with or without excision of the ventral and dorsal roots, including their ganglia, of the corresponding spinal nerves. Such sweating could also readily be brought about in summer by direct exposure of the skin to excessive heat of the sun.

Further, such sweating could be demonstrated in skin strips excised from the body (Fig. 1), for a

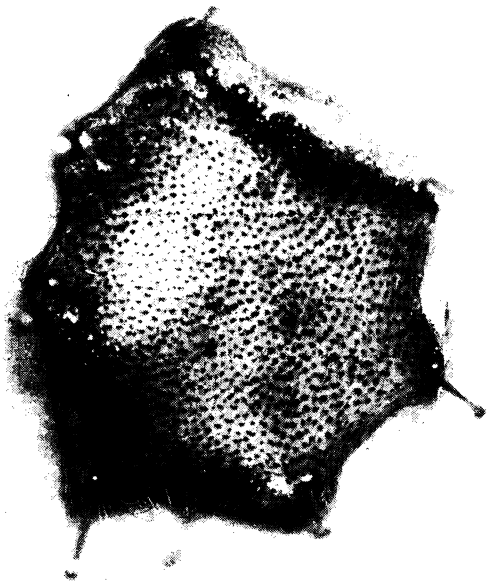

FIG. 1. Sweating response to radiant heat in dog's skin removed from front aspect of the thorax 5 min before heating. Sweating was rendered visible by black spots formed at the openings of hair follicles. Photographed after heating 10 min. (× 2.)

certain length of time after removal from the body.

The fact that local sweating induced by radiant heat in the dog was not inhibited by atropine agrees with observation made by Randall (*5*) upon human skin. Yet the threshold skin temperature at which the sweating began to occur was 38.4° to 38.7° C in three of our dogs, in contrast to 38.4° to 45.5° C measured by Randall in cases of human skin.

Our findings suggest that the sweat glands in the hairy skin of the dog do not participate actively in the central thermoregulatory mechanism, but that they subserve chiefly the protection of the skin from an excessive rise of temperature.

Additional evidence of the secretory activity of the sweat glands in the dog's hairy skin under the influence of the sudorific drugs and radiant heat has been obtained by histological studies.

Recently, Coon and Rothman (*6*) discovered that in the human skin nicotine applied intradermally causes local sweating through the axon-reflex carried by the post-ganglionic sympathetic nerve fibers. Some of their experiments were repeated by one of us (W.) and our colleagues with similar results. In contrast, the hairy skin in most of the dogs showed no sweating response to a local application of nicotine in concentrations of $1:10^3$–$1:10^4$, except in the restricted skin areas of some dogs, where spontaneous sweating, probably reflex in mechanism, was produced. This suggests the possibility that functional integrity of the sudomotor fibers may be judged by the response of the sweat glands to nicotine.

References

1. GURLT, E. F. *Arch. Anat. u. Physiol.*, 399 (1835).
2. CLAUSHEN, A. *Anat. Anz.*, **77**, 81 (1933).
3. WADA, M. *Science*, **111**, 376 (1950).
4. WADA, M., and TAKAGAKI, T. *Tohoku J. Exptl. Med.*, **49**, 284 (1948).
5. RANDALL, W. C. *Am. J. Physiol.*, **150**, 365 (1947).
6. COON, J. M., and ROTHMAN, S. *Proc. Soc. Exptl. Biol. Med.*, **42**, 231 (1939).

The Quantitative Relationship between pH and the Activity of Weak Acids and Bases in Biological Experiments

E. W. Simon and H. Beevers

Department of Agriculture, Oxford University, England, and Department of Biological Sciences, Purdue University, Lafayette, Indiana

The influence of pH on the bactericidal and fungicidal effects of weak acids and bases has been recognized for some time, but the implications of this effect for metabolic studies have frequently escaped attention. Such studies on living cells and tissues often involve the use of weak acids and bases as substrates, inhibitors, or stimulants, and the pH at which they are applied may have an important bearing on their activity. Thus many acids of biochemical importance with pK values of about 4 or 5 are routinely used in solutions in which they are partially dissociated, and their activity can be shown to be influenced in a regular manner by changes in the pH of the medium.

