CHANGES IN ACID AND ALKALI TOLERANCE WITH AGE IN PLANARIANS

WITH A NOTE ON CATALASE CONTENT

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Received for publication July 17, 1920

During a study of the effects of certain typical acids, bases and salts upon living planarians (Planaria dorotocephala, P. maculata and P. velata), observations were made which are here reported on account of the wider interest of their bearing upon tolerance of H+ and OH- ions, upon the relative efficiency of the mechanism for regulation of neutrality in young and old individuals, and upon the problem of acidosis in general.

Methods. A closely graded series of concentration of the acids (hydrochloric chiefly, also sulfuric and acetic) or alkali (sodium hydroxide) is made up from standardized N or 0.1 N solutions by dilution with aerated well water or other (Lake Michigan or Lake Ontario) water in which the worms live and thrive. The well water, which was used chiefly, has a pH value of 7.5 to 7.6 and an ion and gas content to be published with the larger study above mentioned. Both distilled water and strongly chlorinated tap-water are in themselves injurious to these worms and hence could not well be used for the purposes of these experiments; but similar tests are now being made with P. maculata in distilled water, this species being but little affected by distilled water in the period of time required.

Into the series of dilutions of an acid or alkali in 500 cc. or 1000 cc. Erlenmeyer flasks, filled and ready to be plugged with rubber stoppers, are introduced the flatworms, usually ten larger (18 to 20 mm.) and ten smaller (8 to 12 mm.) specimens together, all selected sound from established well-fed cultures. In some cases a similar group of three easily distinguishable sizes was used (22± mm., 15± mm. and 8± mm.). In control flasks all such individuals live practically indefinitely.
The hydrogen ion concentrations, already in a graded series from the
method of diluting the normal solution, are measured and corrected at
critical points by the colorimetric method with appropriate indicators:
thymol-blue, brom-phenol-blue, methyl-red, brom-cresol-purple, phe-
nol-red and phenol-phthalein, and the Hynson, Westcott and Dunning
apparatus (1). Certain difficulties were encountered from the fact that
glassware requires particularly thorough cleaning after each usage, and
because strong acids added to water containing so much carbonate as
do these naturally generate CO₂ and such solutions tend to return gradu-
ally toward neutrality. But these facts have no great significance here
except as they render impracticable a precisely accurate determination
of the actual limits of tolerance, as may be made in distilled water and
by electrometry. In all cases it was the relative rather than the abso-
lute susceptibility that was sought and that is here emphasized.

Extensive physiological studies of planarians have been made by Child
(2), (3), who showed for P. dorotocephala by various means that the smallest
worms (up to about 6 or 7 mm.) consist of but one zooid, while the
medium-sized ones (12 to 14 mm.) usually possess a second zooid region,
and larger specimens (20 to 25 mm. or more) exhibit at the posterior
end a third zooid or group of very small zooids, constituting what is
essentially a “growing tip.” At least the chief, the second and the
third of these zooids were demonstrable physiologically by both “direct”
and “indirect” methods in cyanides, but only after fission do the typical
head structures of the zooids become visibly differentiated morphologi-
cally. The growing tip is evidently involved repeatedly in the reproduc-
tive process; hence as this region grows its zooids become more independent
and acquire a higher rate of metabolic reaction and, like young indivi-
duals, are more susceptible to high concentrations and less susceptible
to low concentrations of lethal agents (KNC, anesthetics, etc.).

Experimental. The chief observations are recorded and summarized
graphically in the accompanying figure. The time records are averages
from repeated tests made of each dilution; such averages for separate
tests show but small deviations from the general average, but individ-
ual deviations are often large and overlapping wherever the curves lie
close together. Dr. C. M. Child, to whom the writer is indebted for
many opportunities and suggestions in this work, has recently used
these experiments as part of a class course at the University of Chicago.

