

caught in the calm "eye" of a hurricane are carried out of normal bounds not so much by the force of the wind as by their constant avoidance of the stronger air currents, so that they are constantly turned back toward the quieter center of a cyclonic storm and thus inevitably are carried along in its track.

An interesting feature is an imaginary journey in which the reader is taken completely around South America, visiting each of the isolated rocks and island groups as far east as Ascension and Gough Island, south to the Falklands and South Georgia and west to the Galapagos. Each of these is described and its seabirds are listed while the accompanying maps, inserted as text-figures, show very clearly the outlines and something of the topography of these important nesting areas.

The major part of the work is devoted to a detailed account of the many seabirds, 183 species and subspecies in all, that occur in the South American sector. These include members of sixteen families, representing five orders (the penguins, petrels and albatrosses, the pelicans and their allies, sundry shorebirds and ducks). The nomenclature of each species is reviewed, its plumages are described, and the known facts relative to its distribution and habits are given. A vast amount of data published and unpublished is critically sifted and set forth in detail. Many of these life histories are exceedingly interesting and afford for the first time a fairly complete picture of the birds' activities. The account of the wandering albatross is a good example. Published errors as to the wing spread are corrected; it is to be not 17 feet, as given by one authority, but instead not over 11.5 feet. The extraordinary habit of the parents in deserting their young before it can fly is shown to be quite normal, for the young bird lives for the last three months of its nestling life entirely without food, depending on the great accumulation of fat gained in the five preceding months while it was being actively fed. The

young do not leave the nest until they are nearly a year old.

Two new forms are described: the Fuegian petrel, *Oceanites oceanicus chilensis*, which migrates northward into the Pacific Ocean in the non-breeding season, and a small race of cormorant, *Phalacrocorax olivaceus hornensis*, from Bertrand Island, Chile. With a sufficient series of skins, Dr. Murphy appears to have solved the puzzling status of the steamer ducks, and shows that instead of one species with volant and non-volant forms, there are in fact three: namely, a flightless form in the Falkland Islands, *Tachyeres brachypterus*; a different flightless bird, *T. pteneres*, inhabiting the continental region from Cape Horn and the Magellanic coasts to Chiloé; and finally a flying species, *T. patagonicus*, inhabiting the entire area covered by the ranges of the two first. Incidentally it may be pointed out that the flightless cormorant (*Nannopterum*) of the Galapagos group is represented by such small numbers that its existence is likely to become precarious, although a saving factor is that it forms only small breeding colonies easily overlooked by visitors.

Altogether this is an outstanding piece of work, carefully done, summarizing the present state of our knowledge of these seabirds and affording a firm basis for any further work in the future. A captious critic might feel that in parts there is even too much detail, and it might have been helpful in finding particular data if side headings had been more freely used. Sixteen colored plates by Jaques help the reader to visualize the settings among which the birds live, but the figures are perhaps too small to do more. Most of the species are further illustrated by a wealth of photographs taken in part by the author. An excellent bibliography and a full index complete this most attractive work.

GLOVER M. ALLEN

MUSEUM OF COMPARATIVE ZOOLOGY
CAMBRIDGE, MASS.

SPECIAL ARTICLES

THE DETERMINATION OF ACIDITY IN HEAVY WATER MIXTURES

WITH the increasing use of deuterium oxide as a research tool in biological chemistry, it becomes important to develop methods for the precise determination of acidity in that solvent and its intermediate mixtures with light water. Ultimately, the colorimetric method will probably receive the most general application by virtue of the ease and rapidity of the technique and the small amounts of material required. However, the quantitative interpretation of colorimetric measurements requires a knowledge of the influence of deuterium substitution upon the ion-product of the solvent as well as the dissociation constants of the acid-base systems employed as buffers

and as indicators—data which can best be obtained by e.m.f. methods.

The e.m.f. measurements available at present indicate that¹ K_w is decreased 6.13 fold at 25° C.; i.e., $C_D \cdot C_{OD} = 0.16 \times 10^{-14}$ and that the dependence upon deuterium content in mixtures of H_2O - D_2O is not linear. This means that if one wishes to construct a pD^+ scale analogous to a pH^+ scale, the characteristic points 0, 7 and 14, referring to molal hydrogen-ion activity, hypothetical neutrality and molal hydroxyl-ion activity, respectively, become 0, 7.4 and 14.8 for molal deuterium-ion activity, neutrality and molal deuterioxyl-ion activity, when pure D_2O is the solvent.

