at right angles, measured with calipers.

From Table 1 it is apparent that the subcutaneous implantation of sarcoma-37 fragments into inbred male albino mice has resulted in a pronounced effect on their average liver catalase levels. From the sixth day after tumor inoculation, the enzyme content in the controls was progressively reduced to a marked extent, while the growth of the established tumors was steady and rapid. However, this depression of liver catalase was prevented, in large measure, by the oral administration of the urinary fractions, and, at the same time, the growth of the sarcoma-37 transplants was appreciably inhibited.

Of the smaller acidic molecules extracted from the urinary concentrates, many would have been able to pass through the dialysis membranes as their sodium salts, while proteins and similar large molecules would either have been precipitated by the additions of ethanol or else adsorbed upon the resultant precipitates. It might therefore be expected that the range of molecular weights contained in the extracts employed for these initial investigations would be restricted to comparatively narrow limits, although they were undoubtedly chemically diverse in nature.

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3. We are greatly indebted to R. F. Fleming for his supervision of the feeding of the mice.

1 April 1957

Water Taste in Phormia

The chemosensory hairs on the labellum of the blowfly *Phormia regina* have been studied extensively. Behavior studies indicate that there are at least two modalities of sensation: acceptable and unacceptable (*1*). Anatomical investigations have shown that there are two neurons associated with the receptor site at the tip of the hair (*2*). Electrophysiological techniques have demonstrated that the two neurons are differentially affected by salt and salt-sugar mixtures in water solution (*3*). Efforts to record the electric response to distilled water and to pure sugar solutions have been unsuccessful. These responses must be found in order to work out a complete theory of chemoreception in *Phormia*.

The present method of recording nerve discharges from single hairs makes it possible to study the response to distilled water and to many types of sugar and/or salt solutions (*4*). Basically, this method is a refinement of the earlier techniques, in which a 50-μ glass capillary, filled with the stimulating liquid, is brought into contact with the tip of the hair. A silver-silver chloride wire, in contact with the stimulating liquid and one input lead of the preamplifier, allows the capillary to be used as the recording electrode. The indifferent electrode is an uninsulated silver-silver chloride wire, which is inserted into the proximal end of the detached labellum of the fly.

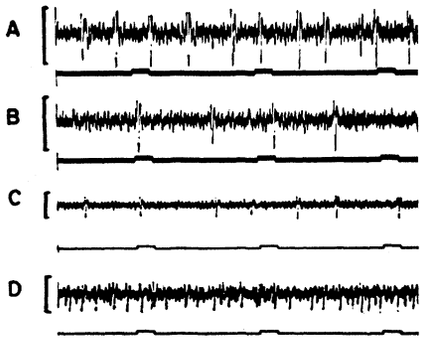

Fig. 1. (*A, B*) Response of a long hair to distilled water. (*C*) Response of a long hair to 0.1*M* *d*-fructose. This hair had no response to distilled water; (*D*) Response of a long hair to 0.1*M* *d*-fructose. This hair had a response to distilled water similar to *A*. In all records the time marks are 0.2 sec, and the voltage calibrations are 200 μv.

Because of the very high resistance (about 10^{10} ohms) of the recording electrode when it is filled with distilled water, a high impedance, negative capacitance preamplifier, designed by MacNichol and Wagner, was used (*5*). The output of this was fed into a conventional direct-current amplifier and oscilloscope for observation and photography. The response was occasionally recorded on magnetic tape, which was later played back and displayed on the oscilloscope for photography.

From behavior studies it is known that distilled water will produce a positive feeding reaction in a thirsty fly. The electric response to distilled-water stimulation consists of large and small impulses intermixed, with a high initial rate of discharge, as is shown in Fig. 1*A*. Adaptation to a lower frequency is rapid and at a different rate for each of the two neurons, as can be seen from a comparison of *A* and *B* in Fig. 1. These are portions of the same record, in which 30 seconds have elapsed between *A* and *B*. The final frequency of the small impulse may become zero in many cases. This response is similar to that obtained from a dilute salt-sugar solution. The character of the response is similar among hairs of the same type on the same labellum but varies from fly to fly. These variations seem to be dependent on the age and nutritional state of the fly. Experiments to explore this relationship are now in progress. Water-satiated flies may have little or no response to water—a single impulse every 5 or 10 seconds.