Acids. Immersed in HCl solutions that kill almost instantly (e.g.,
in acidities greater than about pH = 2) all worms are fixed and pre-
served intact (range of preservation). In lower concentrations, from
pH = 2 down to about pH = 4.5, all individuals are killed and caused to disintegrate, the older somewhat later and more slowly than the younger (range of direct susceptibility and inhibition). In this disintegration all regions of the body are not equally and simultaneously involved, but usually the posterior tip and the head are first attacked and then the regions behind the head in order from in front backward; and, as might be expected from the small differences in direct susceptibility between young and old, the anterior end of a second zoöid is not distinguished. As the acidity approaches pH = 4.5 or pH = 4.6, the age difference in survival time decreases more and more and finally

Fig. 1. Time in hours of distinct initial disintegration of young worms \(--\text{o--o--}\) and old worms \(--\text{x--x--}\) in solutions of different pH values (abscissae) up to pH = 10. Small numbers below abscissae indicate volumes of well water added to one of N/10 HCl (left) or NaOH (right).

Below pH = 2 ± is the range of preservation or fixation. Then follows with increasing dilution up to pH 4.4 ± the range of distinct direct susceptibility which grades off by a transition range into the range of acclimation or indirect susceptibility.

With NaOH the range of preservation is evidently absent, direct susceptibility differences between young and old much more marked, and the indirect susceptibility differences less marked than with acids.
becomes nil, and disintegration does not discriminate between the small and the large. In still more dilute solutions, however (pH = 4.7 to 4.9), where death is delayed for several hours and a certain amount of recovery from the initial inhibitory effects of the agent is possible, the relative susceptibility of young and old individuals is reversed (range of indirect susceptibility and acclimation), and smaller worms disintegrate last or not at all, while larger ones either disintegrate entirely or lose their head region. Posterior zooids are left intact, and the posterior part of the first zooid was never seen to disintegrate before the anterior end. Often the young worms live for days or indefinitely after the old are partially or wholly gone. Recovery tests, made by returning young and old alike to fresh water after various periods of exposure to the agent, are even more delicate in their indication of the sites and degrees of injury, and were used to extend and confirm the results obtained by leaving the animals in the agent up to the end of the experiment.

A more or less definite sequence of changes leads up to the final disintegration with acids. After the initial stimulation, during which the flatworm assumes for a time a slender form, moves rapidly and secretes some mucus, it gradually loses its power of adherence to the glass walls of the container and becomes shortened, cylindrical and swollen. This state is soon followed by discoloration or whitening, the loss of color occurring, as noted, first at the sensory tip, margins and ventral surface of the head and gradually extending backwards, often more rapidly on the ventral surface, and in larger individuals beginning early also at the growing tip. The whitening appears to indicate that semi-permeability or some similar property of the surface layer has been abolished at the approach of death, for following close upon the loss of pigment occur disintegrative changes of a characteristic kind: as the parts become sticky and adherent to glass the regularity of the external contour is interrupted by small breaks in the continuity of the surface, the protoplasmic granules swell and mass into small clumps or liquid spheres and scatter out into the medium until finally little remains of the old body but a soft white shreddy outline composed of the more resistant connective and supporting tissues quite stripped of all the relatively susceptible epithelial parts.

Results differ in no essential way if sulfuric acid in slightly higher concentrations be substituted for the hydrochloric, or if a considerably greater strength of acetic acid be used—the difference probably being necessary to compensate for the lesser dissociation of the organic acid.
A change of response occurs upon addition of acid to the normal medium. The planarians then exhibit a fairly strong negative geotropism, climbing always up the walls of the container, whether in doing so they approach or attain a surface or not. Since strong acids cause a release of CO₂ into the solution, the response may perhaps be considered generally appropriate and adaptive, inasmuch as ordinarily an increase of CO₂ is doubtless associated with an insufficiency of oxygen (to which a similar response is made) and both could doubtless be avoided by rising to a better aerated surface layer.

