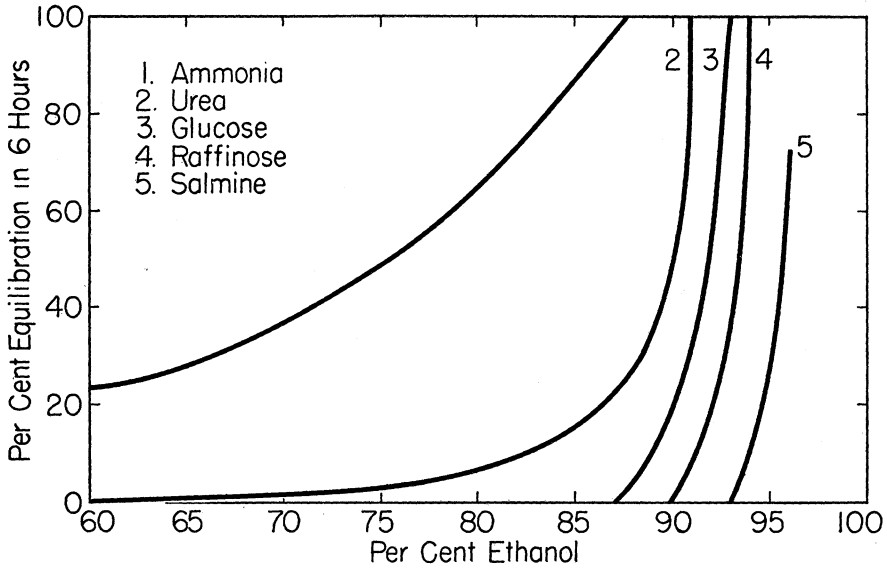

Fig. 1. Permeation of solutes through collodion membranes previously swelled in ethanol-water solutions.

ethanol solution is made by mixing 85 ml of absolute ethanol (water-free) with 15 ml of water; a 95-percent ethanol solution consists of 95 ml of ethanol and 5 ml of water, and so forth. The membranes are kept in the ethanol solutions for 3 hours, preliminary experiments showing that the degree of swelling changes very little after this time. They are then placed in water until they are used.

The determination of the degree of porosity of the membranes was carried out, for the most part, by measuring the rate at which solutes of various sizes diffused through the membranes. In this way, we would know directly whether or not a certain degree of swelling was suitable for a particular separation.

A solution containing the solute to be tested was placed inside a bag-shaped membrane (12 by 100 mm), and water was placed outside. The membrane with solution was placed in a 25- by 100-mm test tube containing 20 ml of water. It was then placed in a thermostat at 25.1°C, and the concentration of the solute which appeared in the outside solution was determined after 6 hours. From the initial volumes and concentrations in the two compartments, the equilibrium concentration was known. From the concentration in the outside solution at 6 hours, the percentage of the equilibrium value was calculated. A summary of results we have obtained using this procedure is shown in Fig. 1.

The curves in Fig. 1 are quite reproducible if such variables as membrane thickness and swelling conditions are controlled carefully. For example, in a given series, ten 90-membranes tested with urea gave an average value of 63.4 ± 2.5 percent equilibration. The variation from series to series, however, is not quite as good. When a particular point on one of the curves is checked with new collodion solutions, new reagents, and so forth, the variation may be as much as 15 to 20 percent from the original value.

For membranes swelled in 94-percent ethanol and higher concentrations, we have used ultrafiltration as a method of characterization. Ultrafiltration of solutions being tested was carried out with the 12- by 100-mm membranes according to the technique of Clegg (*6*). The membranes were placed in nylon bags which were then suspended in 15-ml centrifuge tubes. When centrifugation was carried out at 1000 to 1500 rev/min, liquid collected at the bottom of the tube, the rate depending on the porosity of the membrane. The resulting liquid was then tested for solute.

