tissues had been preserved with 10 percent formaldehyde solution for the 2-month period since the animal's capture; this should have denatured proteins, including any lipoxidases that might otherwise have tended to degrade carotenoids. Thus it was surprising to encounter such a firm, lipid-rich section of liver lacking in these pigments.

The skin of both the thornback ray and the horned shark yielded only xanthophyllic esters, while the uncombined xanthophyll (93 percent of total carotenoids) predominated over its esters in the ray's liver and comprised two-thirds of the carotenoids in the horned shark's liver.

The liver of *Platyrhinoidis* and that of *Heterodontus* each yielded two chromatographically separable esters and the skin of the latter, three ester fractions; but all xanthophyllic material, whether free or esterified, showed the same absorption profile within minor limits of error. The typical values were: 475, 447.3, and an inflection or sloping shoulder at about 424 mμ, but varying between 420 and 425 mμ. All 13 values for the first maximum were located from 474 to 476 mμ except for one fraction which gave a maximum centering at 471, and another, a value of 478 mμ. All loci for the chief maximum not actually at 447 mμ were within 2 or 3 mμ above or below it. Minor departures of this kind, if not indeed within instrumental error, may arise either from a degree of isomerization or from the presence of fatty acid conjugants or incidental lipids.

The spectral absorption maxima of the xanthophyll present in these fishes agreed very closely with those of authentic zeaxanthin: 475, 447, and 422 to 424 mμ in the same solvent (*n*-hexane) and measured with the same instrument. The experimental xanthophyll likewise exhibited the same partition ratio (11 : 89) between hexane and 95 percent methanol as pure zeaxanthin does [as measured by Petracek and Zechmeister (*6*) and by us].

Co-chromatography of a purified sample (from *Platyrhinoidis* liver) with isozeaxanthin, prepared by us from canthaxanthin, showed wide separation between the two xanthophylls. Moreover, Goodwin's test (*7*) for allylic hydroxyl groups resulted in noticeable darkening of isozeaxanthin and reversed the hypophasic behavior, but the shark-liver xanthophyll remained unchanged, as zeaxanthin also does.

Finally, an isolated sample of the xanthophyll, obtained from the hydrolytic treatment of collective carotenoids from *Heterodontus* liver, was co-chromatographed on thin-layered silica gel with some of the authentic, crystalline zeaxanthin (*8*). The two gave identical R_F values on individual chromatograms and a single zone when combined in the same solution, which shows the identity between our xanthophyll and zeaxanthin.

It should be emphasized that contaminating lipids may distort chromatographic zonation as well as behavior on partition and position of absorption maxima. Accordingly, it was necessary sometimes to first adsorb a xanthophyll ester upon powdered MgO or Al_2O_3, discard the supernatant colorless, cloudy solvent, and then rinse the colored powder several times before eluting the carotenoid in preparation for diagnostic examination (*5*).

Our values for the collective liver carotenoid fractions (7.86 and 4.57 mg/100 g, respectively, for the ray and the shark analyzed), although somewhat higher than Fisher's, lie well within the order of magnitude of his analyses, while the skin of the shark yielded more than ten times as much zeaxanthin esters as the skin of the ray. It was surprising that no carotenoid was recovered from the skin of a pelagic shark, *Isurus glaucus*, after a 3-day extraction with ethanol, since Fisher's findings would suggest that carotenoids are present, although in relatively low concentrations, in the livers of all or most sharks, and hence might be expected also, at least in minor quantities, in the integument of this species, as in other fishes (*1*).

Note added in proof: Since this report went to press, Professor C. L. Hubbs kindly made available to us for analysis 70.95 g (wet weight) of liver tissue from a black-skinned chimaerid (*Hydrolagus* sp., being described by him), measuring about 1 m in length, freshly captured off Baja California at a depth of 1200 m. The dark, melanistic, very fatty liver involved less than 0.04 mg of suspected carotenoids, whose recovery was rendered impracticable by the presence of the high concentrations of lipids.

