fixation and reduction is not answered by the evidence presently available. A cautious hypothesis must be limited to this statement: A pteridine related to biopterin acts as a metabolic carrier for a carbon fragment.

Work is in progress on the complete identification of Compound C and its relation to the other pteridines previously described in *Anacystis*. Such information should allow a more precise statement of the metabolic role of these compounds.

Summary.—A labeled pteridine has been obtained from *A. nidulans* during photosynthesis in the presence of $C^{14}O_2$. After acid hydrolysis, this pteridine yielded unlabeled biopterin and a labeled and as yet unidentified fragment. The possible metabolic significance of this and related compounds is discussed.

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*IONIC SEMIPERMEABILITY AS A BULK PROPERTY**

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One of the most fundamental facts of biology is the unequal distribution of K^+ and Na^+ in and outside the cell, K^+ being found in a relatively high concentration inside and Na^+ outside. This unequal distribution is attributed to the function of the cell membrane, to its selective permeability and "pumping action." No generally accepted picture has yet been developed for the mechanism of this hypothetical activity of the membrane, and so it seems justifiable to consider alternate possibilities.

The water molecule has such a distribution of charges, including lone pairs of electrons, as to lend it a tendency to link molecule to molecule in a tetrahedral arrangement. In ice this regular association extends throughout the whole structure. In liquid water the structure is broken up by heat agitation into small fragments, and there is no long-range regularity. The fragments of the crystalline structure are very small and have a temporary existence, associations being constantly formed and broken.

There is strong evidence that around surfaces the small crystallites may join to more extensive lattices, establishing a long-range regularity reaching many hundreds of molecules deep into the fluid.[1] So it is not impossible that most of the water within densely packed biological structure is in a more or less ordered state.

While in K^+ the nuclear charge is screened by three electron shells, in Na^+ it is screened only by two. Moreover, the dimensions of K^+ are very similar to those of the water molecule, which is not the case with Na^+. Consequently we can expect that K^+ does not disturb the regularity of the water structure, while Na^+ does and, by ordering the water dipoles around itself, creates disorder in the water structure; K^+, on the other hand, may readily be substituted for water molecules. This difference is borne out by the temperature dependence of the conductivity of NaCl and KCl as well as by the "B factor" of both cations.[2] When Na^+ is introduced into lattice-ordered water structure, bonds have to be broken which hold the water molecules together, and work will have to be done. Correspondingly, we can also expect the system to tend to eject Na^+ already introduced and to balance its negative charges with preference by K^+. This allows us to construct a theory which picture the ionic semipermeability of cells as a bulk property, while earlier theories ascribed semipermeability to the "membrane" only.

Such a theory suggests experiments and calculations along different lines. First, we can attempt to demonstrate the deranging activity of Na^+ and the inactivity of K^+. Second, we can try to demonstrate actually the preference of biological structures for K^+ in the absence of a membrane. Third, we can calculate whether the dipole forces of water are sufficient to account for the known distribution of K^+ and Na^+ in biological material.

It has been suggested by one of us[1] that the phosphorescent behavior of various fluorescent substances in a frozen watery solution depends on the orderly structure of water and its interaction with the excited molecule. It has also been shown that Na^+ causes a strong disturbance in the light emission of Rhodamin B, while K^+ does not do so. Equally inactive is NH_4^+, the physical properties and dimensions of which are very similar to those of K^+, while Li^+ was found to create even graver disturbance than Na^+. The reaction with Rhodamin was too complex to allow a simple interpretation. The situation is simpler with tryptophan, adenosine triphosphate, and nucleic acid. All these substances are excited to triplets[3] and show in the presence of 0.25 per cent glucose a long-lived phosphorescence if their watery solution is frozen in dry ice and illuminated by ultraviolet light. If their triplet excitations are stabilized by the water structures, then the afterglow must be quenched by any substance which interferes with the regularity of these structures, and we have to expect Na^+ to cause quenching, while K^+ is inactive. The experiment showed that KCl, in physiological concentrations such as 0.1 M, had no effect on the lifetime of the triplet excitation of the above systems and did not materially reduce the duration of the afterglow, while NaCl, in the same concentration, had a strong quenching action.

For the demonstration of the preference of cellular structures for K^+ in the absence of an active membrane, we used glycerinated muscle fibers. According to our experience, glycerination destroys the "membrane," as indicated by the complete absence of electric excitability and conduction. Moreover, in the presence of the high K^+ concentrations employed, the membrane, even if present, could be expected to be inoperative. So we tried to decide in the experiment whether such a glycerinated material is capable of distinguishing between K^+ and Na^+. The difficulty of the experiment lies in the fact that we can expect undisturbed water structures in resting muscle only. and the charge distribution in this material must be greatly in-

fluenced by the tetravalent ATP anions present. However, it is very difficult to produce a glycerinated muscle which is completely relaxed in the presence of ATP. To do this, the so-called "relaxing factors" have to be present and the glycerol treatment has to be of short duration. The objection may be raised that in such a carefully treated material the "membrane" may not completely be destroyed.