¹ Abel, Bratu and Redlich, *Zeits. f. physik. Chemie*, A173: 353, 1935.

Investigations in progress in this laboratory and elsewhere have revealed that deuterium substitution exercises a more pronounced influence upon the chemical properties of acid-base catalysis and acid dissociation constants than might have been predicted on the basis of the changes in physical properties investigated soon after the discovery of deuterium. For example, the ratio of the viscosities of light and heavy water, which exhibits the largest change of any purely physical property, is only 0.79. On the other hand, Table I shows that the effect upon chemical kinetics is much greater.

TABLE I

Property	Ratio $\frac{\text{in H}_2\text{O}}{\text{in D}_2\text{O}}$
Viscosity	0.79 ²
Velocity of nitramide decomposition	5.2 ³
Mutarotation of glucose	3.8 ⁴
Nitroethane neutralization	6.0 ⁵
Inversion of sucrose	0.47 ⁶

TABLE II

Acid	$K_{\text{HA}}/K_{\text{DA}}$	pK (in H ₂ O)	pK (in D ₂ O)	Observer
Water	6.13	14.0	14.8	A. B. and R.
Hydroquinone				
($K = \sqrt{K_1 K_2}$)	3.84	10.58	11.15	L. and K.
Acetic	3.32	4.75	5.27	L. and K.
Salicylic	4.1	3.00	3.61	L. and K.
Chloracetic	2.7	2.8	3.2	L. and S. ⁸
NH ₄ ⁺ = NH ₃ + H ⁺	30.0	9.75	10.7	L. and S.
NH ₄ OH = NH ₄ ⁺ + OH ⁻	1.5	4.75	4.93	L. and S.

The same marked effect has been observed for acidic dissociation, where the ratios of the dissociation constants range between 3 and 6. We have found the

II lists the ratios for the acids which have been reported thus far, together with the corresponding pK values in H₂O and in D₂O.

The effect of deuterium substitution appears to be specific, although in general it is more pronounced for weaker acids.

The potential of the quinhydrone electrode in HCl and DCl solutions is 0.0345 volt more positive in D₂O than in H₂O. This difference arises from the unsymmetric distribution of deuterium in the exchange equilibrium $\text{QH}_2 + 2\text{DCl} = \text{QD}_2 + 2\text{HCl}$ corresponding to $K = 14.64$. Other exchange equilibrium constants are listed in Table III.⁹ It will be noted from No. III that the deuterium-ion concentration in aqueous solution is less than would be expected from its stoichiometric molality as a result of the unsymmetric character of the exchange. This generally unequal distribution of protium and deuterium ions is of fundamental importance in the interpretation of the kinetics of reactions in intermediate mixtures of H₂O and D₂O.^{6, 3}

The success of the quinhydrone electrode as a rapid and reliable means of determining hydrogen-ion activities in biological media where the quantities of material are often severely limited is well known. This electrode reaches a stable equilibrium quickly, and should prove to be as well adapted for biological investigations with heavy water and its intermediate mixtures, as it has for ordinary water. When employing the deuterium gas electrode for intermediate mixtures of H₂O-D₂O, one should recognize that the deuterium content of the gas phase (H₂-D₂) will differ very considerably from the deuterium content

