yields of aflatoxin, although mycelial weight was slightly less (Table III). Palmitic acid supported as much aflatoxin yield as that of control, although mycelial dry weight was reduced to half. With glutaric, lauric, myristic, stearic, oleic and behenic acids, aflatoxin yields were low. In almost all the cases, the amount of aflatoxin in the medium was higher than that in the mycelium. Even in the presence of sucrose in SLS medium, there was no growth or toxin production with acetic, propionic, butyric, caproic, heptoic, caprylic, nonoic, capric and linoleic acids. It may be noted that in the case of sebacic, myristic, palmitic, oleic and behenic acids, aflatoxin production is higher than the sum of the aflatoxin yields obtained on media containing sucrose and the carboxylic acid alone at a carbon level of 8.95 g/l.

Most of the carboxylic acids containing 2 to 10 carbon atoms, as well as linoleic acid, appear to be quite inhibitory. The fungistatic effects of acetic and propionic acids are well known, though their mode of action is not clear. In general, the effects observed with the higher carboxylic acids on aflatoxin production are dependent on the nature and concentrations of these acids. The fatty acids formed by the breakdown of the lipids present in natural substrates thus appear to have a significant influence on aflatoxin production by *Aspergillus*[6].

Résumé. On étudie l'effet de divers acides carboxyliques sur la production de l'aflatoxine en culture agitée d'*Aspergillus parasiticus* en milieu synthétique (SLS). Lorsque le saccharose correspondant à 35.8 g C/l de milieu SLS a été complètement remplacé par des acides gras à équivalence de C, seul l'acide laurique manifeste une croissance satisfaisante avec production d'aflatoxine. Quand le taux en C du milieu a été réduit à 8.95 C/l, les acides sébacique et laurique ont produit plus d'aflatoxine que le saccharose. Ajoutés en supplément à un taux peu élevé de saccharose dans le milieu SLS, les acides sébacique et palmitique ont eu un rendement élevé en aflatoxine.

S. R. GUPTA, H. R. PRASANNA, L. VISWANATHAN[7] and T. A. VENKITASUBRAMANIAN

Department of Biochemistry, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-7 (India),
29 March 1974.

[6] Acknowledgments: This investigation was supported by a PL-480 grant No. FG-In-438.
[7] Present address: National Sugar Institute, Kanpur, India.

Multiple Electrophoretic Bands of Serum Albumin in *Rana tigrina*

Albumin is a major serum protein in all vertebrates with the fastest anodal mobility in electrophoresis. It is believed to have no quaternary structure and in electrophoretic patterns always appears as a single major band. Two such bands appear in a heterozygote. Although genetic variants are known, variation is rare compared to some other polymorphic serum proteins like transferrin[1]. Phylogenetically also serum albumin is conservative in its electrophoretic location[2]. There have been relatively very few problems with non-genetic variation of this protein, although it has the ability to bind a variety of ions, lipids, drugs and hormones. It was thus surprizing that, during our studies on serum proteins of *Rana tigrina*, we have come across an extensive variation in both the location and number of electrophoretic bands of serum albumin. The possible nature of this variation is discussed.

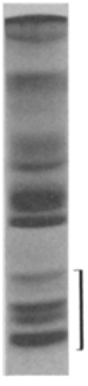

Fig. 1. Disc electrophoretic pattern of serum proteins of *R. tigrina*. The bands in bracket are albumins as identified by their mobility and binding to Evans Blue and hemoglobin.

Frogs (*Rana tigrina*) were caught from ponds and wells in and around the Warangal City, India. Blood was collected by puncturing the aorta close to the heart after mild etherisation of the animal. Serum was separated from the clotted blood the next day and electrophoresis carried out on the fresh sample. A simplified disc electrophoretic technique[3] with 5% acrylamide gel and *tris* buffer of pH 8.1 was used. 3 µl serum sample mixed in 1 ml of 20% sucrose was directly introduced on separation gel. 2.5 mA current per tube was allowed for the first 5 min and then the current raised to 5 mA and the run continued for 20 min. After electrophoresis, gels were stained with 1% amido-black for 30 min and the same were destained with 7% acetic acid overnight. For binding studies graded amounts of Evans Blue and Bromphenol Blue dyes were added to the serum samples and then electrophoresis was carried out. Only Evans Blue stained the albumin bands. Similarly freshly prepared hemoglobin solution was added to the sera and electrophoresis carried out. After electrophoresis, gels were stained with benzidine-peroxide solution[4]. Green bands corresponding to the albumin bands developed in 30 sec.

Figure 1 shows the disc-electrophoretic pattern of the serum proteins of *R. tigrina*. The fast moving components have been identified as albumin bands by their specific bonding with Evans Blue and hemoglobin and also by their electrophoretic behaviour. The 70 frogs examined so far have all shown this multiple banding. Depending on the number and relative positions, 10 patterns can be recognized (Figure 2). Identical patterns were obtained

[1] H. E. SCHULTZE and J. F. HEREMANS, *Molecular Biology of Human Proteins* (Elsevier, Amsterdam 1966).
[2] H. C. DESSAUER, W. FOX and Q. L. HARTWIG, Comp. Biochem. Physiol. *5*, 17 (1962).
[3] J. T. CLARKE, Ann. N.Y. Acad. Sci. *121*, 428 (1964).
[4] O. SMITHIES, Adv. Protein Chem. *14*, 65 (1969).

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.

P. R. RAO and V. LAKSHMIPATI

Department of Zoology, Osmania University Post-Graduate Centre, Warangal-506001 (A.P., India), 13 February 1974.

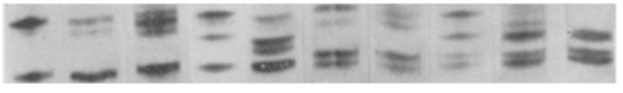

Fig. 2. Disc electrophoretic patterns of serum albumin bands of *R. tigrina*. 3 µl serum sample was applied in each case. The variation in the stain intensity, however, is due to variable protein content in the sera of these frogs.

[5] G. C. ASHTON, Genetics *50*, 1421 (1964).
[6] P. S. CHEN, Experientia *23*, 483 (1967).
[7] H. C. DESSAUER and E. NEVO, Biochem. Genet. *3*, 171 (1969).

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.

[1] E. R. GIBLETT, J. E. ANDERSON, F. COHEN, B. POLLARA and H. J. MEUWISSEN, Lancet *2*, 1067 (1972).
[2] B. B. KNUDSEN and J. DISSING, Clin. Genet. *4*, 344 (1973).
[3] S. H. CHEN, C. R. SCOTT and E. R. GIBLETT, Am. J. hum. Genet. *26*, 103 (1974).
[4] R. HIRSCHHORN and N. G. BERATIS, Lancet *2*, 1217 (1973).
[5] H. NISHIHARA, S. ISHIKAWA, K. SHINKAI and H. AKEDO, Biochim. biophys. Acta *302*, 492 (1973).
[6] B. A. CROY and D. OSOBA, Cell. Immun. *9*, 306 (1973).
[7] S. P. FLANAGAN, Genet. Res. *8*, 295 (1966).