CELL PENETRATION BY ACIDS.

V. Note on the Estimation of Permeability Changes.

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1. Effects of Tension.

In view of the fact that qualitative studies regarding permeability have sometimes been based upon experiments involving intense muscular activity in the tissue examined, I thought it worth while to measure the effect of stretching upon the rate with which acids penetrate the indicator-containing tissue of the nudibranch *Chromodoris* (*cf.* Crozier, 1916 *a*).

A piece of the mantle fold, about 2 cm. in length by 0.5 cm. in breadth, was freshly secured by cutting from an individual sufficiently large to supply several such pieces. The piece was then adjusted in a holder, sketched in Fig. 1. This was accomplished by tying to either end a short ligature of waxed thread. One of the ligatures was made fast at *a*, while the other, longer, one was passed over the groove *c*. The longer thread carried at its free end a mass which in the different tests was varied from 5 to 50 gm. The tissue, being fixed in this manner, was lowered into the trough, *A*, containing an acid solution. The time required for the internal color change to be brought about was measured with a stop-watch, while the first evidence of pigment loss could be accurately recognized by watching the edge of the tissue through a horizontal microscope. A slip of milk-glass, mounted back of the tissue, increased the precision of the observations.

By employing a strip of the mantle about 2 cm. in length, there was secured an area in the center of the strip which was free from complications due to the presence of tight ligatures at either end. This was checked by experiments in which the tissue, with the threads made fast, was allowed to hang freely without any attached weights.

The crushed regions about the threads were almost instantly changed to a pink color by acids, but the central part gave penetration times agreeing absolutely with those found for control pieces devoid of ligatures. With a little practice, the strip of tissue could be adjusted without introducing any twist. It was found that these strips of mantle tissue could in all cases support, over the period involved in an experiment, a mass of about 50 gm. Beyond 55 gm., tearing effects became evident.

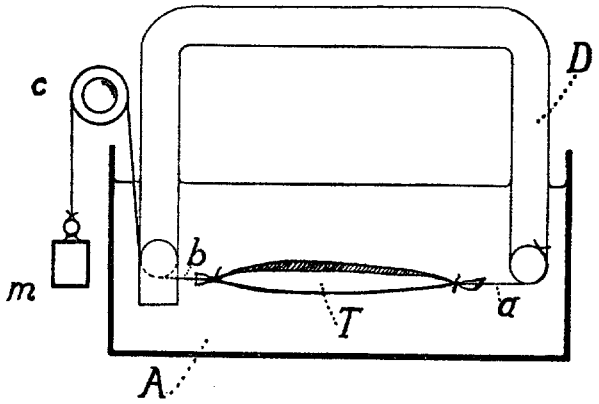

FIG. 1. Side view of device for subjecting to tension, in an acid bath, *A*, a strip of tissue cut from the mantle fold of *Chromodoris*. *T*, tissue; *a*, ligature made fast to glass rod (*D*); *b*, another ligature passing under hook on end of glass rod; and *c*, smooth metal groove (greased), over which ligature from *b* passes to the mass *m*.

The acid chosen for most of the measurements was dichloroacetic, since up to 30 or 40 gm. tension the stronger mineral acids, with higher rates of penetration, give figures which could not always be relied upon as different from those obtained with unstretched tissue. With dichloroacetic, however, the effect of stretching was easily shown.

Experiment 9.26a.—Mantle tissue from a *Chromodoris* 8.5 cm. long; 0.01 N dichloroacetic acid; 25°C.

	min.
(*a*) Control, unstretched, penetration time	6.0
(*b*) With a mass of 10 gm. attached, penetration time	5.5
(*c*) " " " " 20 " " " " "	3.7
(*d*) " " " " 40 " " " " "	2.8

From a number of tests of this kind, data were secured for the construction of Fig. 2. It is apparent that even moderate tension exerts a very decided effect upon the penetrability of this tissue for 0.01 N dichloroacetic acid. A similar result was had with other weak acids; the coagulative action of the stronger acids tends to obscure the phenomenon.