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Gastric Secretion: Mechanism for Production of Hydrogen Ions

Abstract. *The passage of a direct electric current across a fixed-charge membrane interposed between neutral electrolyte solutions can give rise to the production of hydrogen and hydroxide ions at the solution-membrane boundary in equivalent amounts, each of which can approach and equal the number of faradays passed. At this unique boundary, the electric field can inhibit the rate of recombination of hydrogen and hydroxide ions strongly enough to increase the dissociation constant of water and other weak electrolytes by several orders of magnitude. These observations lead to a model of the gastric secretory process wherein the gastric potential, primarily due to the activity of a chloride-ion pump, is applied across an adjacent ion-selective membrane also present in the mucosa. The dissociating weak electrolyte can be water, to produce hydrogen ions in the stomach and hydroxide ions which combine with carbon dioxide in the blood, or it can be carbonic acid to produce bicarbonate directly.*

Gregor and Peterson (*1*) have used a new experimental approach to the study of the production of acid and base at the boundary between a neutral electrolyte solution and an ion-exchange membrane under conditions of electrodialysis. These studies were suggested by observations of Bethe and Toropoff (*2*) who measured small *p*H shifts when studying electroosmosis.

Gregor and Miller (*3*) have treated this problem theoretically, and we now propose a simple mechanism for hydrogen-ion secretion. As an example, hydrogen-ion secretion in the stomach may be the result of a hydrolysis, induced by an electric field, of either water or carbonic acid at an ion-selective membrane-solution boundary; the primary potential probably arises from active transport of chloride ions into the stomach.

At the boundary between a neutral solution of potassium chloride and a cation-exchange membrane (as an example), as the current increases to a critical current density there is a greatly increased transport of hydrogen ion across the membrane, with an equivalent amount of hydroxide appearing in the adjacent solution (*1*, *3*). Below a critical current density the transport number of the hydrogen ion is extremely low; at this density, it rises very sharply to 0.8 and higher (Fig. 1). The explanation of these phenomena is based first upon the ordinary concentration polarization which occurs in these systems, and the resultant increase in the field at the solution-membrane boundary. A new kind of Wien effect (deviation from Ohm's Law in electrolyte solutions) now occurs, which affects the kinetics of processes involved in the dissociation of weak electrolytes. The application of a potential to homogeneous solutions does not change the rate of recombination of weak electrolytes (including water) because here the probability for increasing the rate of recombination is equal to that for decreasing it, as was shown by Onsager (*4*). However, at the surface of a membrane which is permeable only to positive ions and therefore does not contain appreciable quantities of mobile negative ions, the field in the adjacent solution phase can act only to retard the rate of recombination, so that hydrogen ions are pulled into the membrane phase and hydroxide ions (in the case of water) are pulled away from it. In the boundary region, the probability that a dissociated water molecule will recombine is lowered by the field; this region is but a few angstroms thick. A parallel phenomenon occurs at the boundary of an anion-permeable membrane with the hydroxide ions entering the membrane phase and the hydrogen ions moving away from it. These phenomena are strongly field-dependent; a field of 1000 volt cm^{-1} increases the dissociation constant of water by six orders of magnitude. It might appear that such conditions would not occur readily at the low potentials (and corresponding low currents) which prevail across the stomach (*5*, *6*), but one of us has demonstrated (*7*) that a current density of 0.1 ma cm^{-2} readily produces a flux of hydrogen ions nearly equal to the total current. The theory predicts (and experiments confirm) that the production of acid and base will increase with dilution of the ambient solution, with a decrease in the rate of stirring, which increases the hydrodynamic boundary-layer thickness, and when salt ions of low mobility are present.

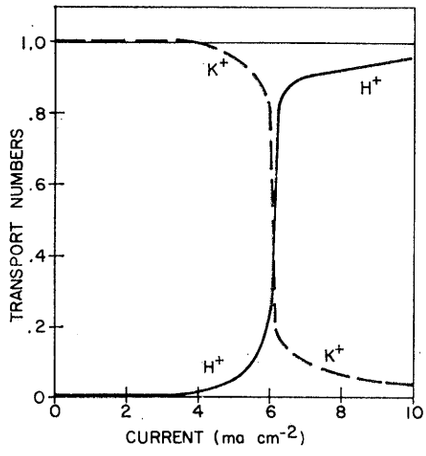

Fig. 1. Transport number of potassium and hydrogen ions across a cation-exchange membrane separating 0.005*M* potassium chloride solutions, when the system was well stirred, to produce a boundary-layer thickness of 30.1 μ (*1*).

