The lack of increase in ambulatory activity among drug treated BALB/cJ mice during the first 4 days does not mean that these animals were not hyperactive; some of them were actually quite hyperactive in their home cages (although not as much as C57BL/10J) but 'froze' when they were put in the open field. They became hyperactive in the open field only after 5 days of treatment, which might be due to an altered response to the drug after its chronic administration or to psychological habituation to the open field caused by repeated exposure to it.

Several authors have shown that amphetamine has different effects in mice of different genotypes: upon body temperature[7], upon temperature and lethality[8], and upon measures of emotionality[9]. This experiment shows that genetic factors are also important determinants of the response to amphetamine as measured by the open field test.

The different behavioral response to amphetamine of these 2 strains of mice suggests a difference in the brain aminergic mechanisms through which amphetamine acts. It would be of value to study the comparative effects of amphetamine on brain catecholamines and serotonin in BALB/cJ and C57BL/10J mice.

Résumé. Chez des souris C57BL/10J, on constate une augmentation de l'activité locomotrice durant tous les 7 jours qui suivent leur injection avec la d-amphétamine. Chez des souris BALB/cJ, cette augmentation n'apparaît pas, sauf légèrement après le quatrième jour. Des facteurs génétiques jouent un rôle important dans la détermination de la réponse comportementale à la d-amphétamine.

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Serological Relationships of Frogs (Ranidae) and Toads (Bufonidae)

The two familiar tailless amphibians, the frogs of the family Ranidae and the toads of the family Bufonidae, are not as closely related as popularly imagined. Indeed, the horny-skinned toads are evolutionarily more allied to the small tree frogs (Hylidae) than to the true frog family (Ranidae). The fossil record suggests that the Ranidae and the Bufonidae diverged from some common ancestor in Jurrasic times, 150 million years ago, and the two groups have long since evolved along separate lines[1]. The long-standing separation of the frog and toad lineages is reflected in the near absence of common antigenic components in their serum proteins, as revealed by the study presented herein of immunoelectrophoretic patterns of representative American frogs and toads. The basic assumption is that the degree of differences in antigenic constituents is a function of the length of time two lineages have been apart[2,3].

Materials and Methods. Sera were obtained from adult individuals of the common American toad, *Bufo americanus*, from Wisconsin (USA); the Gulf Coast toad, *Bufo valliceps*, from Louisiana (USA); Fowler's toad, *Bufo fowleri*, from Louisiana (USA); and the common leopard frog, *Rana pipiens*. Because of the extensive morphological and physiological geographical variation in *Rana pipiens*[4], members of this wide-ranging species (or species complex) were obtained from 4 geographical areas: Wisconsin (USA), Vermont (USA), Louisiana (USA), and the province of Tamaulipas in Mexico. Specific antiserum against the serum of each of the 7 representative anurans was produced in adult, male, New Zealand rabbits. The immunological techniques used for comparing serum antigens and their respective rabbit antisera were immunodiffusion by the OUCHTERLONY[5]

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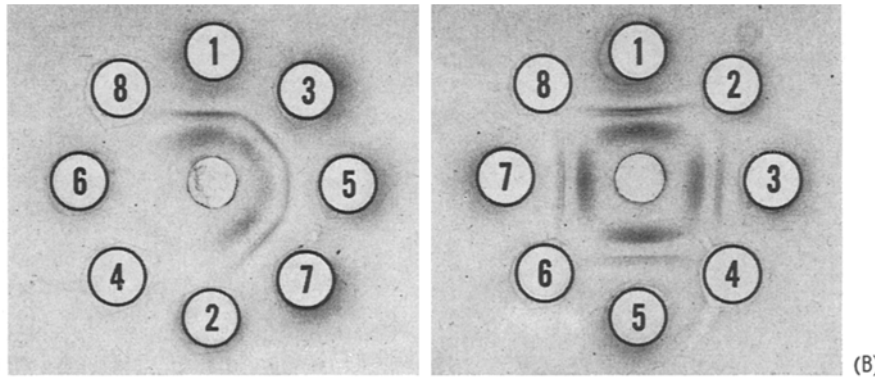

Fig. 1. Immunodiffusion patterns between frog and toad antigens and anti-*Rana pipiens* rabbit serum. Central well contains anti-*Rana pipiens* (Louisiana) rabbit serum and peripheral wells contain normal sera of 1 Louisiana *Rana pipiens;* 2 *Bufo valliceps;* 3 Vermont *Rana pipiens;* 4 *Bufo americanus;* 5 Wisconsin *Rana pipiens;* 6 *Bufo fowleri;* 7. Mexican *Rana pipiens;* and 8. human (*Homo sapiens*). Different arrangements of the 8 antigens distinguish (A) and (B).

