THE COMPARATIVE SENSITIVITY OF THE MALE TOAD PREGNANCY TEST AND THE FRIEDMAN TEST

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Comparative Series

A series of 452 comparisons was made in this laboratory between the Galli Mainini pregnancy test, using the English male toad, Bufo bufo, and routine Friedman pregnancy tests. The latter were made in this laboratory by Dr. A. L. Taylor, and in the University of Bristol Department of Preventive Medicine by Professor K. E. Cooper. The series covered the period from March, 1950, to September, 1951, the majority of tests being done in the 12 months from July, 1950, to July, 1951. The technique used for the male toad test was that described by Klopper and Frank (1949). The toad was first catheterized, then 2 ml. of the patient’s untreated urine was injected into the dorsal lymph sac. The toad was again catheterized after two hours, and, if no spermatozoa were seen in the urine, again after four hours. In the earlier tests two negative results up to a period of four hours were considered adequate; later the period was extended to six hours, but in all cases two negative examinations were required before a toad was reported negative.

Results obtained with the 452 comparisons are shown in the Table. Further reports were obtained a total of 205 positive results (113 + 62 + 13 + 9 + 7 + 1), of which 197 (96%) were detected by the Friedman test, and 129 (63%) by the Galli Mainini toad test.

Practical Use of the Male Toad Test

The above results confirm the observations of Klopper and Frank (1949) that the test is not in general so sensitive as the Friedman test in cases with low levels of chorionic gonadotrophin, so that negative results with the Galli Mainini test may require repeating, either at a later date using the same test, or immediately using a concentration technique, such as the modification of the Scott (1940) method described by Law (1949), or with the Friedman test. In spite of the slight extra trouble involved in concentration, the cheapness, simplicity, and speed of the toad test are such that it may be expected to replace the Friedman test in many laboratories.

If a laboratory is considering replacing the Friedman by a toad test, the question must be considered of the comparative advantages of the Galli Mainini test, using the English male toad, and the Hogben test with the female African toad, Xenopus laevis, which is used in the larger pregnancy diagnostic centres at Edinburgh and Watford (Schwabacher, 1950). In either case a concentration technique will be necessary in some cases, and, though the male Bufo is somewhat more sensitive than the female Xenopus, requiring according to Frazer and Wohlzogen (1950) about 8 i.u. chorionic gonadotrophin in 1 or 2 ml. injection as against 70 i.u. in 7 ml., nevertheless, if a concentration technique be used, the accuracy in either case is so great that the difference in sensitivity is negligible. The choice of test will then depend on matters of convenience in supply, maintenance, and handling.

In either case it appears necessary to lay in a stock during the breeding season which will be adequate for at least a year. Xenopus is almost
impossible to obtain except direct from Africa, and the question of breeding in this country has not yet been satisfactorily solved. Male English toads were easily obtained from a number of dealers during the spring and early summer of 1950, but during the autumn and winter they were found to be quite unobtainable. In March and April, if a breeding-pond is known, almost any mature, though it takes at least one month's interval between tests with Xenopus. Frazer and Wohlzogen (1950) allow an interval of one week with Bufo, and reject the animal after four injections. But it was found in this laboratory that, though a toad injected at weekly intervals with positive urine may give a weak or negative reaction after four or more weeks, yet, if the interval between injections be lengthened to a month, the same toad will regain the ability to give a strong positive reaction, so that with monthly injections Bufo can, like Xenopus, be used repeatedly throughout its life.

For maintenance, Xenopus requires a slightly warmed tank, but it is simple to feed, needing only chopped liver at weekly intervals. Bufo can be kept at room temperature in boxes or cages with earth or peat, with the provision of a dish of water, but it requires live food (also at weekly intervals). Most insects, woodlice, and small worms are suitable, and the difficulty may be largely solved by the establishment of a colony of mealworms.

