a *trans*,5–5′,6–6′ type, which corresponds to Isomer II of the four possible isomers of thymine dimer reported by WULFF AND FRAENKEL[6].

Methylation of the N-3 positions of the photodimer obtained here would lead to an *N*,*N*′-dimethylthymine photoproduct which could then be compared to the two photoproducts isolated by WULFF AND FRAENKEL[6]. Such a comparison would help to identify some of the four possible isomers[6].

In the hydrogen-bonded complex of 1-methylthymine and 9-methyladenine, the thymine molecules are also stacked over one another but in this structure the 5,6 double bonds of adjacent 1-methylthymines are separated by 4.8 Å. Thus it was to be expected that no dimer would be formed by ultraviolet irradiation.

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Department of Chemistry, California Institute of Technology, Pasadena, Calif. (U.S.A.) ROBERT F. STEWART*

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* Predoctoral Fellow of the U.S. Public Health Service. Present address: Department of Chemistry, University of Washington, Seattle, Wash. (U.S.A.).

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Equivalent pore radius in the frog gastric mucosa

The permeability of the frog gastric mucosa to water and potassium has been previously studied[1]. Two barriers, arranged in series, were proposed to explain the action of histamine. A first barrier, insensitive to histamine, was apparent in the water-diffusion experiments, and a second, affected by addition of histamine, in the water filtration and in the potassium diffusion experiments.

In the present study the diffusion of tritium-labelled water and of some non-electrolytic molecules of graded size, with low lipid solubility, labelled with ^{14}C was studied. These experiments were performed in order to characterize the mucosa in terms of the pore hypothesis.

The isolated frog gastric mucosa was mounted between two lucite chambers, with facilities for sampling. These chambers were similar to those described by USSING AND ZERAHN[2]. The area of the mucosa between the chambers was 1.13 cm^2. The volume of each chamber was 10 ml. The chamber in contact with the serosal face of the mucosa was filled with nutrient solution and stirred by bubbling with 95 % O_2 and 5 % CO_2. The other chamber, in contact with the mucosa face, was filled with secretory solution and stirred by bubbling with O_2. The composition of these solutions has already been described[1]. Bubbling was regulated to obtain homogeneous mixing in each chamber, within 15 sec. The rate of diffusion of water, urea, acetamide, ethylene glycol, methyl urea, glycerol, sucrose and inulin from the nutrient towards the secretory solution was measured. A single probing molecule was added to the nutrient solution in each experiment. Samples of 0.1 ml were taken immediately after addition of the tracer and every 30 min thereafter, for 5 h. After an initial equilibration period, the diffusion flux of the labelled substances became constant. The 1st h was not considered in the calculation. Histamine diphosphate was added, to a concentration of $2 \cdot 10^{-5}$ M, at the end of the 3rd h. Activity of the samples was measured in a liquid scintillation spectrometer (Packard 314 DC), using the scintillation mixture described by VILLEGAS AND VILLEGAS[3], or in a proportional gas-flow counter (Nuclear Chicago D47).

For non-charged molecules, and in the absence of any osmotic gradient, the ratio between the total area for diffusion and the length of the diffusion path ($A/\Delta x$) could be defined according to Fick's equation, as follows:

$$A/\Delta x = \Phi_{ns}/c_n D$$

where Φ_{ns} is the unidirectional diffusion flux from the nutrient to the secretory solution per unit time; c_n is the concentration of the probing molecule in the nutrient solution; and D is the diffusion coefficient. The concentrations of the probing molecules, c_n, used in the experiments, are shown in Table I. They were always sufficiently high with respect to the concentration of the same species in the mucosa. Thus, dilution of the tracer in the mucosa could be neglected in the calculations. Care was taken, however, to avoid osmotic effects. The total flux of the tracer was so small that the back flow could also be ignored in the calculations. The diffusion coefficients, D, were considered equal to those in free solution and are shown in Table I. The values used for water, urea, sucrose and inulin were those of DURBIN, FRANK AND SOLOMON[4], corrected for a temperature of 22°. Values for acetamide, ethylene glycol, methyl urea and glycerol were taken from LONGSWORTH[5]. Water molecular radius was considered 1.5 Å according to MORGAN AND WARREN[6]. Inulin radius, 15.2 Å was taken from DURBIN, FRANK AND SOLOMON[4]. The radii of all other molecules were estimated from constructed models[7,8] using Catalin atom models.

