SHORT COMMUNICATIONS

THE STATISTICAL TREATMENT OF HYDROGEN ION CONCENTRATION AND pH

BY R. G. BOUTILIER AND G. SHELTON

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ

A certain amount of confusion exists amongst respiratory physiologists about the statistical treatment of pH measurements taken from animals during replicated experiments. In some cases average pH (with standard deviations or standard errors as appropriate) is calculated directly whilst in others, pH determinations are converted to corresponding hydrogen ion concentrations before the statistical calculations are carried out. Values for pH may or may not then be recalculated but, if they are, the result of these manipulations is an asymmetry of standard deviations or errors about the mean which is neither useful nor acceptable to the statistician. Our interest in this subject was the result of a mild irritation on being told by a referee of one of our papers that we made the common error of taking simple pH averages. Further enquiry and thought led us to the present paper.

Arithmetic averages of pH values have for many years been criticized because the logarithmic pH notation is considered not to be a natural quantity of biological importance but merely a convenience of physiologists (Davenport, 1947; Fenn, 1969). This view has not to our knowledge been justified by experiment and there seems to be no good *a priori* reason for rejecting a logarithmic scale (i.e. pH) in favour of a linear one (i.e. H^+ ion concentration). Sensory systems in animals often detect parameters on a logarithmic scale and though the Weber–Fechner law (the intensity of sensation is proportional to the logarithm of the stimulus intensity) is not universally applicable (Stevens, 1971), it does seem to provide a 'convenient rule of thumb' (Granit, 1955). Additionally, if at some stage the $[H^+]$ gradient across a membrane is in any way responsible for a generator potential as part of a causal sequence in the production of nerve impulses, then the Nernst equation again suggests a logarithmic relationship.

There seems therefore to be good reason to suspect that receptor systems in animals could be measuring hydrogen ion concentrations on a logarithmic basis, and that regulation of the concentrations would be on the same basis. If so, one might expect the variation around the regulated variable to have a symmetrical distribution. It is the fundamental principle of the central limit theorem that, if we are to make mean calculations of any parameter, we assume the averaged quantity to have a normal distribution. We therefore collected a large number of pH measurements on an amphibian and in man to see which relationship (linear or logarithmic) best fitted a theoretical normal distribution.

Blood samples for pH measurements were obtained by finger puncture from 62

Table 1. *Statistical parameters associated with direct arithmetic treatment of pH measure-ments and with the calculation of pH by mathematical transformation to $[H^+]$ and reconversion to pH*

(Data are for arterialized blood. N = number of animals.)

	Human (N = 63)			Toad (N = 53)		
	Direct pH	[H+], nmoles/ litre	Reconverted pH	Direct pH	[H+], nmoles/ litre	Reconverted pH
Mean	7·427	37·4	7·427	7·809	15·7	7·804
Standard deviation	±0·023	±1·97	+0·023 −0·022	±0·052	1·92	+0·056 −0·050
Median	7·425	37·6	7·425	7·810	15·5	7·810
Range	7·380–7·495	32·0–41·7	7·380–7·495	7·646–7·955	11·1–22·6	7·646–7·995

healthy male and female undergraduates during the course of an ordinary laboratory class. A large drop of blood usually formed within seconds of the puncture and was taken into a 150 μl heparinized capillary tube which had a small magnetic bar inside. When completely filled, each tube was then sealed with wax (within 15–20 s of puncture) and put on melting ice for a period of 30 min to 1 h. Blood samples were rejected if the blood contained bubbles or if the tubes were incompletely filled. Just before measurement, each tube was allowed to warm to room temperature (20–22 °C) whilst the blood was being thoroughly mixed by passing a magnet back and forth along the tube. The sample was then injected into a thermostatically controlled (37 ± 0·1 °C) Radiometer microelectrode unit (type E5021a) and the output displayed on a Radiometer PHM 71 acid-base analyser. The instrument was frequently standardized with two point calibrations using Radiometer precision buffer solutions (ampoules S1500, pH 7·383 and S1510, pH 6·841).

In addition, a considerable number of arterial blood pH values were available to us from previous studies on the toad, *Bufo marinus*. Data were selected from 53 animals which had been treated under identical conditions at 25 °C, as background studies made before further experimental procedures. A number of these data are already published (Boutilier *et al.* 1979 *a–c*; McDonald, Boutilier & Toews, 1979) along with the details of blood collection (indwelling cannulae) and measurement (Radiometer electrodes and display meters).