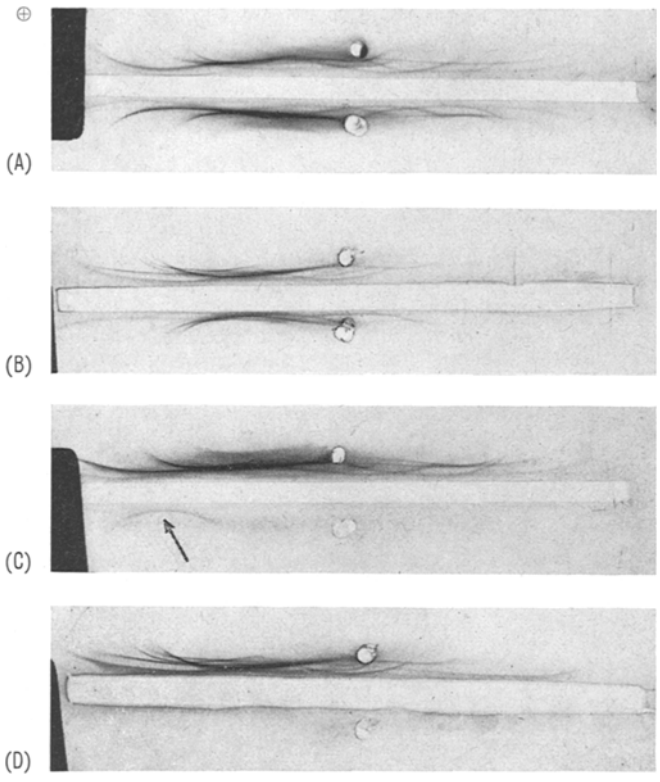

Fig. 2. Immunoelectrophoretic patterns. (A) *Rana pipiens* (Vermont) serum (top) and *Rana pipiens* (Louisiana) serum (bottom) reacting against anti-*Rana pipiens* (Vermont) rabbit serum. (B) *Bufo americanus* serum (top) and *Bufo valliceps* serum (bottom) reacting against anti-*Bufo americanus* rabbit serum. (C) *Rana pipiens* (Wisconsin) serum (top) and *Bufo americanus* serum (bottom) reacting against anti-*Rana pipiens* (Wisconsin) rabbit serum. Arrow is directed to a slight precipitate line, which suggests some cross reactivity. (D) *Bufo valliceps* serum (top) and *Rana pipiens* (Louisiana) serum (bottom) reacting against anti-*Bufo valliceps* rabbit serum.

method (employing an LKB 6800A-7 Standard Immunodiffusion Set), immunoelectrophoresis after GRABAR and WILLIAMS[6], and passive hemagglutination after BOYDEN[7] (employing Micro-titer 'U' plates).

Results. A typical outcome of immunodiffusion is shown in Figure 1, in which anti-*Rana pipiens* rabbit serum was placed in the center well of the agar template and 8 test antigens were placed in the peripheral wells. The test antigens were the normal sera of the 7 anuran representatives and normal human serum. The 8 antigens were arranged in 2 different fashions, as illustrated by Figures 1A and 1B. When, as in Figure 1A, the antigens of the 4 *Rana pipiens* representatives were placed in wells adjacent to each other, their bands of precipitate fused. The converging arcs reveal the so-called 'reaction of identity', indicating that the 4 geographical forms of *Rana pipiens* share antigenic determinants. There is no discernible cross reactivity between anti-*Rana pipiens* rabbit serum and the antigens of *Bufo valliceps*, *Bufo fowleri*, or, of course, man. A slight precipitate line with *Bufo americanus* normal serum is suggestive of some cross reactivity with anti-*Rana pipiens* rabbit serum. The limited, if any, cross reactivity between a ranid and bufonid is evident when, as in Figure 1B, the frog and toad antigens are placed in such a manner that a well containing a particular frog antigen alternates with a well containing a particular toad antigen. In this case, the precipitate lines of the 4 frog groups do not fuse because the adjacent toad samples do not contain antigenic determinants in common. The results were invariably the same when the central well contained anti-*Rana pipiens* rabbit serum from the 4 different sources (Vermont, Wisconsin, Louisiana, or Mexico anti-*Rana pipiens* rabbit serum).