Alkali. In alkaline solutions the same general results are obtained with certain more or less significant modifications. In the hydroxide (NaOH) stimulation is evidently more marked than in acids, and both whole worms and surviving parts of any size are more active both spontaneously and upon mechanical stimulation up to the very point of death. Alkalis also cause the secretion of a very excessive amount of mucus, which collects, as often as removed, in the bottom of the vessel.

Even quickly killing concentrations do not produce a definite fixation and preservation. Disintegration, if rapid, occurs, by a rather violent process of splitting and bursting of the dorsal surface in darkened lines; if slow, it begins at the margins and dorsal surfaces of the head and the posterior tip, and in larger specimens may also appear at what is presumably the anterior end of the second zooid.

Results with NaOH differ from those with HCl and resemble more nearly those with KNC in one respect—in the slowly acting concentrations, allowing partial acclimation of the larger animals, death sometimes begins at the posterior end of the first zooid and proceeds forward, while the head region of the first zooid and all of the posterior zoöids remain intact for some time or indefinitely. In short the details of disintegration with this alkaline agent resemble those with most “acid dyes,” while “basic vital dyes” rather resemble acids in their effect (unpublished work).

By the method of direct susceptibility there is much greater difference in survival time of young and old with NaOH than with HCl, the old surviving about twice as long as the young. The effective range of concentrations for indirect susceptibility, on the other hand, is less extended than with acids.

It will be noted that the range of critical concentrations, within which young animals and young parts only are able to regulate slight H⁺ ion alterations, is a comparatively limited one and lies just beyond the limits resisted by all alike. Thus increase of H⁺ ion up to pH =
4.9 on the one side or of OH− ion to about pH = 9.1 on the other come within the normal range for all members of the species; slight additional changes (from pH = 4.9 to pH = 4.8 or 4.7 and from pH = 9.1 to pH = 9.2 or 9.3) can be met by the young individuals and parts alone; still greater changes are beyond the powers of acclimation of any, though the old resist the longer.

The greater tolerance by younger planarians and the posterior zoöid region of such dilute acid and alkaline solutions is almost certainly only another example of the greater power of acclimation to mildly depressing conditions associated so generally with more active metabolism (3). In fact the general principle underlying the indirect susceptibility method is founded on the discovery that organisms or parts of organisms possessing an intenser metabolism can acclimate or acquire tolerance more quickly and more completely than less active organisms or parts to low concentrations of cyanides, narcotics, etc. Child also showed later that the anterior, ventral and median regions (the regions of high direct susceptibility and presumably of most rapid metabolism) in Echinoderm and Annelid embryos, developing in low concentrations of NaOH, alcohol or HCl in sea-water, acclimated or acquired tolerance, or after temporary exposure recovered most quickly and underwent a proportionately accelerated and increased development in the larvae (4).

The explanation of this power of acclimation is not known, but may be in some way associated, as regards acids and alkalies, with differences in protoplasmic conditions, such as the higher percentage water content of metabolically active parts or individuals. If this water carries, as seems probable, at least an equal proportional and a greater total salt content, such inorganic salts of these as are buffer-acting substances (carbonates, phosphates, etc.) would act here much as in mammalian blood, to increase resistance to additional H+ and OH− ions in the medium. Or, if a greater proportion of mid-products of protein metabolism, or more ionized protein, be present during rapid metabolism, then these amphoteric substances may serve as acids or bases according as there are excess bases or acids in the medium. Naturally such buffers and metabolites would be protective only against slightly and slowly injurious concentrations; with higher concentrations the projective action is quickly overcome and the agent may diffuse and act most rapidly in the parts with greatest water content.