Results of the calculations of mean (± S.D.) pH for *Bufo* and man by the direct and conversion methods are given in Table 1. In view of the linear-log transformations associated with the reconversion from $[H^+]$ to pH, we expected that some discrepancies would be observed between the two methods. It is clear that as the logarithmic range expands, the reconverted mean pH will tend to move further from and be lower than the direct arithmetic average. Similarly the asymmetry of the standard deviation will become more marked and will show its larger variation on the high pH side of the mean. These patterns emerge in the present study only in the case of *Bufo*, the range of pH measurements in this animal being approximately three times greater than that seen in man (Table 1). Even so, the discrepancies between both methods of calculation in *Bufo* are restricted to the third decimal place. In man, the range of pH is so restricted that the differences between the two methods of calculation are negligible.
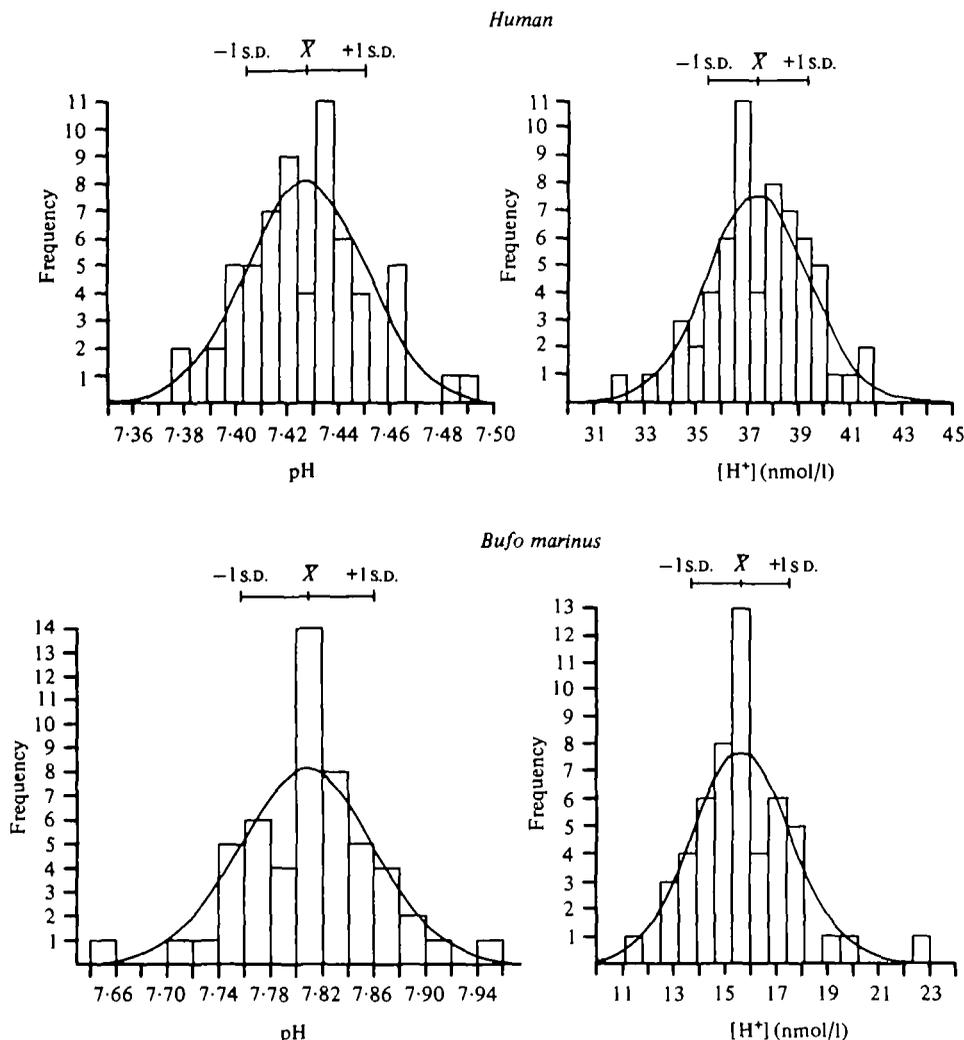
Fig. 1. Frequency histograms of pH measurements and, their calculated equivalents on the [H$^+$] scale in arterial blood of 62 human subjects and 53 *Bufo marinus*. Superimposed on each histogram are theoretical normal distribution curves having the same mean and standard deviation (shown at the top as $\overline{X} \pm$ s.D.) as each set of observations.