Our experiments were performed in the following manner: 2–3 mm. thick bundles of the psoas muscle of freshly killed rabbits were tied to applicator sticks and immersed for 3–6 hours in 50 per cent glycerol at 0° C. Then the muscle fascicles were transferred into a muscle extract prepared according to Bendall.[4] The muscle fascicles remained in this extract for another 2 hours, in order to provide them with a sufficient amount of relaxing factor to prevent their contraction in the next bath, which consisted of the same extract diluted with an equal volume of salt solution. Here the final concentration of KCl and NaCl was 0.08 *M*, that of $MgCl_2$ was 0.004 *M*, and that of ATP was 4 m*M*. After 1-hour incubation at room temperature the muscle was decomposed into 0.1–0.2-mm. thin fiber bundles which were left in this liquid for another 20–40 minutes. After this time a number of fiber bundles (20–40) were liberated from adhering fluid by placing them on filter paper, then dissolved in concentrated nitric acid and analyzed for K and Na on the flame photometer. The results are summarized in Table 1. As the table shows, no significant difference was found between the relative concentrations of K and Na in the bath and in the fibers.

TABLE 1

Experiment No.	K/Na Ratio in Bath	K/Na Ratio in Muscle
I	0.96	0.98
II	1.09	1.14 1.12
III	1.12	1.10

As is well known, Ca plays a dominant role in muscle function and ionic distribution. In order to maintain its normal ionic K/Na balance, the muscle must be provided with Ca^{2+}. For this reason, the above experiments were completed by adding $CaCl_2$ in 0.5 m*M* final concentration to the bath. In the first experiment the intracellular K/Na ratio rose within 5 minutes from 1.07 to 1.31. In the second experiment the quotient rose within 3 minutes from 1.07 to 1.33. After 4 minutes it went up to 1.8. In the third experiment the quotient rose within 4 minutes from 1.10 to 1.52 and within 9 minutes to 1.77. In a fourth experiment the fibers were immediately placed in a Ca-containing bath; the K/Na ratio was found within 4 minutes to be 1.30, in 12 minutes, 1.35, while in the bath it was 1.05.

On more prolonged incubation the quotient dropped again, which may have been due to the disorganizing effect of the contracture induced by the addition of Ca. It seems that two effects were counteracting one another: the Ca tended to increase the quotient of K/Na, while the developing contraction tended to equalize the ionic distribution. It is unfortunate that no relaxed state could be maintained in the presence of Ca.

The experiment is a rather imperfect one, and the quotients obtained are still far from the physiological quotient 10; nevertheless, the experiment definitely shows an unequal distribution, a trend toward eliminating Na^+ and retaining K^+.

The results discussed suggested an attempt to calculate the free energy of the ordering of water molecules. As is well known, the membrane potential of cells is about 0.1 volt. Therefore, the question arises whether the free energy of the ordering of water molecules is equal to or somewhat greater than 0.1 electron volt, which free energy would be necessary, according to the proposed mechanism, to produce the unequal ionic distribution found in cells.

Quantitative treatment of the ordering aspects of molecules in condensed phases is not easy. Whereas the distances between the molecules are largely determined by attractive and repulsive central forces, orientational forces also play an important role for the ordering of the molecules. To obtain an estimate of the free energy due to the orientational forces, we will consider only the interaction of nearest neighbors, for which we take only dipole-dipole interaction. Recently, Pople[5] has studied, on the basis of statistical mechanics, systems with noncentral force fields and has been able to derive an expression for the orientational free energy. His expression reduces, for the case of point-dipole interaction, to

$$F^{(or)} = -\frac{1}{6}\frac{z}{kT}\left(\frac{\mu^2}{a^3}\right)^2,$$

where $F^{(or)}$ represents the orientational free energy per molecule, z the number of nearest neighbors of a molecule, μ the dipole moment of a molecule, a the nearest-neighbor distance, T the absolute temperature, and k the Boltzmann constant.

Since the water molecules tend to arrange the other water molecules around themselves in a tetrahedron, we will take $z = 4$. The value of a in water is 2.9 A (Morgan and Warren[6]). Substituting these values together with $\mu = 1.85 \times 10^{-18}$ esu (Sänger and Steiger[7]) and the value of k, we find, at $T = 300°$ $F^{(or)} = -0.193$ electron volt (which, of course, is of the order of magnitude of the energy of the H-bond). It therefore seems that there is enough energy already in the locally ordered structures of molecules in liquid water, even taking into account only orientational forces and dipole-dipole interactions, to sustain electrical potentials of the order of 0.1 volt. If the order were more extensive than in liquid water, the energy would certainly be enough to account for the order of magnitude of bioelectrical potentials.

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