Fig. 1, in which the concentrations of weak acid required to produce a standard response are plotted, shows the magnitude of this influence of pH. It is based on a study of graphs from 90 pH experiments

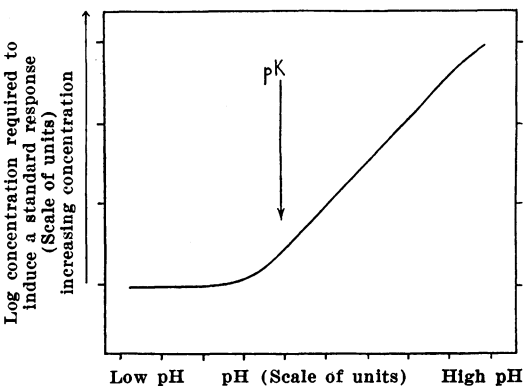

FIG. 1. The effect of pH on the concentrations of a weak acid that are required to give a standard response from the test organism. The corresponding graph for a weak base is obtained by reversing the pH scale. The curve is derived from a study of graphs from 90 pH experiments (*1*).

involving a wide range of acids, test organisms, and responses, and it thus represents a relationship between pH and activity that is of general occurrence (*1*). The construction of individual curves in which the concentrations of weak acid bringing about the selected response (e.g., a standard respiratory inhibition) are plotted against pH, and the derivation of the typical curve shown here, are described in detail elsewhere (*1*). It will be seen that the effect of a given pH change depends on the pK of the compound under investigation. Below pK, changes of pH are of little consequence, but as the pH is raised above pK the rising curve shows that the activity of the acid falls off by as much as three times for each pH unit. It should be noted that a decrease of ten times per pH unit would be expected if activity were confined entirely to undissociated molecules (*1*).

Since activity does vary so markedly with pH, a statement of a response to a weak acid or base should always be accompanied by a statement of the pH at which it was measured—e.g., 0.001 *M* iodoacetic acid gave 50% inhibition of respiration at pH 6.5, or *p*-amino benzoic acid was not effective below 10^{-8} *M* at pH 7.0.

It is clear that rigid control of the pH of the medium is necessary in experiments with weak acids and bases. Neglect of this precaution may result in misleading conclusions. Thus it has been shown that the Lundsgaard differentiating effect of iodoacetic acid may in some cases result from a decrease in the pH of the medium in the fermentation vessel where respired CO_2 is not absorbed (*2*). Again, Schroeder *et al.* (*3*) were able to disprove a claim that yeast poisoned with iodoacetic acid could be reactivated by the addition of certain amines by showing that the apparent reactivation was simply the result of the alkalinity of the reaction mixtures containing amines. Caution is required in interpreting the results of experiments designed to study the effect of the addition of a second acid to a tissue immersed in an unbuffered solution of an acid substance (e.g., the addition of malic acid to *Avena* coleoptile sections in 2,4-dichlorophenoxyacetic acid [*4*]).

The use of buffers for the control of pH is now a standard procedure, but, clearly, a buffer should be chosen which is not toxic to the test organism or tissue. This is particularly important in experiments designed to find the optimum pH level for the growth of an organism. If the acid used is toxic it will be most toxic under acid conditions, and this will influence the result obtained. Thus the optimum for the growth of *Absidia orchidis* was found (*5*) to be pH 4 when phosphate or citrate was used as a buffer; but with oxalate, which was slightly toxic, the optimum rose to pH 5, and with acetate it was pH 6. The more toxic the acid the more it will inhibit growth at a low pH and the higher the apparent optimum will become. Similar considerations would apply to other experiments in which the tissue under investigation is immersed in solutions of different pH.

The choice of pH in experiments has often been made without thought of its effect on the activity of any weak acids or bases that may be present, and it is sometimes necessary to make comparisons between the experimental results of authors who have measured the activity of the same acid (inhibitor, metabolite, etc.) but at different pH levels (*6*). The accompanying graph provides a quantitative basis for such comparisons. Suppose, for instance, that one author reports that the respiration of tissue A is reduced by 50% by 0.001 *M* azide at pH 4.0, and that another author is unable to demonstrate similar inhibition of the respiration of tissue B with 0.005 *M* azide at pH 7.0. Reference to the graph shows that the change from pH 4.0 to 7.0 would reduce the activity of azide (pK 4.7) by 33 times. It follows that the work of the second author does not demonstrate the absence of an azide-sensitive respiration. If the two tissues were equally sensitive to azide then a concentration of 0.033 *M* would be required for 50% inhibition at pH 7.0.