As a first approximation of the form that the pH and [H$^+$] distributions take, we examined the mean–median difference since in data that are normally distributed these values coincide. Once again, the differences were minimal (Table 1) so we undertook a more rigorous statistical examination using the Kolmogorov–Smirnov (Campbell, 1974) one sample test. This test's sensitivity to departures from normality is based on comparisons between the cumulative proportional frequencies of the normalized observations with those of the theoretical normal distribution. The test statistic, the largest value of the differences between the two sets of cumulative proportional frequencies (human pH 0·1079, [H$^+$] 0·1109; *Bufo* pH 0·1188, [H$^+$] 0·1604), when

tested at five per cent significance, gives no reason to doubt that the within level distributions of both pH and [H$^+$] of either *Bufo* or man approximate to the normal.

To illustrate the distributions in a standard form, theoretical normal distribution curves having the same mean and standard deviation as each set of observations were superimposed on the frequency histograms of the original data (Fig. 1). Because of the conversion from pH to hydrogen ion concentration, the corresponding distributions are to some extent approximate mirror images of one another though the column heights do not correspond.

The evidence presented in this paper clearly does not conflict with our original hypothesis that determinations of pH in the arterial blood of animals were just as likely to be normally distributed as their equivalents on a linear scale (i.e.: the hydrogen ion concentrations). Unfortunately, the statistical tests on the populations we have studied do not allow us to make judgements about the linear and logarithmic scales as to the best fit to a normal distribution; both seem to be equally good and therefore the mean of pH is as valid a statistic as that of hydrogen ion concentration. This was not surprising in the case of the mammal where regulation is precise, the range of pH (or [H$^+$]) is small, and linear-logarithmic transformations have little effect. Even in *Bufo*, however, where the range is much larger, no clear discrimination emerges, any differences (Table 1) appearing at the limits of resolution of the measuring instrument. We cannot therefore make any suggestions about the characteristics of the regulating systems in these animals but we can say, with some certainty, that statistics on pH are as acceptable as those on hydrogen ion concentrations.

We venture to suggest that this conclusion is applicable to most experiments, not only on *Bufo* and man, but on all vertebrates which show a fairly precise pH (or [H$^+$]) regulation. It should further apply to situations where N is smaller, providing that the individuals are selected randomly from a large parent population. The statistical analysis notwithstanding, a slight predisposition towards pH may be detectable in this paper, based on the rather slender theoretical arguments presented here and elsewhere (Gaddum, 1945) and the more convincing experimental grounds that this is the parameter measured, using an electrode system with logarithmic characteristics (Waddell & Bates, 1969).

REFERENCES

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1979a). Acid-base relationships in the blood of the toad, *Bufo marinus*. I. The effects of environmental CO$_2$. *J. exp. Biol.* (in the Press.)

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1979b). Acid-base relationsips in the blood of the toad, *Bufo marinus*. II. The effects of dehydration, *J. exp. Biol.* (In the Press.)

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. and TOEWS, D. P. (1979c). Acid-base relationships in the blood of the toad, *Bufo marinus*. III. The effects of burrowing. *J. exp. Biol.* (In the Press.)

CAMPBELL, R. C. (1974). *Statistics for Biologists*. Cambridge University Press.

DAVENPORT, H. W. (1947). *The ABC of Acid-Base Chemistry*. University of Chicago Press.

FENN, W. O. (1969). The physiological effects of hydrostatic pressure. In *The Physiology and Medicine of Diving and Compressed Air Work*. (ed. P. B. Bennett and D. H. Elliott), pp. 36–57. Baltimore: Williams and Wilkins.

GADDUM, J. H. (1945). Lognormal distributions. *Nature, Lond.* **156**, 463–466.

GRANIT, R. (1955). *Receptors and Sensory Perception*. New Haven: Yale University Press.

McDONALD, D. G., BOUTILIER, R. G. and TOEWS, D. P. (1979). The effects of enforced activity on ventilation, circulation and blood acid-base balance in the semi-terrestrial anuran, *Bufo marinus. J. exp. Biol.* (In the Press.)

STEVENS, S. S. (1971). Sensory power functions and neural events. In *Handbook of Sensory Physiology*, vol. I, ch. 7 (ed. W. R. Loewenstein). New York: Springer.

WADDELL, W. J. & BATES, R. G. (1969). Intracellular pH. *Physiol. Rev.* **49**, 285–329.