even after repeated electrophoresis of the same sample. Freeze-thawing or storage of the serum over long periods did not alter the patterns as reported in some other cases[5].

CHEN[6], in a comparative study of electrophoretic pattern of serum proteins of several amphibians, found two serum albumin bands in a female *R. esculenta*. He analyzed only 1 frog serum and no further work was undertaken to confirm polymorphism of this protein in this species. Some other reports on serum proteins of frogs always found a single albumin band, whereas transferrin was found to be polymorphic in the same species[2,7]. The constancy of the specific pattern of bands in several runs of the same sample, even after long storage and freeze-thawing, and the finding of a given pattern in number of organisms, strongly indicates that we are not dealing with an artifact produced during serum processing or electrophoresis. However, the patterns are too complicated to give an easy interpretation depending on the phenotype alone. Further genetic and biochemical studies are needed to elucidate the nature of these bands. It is probable that there are several alleles producing serum albumin in these frogs which differ only slightly in electrophoretic location, and since it is well established that albumin has no quarternary structure, the multiple bands specific to each pattern are probably due to the binding of the smaller molecular substances to the different allelic products. It would also be interesting to see if the frogs of this species from other locations have the same pattern of albumin bands or whether these patterns are influenced by local environmental conditions.

Zusammenfassung. Elektrophoretische Untersuchungen der Serumproteine von *Rana tigrina* ergaben verschiedene Bandenmuster des Albumins. Die genetisch-biochemische Bedeutung dieser multiplen Banden wird diskutiert.

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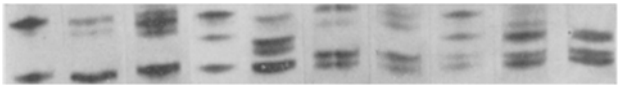

Fig. 2. Disc electrophoretic patterns of serum albumin bands of *R. tigrina*. 3 µl serum sample was applied in each case. The variation in the stain intensity, however, is due to variable protein content in the sera of these frogs.

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Adenosine Deaminase Activity in Livers of Congenitally Athymic Nude Mice

Congenital defects of immunity in man are divided into 3 groups in which the causal factors are different. These are pure defects of T-lymphocyte function, abnormalities of immunoglobulin synthesis and a combined defect of both cellular and humoral immunity. A severe form of combined immunodeficiency in man recently has been associated with a deficiency of adenosine deaminase (ADA), EC 3.5.4.4 in all tissues studied, including red cells, lymphocytes, liver, and fibroblast cultures[1-3]. This enzyme functions in the catabolism of nucleic acids by 'salvaging' adenosine through deaminating it to inosine. In the human, various 'tissue specific' isozymes of ADA have been described. The red cell enzyme is polymorphic and is also the major isozyme in lymphocytes. HIRSCHHORN and BERATIS[4] have presented evidence that ADA is the product of a single genetic locus and the various tissue isozymes are the result of post-translational modifications of ADA. These alterations in ADA are probably due to tissue specific conversion factors[5].

Nude mice are homozygous for a mutation in the 7th linkage group and are characterized by deficient thymic development and a cellular and humoral immunodeficiency[6,7]. The association between ADA deficiency and combined immunodeficiency syndrome in man prompted a study of ADA activity in individual livers of immunodeficient nude mice and their normal littermates.

Materials and methods. Outbred nudes and their normal littermates were used throughout. These mice were the progeny of matings between heterozygous parents, one of which was partially backcrossed to strain C57/Bl, and the other, Balb/c. Dissected livers from 7 nude and 4 normal mice, age 5 to 7 weeks, were weighed and homogenized in a 0.25 *M* sucrose with 0.01 *M* $MgCl_2$ solution. The homogenate was centrifuged at 13,000 *g* for 20 min in a refrigerated centrifuge. The supernatants were stored at −20 °C and aliquots were taken for

Mean deaminase activity of liver extracts

Mice[a]	µmoles NH_3/mg protein	µmoles NH_3/g liver
Normal (4)	0.542 ± 0.096	1.440 ± 0.167
'Nude' (7)	0.453 ± 0.061	1.516 ± 0.397
t	1.702	0.327
P	0.13	0.75

[a] These mice were the progeny of matings between heterozygous parents, 1 of which was partially backcrossed to strain C57/Bl, and the other, Balb/c. Ammonia evolved from adenosine during the incubation with liver extracts was determined according to the method of SELIGSON and SELIGSON[8], as described in the text under Materials and Methods. Adenosine deaminase activity is expressed in µmoles ammonia released in 30 min at 37 °C.

