Seasonal Variation in Sensitivity of *Rana pipiens* to Chorionic Gonadotropin

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Early in 1948, Wiltberger and Miller (*5*) and Robbins and Parker (*3*) independently announced the usefulness of the male *Rana pipiens* as a test animal for detection of chorionic gonadotropin of pregnancy. Both of these reports cited the use of small amounts of native urine. Subsequently Cutler (*2*), Brody (*1*), and others have reported the necessity of using concentrates prepared from large amounts of urine (20–50 ml) in order to obtain satisfactory results.

TABLE 1

SEASONAL VARIATION IN RESPONSE OF *Rana pipiens* TO CHORIONIC GONADOTROPIN

Season (1949)	No. of tests	Refractory animals	
		No.	%
Winter	866	2	0.2
Spring	1376	14	1.0
Summer	367	41	11.2
Fall	620	3	0.5

Our experience with the *Rana pipiens* test, which covers several thousand cases, correlates these discrepant recommendations. The animal is quite sensitive to chorionic gonadotropin during the fall, winter, and spring seasons. In this period it responds to between two and five international units of the hormone. The injection of a small amount of native urine is then sufficient, in most cases, to yield a positive response. In the summer, on the other hand, the animal is relatively insensitive, requiring from 10 to 25 units of gonadotropin for stimulation of spermatogenesis. Under these circumstances, the concentration method is essential to detect the chorionic gonadotropin, if present in low titer.

It is always necessary, and especially during the summer months, to test with known gonadotropin all animals which give a negative response in a test. For this purpose 1 ml of a concentrate prepared by the Scott (*4*) method from pooled pregnancy urine, containing about 50 I. U. of chorionic gonadotropin, is used in the summer season. At other times, 0.2 ml, diluted with saline to 1 ml, suffices. During the summer about 11% of the animals are refractory to gonadotropin, requiring retesting of the unknown specimen with another animal. In other seasons only about one animal in two hundred is refractory.

References

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Behavior of Hydrogen Ion in Exchange Reactions

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The anomalous behavior of hydrogen in ion-exchange reactions is well known. For example, when the adsorption energy of hydrogen ion is compared with other monovalent ions in clays and synthetic resins, it is found that hydrogen occupies a position which is quite abnormal in view of its charge and atomic weight. Moreover, no ion-exchange formulations are found to describe satisfactorily the reactions of hydrogen ion.

A possible explanation of these facts is that adsorbed hydrogen in the exchange materials is partly bound by covalent as well as electrovalent bonds. It is therefore of considerable interest to attempt the calculation of the surface dissociation constant for this ion.

Let us imagine an adsorbent in its hydrogen form treated with an electrolyte solution such as KCl. Then the ideas just stated lead to the following equation:

$$\mathrm{RH} \xrightarrow{\qquad} \mathrm{R}^- + \mathrm{H}^+\,(\text{e.a.})^1 \ \text{(surface dissociation)}$$

and

$$k_{\mathrm{RH}} = \frac{[\mathrm{R}^-][\mathrm{H}^+(\text{e.a.})]}{[\mathrm{RH}]} \ . \ . \ . \qquad (1)$$

The quantities in brackets may be expressed in terms of milliequivalents per unit volume of suspension.

Let us now consider the exchange between the K ions and electrovalently bound hydrogen ions. That is,

$$\mathrm{H}^+(\text{e.a.}) + \mathrm{KCl} \xrightarrow{\qquad} \mathrm{K}^+(\text{ad}) + \mathrm{HCl}$$

and applying the mass action law:

$$\frac{[\mathrm{HCl}][\mathrm{K}^+(\text{ad})]}{[\mathrm{H}^+(\text{e.a.})][\mathrm{KCl}]} = \text{constant} = C \ . \ . \ . \qquad (2)$$

We assume here by analogy with weak acids and their salts that all potassium adsorbed is held electrovalently. Then from equation 2,

$$[\mathrm{H}^+(\text{e.a.})] = \frac{1}{C} \cdot \frac{[\mathrm{K}^+(\text{ad})]}{[\mathrm{KCl}]} \cdot [\mathrm{HCl}]$$

$$= \frac{X}{C}$$

where

$$X = \frac{[\mathrm{K}^+(\text{ad})]}{[\mathrm{KCl}]} \cdot [\mathrm{HCl}]$$

The quantity X can be obtained experimentally. Substituting the value of H^+(e.a.) obtained from equation 2 in equation 1,

$$k_{\mathrm{RH}} = \frac{\frac{X}{C}\left[\frac{X}{C} + \mathrm{K}^+(\text{ad})\right]}{[T - \mathrm{K}^+(\text{ad})] - \frac{X}{C}}$$

$$= \frac{X}{C} \quad \frac{[X + C \cdot \mathrm{K}^+(\text{ad})]}{[C(T - \mathrm{K}^+(\text{ad}) - X]} \qquad (3)$$

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[1] H^+(e.a.) refers to the amount of electrovalently adsorbed hydrogen in the adsorbent phase.