the sp. gr. reading a coefficient is obtained which is of use in reducing sp. gr. readings to salt content. For the range of concentration likely to be seen at Wood's Hole, *i. e.*, when sp. gr. 15° C./4° C. = 1.0210 to 1.0245 corresponding to a total salt content of 2.84 per cent. to 3.29 per cent., the salt content is obtained with a probable error less than 2 in the second decimal place by multiplying the sp. gr. reading by the factor *134.5*.

RODNEY H. TRUE
U. S. DEPARTMENT OF AGRICULTURE

ON CELL PENETRATION BY ACIDS[1]

Preliminary Note

1. THE water-soluble blue pigment[2] in the cells of a nudibranch, *Chromodoris zebra* Heilprin, is a sufficiently delicate indicator to justify its use for the study of cell penetration by acids. Water extracts of the animal, containing this pigment and other cell materials expressed by grinding, change from a deep blue color with reddish-purple fluorescence to a delicate pink hue at a hydrogen ion concentration of $p_H = 5.6$[3]; the acidity of the body fluids of *Chromodoris* averages $p_H = 7.4$ (27°). The indicator promptly flocculates, in the form of a greenish-blue precipitate, leaving a blue solution, at $p_H = 7.6$. Within the epidermal cells the pigment is also turned green, so that it may be used to measure the penetration of alkalies; it gives results concordant with those obtained with a great variety of tissues by the neutral red method (Harvey[4]), and with neutral red-stained *Chromodoris* cells lacking the blue pigment.

The pigment occurs in two forms: as granules scattered through the superficial and deeper tissues, and dissolved in clear globular bodies located within the cells of the outer epithelium, especially along the edges of the mantle and foot. It is totally insoluble in anhydrous acetone, ether, chloroform, xylol and oils. The globules containing it do not stain with fat dyes. I conclude that the pigment is held naturally in water solution.

2. Direct measurements of the speed with which acids penetrate protoplasm were first given by Harvey,[5] who determined the time required for the testis of *Stichopus ananus* to change in color when immersed in 0.01 *N* solutions of a number of acids. I have used pieces of the lateral mantle edge of *Chromodoris* in a similar way, precautions being taken to insure comparative uniformity of the pieces in the different tests, and find that *at this concentration* (0.01 *N*) the acids employed when arranged in the order of increasing penetration-time form the series shown in Table I. Comparison of this list with

TABLE I

Penetration of Acids from 0.01 N Solutions

No.	Acid.	Time, Minutes.	
		Chromodoris. Mantle Edge. 27°.0.	*Stichopus ananus, testis* (Harvey).[6]
1	Valeric (Iso-)..... ...	1.9	2–4
2	Salicylic.....	3.5	0.25
3	Formic.....	4.5	2–4
4	Hydrochloric.....	7.6	9–11
5	Nitric[7].....	8.4	
6	Sulphuric.....	8 5	
7	Lactic	8.6	
8	Oxalic.....	8.8	12–15
9	Tartaric.....	13.5	30
10	Citric.....	16.0	40
11	Butyric.....	19.0	45–60
12	Acetic.....	75.0	

[1] Contributions from the Bermuda Biological Station for Research, No. 39.

[2] Crozier, W. J., 1914, *Journal of Physiology*, Vol. 47, p. 491.

[3] This point changes somewhat with the age of the extract, in the case of alcohol (95 per cent.) and other permanent solutions of the pigment. The p_H values given were obtained by titration with phosphate and acetate mixtures, checked by gas chain measurements on alcohol and formalin solutions of the pigment.

[4] Harvey, E. N., 1914, Papers from the Tortugas Lab., Vol. VI., p. 133.

[5] Harvey, E. N., 1914, SCIENCE, N. S., Vol. XXXIX., p. 947.

[6] Only those acids which I have studied have been taken from Harvey's table, which includes a number of others.

[7] The differences in penetration-time for Nos. 5–8 are slight at this concentration, but their separation is justified on the basis of the dilution curves.

that of Harvey discloses that the relative penetrating power of the acids at this concentration is practically identical in the two cases. Some of the differences may be due to the temperatures at which the two sets of experiments were made. The figures for the *Chromodoris* tissue represent the mean of ten concordant experiments at a uniform temperature of 27°.0.

3. Examination of the penetration time of these acids over a *range of concentrations* (0.1 *N* to 0.001 *N*) shows, however, that the series established at the single concentration (0.01 *N*) gives an entirely misleading picture of the penetrating powers of the different acids, which is better judged by the nature of the penetration curves as a whole. According to this view the order arrived at is seen in Table II. The acids studied may be arranged

TABLE II

Penetration Power of Acids

Acid	Ionization Constant (*K*)
Group I:	
Hydrochloric	(100)
Sulphuric	(100)
Oxalic	3.8
Nitric	(100)
Group II:	
Formic	0.0214
Salicylic	0.102
Valeric (iso-)	0.0017
Lactic	0.0138
Tartaric	0.1000
Citric	0.0870
Butyric	0.00149
Acetic	0.00180

in two groups on the basis of the character of their penetration-dilution curves.[8] The curves of the second group are all more or less parallel and uniformly concave toward the axis of penetration-time, whereas the curves of acids of the first group (up to 0.002 *N*) are from the beginning concave toward the axis of dilution. The curves of the two sets cut across one another, as do also some of the curves within each set. The acids of the first set give visible evidence of penetration at higher dilutions (*n*/750) than do those of the second group.

The separation of these two groups of acids is further warranted by the fact that, within certain time-limits, a preliminary exposure of the *Chromodoris* tissue to the action of acids of the second group does not hasten the penetration of acids of the first series, but does that of other acids of the second set.

4. The acids included in my group I. of Table II. are all acids of strong ionization, while those of the other group are of low acid strength. To this extent the rôle of ionization in determining permeability toward acids is made clearer than has hitherto been the case, and it seems probable that these two kinds of penetration curves represent at least two different and distinct methods whereby acids may gain access to the interior of cells. Within each of the two sets of acids the degree of ionization is less important in controlling the speed of penetration. Formic acid occupies a somewhat peculiar position, as do also butyric and valeric; the first substance shows a dilution curve more nearly approaching that of the strong acids, agreeing with its constitution, but the relative positions of butyric and valeric in the series are more likely to be accounted for by their rather high solubility in lipoids.

All the evidence so far available indicates that acids penetrate and combine to various degrees with one or more of several constituents of the cell surface. It is certain, at any rate, that the "lipoid theory" of permeability is not even approximately complete as an explanation. Further attempts to elucidate the significance of the apparently quite general uniformity in the order of cell penetrability for various acids in different animals must await the study of a larger series of substances, especially with reference to the action of acids on penetrability for other acids.[9]

W. J. Crozier

Agar's Island, Bermuda

[8] This series has an important bearing on the interpretation of sensory stimulation by acids, a matter which first turned my attention to this problem.

[9] Details, covering additional points not here considered, will be found in a paper to appear in the *Journal of Biological Chemistry*.