The model (Fig. 2) shows the process either at a cation-permeable membrane or at a paired-cation-and-anion-permeable membrane system. The negative potential at the secreting side of the mucosa (relative to that at the nutrient side) causes the dissociation of water (as an example) at the nutrient side of the cation-permeable membrane or between the paired membranes. The rate of acid production is governed by the current; a current of 0.1 ma across 1 cm^2 of active membrane area can result in 0.4 μl of 0.16*N* hydrochloric acid per minute. The concentration of hydrochloric acid is governed by the secretion of water or by electroosmotic transport of water; the upper limit of acid concentration attainable, at least with synthetic ion-exchange membrane systems (*7*), is considerably higher than that reached in the stomach.

The well-known facts on acid secretion are generally compatible with the mechanism we propose. Our model explains only the production of hydrogen (and hydroxide or bicarbonate) ions; it suggests that the driving mechanism is a chloride pump, but does not explain the working of the pump itself. It describes a process for producing relatively concentrated acid and base from neutral electrolyte without recourse to an oxidation-reduction process at electrodes. The lack of this kind of mechanism caused Davies and Ogston (*14*) to argue for the generation of hydrogen ions by a chemical reaction in primary secretion. James (*5*, p. 89) concluded "acid can be formed by electrolysis, and in the inorganic world the electrical power can be obtained from mechanical processes; but in the 'wholly aqueous' medium of which cells consist any electromotive force capable of performing work can only be derived from machinery which can separate ions; any theory of gastric secretion, therefore, which postulates an e.m.f. as the prime mover only shifts the problem; the primary concentration must be chemical concentration of a neutral compound from which the ions are derived." This reasoning was quite correct before the work of Gregor and Miller (*3*).

Extensive studies have been made of the potential across the stomach wall, particularly across the gastric mucosa, with external electrodes connected by salt bridges to impress a current or to short-circuit the mucosal cell. The equivalence of acid and base (as bicarbonate) production was observed. The isolated mucosa of the bullfrog and that of the dog stomach showed a spontaneous negative (with respect to the serosal surface) potential of from 30 to 80 mv in both the resting and secreting state. The potential arises in the mucosa itself and can generate continuously a short-circuited current of from 0.1 to 1 ma cm^{-2} and spontaneously secrete hydrochloric acid when nutrient and oxygen are present in the serosal medium (*8*). In the dog, the resting stomach showed a potential of 60 to 80 mv; the secreting potential was 60 mv (*9*). The potential fell either with secretion or short-circuiting in the external circuit (*10*).

When an external potential was applied and a current (positive) flowed, current from serosa to mucosa resulted in a sharp increase in H^+ secretion, whereas a current from mucosa to serosa makes for a correspondingly sharp drop in acid secretion, to the point of acid consumption (*11*). The current from serosa to mucosa causes a decrease in chloride production; a current in the reverse direction led to variable results, and Rehm concluded that H^+ and Cl^- were produced at separate sites (*12*).

Rehm defined an "electrogenic" ion pump as one which transports an ion against its electrochemical potential gradient with net charge transport across the membrane at the pump site (*13*). The return (or electrically compensating) circuit would be at another site (or sites) in the mosaic membrane. When the chloride was replaced with sulfate in a frog mucosal system, the secretion of acid was greatly diminished, and there was a small positive potential at the mucosa. The resistance of the membrane system greatly increased. When an external voltage was applied (voltage clamping) at increasing levels up to that in the normal chloride state, H-ion secretion was increased in proportion to the applied voltage, but at a lower rate than that obtained by the normal (chloride) potential.

The interpretation of these findings in relation to our model requires a careful examination of the biological systems investigated and the methods used. The gastric potential, as it is usually measured, is about 60 mv. However, in a complex, mosaic membrane system the potential difference between nutrient and secretory sides may be substantially lower than that at the site of the chloride pump. The chloride-pump potential and the measured potential will be the same only if there are no other components in the circuit, that is, no interposed resistances or sources of potential. These are present in the systems which have been studied. There is certainly a diffusion potential opposite in sign to that of the chloride pump, one which arises from the concentration gradient of sodium or potassium or both. The work of Harris and Edelman (*15*) emphasizes the effects of chemical concentration gradients on the measured electrical properties of gastric mucosa. In addition, the hydrogen-ion secretory mechanism does involve a short-circuit (*13*). Potentials can also arise from the diffusion of hydrochloric acid away from the mucosal surface, and a similar potential may arise from hydroxide or bicarbonate movement towards the serosa; both potentials will be opposite in sign to that of the chloride pump and will decrease the measured potential. Several equivalent circuits can be drawn, some of which will be similar to that of Ussing and Zerahn (*16*). But one cannot yet determine site potentials in a complex mosaic membrane system. Further, one would not expect a stoichiometric relation between acid secretion by an applied current and the number of faradays passed, because "leaky" membranes undoubtedly exist in the mucosa mosaic, membranes where exchange-diffusion of H and Na ions occurs with water transport, either passively or by electro-osmosis or anomolous osmosis (*17*).