Using this procedure, we found that the 94-percent membranes just held back lysozyme, the 96-percent membranes just held back egg albumin, and the 97-percent membranes just held back serum albumin. This is about the upper limit in porosity that can be obtained with this method. Membranes swelled in 98-percent ethanol for 30 minutes are very weak, and 99-percent ethanol dissolves the film completely in a very short time.

To test these membranes further, separations of two different substances by dialysis have been attempted by placing a solution of the two substances inside a given membrane and water outside. With a membrane swelled in 90-percent ethanol and urea and sucrose as the solutes, it was found that in 36 hours the urea concentration was reduced to 10 percent of its original value, while the sucrose concentration was unchanged. With a membrane swelled in 87.5-percent ethanol and urea and glucose or urea and the tripeptide, glycylglycylglycine as the solutes, it was found that when the urea concentration was reduced to 5 percent of its original value, there was a 10 to 15 percent loss of the larger solute in the dialyzate. In these instances when the ratio of the molecular weights of two solutes is as low as 3/1, a complete separation is not obtained. Nevertheless, such separations could still be practical, especially if fractional dialysis were employed. Craig and King (*7*) have recently shown that fractional dialysis with cellophane membranes can be quite useful in estimating the size and homogeneity of unknown solutes. The availability of a series of graded membranes covering the smaller solute range should make this technique even more useful.

We have made practical use of these membranes in the separation of salts from low-molecular-weight proteins and polypeptides by means of electrodialysis. In addition, we have been able to make studies on the equilibrium dialysis of solutes which diffuse through the conventional dialyzing membranes (*8*).

CHARLES W. CARR
DANIEL ANDERSON
IRVING MILLER
Department of Physiological Chemistry, Medical School,
University of Minnesota, Minneapolis

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22 March 1957

Liver Catalase and Tumor Inhibition by Urinary Extracts

The influence of tumor growth on the liver catalase content has been noted by many workers; in particular, Adams (*1*) has reported on the changes in this enzyme that follow the injection into mice of homogenized sarcoma-37 tissue.

Within about 4 days, the liver catalase was found to diminish rapidly—a fall which was not characteristic of tumor growth (*2*)—and then to rise almost to the original normal value. Later a somewhat more gradual reduction was observed, coincident with the growth of the established tumors, the effect being more marked in male animals. In view of the afore-mentioned findings, tumor inhibitory extracts of urinary concentrates were examined also for their effect on the depression of liver catalase produced by the growth of sarcoma-37 tumors in inbred male albino mice.

For the preparation of the extracts, acidified tenfold concentrates of pooled human male urine were treated with absolute ethanol in amounts equal to one-half of their volumes; the resultant precipitates were discarded, and the filtrates were concentrated seven to eight times under reduced pressure; then further equal volumes of ethanol were added. Again, the precipitates that resulted from the incorporation of the ethanol were filtered off and discarded; the evaporation of the solvents from the filtrates, under reduced pressure, was continued in these instances to approximately a 15-fold concentration. From these residues it was difficult to distill any more water.

The additions to the viscous brown residues of absolute ethanol (of half the volumes previously employed for precipitation) resulted in solutions that contained most of the urinary solids; these were repeatedly extracted with ether. Evaporation of the ether and ethanol from the bulked extracts provided very thick oils, which constituted one part of the extracts under investigation.

Further tumor inhibitory material was obtained, from the small, aqueous residues that remained from the ether extractions, by a third treatment with equal volumes of absolute ethanol. The resultant precipitates were again discarded, and the filtrates were evaporated to dryness, under reduced pressure, to yield the other parts of the combined urinary fractions that were employed.

The total dark brown, gummy material thus extracted was brought into aqueous solution by the addition of excess 5*N* sodium hydroxide, at room temperature. The dialysis of these solutions was accomplished in Visking synthetic cellulose casings against running tap water for 3 hours, during which time much water entered the casings. The products remaining within were then concentrated, under reduced pressure, to volumes suitable for their homogeneous incorporation into sufficient rat cake to provide a daily ration of 2.5 g per mouse (*3*); this amount was also given, unadulterated, to the control animals. In each experiment, the average daily mouse dose of these urinary fractions corresponded to the amount excreted in 400 ml of freshly voided, pooled male urines.