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protein and enzyme determinations. 0.1 ml of a stock adenosine solution (50 µmoles adenosine/ml H_2O), 0.15 ml of a 0.1 *M* potassium phosphate buffer pH 7.4, and 0.1 ml of H_2O were incubated for 30 min at 37 °C with 0.3 ml of the supernate. Ammonia released from adenosine by deamination was determined by the method of SELIGSON and SELIGSON[8]. Protein concentrations in the supernates ranged from 12.5 to 18.9 mg/ml[9].

Results and discussion. As shown in the Table, easily measurable levels of ADA activity were found in each individual liver extract. No significant differences in this enzyme activity were found between genotypes of animals of ages less than 2 months. These findings indicate that the immunological impairment in nude mice is not associated with ADA deficiency and is not a model for this form of human combined immunological deficiency disease. Gross examination of the liver samples showed no noticeable abnormalities. Microscopy performed on the livers taken from nudes of the same age raised under identical conditions did not show necrotic foci with granulocytic infiltration that have been observed in livers of older animals. It is noteworthy that PANTELOURIS and MACMENAMIN[10] have reported an increase in L-tryosine and L-glutamine acid decarboxylase activity in the livers of nude mice greater than 2 months of age[10]. However, adult nude mice have a high incidence of liver disease and the reported enzyme differences in older mice may be due to hepatic pathology rather than related directly to the mutation[7]. In any case, mice with a selective deficiency in cellular and humoral immunity have ADA activity that is similar to their immunologically intact littermates.

Résumé. Une forme de maladie humaine d'immuno-insuffisance grave combinée est associée à une insuffisance d'adénosine-déaminase dans les cellules de lymphe et dans d'autres tissus comprenant le foie, la rate et les érythrocytes des cultures de fibroblastes de la peau. Un modèle animal d'immuno-insuffisance combinée a été récememt créé dans une race de souris sans poils. Nous avons montré que chez ces souris immunologiquement déficientes l'activité de l'adénosine-déaminase est normale.

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Inter-Specific Relationships of *Leiopelma* (Amphibia: Anura): Further Karyological Evidence

The genus *Leiopelma*, because of its long period of geographical isolation and its retention of a relatively large number of primitive anatomical and morphological characteristics, is of general biological interest and evolutionary importance. Among the many questions surrounding the genus are those concerning its degree of affinity with the North American amphicoelous frogs of the genus *Ascaphus* Stejneger and the inter-specific relationships within the genus *Leiopelma* itself. The present karyological investigation was undertaken in the hope that it would assist in taxonomic clarification.

Arm ratios, centromere indices and relative lengths of chromosomes of *L. hamiltoni*

Pair No.	Arm ratios[a]	Centromere indices[a]	Relative lengths[a]
1	1.18 ± 0.05	45.86 ± 1.14	1.00
2	2.12 ± 0.18	31.74 ± 0.37	0.87 ± 0.02
3	1.13 ± 0.02	46.86 ± 0.58	0.89 ± 0.02
4	1.16 ± 0.03	46.41 ± 0.53	0.81 ± 0.02
5	2.81 ± 0.08	26.37 ± 0.54	0.69 ± 0.02
6	1.22 ± 0.04	45.00 ± 0.75	0.50 ± 0.01
7	1.19 ± 0.02	45.72 ± 0.41	0.43 ± 0.01
8	1.03 ± 0.02	49.20 ± 0.34	0.39 ± 0.01
9			0.29 ± 0.01

[a] Mean ± standard error. Figures are based on measurements from 10 metaphase spreads. Lengths have been calculated in relation to the length of the longest pair. The chromosomes are shown in the same order as in Figure 2.

MORESCALCHI[1] first described the chromosomes of *Leiopelma hochstetteri* Fitzinger, the most widely distributed species of the sole endemic New Zealand genus of frogs, from 2 female specimens. Both of these had 5 pairs of metacentric or submetacentric chromosomes and 6 smaller pairs of acrocentrics but, in addition to this basic complement of 22, the karyotype of 1 specimen included 12 microchromosomes while the other had only one. Later, the present authors[2] demonstrated that the microchromosomes of *L. hochstetteri* appear to function as supernumeraries and that they show considerable individual variation in number and may even be absent.

The karyotype of a second species, *L. archeyi* Turbott, was concurrently described[2] from 2 female specimens. The total chromosome number was 18 and only the smallest pair was acrocentric. No microchromosomes were present.

Until now, the karyotype of the third known species, *L. hamiltoni* MCCULLOCH, has not been described. The occurrence of *L. hamiltoni* was originally reported from Stephens Island in Cook Strait[3] where the frog has an extremely restricted distribution[4]. In 1957, another population of animals resembling *L. hamiltoni* was discovered on Maud Island in Pelorus Sound[5] and the

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