

after third exposure (Table). BANNIKOVA and PYATNITSYNA<sup>4</sup> reported an increase in acid production by UV-irradiation in *S. lactis*, while KUILA et al.<sup>6</sup> observed decrease in acid-producing ability in a strain of *S. diacetilactis* by the same method of treatment. The nisin-producing character thus appears to be independent of acid-producing ability of the culture.

*Streptococcus lactis*-6 exhibited a higher percentage of survival namely, 5.2% after third exposure as against 0.17 and 0.26% after first and second exposures respectively, thereby indicating development of higher percentage of resistant population in the culture although the dosage was kept constant.

**Zusammenfassung.** Isolierung von Mutanten mit erhöhter Nisin-Produktion aus einem weiteren *Streptococcus lactis*-6 Stamm durch UV-Bestrahlung (9000 erg/mm<sup>2</sup>). Die Produktion des Antibiotikums nahm nach der ersten Bestrahlung um 50%, nach der zweiten Bestrahlung um 100% zu, während nach der dritten eine Abnahme beobachtet wurde.

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## Effects of d-Amphetamine upon Open Field Behaviour in Two inbred Strains of Mice

BALB/cJ mice are regarded as more emotional than C57BL/10J mice because they defecate more and ambulate less in the open field<sup>1,2</sup>. They also have a higher concentration of serotonin and norepinephrine in brain stem and lower concentration of norepinephrine in the hippocampus and the pyriform cortex as compared with C57BL/10J<sup>3-5</sup>.

In order to elucidate some of the relationships between these differences in brain chemistry and the behavioral differences of these two strains of mice it would be of value to test the effects upon behavior of a drug such as amphetamine that acts upon release and uptake of brain monoamines (c.f. COSTA and GARATTINI<sup>6</sup>).

This paper reports differences between BALB/cJ and C57BL/10J mice in their response to d-amphetamine as measured by the open field test.

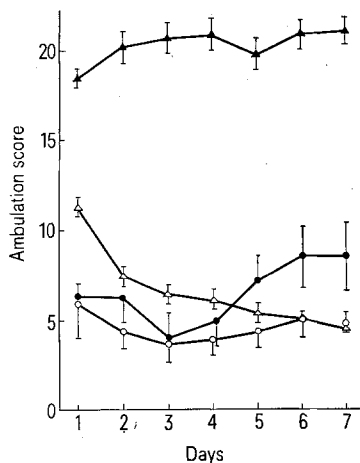
**Methods.** Male mice of the strains BALB/cJ and C57BL/10J were received from Jackson Laboratory at weaning age and housed 5 to a cage for 3 to 4 weeks to acclimate. Treatment was started at the age of 40 to 50 days. The open field used was a square surface 24 × 24 inch surrounded by walls 8 inch high; the walls and floors are white, and the floor is divided into 36 squares by thin lines. 12 BALB/cJ and 14 C57BL/10J mice were given an i.p. injection of 5 mg/kg of d-amphetamine

sulfate (in 0.1 ml 0.9% NaCl). The controls (15 BALB/cJ and 13 C57BL/10J) received an injection of the same volume of saline solution. Each animal was tested individually 15 min after injection; mice were placed into one corner of the open field and allowed to explore it for 3 min, and the number of lines crossed during that time was recorded as the ambulation score. The open field was wiped clean with a wet sponge and paper towel after testing each mouse. All tests were done between 09.30 h and 12.30 h. This whole procedure was repeated daily for each mouse on each of 7 consecutive days. Ambulatory activity was expressed as the square root of the number of lines crossed during a 3 min period.

**Results.** C57BL/10J mice ambulated more than BALB/cJ mice on the first 4 days (Figure), in agreement with results of other authors<sup>1,3</sup>. The difference was largest on day 1 and became gradually smaller until there was no significant difference in ambulation between the controls of the 2 strains on days 5 to 7. This was due to the fact that C57BL/10J mice became habituated to the open field and explored less after the first day while BALB/cJ did not.

Amphetamine caused a highly significant increase (between 300 and 1000%) of ambulatory activity of C57BL/10J mice with respect to C57BL/10J controls on all 7 days ( $p < 0.001$ ); the rate of locomotion remained constant throughout the 7 days of treatment, and there was no sign of habituation to the novel environment. Amphetamine treated BALB/cJ mice did not show greater ambulatory activity during days 1 to 4 than their controls, but there was a moderate increase in their ambulation (between 200 and 300%,  $p < 0.05$ ) during days 5, 6, and 7.