For the observation of the variations in the liver catalase levels, groups of from 4 to 6 control mice and from 5 to 7 experimental mice (all 6 weeks old at the beginning of the study) were sacrificed at intervals of 2 to 3 days (excluding weekends), commencing from a date 6 days after implantation of small fragments of actively growing sarcoma-37 tissue. Enzyme estimations on the liver homogenates were carried out in duplicate by the titration method of Adams (*1*), but 0.042*M* hydrogen peroxide in phosphate buffer *p*H 6.8 was used, in order that the fraction of the substrate destroyed should not be unduly high. Units of catalase action were calculated, as by Adams, in relation to liver nitrogen. Tumor areas were derived from the product of two diameters of the tumors

Table 1. Liver catalase levels in inbred male albino mice bearing growing sarcoma-37 transplants, during oral treatment with urinary extracts. (Tumor areas are calculated from the product of two diameters of the tumor, taken at right angles. Liver catalase was measured by the method of Adams, *1*.)

Days after tumor inoculation	Controls		Treated mice	
	Avg. tumor area (mm^2)	Liver catalase (units)	Avg. tumor area (mm^2)	Liver catalase (units)
		Experiment 1		
0	0	106	0	106
6– 7	20	89	20	75
8– 9	41			82
10–11	101	58	47	
12–13			90	82
14–15	220	55		
		Experiment 2		
0	0	101	0	101
6– 7	24	89	15	102
8– 9	43	85	27	103
10–11	66	72	40	
12–13	100			
14–15	126	60	80	85
16–17	148	44	106	
18				55
		Experiment 3		
0	0	95	0	95
6– 7	11	90	8	106
8– 9	32	72	22	116
10–11	60	65	38	105
12–13	100		65	88
14–15	130	43	65	70
16–17	155	41		77
		Experiment 4		
0	0	95	0	95
6– 7	11	90	5	83
8– 9	32	72	14	124
10–11	60	65	18	
12–13	100		25	78
14–15	130	43	40	85
16–17	155	41	56	96
		Experiment 5		
0	0	99	0	99
6– 7	17	94	13	92
8– 9	38	75	31	103
10–11	64	81	50	90
12–13	109		76	
14–15	150	43	70	77
16–17	201	29	83	57
		Experiment 6		
0	0	99	0	99
6– 7	17	94	17	101
8– 9	38	75	26	108
10–11	64	81	51	
12–13	109		85	85
14–15	150	43	88	82
16–17	201	29	100	54

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.

CONSTANCE B. BROWN
C. L. WALTERS
Hosa Research Laboratories, Sunbury-on-Thames, Middlesex, England

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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.

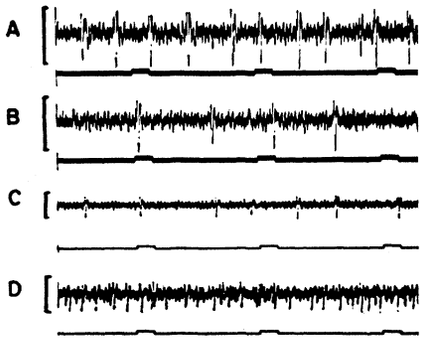

Fig. 1. (*A, B*) Response of a long hair to distilled water. (*C*) Response of a long hair to 0.1*M* *d*-fructose. This hair had no response to distilled water; (*D*) Response of a long hair to 0.1*M* *d*-fructose. This hair had a response to distilled water similar to *A*. In all records the time marks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

MYRON L. WOLBARSHT
Department of Biology, Johns Hopkins University, Baltimore, Maryland

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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-