates the order of magnitude with which the method can be safely used. Even if the actual DNA values obtained by microspectrophotometry of the Feulgen stain may sometimes show variations up to 15 percent from the theoretical ratios of 1:2:4:8 DNA, such a difference would hardly be capable of obscuring the multiplicity of the DNA values.

On the other hand, the question of the significance of *intermediate* DNA values occurring in nuclei cannot be so readily answered. Although there does not seem to be any doubt about the constant occurrence of such intermediated DNA values in nuclei of proliferating tissues (*3, 8*) it is felt that much more work and probably more exact methods are needed to interpret the degree of accuracy of the intermediate values.

On the basis of the data presented in Table 1, it seems justifiable to say that microspectrophotometry of Feulgen stain can be utilized as a reliable tool for the comparison of relative amounts of DNA in nuclei of different cells, provided that the differences to be detected are sufficiently large.

In support of the validity of the method, it may be added that microspectrophotometry of Feulgen stain also permits an excellent reproducibility of the DNA results, if preparation of nuclei and measurements are done under carefully standardized and controlled conditions. In this laboratory, a group of 12 trained workers, using four different microspectrophotometric setups within a period of more than 4 years, obtained nearly identical DNA results in the same tissues, the largest variation ever encountered being 10 percent. Furthermore, results from other laboratories are essentially in accordance with the data presented in Table 1 (*2–4*).

Taking into consideration the reproducibility of the DNA data obtained by Feulgen microspectrophotometry, their good correlation with chromosomal counts, with ultraviolet microspectrophotometry, and with the biochemical analysis, one can hardly escape the conclusion that the method has its merits and can be used safely for DNA determination at this level of investigation. This, of course, does not exclude the necessity of cautious interpretation, of checking the DNA data by other methods, and of the possible interference of other factors (*5*), if the method is used for the detection of smaller differences in DNA content.

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Hexokinase Reversibility Measured by an Exchange Reaction Using C^{14}-Labeled Glucose

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In the hexokinase reaction, a phosphate group is transferred from adenosine triphosphate (ATP) to glucose to form glucose-6-phosphate:

$$\text{ATP} + \text{glucose} \rightleftharpoons \text{glucose-6-phosphate} + \text{ADP}.$$

The reaction from left to right is associated with a large loss of free energy, and hence equilibrium is far to the right. Net synthesis in the reverse direction has not been demonstrated. In the present study (*1*) evidence of reversibility was obtained by observing an exchange between glucose and glucose-6-phosphate with the aid of C^{14}-labeled glucose. The exchange reaction was found to require catalytic amounts of either ATP or ADP.

The enzyme used in this study was purified from yeast using the method of Berger *et al.* (*2*), the preparation, essentially fraction V, contained 800 units per milligram of protein and, hence, was considered 26-percent pure. Phosphohexose isomerase was present as a significant impurity. During the prolonged reaction times used in this study, this latter enzyme functioned to bring the glucose-6-phosphate $\rightleftharpoons$ fructose-6-phosphate reaction to its equilibrium and, thus, to convert approximately one-third of the glucose phosphate to its fructose isomer. Phosphoglucomutase was also present as a contaminating enzyme. Phosphatase was not detected. The hexokinase proved durable, re-

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.

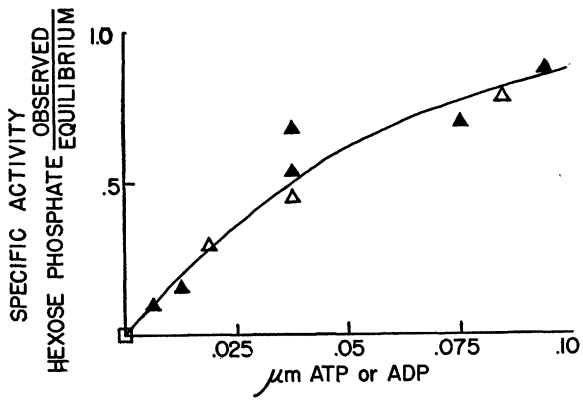

Fig. 1. Hexokinase exchange reaction. The reaction mixture consisted of 250 units of hexokinase, 2 μM of glucose, 2 μM of glucose-6-phosphate, and 0.05 μc (5000 counts/min) of C^{14}-labeled glucose (0.1 mg) in 0.01*M* $MgCl_2$ and 0.03*M* "Tris" buffer (*p*H 7.6). The total volume was 0.3 ml. The mixture 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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Effects of Light Intensity and Nitrogen Growth Source on Hydrogen Metabolism in *Rhodospirillum rubrum*

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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