The acid or alkali effects may of course be produced through injury to some enzyme or enzymes essential to continuance of metabolic processes. Inasmuch as the almost universally occurring enzyme, ceta-
lase, may eventually be shown to play some rôle in metabolism generally and in oxidation in particular (5), the writer wishes to record here the results obtained from numerous experiments to determine the catalase content or activity of planarians of different ages. It was found that equal weights of crushed young worms (8 to 15 mm.), maturer worms (18 to 20 mm.), and of very old worms (25 to 30 mm.) liberated in 15 minutes at 22°C. from 1 3/4 per cent unneutralized hydrogen peroxide the following quantities of oxygen respectively per gram weight of tissue: 653.3 cc., 460.4 cc. and 317.6 cc. Without a single exception, in many repetitions of the experiment, the rule was found to hold that the larger (older) the worm the lower is the catalase content. It is of interest to note that the oxygen consumption of young planarians has been found to be from 15 per cent to 100 per cent greater than that of old ones (6), showing a higher basal metabolism, just as the Benedict method does for man.

Some significance should be attached to the fact that though there are such large differences in direct susceptibility of young and old with alkalies, these differences are small with acids; while, on the contrary, though the differences by indirect susceptibility are small with alkalies, they are larger with acids. The young are evidently comparatively and absolutely less resistant to alkalies, but relatively more resistant to acids. With advancing age there would appear to be a decreased relative resistance to acids and an increased relative resistance to alkalies—a set of changes such as would result from a gradual onset of a state of acidosis and the more or less incomplete oxidation of the larger-acid products left from a state of lowered metabolism. MacNider (7) has shown that as age advances the acid-base equilibrium of mammals is more and more easily disturbed or overtaxed; that uranium nitrate, for instance, is more toxic to the old than to the young and produces sooner in the old a condition of true acidosis (8), characterized by a depletion of reserve carbonates in the blood, etc.; and that the aged, after uranium treatment, received intravenously without injury considerably more alkali than did the young.

For all the species used by Child (4) in controlling form and proportions of developing embryos, he found that “the agents which are most effective in producing the differentially inhibited type of form are least effective in producing the types of form characteristic of differential acclimation, and vice versa,” his series being effective in producing acclimation types in the order: HCl, alcohol, NaOH, NH₄OH, KNC. Thus acclimation was rapid in acids and alcohol, slow in NaOH and...
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NH₄OH, and exceedingly slow in KNC. A similar contrasting physiological effect between HCl and NaOH is here shown by other means for fresh-water planarians.

Comparing allied species with *P. dorotocephala* it may be said that in general *P. maculata* has a slightly wider range of normal tolerance, while *P. velata* is distinctly less resistant to acids and more resistant to alkalies.

**SUMMARY AND CONCLUSIONS**

1. *Planaria dorotocephala* of all ages used tolerate HCl up to about pH 4.9 and NaOH up to about pH 9.2 in the well water (pH = 7.5 to 7.6) in which they live, i.e., they tolerate a range of pH from 4.9 to about 9.2.

2. Smaller, physiologically younger, individuals are on the average tolerant of a slightly wider range of hydrogen ion concentration (from pH = 4.7 to pH = 9.3) than are larger, physiologically older individuals, this difference of susceptibility being apparently somewhat greater on the acid than on the alkaline side of neutrality. The young possess a greater power of neutrality-regulation than do the old, explanatory suggestions for which are offered.

3. In concentrations of alkali which kill within a few hours susceptibility is reversed in relation to age, the young being very much more susceptible than the old. In similar concentrations of the acids young specimens are likewise on the average more susceptible, but only slightly more so. In other words, high concentrations of OH⁻ tend to increase and high concentrations of H⁺ tend to diminish differences in direct susceptibility between young and old individuals. This suggests a possible increasing average acidity with senescence and decreasing metabolism.

4. Young planarians have about double the catalase content of old planarians per gram weight of tissue.

5. In acid solutions liberating CO₂, in which worms live for some time, they commonly assume, as in conditions of oxygen deficiency, a negative geotropism of an obviously adaptive nature as a normal means of escape from excess CO₂.

6. These facts indicate the necessity of taking into account the factors of age, size and metabolism in defining range of tolerance to agents and conditions.
BIBLIOGRAPHY

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