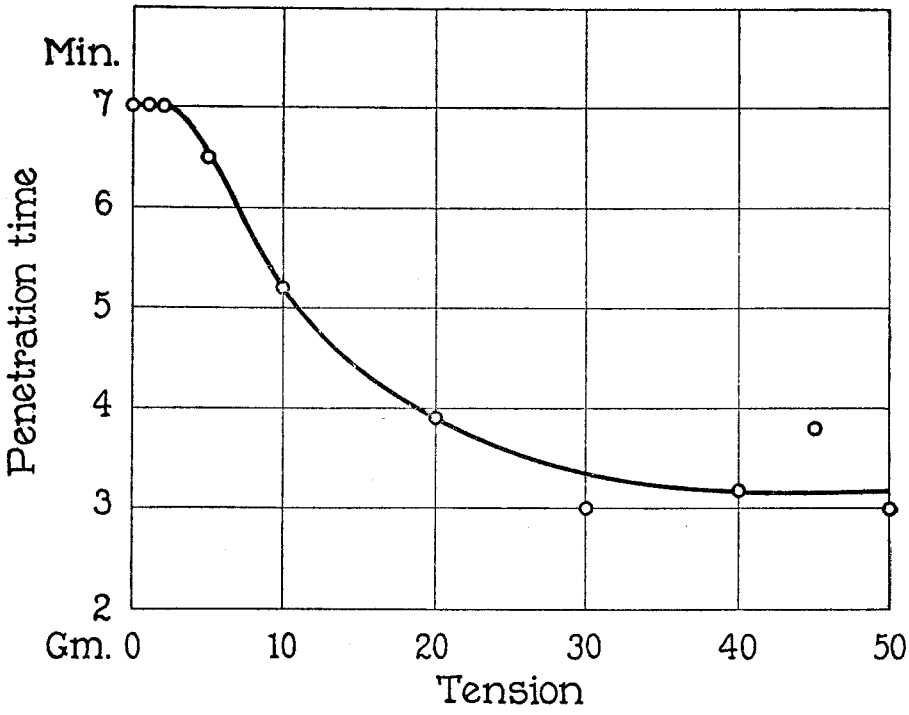

FIG. 2. Penetration of tissues under tension, by 0.01 N dichloroacetic acid; 25°C. Ordinate, penetration time, minutes; abscissa, tension, gm.

Since pieces of mantle tissue subjected for 10 minutes to tensions of 30 to 40 gm., in sea water, quickly regained about their normal penetrability when the traction was removed, the effect of tension is (within limits) reversible.

The effect of tension noted in these experiments cannot be ascribed to manipulation of the tissue during its preparation. Undue

exposure to the air has a very marked influence in the direction of *decreasing* its penetrability. The penetrability of isolated fragments of the *Chromodoris* mantle kept in sea water remained very nearly constant for some 24 hours after their removal.

The influence of tension is equally apparent in relation to diffusion of pigment from the mantle tissue. In one set of experiments, which may be cited as typical, the following observations were recorded:

Experiment 9.26b.—Mantle tissue from two nudibranchs 8.5 cm. long; 0.01 N dichloroacetic acid; 25°C.

Animal.	Stretching mass.	Penetration time.	First sign of pigment loss.
	gm.	*min.*	*min.*
A	0	7.0	15.0
	10	5.5	10.0
	20	3.7	4.0
B	0	7.1	12.0
	30	3.2	4.0
	50	3.0	2.5

The observations on the outward diffusion of pigment are difficult to summarize, because it is necessary to choose for comparison individuals with closely agreeing pigmentations. There was no doubt of the qualitative result, however, which was clearly that the rate of spontaneous pigment diffusion, in acid, is greater when the tissue is stretched. Moreover, the effect of tension was usually greater with respect to pigment diffusion than in relation to penetration of acid (as evidenced by color change). The outcome of these tests may be held to indicate that under tension the resistance of a protoplasmic surface to the diffusion of electrolytes undergoes a (more or less reversible) decrease.[1] The experiments may then have an interesting connection with Carlson's (1907) finding in the

[1] What rôle may be occupied by an actual thinning-out of the cells of the tissue stretched in the present experiments, it is difficult to decide; my chief purpose is to point out, merely, the complication introduced into "permeability" studies by the presence of tractive forces.