The response to a pure sugar solution may show a discharge from only one neuron or from two neurons, as is shown in Fig. 1*C* and *D*. In cases where only one neuron discharges, there is little or no response to stimulation by distilled water alone. A two-neuron response to pure sugar solution is usually found in a hair where there is a vigorous water response. This suggests that the sugar stimulates only one neuron and that the discharge of the second neuron is from the stimulation by water. In this way, the response to any solution can be analyzed into two parts: (i) the response to water and (ii) the response to the solute. Of course, if the hair has little or no response to distilled water, then its response to a solution will be almost entirely attributable to the solute. The question of whether or not the water stimulation sums linearly with that of the solute is being investigated in detail for different types of solutes.

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4. I wish to thank V. G. Dethier, for his help and suggestions, and E. F. MacNichol, Jr., for the loan of some of the equipment used. These studies were aided by a contract between the Office of Naval Research, U.S. Department of the Navy, and Johns Hopkins University. (N.R. 160–353).
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26 April 1957

Equilibria as Origin of Differences in Spectra of Chlorophyll in Different Solvents

In the studies of the absorption spectra of solutions of chlorophylls from room temperature to the temperature of liquid nitrogen (*1*), the existence of several molecular species in equilibria became evident. These species were later (*2*) identified as solvates. It was surmised that the chief differences in the well-known spectra of a given chlorophyll from one solvent to another at room tem-

perature arose from different relative amounts of solvates.

This discussion is limited to chlorophyll *b*; chlorophyll *a* and some chlorophyll derivatives had unmistakably the same behavior. In Fig. 1 are given the absorption spectra of chlorophyll *b* in ethyl ether and in methanol at room temperature (300°K) according to Harris and Zscheile (*3*). The inset at the upper right corner of Fig. 1 shows the change in its Soret band at about 4500 A with temperature at a greater dispersion of wavelengths. The solvent of chlorophyll *b* at room temperature (300°K) was 20 percent propyl ether and 80 percent hexane. At the three lower temperatures, it consisted of 20 percent propyl ether, 40 percent propane, and 40 percent propene. As may be seen, the spectrum of the chlorophyll in ether at room temperature is very similar to that in the ether at 75°K, except for a shift toward longer wavelengths. (It has been repeatedly shown that the spectra of chlorophyll in ethyl ether and in propyl ether are indistinguishable, and likewise no change is produced by the substitution of hexane for propane and propene, which are gases at normal temperature and pressure.) The maxima shown at 180°K represent the two solvates, probably the monoetherate and the dietherate. The latter has its maximum at a longer wavelength than does the former. Notice that, at the extremes of temperature, the shape of the bands of these two solvates is roughly the same except for greater detail, chiefly a shoulder toward the long wavelength at the lowest temperature. It was the resemblance of the rather symmetrical shape of the composite spectrum of the two coexisting solvates in ether at 180°K to the shape of the spectrum of chlorophyll in methanol at room temperature which suggested that, in this solvent also, two solvates were present in appreciable concentrations. The presence of two maxima in the composite band at the lower temperature and their absence at the higher would be connected with the improved resolution usually achieved at lower temperatures.