For the actual injection Bufo is the more convenient to handle, for Xenopus is a slippery creature to hold, but either may be controlled and injected single-handed with the aid of a duster, or with a simple device which consists of an elliptical loop of stiff wire, about 5 by 3 in., covered in calico with a transverse slit in the centre, through which the injection is made into the dorsal lymph sac. Xenopus has the advantage that no further manipulation or microscopic examination is necessary, the test jar being simply examined for ova up to an interval of 18 hours, whereas Bufo requires catheterization and microscopic examination of its urine. Bufo has the advantage of speed (two to six hours as against 18 hours), and this makes it a convenient animal if a preliminary examination is carried out before concentration methods are used.

**Seasonal Variation in Sensitivity**

Frazer and Wohlzogen (1950) state that Bufo bufo is sensitive to about 8 i.u. chorionic gonadotrophin, and that this sensitivity does not vary significantly throughout the year in animals kept in a warmed animal house. Toads used in this laboratory were not kept in a warmed room, and considerable variation in sensitivity was found, as will be seen by reference to Fig. 1, which shows a seasonal variation both in comparisons with the Friedman test and in sensitivity tests with standard chorionic gonadotrophin. The lower part of the figure gives the percentage disagreement in each month's comparisons with the Friedman. Perpendicular shading indicates the percentage results in which the toad was more reactive than the rabbit, and diagonal shading those in which the rabbit was more reactive. The complete agreement between the results of the two tests in March does not indicate a fall in the numbers of comparisons, and may possibly suggest an increased sensitivity in both test animals. Apart from that, there is little difference throughout the year in the numbers of more sensitive Friedman tests, but the more
reactive toads are strictly confined to the first six months of the year, with a peak in the breeding season in April.

The upper part of the figure gives the results of sensitivity tests done in different months on batches of seven to 19 toads. With one exception, the November results, these figures were all obtained by the same technique, the repeated injection of 2 i.u. chorionic gonadotrophin (Pregnyl-Organon) in distilled water at two-hourly intervals until a positive result was obtained, the toad then being considered sensitive to the sum total of gonadotrophin given to it. The figures for November were obtained from a series of 16 toads in which the first injection was 4 i.u. chorionic gonadotrophin, followed at four hours by a further 4 i.u. for all non-reactors, and a further 10 i.u. for all non-reactors two hours later. Only one toad out of the 16 reacted with 4 i.u., and it is assessed as requiring 4 i.u. to react. All those which took 4 + 4 i.u. are assessed as requiring 6 i.u. (some probably needed the full 8 i.u.), and the others, which were given 4+4+10 i.u., are assessed at 10 i.u. Thus, except for the one toad assessed at 4 i.u., they are all considered to require only 2 i.u. above the non-reactive dose, thus ensuring that any weighting of these figures as against those obtained by the standardized method will be on the side of increased sensitivity. In spite of this, the figures agree well with the other low-sensitivity results obtained for August and January, and the curve indicates a marked increase in sensitivity from March to July, and a nearly constant low level from August to January. This lower level, of approximately 7 i.u. chorionic gonadotrophin, agrees well enough with the finding of Frazer and Wohlzogen of a sensitivity of approximately 8 i.u., but in their experiments on toads kept in a warmed animal house this sensitivity is constant throughout the year, instead of showing the present rise to a level of between 3 and 5 i.u. from March to July.