The results of the diffusion experiments are shown in Table I as values of $A/\Delta x$. Since no difference was observed in the values obtained in spontaneously secreting mucosae, or in those stimulated by histamine, each value represents the mean

TABLE I

DIFFUSION OF NON-ELECTROLYTIC MOLECULES IN FROG GASTRIC MUCOSA

Molecular species	*Radius (Å)*	*D at 22° (cm^2/sec × 10^{-5})*	*No. of expts.*	*C_n (mM)*	*$A/\Delta x$ (cm/cm of mucosa)*
Water	1.50	2.45	5	$55.6 \cdot 10^3$	1.83 ± 0.11
Urea	2.17	1.27	5	20	0.26 ± 0.03
Acetamide	2.22	1.15	4	2	1.00 ± 0.09
Ethylene glycol	2.25	1.06	6	2	0.49 ± 0.09
Methyl urea	2.39	1.08	6	2	0.23 ± 0.02
Glycerol	2.83	0.87	4	2	0.19 ± 0.02
Sucrose	4.59	0.48	3	2	0.03 ± 0.02
Inulin	15.2	0.15	3	2	0.04 ± 0.06

± standard error of mean of all the measurements realized after the 1st h equilibration period.

Fig. 1 presents the experimental values of $A/\Delta x$ plotted against the radius of the probing molecules. Following Eqn. 10 of RENKIN[9] continuous lines representing equivalent pore radius of 3 and 4.5 Å were drawn. As shown in Fig. 1, almost all experimental points fit between these two lines. The values for acetamide correspond to pores with an equivalent radius of 7 Å. Discrepancies observed in the equivalent pore radius fitted by the different molecules could be considered as a consequence of their different lipid solubility, as already discussed[7].

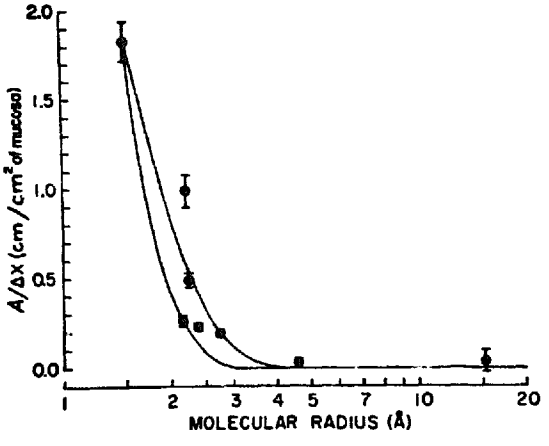

Fig. 1. Diffusion permeability of the frog gastric mucosa to graded-size non-electrolytic molecules. The fractional area for diffusion per unit path length, $A/\Delta x$, is presented as a function of the test molecule radii. Each point represents the mean ± standard error of mean. The continuous lines were calculated for effective pore radii of 3 and 4.5 Å, as described in the text.

In summary, the present results show: (i) that the restriction offered by the frog gastric mucosa to diffusion of non-electrolytic molecules with low solubility in lipids may be explained by the existence of a barrier with pores with an equivalent radius between 3 and 4.5 Å; (ii) that the restriction offered by this barrier is not affected by histamine stimulation; (iii) that there exists no basis, from the present experiments, to consider the existence of two different systems of pores arranged in parallel in this diffusion barrier to non-electrolytic molecules, and (iv) that another

barrier, sensitive to histamine, arranged in series (see p. 131), could explain the changes in water filtration and in potassium diffusion during histamine stimulation described previously[1].

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Departamento de Biofísica,
Instituto Venezolano de Investigaciones Científicas (IVIC), LEOPOLDO VILLEGAS
Apartado 1827, *Caracas (Venezuela)*

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