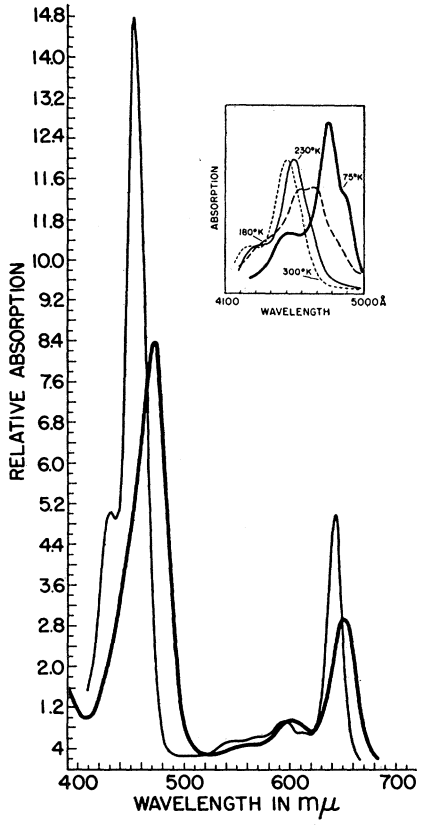

Fig. 1. Absorption spectra of chlorophyll *b* at room temperature. The thin-lined curve with maxima at shorter wavelengths represents a solution of chlorophyll in ethyl ether; the thick-lined curve gives the spectrum when the solvent is methanol. (Inset) Changes with temperature of the absorption spectra of chlorophyll *b* in solution. At 300°K, the solvent was 20 percent propyl ether and 80 percent hexane. At the three lower temperatures, it was 20 percent propyl ether, 40 percent propane, and 40 percent propene.

This interpretation is amply confirmed by the following observations. The left of the lower pairs of curves of Fig. 2 gives the Soret band of chlorophyll *b* in pure propanol at room temperature. When the alcohol is diluted to 9 percent by volume with hydrocarbons, the spectrum becomes that on the right side of the lower pair of Fig. 2. The characteristic shoulder about 150 A toward short wavelengths has appeared, and the main peak is now at shorter wavelengths. This shoulder with its maximum has proved to be a criterion (*2*) that, predominantly, a single solvate of the chlorophyll is present. In Fig. 1 it is an etherate; here it is presumably a monoalcoholate. The left of the upper pair of curves of Fig. 2 shows the spectrum of chlorophyll *b* in a solution 14 percent in propanol with the rest consisting of methlycyclopentane and methylcyclohexane in about equal proportion. The chlorophyll is here mostly in the form of a single solvate—that is, the shoulder toward shorter wavelengths is in evidence. When the temperature was lowered to 231°K, the Soret band of this solution took on reversibly the more symmetrical form *at longer wavelengths* depicted at the right side of this figure, which clearly is a composite of the Soret bands of the two alcoholates and is practically identical

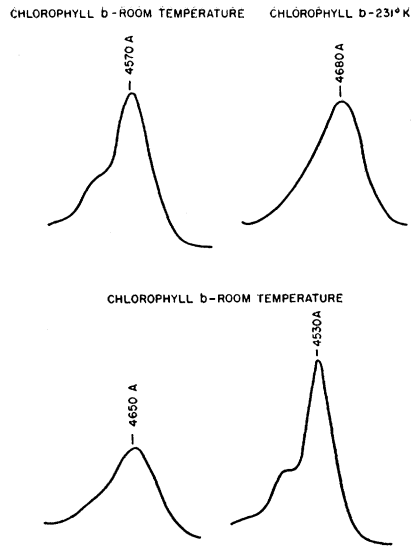

Fig. 2. (Bottom) Curves giving spectra of solutions of chlorophyll *b* at room temperature, in 100 percent n-propanol at left, and in 9 percent n-propanol, 45.5 percent methyl cyclopentane, and 45.5 percent methyl cyclohexane at right. (Top) Curves giving spectra of solution of chlorophyll *b* in 14 percent n-propanol, 43 percent methyl cyclopentane, and 43 percent methyl cyclohexane, at room temperature at left, and at 231°K at right.

with the Soret band of chlorophyll *b* in pure alcohol at room temperature in shape and wavelength. As was to be expected, the increased concentration of the higher solvate could be achieved either by a reduction in temperature or by an increase in concentration of the polar solvent at room temperature (*4*). By means of these two variables, it was possible then, by starting with a given solvent at a given temperature, to bring about a match in the spectra which chlorophyll exhibited when it was in either of the pure solvents at room temperature (*5*).

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4. The effect of the increase in concentration of alcohol is already observable in the spectrum of the solution 14 percent in propanol compared with that 9 percent in propanol. While the Soret band of the former exhibits a sloping shoulder toward short wavelengths, the shoulder of the latter still possesses the maximum characteristic of a single solvate.
5. This research was performed under the auspices of the U.S. Atomic Energy Commission. I am indebted to Donald G. Miller for assistance in taking the spectra.

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