The above findings were substantiated in the second set of immunodiffusion experiments in which the central well contained anti-*Bufo* rabbit sera, either anti-*Bufo americanus*, anti-*Bufo fowleri*, or anti-*Bufo valliceps*. The fusion of bands of precipitate, indicative of likeness of antigens, occurred only when the antigens of the 3 toad groups were placed in adjacent wells. When the antigens of the frog and toad groups were in alternate wells, there was no indication of a cross reaction between the antigens of any of the frog representatives and any of the anti-*Bufo* rabbit sera.

The results of immunodiffusion were somewhat surprising, since the expectation was that the 2 anuran groups being compared (frogs and toads) would possess some common antigenic determinants. Nevertheless, the outcome of immunoelectrophoresis experiments also revealed a vast disparity in the serological patterns of the frogs and toads. Results that typify this phase of the study are shown in Figure 2. In Figure 2A, the trough was filled with anti-*Rana pipiens* (Vermont) rabbit serum, and the 2 circular wells contained normal sera of Vermont frogs (upper well) and Louisiana frogs (lower well). Numerous arcs reveal the great complexity in the protein composition of the normal serum of *Rana pipiens*. No differences in the reactivity of Vermont and Louisiana normal sera could be detected. For that matter, none of the 4 geographical forms of *Rana pipiens* could be distinguished by their immunoelectrophoretic patterns. Figure 2B represents the reactions of anti-*Bufo americanus* rabbit serum against the antigens of *Bufo americanus* and *Bufo valliceps*. The pattern of *Bufo americanus* normal serum is representative of all 3 groups of toad species tested. The immunoelectrophoretic pattern of the toad apparently is less complex than that of the leopard frog; a noteworthy feature is the fewer bands in the globulin regions of the toad serum. Figure 2C shows the

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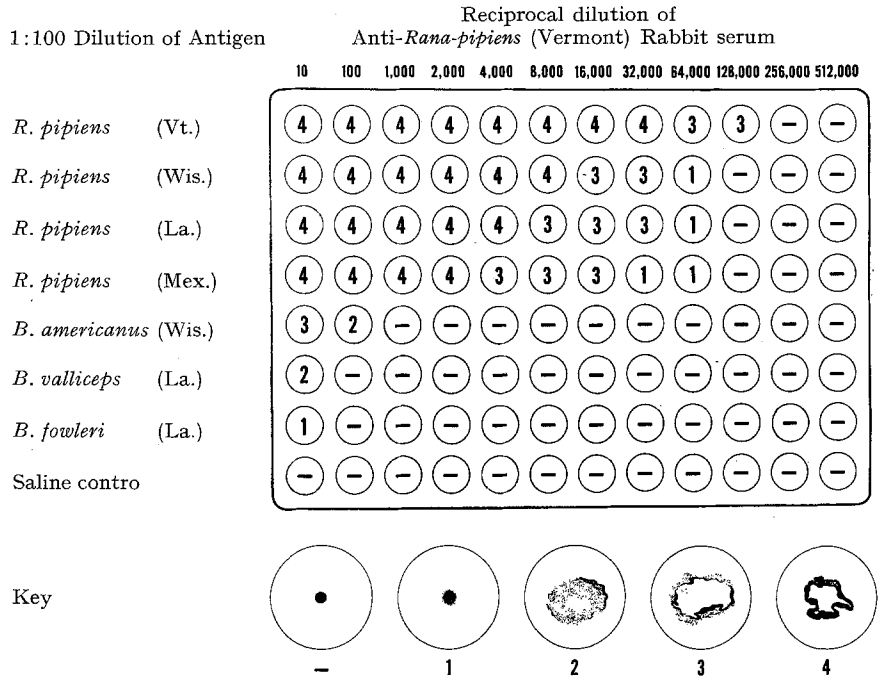