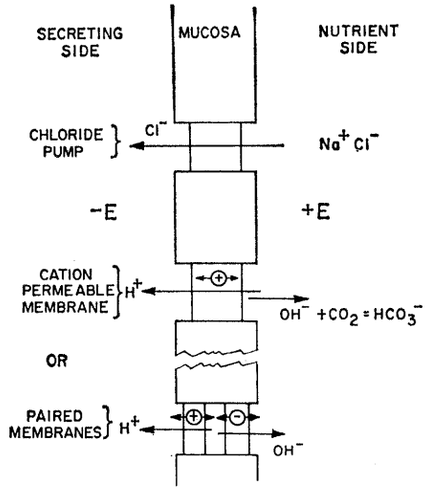

Fig. 2. Model of gastric mucosa with chloride pump and resulting potentials (*E*), and with two kinds of weak electrolyte dissociating systems, a cation-permeable membrane and paired-cation-and-anion-permeable membrane.

The interpretation of prior results on biological systems with respect to our model requires also consideration both of the *p*H at the generating site and of the primary source of hydrogen ions. If the *p*H is 7, then the reversible potential (the thermodynamic minimum) must obviously be about 360 mv. One can only speculate whether the chloride pump does deliver this potential. However, the *p*H at the local site can be substantially lower than in the ambient blood stream. In Donnan systems, for example, *p*H changes of several units are observed. A local *p*H of 3 would require but 120 mv. If the local *p*H is 3, then the available electrical free energy may suffice only to produce hydroxide ions at very low concentrations. However, the resulting chemical reaction with carbon dioxide would provide sufficient additional free energy to produce bicarbonate at the required concentration.

The most likely primary source of the hydrogen and bicarbonate ions formed in gastric secretory processes is carbonic acid. First, being a relatively strong acid compared to water, the reversible free-energy requirement for its dissociation is much lower than for water. To calculate the potential required to produce 0.1*M* acid, the focal concentration of carbon dioxide and the *p*H must be known. Since carbon dioxide is one of the primary products of the metabolic processes which must accompany secretion, its concentration may be considerably higher than that in the blood. Davenport has shown that carbonic anhydrase in relatively high concentration is present in the parietal cells of several mammalian species (*18*), and Janowitz, Colcher, and Hollander have shown that a potent carbonic anhydrase inhibitor could inhibit gastric secretion in dogs (*19*).

The action of certain drugs or other agents in stimulating or inhibiting secretion may have a "gate" effect because they open or close the circuit by a separate mechanism not directly related to the chloride pump or to our acid-secretion model. The operation of the chloride pump to produce the potential occurs in the presence of nutrient. Agents which stimulate secretion lower the resistance across the stomach wall, and in this manner increase the current and the production of acid and base. The fact that so many stimulants of gastric secretion are amines or bases (histamine, acetylcholine) may be an important clue as to the nature of the ion-selective membrane itself. A clue of greater significance can be found in the work of Ingraham and Visscher (*20*) who injected solutions of dyes into the bloodstream of dogs and collected gastric and pancreatic juices under histamine stimulation; anionic dyes appeared in the pancreatic juice, not in the gastric juice. Since cationic dyes appeared solely in the gastric juice, a single cation-permeable membrane may be present.

Our proposed mechanism for the gastric generation of hydrogen ions supports Rehm's concept of an electrogenic chloride pump (*13*) as the primary cell, with the ion-selective membrane model supplying acid and base to complete the circuit.