**Discussion.** The increase in ambulation induced by d-amphetamine in C57BL/10J mice seems to reflect locomotor hyperactivity rather than an increase in exploration, because the animals were about as hyperactive in their home cages as in the open field and because there was no drop in activity, no habituation to the open field, throughout the seven days of testing.



Ambulation in the open field of male BALB/cJ and C57BL/10J mice after an injection of d-amphetamine (5 mg/kg). Data are square roots of the numbers of squares crossed during 3 min. Vertical bars represent standard errors of the mean. BALB/cJ: amphetamine ●; saline ○. C57BL/10J: amphetamine ▲; saline △.

<sup>1</sup> N. D. HENDERSON, *Anim. Behav.* 15, 364 (1967).

<sup>2</sup> J. C. DEFRIES and J. P. HEGMANN, in *Contributions to Behavior-Genetic Analysis-The Mouse as a Prototype* (Eds. G. LINDZEY and D. D. THIESSEN; Appleton-Century-Crofts, New York 1970), p. 23.

<sup>3</sup> J. W. MAAS, *Science* 137, 621 (1962).

<sup>4</sup> J. W. MAAS, *Nature*, Lond. 197, 255 (1963).

<sup>5</sup> H. S. SUDAK and J. W. MAAS, *Nature*, Lond. 203, 1254 (1964).

<sup>6</sup> E. COSTA and S. GARATTINI, *International Symposium on Amphetamines and Related Compounds* (Raven Press, New York 1970).

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<sup>7</sup>, upon temperature and lethality<sup>8</sup>, and upon measures of emotionality<sup>9</sup>. 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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<sup>7</sup> J. P. SCOTT, C. LEE and J. E. HO, *J. comp. Physiol. Psych.* 76, 349 (1971).

<sup>8</sup> E. DOLFINI, S. GARATTINI and L. VALZELLI, *Eur. J. Pharmac.* 7, 220 (1969).

<sup>9</sup> K. P. SATINDER, J. R. ROYCE and L. T. YEUDALL, *J. comp. Physiol. Psych.* 71, 443 (1970).

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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 Jurassic times, 150 million years ago, and the two groups have long since evolved along separate lines<sup>1</sup>. 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<sup>2,3</sup>.

**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*<sup>4</sup>, 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<sup>5</sup>

<sup>1</sup> M. K. HECHT, *Syst. Zool.* 12, 20 (1963).

<sup>2</sup> S. N. SALTHER and N. O. KAPLAN, *Evolution* 20, 603 (1966).

<sup>3</sup> H. C. DESSAUER and W. FOX, *Science* 124, 225 (1965).

<sup>4</sup> J. S. MECHAM, *J. exp. Zool.* 170, 169 (1970).

<sup>5</sup> O. OUCHTERLONY, in: J. F. ACKROYD, *Immunological Methods* (Blackwell Scientific Publications, Oxford 1964), p. 55.

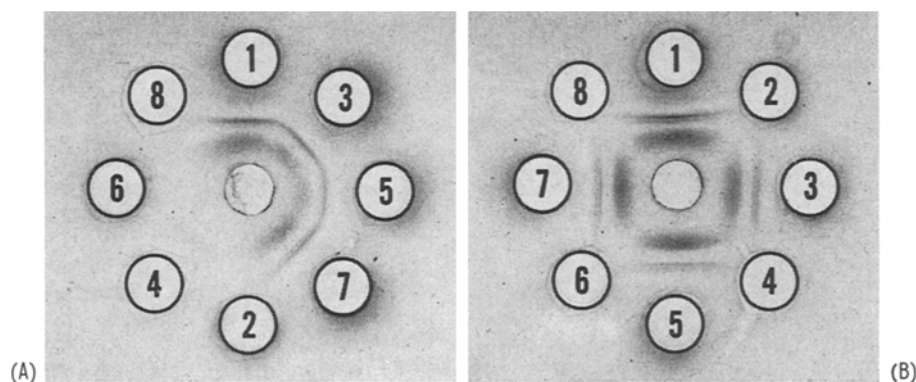


Fig. 1. Immunodiffusion patterns between frog and toad antigens and anti-*Rana pipiens* (Louisiana) rabbit serum. Central well contains anti-*Rana pipiens* (Louisiana) rabbit serum and peripheral wells contain normal sera of 1 Louisiana *Rana pipiens*; 2 *Bufo valliceps*; 3 Vermont *Rana pipiens*; 4 *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).