TABLE III
EXCHANGE EQUILIBRIA IN SOLUTION

No.	Process	$E^\circ = (.059/n) \log K$	K	Observer
I	$2\text{DCl} + \text{QH}_2 = 2\text{HCl} + \text{QD}_2$	0.0345	14.64	L. & K.
II	$\text{QH}_2 + \text{D}_2\text{O} = \text{QD}_2 + \text{H}_2\text{O}$		0.96	H. & L.
III	$2\text{DCl} + \text{H}_2\text{O} = 2\text{HCl} + \text{D}_2\text{O}$		15.3	I - II
IV	$2\text{DCl} + \text{H}_2(\text{g}) = 2\text{HCl} + \text{D}_2(\text{g})$	.0034	1.30	A. B. & R.
V	$\text{D}_2(\text{g}) + \text{H}_2\text{O} = \text{H}_2(\text{g}) + \text{D}_2\text{O}$		11.8	III - IV
VI	$\text{QH}_2 + \text{D}_2(\text{g}) = \text{QD}_2 + \text{H}_2(\text{g})$		11.26	I - IV
VII	$2\text{H}_2\text{O} + \text{D}_2(\text{g}) + 2\text{NaOD} = 2\text{D}_2\text{O} + \text{H}_2(\text{g}) + 2\text{NaOH}$	.0431	28.58	A. B. & R.
VIII	$\text{H}_2\text{O} + 2\text{NaOD} = \text{D}_2\text{O} + 2\text{NaOH}$		2.42	VII - V
IX	$\text{HCl} + \text{NaOD} = \text{DCl} + \text{NaOH}$		0.4	$\frac{1}{2}(\text{VIII} - \text{III})$
X	$2\text{NaOD} + \text{H}_2(\text{g}) = 2\text{NaOH} + \text{D}_2(\text{g})$		0.21	VII - 2V
XI	$\text{D}^+ + \text{OD}^- + \text{H}_2\text{O} = \text{H}^+ + \text{OH}^- + \text{D}_2\text{O}$	.0233	6.13	$\frac{1}{2}(\text{VII} + \text{IV})$

quinhydrone electrode to be satisfactory for making such measurements. The dissociation constants obtained with this electrode in D₂O prove to be in very close agreement with the conductivity measurements of acetic acid solutions in H₂O-D₂O mixtures.⁷ Table

² Baker and La Mer, *Jour. Chem. Phys.*, 3: 406, 1935.

³ La Mer and Greenspan, unpublished results.

⁴ Hamill and La Mer, *Jour. Chem. Phys.*, 4: 294, 1936.

⁵ Wynne-Jones, *Jour. Chem. Phys.*, 2: 381, 1934.

⁶ Hamill and La Mer, *Jour. Chem. Phys.*, 4: 294, 1936.

⁷ Unpublished work of James P. Chittum in this laboratory.

⁸ G. N. Lewis and Schutz, *Jour. Am. Chem. Soc.*, 56: 1913, 1934. These data were obtained upon very small quantities of D₂O and may require revision.

of the liquid phase by virtue of No. V, Table III. This circumstance complicates the ready use of the gas electrode. On the other hand, the exchange equilibria between the proto- and deuterioforms of hydroquinone and the corresponding components of the solution are set up in a homogeneous system; hence the distribution of deuterium in the quinhydrone is automatically adjusted to the value corresponding to complete equilibrium with the solvent. The normal potential of the quinhydrone electrode exhibits a minimum value

⁹ Detailed account to appear in the *Journal of the American Chemical Society*.

(about 2 mv. less than for H_2O) at 5 per cent. D_2O . For higher D_2O content E° is an almost linear function of the composition of the solvent.

VICTOR K. LA MER
SAMUEL KORMAN

DEPARTMENT OF CHEMISTRY,
COLUMBIA UNIVERSITY

THE INACTIVATION OF CRYSTALLINE TOBACCO-MOSAIC VIRUS PROTEIN

THE isolation of a crystalline protein possessing the properties of tobacco-mosaic virus has been described.¹ It has now been found that treatment of this active protein with hydrogen peroxide, formaldehyde, nitrous acid or ultra-violet light produces inactive native proteins that, although slightly altered, retain certain chemical and serological properties characteristic of the virus protein. These inactive proteins do not cause the mosaic disease nor the production of a high molecular weight protein on inoculation to Turkish tobacco plants, and do not produce local lesions on inoculation to *Nicotiana glutinosa* L.