Limulus heart, where even moderate traction applied to a cardiac ganglion was seen to increase the intensity of nervous discharge as reflected in the heart beat. And it is obvious that in experiments concerning permeability (*cf.* Lillie, 1909) the presence or absence of relatively intense muscular activity, itself employed as an index of excitation, may introduce a complication difficult to evaluate.

2. Effects of Stimulation.

Pieces of the mantle of the nudibranch were cut from individuals of medium size. The fragments excised were about 1.5 cm. long. Non-polarizable electrodes, 4 mm. apart, were applied to the pigmented surface. Current was obtained from a Harvard inductorium with two Leclanché cells in parallel in the primary circuit, the secondary coil being at "5." The stimulation was purposely made rather severe. The mantle fragments became contracted when stimulated, since they contained smooth muscle fibres. Considerable quantities of slimy secretions were expelled from the stimulated surface. After being stimulated the piece of tissue was immersed in an acid solution, and the penetration time was compared with that exhibited for a control piece previously unstimulated.

Experiment 8.33.2.—Four pieces of tissue, each stimulated for 30 seconds, and four control pieces, unstimulated; 0.10 N HNO_3; 21°C.

	Penetration time.	
	Unstimulated.	Stimulated.
	min.	*min.*
(*a*)	6.5	3.2
(*b*)	6.0	2.5
(*c*)	6.0	4.0
(*d*)	6.5	5.0
Mean.	6.3	3.7

The pronounced increase of penetrability demonstrated in such tests was found to hold for other acids as well.

The increase of penetrability is somewhat augmented according to the duration of the stimulation.

Experiment 8.33.3.—Individual A, 6 cm. long; B, 11 cm. long; 0.05 N HNO_3; 20°C.

Tissue from animal.	Stimulation.	Penetration time.	
		Unstimulated.	Stimulated.
	min.	*min.*	*min.*
A	0.5	2.45	1.5
	0.5	2.5	1.3
	1.0	2.5	0.9
B	0.5	5.5	5.5
	1.0	5.5	3.7
	1.0	5.5	3.0
	1.5	5.6	3.7

This effect can hardly be referred to the contraction of the tissue; the mantle fragments relaxed when the electrodes were removed, before being placed in acid; and they always contracted upon immersion in acid, whether previously stimulated or not. Nor can the extruded mucus be directly involved, for the same reason. Moreover, as pointed out beyond, mucus extrusion also occurs under the influence of chloroform, which has an opposite effect upon penetrability.

When a small spot upon a fair sized piece of the nudibranch integument was stimulated, and the whole then placed in acid, the increased penetrability was manifest merely upon the immediate site of excitation. Thus, in one experiment with 0.05 N HNO_3, a stimulated area turned pink in 2.45 minutes, while the general surface of the tissue did not do so until 3.20 minutes had passed, in spite of the fact that mucus discharge had occurred over the entire surface.

The method of observation is such that the results may not be interpreted in terms of "permeability," since the intracellular color change serving as an index of acid penetration is dependent upon the relation of the pigment to the rest of the cell contents; this relation might be altered by excitation of the epithelium. Nevertheless, it is entirely probable that in these tests the penetrability of the protoplasm toward acid is markedly increased as the result of faradic stimulation.

3. *Anesthetics.*

Ether, ethyl alcohol, chloroform, and $MgSO_4$ all produce a very decided increase in the apparent time of penetration of acids.

Experiment 8.54.1.—Tissue from several animals of the same size; acid, 0.05 N HCl. Pieces of mantle immersed for the intervals stated in sea water containing M ethyl alcohol, then transferred to acid.