It is to be expected that any increase in sensitivity in spring would be related either to the rise in temperature, or to the increased hours of daylight. Since the obvious difference in the present experiments from those of Frazer and Wohlzogen is the variation in temperature, and in view of the nocturnal or crepuscular habits of the toad, it seemed likely that the temperature effect was the more important, and an attempt was made to correlate this with sensitivity. Fig. 2 shows the results of the sensitivity test plotted against mean monthly temperatures, the figures for these being calculated from the mean monthly maxima and minima from the records of the Air Ministry meteorological station at Felton, Bristol. The sensitivity results have been expressed, not in i.u. gonadotrophin per toad, but in weight in grammes sensitized by 1 i.u. gonadotrophin, since results suggested an inverse relationship between sensitivity and body weight. Thus, if a 30 g. toad reacts with 5 i.u. and a 24 g. toad with 4 i.u. gonadotrophin, the reactivity figure is in both cases 6 g. per i.u. This method has also the advantage that it gives a direct measure of sensitivity, so that the peak on the graph in May corresponds to a peak in sensitivity. It will be seen that this peak sensitivity corresponds, not to the peak temperature, but to about the mid-point of the upward rise, and by the time the peak temperature is reached sensitivity has dropped to the base figure, where it remains during the period of the temperature drop throughout autumn and winter.

Experimental

Experimental attempts to correlate temperature and sensitivity give a somewhat similar picture. Fig. 3 is the record of two batches of eight toads of as nearly as possible matched weights and sensitivities, which were subjected to artificial changes in temperature by moving the cages from a cool basement to progressively warmer rooms or vice versa, the cage in the warmer room always being heavily screened to ensure that it never received more light than in the colder basement. The figure shows mean temperatures of the cages over seven-day periods, and sensitivities in g./i.u. gonadotrophin for both batches of toads. In the first period after separation both experienced a steady rise in temperature and show increased sensitivity, which is somewhat greater for batch Y, for which the temperature rise is also greater. During the next period batch X experienced a further rise in temperature, and again shows increased sensitivity, whereas Y underwent a marked fall in temperature and shows a corresponding decrease in sensitivity. During the final period both batches experienced a slight overall rise in temperature, but batch X, which starts this rise at a higher level, shows a slight decrease in sensitivity, whereas Y, with a parallel rise at a lower level, shows a marked increase. This picture agrees with that given by the natural seasonal variation in sensitivity, where the peak coincides approximately with the crossing of the 50° line on the upward temperature curve. This picture also agrees with that obtained by comparing dates for the breeding times of Bufo bufo in different parts of Europe with the mean
FIG. 2.—Sensitivity of *Bufo bufo* and temperature. X—Average sensitivity in g. per i.u. chorionic gonadotrophin. O—Mean monthly temperature at Filton.

FIG. 3.—Effect of temperature changes on sensitivity. X—Mean sensitivity of batch X in g./i.u. gonadotrophin. Mean temperature of cage X at seven-day intervals. O—Mean sensitivity of batch Y in g./i.u. gonadotrophin. O—Mean temperature of cage Y at seven-day intervals.
monthly temperatures, as given by Kendrew in *The Climates of the Continents*. In England the toad breeds at the beginning of April, corresponding to mean monthly temperature at Kew of 42.5° in March and 47.4° in April. In England, Rostand (1934) gives the breeding season as the end of March, and Kendrew (1922) gives Paris means as 43.2° for March and 50.5° for April. Malcolm Smith (1951) quotes April 6–27 for Edinburgh, for which the temperatures are 44.9° for April and 50.1° for May. Finally, Boulenger (1897–8) gives the beginning of May as the breeding time in Norway, and mean temperatures at Bergen are given as 42.1° in April and 48.9° in May. These few times correspond to approximately the same mean temperature of somewhat below 50° F. Whether this figure holds good over the whole of the animal's range is uncertain, for it extends south as far as the Mediterranean, and may inhabit regions where the mean temperature does not drop below 50° F.

Effects of light and humidity were not investigated, but the rise in temperature up to about 50° F. would appear to be the dominant factor in increasing sensitivity and determining breeding time, for the increase of light is greater in the more northerly regions of the animal's range, where breeding takes place later than further south.

**Summary**

A comparative series of 452 pregnancy tests gave a total of 205 positive results, of which 197 were detected by the Friedman and 129 by the Galli Mainini toad test.

The use of the male toad as a test animal is discussed, and curves are given showing a seasonal variation in sensitivity. An account is given of an experimental attempt to correlate sensitivity with rise in temperature.

**References**

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