Although the inactive proteins are native and may be taken into solution and crystallized, their solutions are more opalescent than are those of active protein, and they tend to denature more readily than does active protein. The optical rotation of solutions of protein previously treated with ultra-violet light, hydrogen peroxide or formaldehyde is practically unchanged, while solutions of protein inactivated by nitrous acid possess a considerably lower laevo rotation than before treatment. The isoelectric point of protein inactivated by ultra-violet light or by hydrogen peroxide is practically unchanged, while that of protein treated with formaldehyde or nitrous acid is shifted towards the acid side. The amino-nitrogen content of protein inactivated by means of hydrogen peroxide or formaldehyde is considerably lower than that of active protein, and, as would be expected, the protein treated with nitrous acid contains practically no amino-nitrogen. A preliminary determination of the sedimentation constant of protein inactivated by ultra-violet light, kindly made by Dr. Wyckoff and Mr. Biscoe, indicates that no marked change has occurred in the molecular weight; the more diffuse boundary they observe, however, is indicative of a decreased molecular homogeneity. Under the microscope the crystals of the inactive proteins are indistinguishable from those of active protein, and in a preliminary analysis of oxidized protein, kindly made by Drs. Wyckoff and Corey, no conspicuous difference in the x-ray diffraction pattern was found. Mixtures containing varying amounts of active and inactive protein may be prepared, crystallized and recrystallized. The crystals are indistinguishable from those

of active protein, but they possess an activity which is slightly less than that which would be proportional to the amount of active protein that they contain. Crystalline protein possessing any desired activity less than that of the regular active protein may be prepared by mixing the active and inactive or by partial inactivation of active protein using any of the four methods mentioned.

In a typical experiment, inactivation of a 1 per cent. solution of virus protein occurred after standing for 5 hours at 27° C. with 5 per cent. formaldehyde at pH 7, 5 per cent. hydrogen peroxide at pH 7, or with 2 per cent. sodium nitrite at pH 3. The amino-nitrogen content was found to have been decreased 60 per cent., 60 per cent. and 99 per cent., respectively, as a result of the treatments. Irradiation of a 0.5 per cent. solution with the full light of a laboratory mercury vapor lamp for 8 hours caused inactivation. The preparations were dialyzed against water at pH 7 immediately after the treatments and were then tested for virus activity.

The sera of animals injected with virus preparations give a precipitate when mixed with a solution containing as little as 10^{-5} gm per cc of inactive protein, and the serum of an animal injected with a solution of inactive protein gives a precipitate when mixed with solutions containing but 10^{-5} gm. per cc. of either active or inactive protein. Thus the precipitin reaction, which has been used as a measure of virus activity,² may not be used unreservedly for this purpose, for in the case of inactive protein there is no correlation between precipitin titer and virus activity. Positive precipitin reactions between anti-sera to virus preparations and apparently inactive material have been reported.³ The serum of an animal injected with protein inactivated by ultra-violet light has a neutralizing effect on tobacco-mosaic virus, which appears to be similar to that previously reported for the sera of animals injected with sap from mosaic-diseased plants.⁴

Vigorous treatment of the virus protein, such as denaturation by means of acids, alkalis or heat, oxidation with potassium permanganate, chromic acid or chloramine-T, or prolonged treatment with concentrated nitrous acid, causes not only loss of virus activity, but also loss of the characteristic properties of the protein, and it is only by means of comparatively mild treatments that inactive native protein, retaining

² T. Matsumoto and K. Somazawa, *Jour. Soc. Trop. Agr.*, 2: 223, 1930; *Ibid.*, 6: 671, 1934; J. M. Birkeland, *Bot. Gaz.*, 95: 419, 1934; K. Starr Chester, *Phytopath.*, 25: 702, 1935; E. T. C. Spooner and F. C. Bawden, *Brit. Jour. Exp. Path.*, 16: 218, 1935.

³ T. Matsumoto and K. Somazawa, *Jour. Soc. Trop. Agr.*, 3: 24, 1931; F. C. Bawden, *Brit. Jour. Exp. Path.*, 16: 435, 1935; F. C. Bawden and N. W. Pirie, *ibid.*, 17: 64, 1936.

⁴ Helen A. Purdy, *Jour. Exp. Med.*, 49: 919, 1929; Kenneth S. Chester, *Phytopath.*, 24: 1180, 1934.

¹ W. M. Stanley, *SCIENCE*, 81: 644, 1935; *Phytopath.*, 26: 305, 1936.