One caution is necessary in the use of the graph. In a few clearly defined instances this relationship between pH and activity does not apply (*7*). Thus the effect of pH may be masked if a small quantity of active material is applied to a relatively large test object, as in pharmacological tests with laboratory animals, the *Avena* curvature test, or herbicide spraying experiments. Masking results because the pH of the applied solution affects the penetration of weak acids and bases only into those cells which are bathed by it. The degree of such masking, then, will be determined by the proportion of cells with which the external solution comes into contact; in bulky tissues this proportion may be very small, and the active substance will reach cells remote from the surface only after passage through other cells or intercellular fluids. The results from such tests are not therefore directly comparable to those obtained from experiments on thin slices of tissue or individual cells, in which all the cells whose responses are being measured are in direct contact with the test substance at the pH of the external medium.

It should be emphasized that in contrast to the behavior of weak acids (whose activity decreases with rising pH) and that of weak bases (whose activity increases with rising pH) the activity of a nonelectrolyte is not affected by pH changes. Hence the relative activities of weak acids, weak bases, and nonelectrolytes are determined by the pH at which the measurements are made, and the same is also true for two weak acids with different pK values. These considerations may be of prime importance in experiments designed to measure relative toxicity or the relative effectiveness of synthetic growth substances, vitamins, inhibitory analogs, etc. It has been shown (*8*) that the measure of activity most appropriate for studies of the relationship between activity and chemical structure or physical properties is the activity measured at a pH at which there is little or no dissociation. If the measurement is made at a pH at which the compounds are much dissociated, the pH

factor will obscure the difference in activity due to other factors. A pH two or more pH units below pK was recommended (*8*) for such measurements, but this may not always be practicable. Thus there are few organisms that could be used to measure the activity of a carboxylic acid of pK 4 at a pH of 2. In such a case it may be best to make the measurements at a convenient pH, say, pH 7, and then use the quantitative relationship between pH and activity shown in Fig. 1 to make a correction for the effects of the varying degrees of dissociation of the different compounds. It may be noted that an alternative form of correction has been used by some authors (*9*), who measured activity in terms of the concentration of undissociated molecules instead of the total concentration; this procedure is open to criticism because it is based on the unjustified (*1*) assumption that only undissociated molecules are active.

References

1. SIMON, E. W., and BEEVERS, H. *New Phytologist,* Pt. 1 (in press).
2. BEEVERS, H., and SIMON, E. W. *Nature,* **163**, 408 (1949).
3. SCHROEDER, E. F., WOODWARD, G. W., and PLATT, M. E. *J. Biol. Chem.,* **100**, 525 (1933).
4. KELLY, S., and AVERY, G. S. *Am. J. Botany,* **36**, 421 (1949).
5. DAGYS, VON J., and KAIKARYTE, O. *Protoplasma,* **38**, 127 (1943).
6. BENNET-CLARK, T. A. *Ann. Rev. Biochem.,* **18**, 639 (1949); THIMANN, K. V., and BONNER, W. D. *Ann. Rev. Plant Physiol.,* **1**, 75 (1950).
7. SIMON, E. W., and BEEVERS, H. *New Phytologist,* Pt. 2 (in press).
8. SIMON, E. W., and BLACKMAN, G. E. *Symposia Soc. Exptl. Biol.,* **3**, 252 (1949).
9. BONNER, D. M. *Botan. Gaz.,* **100**, 200 (1938–39); WENT, F. W., *Proc. Acad. Sci. Amsterdam,* **42**, 731 (1939); CAVILL, G. W. K., PHILLIPS, J. N., and VINCENT, J. M. *J. Soc. Chem. Ind. (London),* **68**, 12 (1949).