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Alternative Forms of Triosephosphate Dehydrogenase in Chromatium

Abstract. *Triosephosphate dehydrogenase was purified extensively from the obligately phototrophic bacterium* Chromatium. *Enzyme prepared from photolithotrophically grown cells differed in several properties from enzyme prepared from photoorganotrophically grown cells. Either form of the enzyme could be transformed in vitro to the other by mild oxidation or reduction, which effected both Michaelis constants and reactive —SH contents of the proteins.*

Numerous studies on the intracellular and phylogenetic distribution of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP)-linked triosephosphate dehydrogenase (TPD) lend evidence to the hypothesis that the NADP enzyme functions primarily in photosynthesis (*1–3*). However, the photosynthetic bacteria apparently lack NADP-linked TPD activity (*4*). We have studied the properties of the NAD-dependent TPD of the obligately phototrophic bacterium *Chromatium* in order to clarify more fully the function of the NAD-linked TPD in bacterial photosynthesis.

Chromatium, strain D was grown either photolithotrophically with CO_2 as the sole source of carbon and $Na_2S_2O_3$ as reductant, or photoorganotrophically with sodium malate as source of both carbon and reductant (*5*). Cells were harvested by centrifugation, washed once, and suspended in 0.10*M* potassium-phosphate buffer at *p*H 8.4. The cells were disrupted by high-frequency sound, and whole cells and debris were removed by high-speed centrifugation. Most of the TPD activity was then precipitated by $(NH_4)_2SO_4$ between 0.50 and 0.80 saturation. This precipitate was suspended in the phosphate buffer and a particle-bound pigment fraction was removed by centrifugation at 144,000*g* for 90 minutes. The supernatant fluid was then passed through a 150-ml column (2.8 by 25.0 cm) of Sephadex G-200. The active eluant fraction was brought to 0.50 saturation with $(NH_4)_2SO_4$, and crystallization of TPD was effected. This product gave a monodispersed peak in the analytical ultracentrifuge, although most preparations showed a marked tendency to dissociate. A sharp symmetrical peak of activity was eluted from Sephadex G-200. The purified enzyme migrated as a single protein band during electrophoresis on acrylamide gel. The specific activity of twice recrystallized enzyme was no higher than that of the Sephadex eluate and the latter was used as a source of enzyme in most experiments.

The TPD activity was measured in the reductive direction from 1,3-diphosphoglyceric acid to glyceraldehyde 3-phosphate (G-3-P) by the method of Heber, Pon, and Heber (*3*) except phosphoglyceric acid kinase was added in at least twofold excess in all cases. Activity in the oxidative reaction from G-3-P to 1,3-diphosphoglyceric acid was measured as described (*2*).

Under conditions of saturation, the assay showed that enzyme from either photolithotrophically or photoorganotrophically grown cells had a *p*H optimum between 8.3 and 8.5. The specific activities of the enzyme from both sources were equal. The molecular weights, as judged by ultracentrifugation and elution from Sephadex G-100 and G-200 columns (*6*), were 120,000, which is identical to that of TPD prepared from other sources. The enzymes also had similar Michaelis constants (K_m) for NAD and for NADH (Table 1). The enzyme from photolithotrophically grown cells, however, had affinities three times higher for G-3-P and 1,3-diphosphoglyceric acid than did the enzyme prepared from photoorganotrophically grown cells. These observations led to the question of whether *Chromatium* contains two different NAD-specific TPD's or a single TPD, the properties of which are controlled by growth conditions and the metabolic requirements of the cell. Since growth medium containing $Na_2S_2O_3$ and CO_2 has a lower redox potential than does the sodium malate medium, the effect of mild oxidation and reduction on the properties of purified TPD from *Chromatium* were examined.

The TPD prepared from photoorganotrophically grown cells was reduced by treatment with 0.10*M* sodium ascorbate for 1 hour. The TPD from photolithotrophically grown cells was oxidized by overnight dialysis against 0.10*M* potassium phosphate buffer, *p*H 8.0, which removed the cysteine used to protect the enzyme. Reduction of the TPD from cells grown on sodium malate reduced the K_m for 1,3-diphosphoglyceric acid from $10^{-2}M$ to $4 \times 10^{-3}M$ (Table 1), approximately equal to the enzyme prepared from cells grown on CO_2. Conversely, oxidation of the enzyme prepared from cells grown on CO_2 raised the K_m for the 1,3-diphosphoglyceric acid to 9×10^{-3}, a value approximately equal to enzyme from cells grown on sodium malate.

The number of active —SH groups