Fig. 3. Results of passive hemagglutination test using anti-*Rana pipiens* (Vermont) rabbit serum and antigens of various frogs and toads at 1:100 dilution. Results are expressed in terms of numerals which reflect the degree of alteration of the button pattern of settled cells.

response of anti-*Rana pipiens* (Wisconsin) rabbit serum to its own antigens and to those of *Bufo americanus*. Little cross reactions are evident; at best, only 1 band is shared between *Bufo americanus* and *Rana pipiens* normal sera. As seen in Figure 2D, *Rana pipiens* (Louisiana) antigens fail to react with anti-*Bufo valliceps* rabbit serum. In fact, a complete lack of response of *Rana pipiens* antigens was seen also when their antigens were exposed to anti-*Bufo fowleri* rabbit serum.

The passive hemagglutination test provides a sensitive index of the antibody content of sera, as well as furnishes a good gauge of antigen similarities and dissimilarities. Figure 3 shows representative results of the reactions of frog and toad antigens (at dilution 1:100) against anti-*Rana pipiens* (Vermont) rabbit serum. The end point was the highest dilution of rabbit antisera that caused a conspicuous alteration of the button pattern of settled unagglutinated cells (value of 2 or higher; see 'key' in Figure 3). The strongest reaction, as expected, was between normal *Rana pipiens* (Vermont) serum and anti-*Rana pipiens* (Vermont) rabbit serum (end point = serum dilution of 1:128,000). Titer values were lower for the other geographical forms of *Rana pipiens*, with Mexican antigens (end point = 1:16,000) being lower than either Wisconsin or Louisiana (end point = 1:32,000). There are, accordingly, subtle differences among the serum antigens of the 4 geographical forms of *Rana pipiens*. The same kinds of minor differences were found at various dilutions of frog antigens (1:50, 1:200, and 1:400). Once again, we direct attention (Figure 3) to the exceedingly little cross reactivity between *Rana* and *Bufo* species. Positive reactions (value of 2) were obtained only at such extraordinarily low titers as 1:100 (*Bufo americanus*) and 1:10 (*Bufo valliceps*).

A comparable picture emerged when frog and toad antigens were exposed to anti-*Bufo americanus* rabbit serum. Strong reactions were observed only when this toad antiserum was tested against its own specific antigen or related antigens (*Bufo fowleri* and *Bufo valliceps*). This sensitive test revealed slight differences in the serum antigens of the 3 toad species, but there were no indications that *Bufo* and *Rana* have many antigens in common.

Conclusions. GRIFFITHS[8] has stated that the tailless amphibians (Anura) '...are the most difficult order in which to analyse phyletic trends...'. Based on osteological characters, anuran phylogeny is traceable along 2 distinct lines: families with the more primitive 'arciferal' type of pectoral girdle, and families with the more advanced 'firmisternal' type of shoulder girdle[9]. The Bufonidae fall into the former group, while the Ranidae belong to the latter category. Our study reveals a striking dearth of common antigenic determinants in the serum proteins of ranids and bufonids. Our data suggest an early separation of the two lineages as well as appreciable genetic divergence during the period of separate existence.

Zusammenfassung. Das Ergebnis der immunoelektrophoretischen Untersuchungen zeigt einen gewaltigen Unterschied in den serologischen Mustern der Frösche (Ranidae) und Kröten (Bufonidae). Die amerikanischen Vertreter der Ranidae und der Bufonidae haben sehr wenig gemeinsame Serumantigene. Der Mangel an gemeinsamen antigenischen Bestandteilen in ihren Serumproteinen weist sowohl auf eine frühe Evolutionstrennung des Frosches und der Kröte wie auch auf ein merkliches genetisches Auseinandergehen der zwei Stämme während der langen Zeitspanne isolierter Existenz hin.

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