Loose Carbonate Accretions from Carlsbad Caverns, New Mexico

Donald M. Black

Carlsbad Caverns National Park, New Mexico

The external shapes of loose carbonate accretions often indicate the type of core or "seed" around which they have grown. Three distinctive groups of loose accretions have been observed in Carlsbad Caverns. Undoubtedly more will be recognized as this study progresses; however, it is believed that these three represent the greater percentage of loose carbonate accretions to be found in the caverns. For simplicity, they will be referred to as the "spherical," "prismatic," and "irregular" groups, as shown in Fig. 1.

Spherical accretions. Small spherical accretions, or "cave pearls," usually have a very small calcareous fragment as a seed (II); this fragment is seldom more than 10% of the pearl's volume. Apparently, each accretion layer is nearly consistent in thickness over the entire surface of the previous layer. Pearls larger than a centimeter in diameter do not necessarily have a small seed.

Prismatic accretions. This group contains the forms

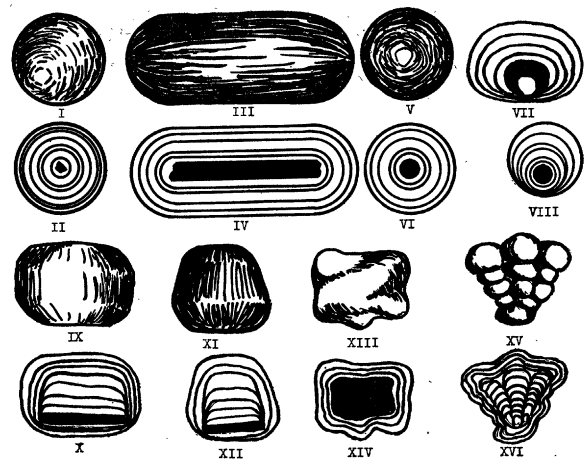

FIG. 1. I, Cave pearl, × 1.5. II, Cross section. Angular spot in center represents sand grain; concentric circles, series of minute, irregular growth rings. III, Accretion with stalactite core, × 1.5. IV, Transverse section. Heavy shaded center represents stalactite; lines represent series of minute, irregular growth increments. V, End view. VI, Cross section. Heavy shaded center represents stalactite; concentric circles represent series of minute, irregular growth increments. VII, Cross section of nonturning accretion with a stalactite fragment core (dark center), × 1.5. VIII, Cross section of nonturning accretion with a cave pearl core (dark circle), and growth increments, × .75. IX, Truncated prism accretion with a scale core, × .75. X, Cross section showing scale core (dark area), and growth increments. XI, Truncated cone accretion with scale core, × .75. XII, Cross section showing scale core (dark area), and growth increments. XIII, Irregular accretion with bedrock core, × .75. XIV, Cross section showing bedrock core (dark area). XV, Irregular accretion with "popcorn" fragment core, × .75. XVI, Cross section showing "popcorn" fragment as a triple series of vertical growth increments.

having fragments of stalactites or flat fragments as a core (III–XII). The stalactite core usually has the accretion layers concentric with its diameter (VI); however, at each end of the stalactite fragment the accretion growth tends to seal the ends and become convex in outline (IV). A scale or flake permits a faster rate of growth on its top surface than on the side or bottom (X, XII). After growth once stabilizes, the accretion formed around a scale or flat fragment will resemble a truncated cone or prism (IX–XII).

Irregular accretions. Irregularly shaped accretions usually have formed around fragments of bedrock or pieces of broken formations (XIII–XVI).

Spheres or pearls. These require constant turning while growing; if rotation ceases and growth continues, they will become elongated. Dripping water that causes rotation is most conducive to the forming of pearls; core or seed of such an accretion is usually a very small calcareous fragment.

Prismatic. Very elongated accretions usually form around a section of a broken stalactite. If this section is round in cross section, it rolls readily and forms concentric rings of growth (VI). If it cannot rotate, one side will be flat (VII, VIII).

Truncated prisms or cylinders represent growth on a flat fragment; many such fragments peel or spall from decomposing stalagmites. Growth on these fragments is primarily upward from the top surface; very little growth accumulates on the sides or bottom as