Tissue from animal.	Time in alcohol solution.	Penetration time.	
		Alcohol.	Control.
	min.	*min.*	*min.*
A	3	5.2	5.0
	3	7.0	5.0
	10	10.0	5.0
	20	10.0	
	20	9.1	
B	2	3.5	2.5
	10	5.0	2.6
	20	9.1	

A number of tests showed essentially similar conditions under the action of other anesthetics. The primary effect of these materials is to decrease the penetrability of the tissue toward acids. Ether, one-half saturated in sea water, and chloroform, one-fifth saturated, decreased the apparent penetration rate of even 0.1 N HCl by 50 per cent, in 2 minutes and in 5 minutes respectively.

4. *Pigment Diffusion.*

The outward diffusion of intracellular coloring matters has frequently been employed as a criterion of increased permeability to the pigment involved, and by inference to other substances also. In addition to other sources of error, this method of observation tends to ignore the fact that the pigment concerned may be held in the cell, not by the state of the cell surface primarily, but by the relation of the pigment, especially when in the form of droplets, to the cytosome as a whole.

As bearing upon the value of pigment diffusion as an index of permeability increase, I would cite experiments showing that the

penetrability of the mantle epithelium of *Chromodoris* toward acids may be caused to vary in the direction either of increase, or of diminution, while the spontaneous diffusion of pigment (uncomplicated by muscular contractions, in this instance,) simultaneously changes in a directly contrary manner. The pigment involved serves as indicator of internal acidity and is at the same time the freely water-soluble coloring matter concerned in visible outward diffusion. For comparative tests it is necessary to use tissues colored in precisely the same way by this pigment.

min.

Experiment 8.34.3.—(*a*) Two pieces of mantle placed in 0.1 N HCl; penetration time (24°) 2.16
2.05

(*b*) Two similar pieces placed in sea water one-half saturated with $CHCl_3$, for 5 minutes; then put into 0.1 N HCl; penetration time (24°) 2.25
3.75

In (*a*) loss of pigment was evident in 4 and 10 minutes, respectively; in (*b*), within 2 minutes in both cases.

Experiment 8.42.1.—Four pieces of mantle tissue from a single individual were used; two were placed in 0.1 N HNO_3, the penetration times being 3.25 and 3.50 minutes, respectively; two others were placed for 10 minutes in sea water containing 0.1 M urea, then transferred to 0.1 N HNO_3—the penetration times being now 0.5 and 0.3 minutes. In the first case pigment diffusion was observed in 7 minutes, in the second in 9 minutes.

These tests, typical of a number made, show clearly that the condition of the tissue used may differ markedly when regarded from the standpoints (1) of penetrability for acids, and (2) of the readiness with which spontaneous loss of pigment occurs. A long series of observations upon the penetration of various acids showed, in addition, that practically no correspondence obtains between the relative ease of penetration and the speed with which pigment is lost to the surrounding solution. With mineral acids, pigment loss in general followed the internal color change indicative of penetration; while with weak acids, and especially with fatty acids, even in the case of relatively concentrated solutions penetrating with high velocity, loss of pigment usually preceded the internal sign of acid penetration. Quite apart from the fact that it seems inadvisable to speak of "permeability" as a general property, having reference indis-

criminately to all classes of substances, the facts already enumerated make it hazardous to lay much weight upon pigment diffusion as an index of permeability changes (Crozier, 1916 *b*). Thus in Lillie's extensive operations with *Arenicola* larvæ (1913), the fact that in the presence of anesthetics pigment diffusion is retarded, has been urged as evidence for the restraint by anesthetics of permeability-increasing agencies; on the other hand, when mantle tissue of *Chromodoris* is subjected to the action of anesthetics, pigment is lost much more readily than otherwise, even though the resistance of the tissue toward the diffusion of acids is by this action markedly enhanced.

SUMMARY.

The penetration of acid into mantle tissue of *Chromodoris zebra* is accelerated after local faradic stimulation, and is retarded by brief treatment with anesthetic solutions. The spontaneous outward diffusion of intracellular pigment is an inadequate criterion of "permeability." Outward diffusion of pigment and penetration of acid are both facilitated when the tissue is artificially put under tension.

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