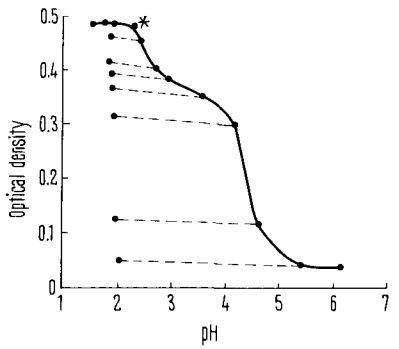
niques described by HARBISON, BOERTH, and SPRATT[14] with modification by LAGE and SPRATT[15]. A diazotized conjugate sample and a sample of diazotized non-conjugated bilirubin were placed in separate solutions containing 95% ethanol, *Tris* buffer, pH 8.0 at 25 °C, and distilled water. This procedure was performed in duplicate each time. Each of a pair of non-conjugated and conjugate samples were placed in a dark area, away from all light. The other sample of each pair was placed on a flash evaporator, without vacuum, in a hood exposed to three 30 watt fluorescent bulbs (General Electric 'Cool White' F30T8) about 2.5 feet from the sample. Samples were rotated under such conditions for 5–6 h, since this approximated the time necessary for the extraction and chromatographic separation of samples. All solutions were then read at 535 nm.

Data obtained from exposure of non-conjugated and conjugated diazotized bilirubin to artificial light is expressed as a percentage of the optical density of the sample kept in the dark (mean ± standard error and n = number of trials). For non-conjugated bilirubin this was 100.1 ± 1.2 (n = 3) while the conjugated bilirubin has a mean of 98.8 ± 2.2 (n = 4).



Effects of acid and base on diazotized bilirubin. Data shown as the mean for 3 experiments. All pH readings were within 0.03 pH units (S.E.) for the reported values. All optical density readings were within 0.015 units (S.E.) for the reported values. The control sample (designated by an asterisk, *) had an O.D. of 0.48 at pH 2.3. The solid line to the left of this control value represents results from the addition of HCl. The solid line to the right represents addition of NaOH. The dashed lines represent results from the addition of HCl in an attempt to reverse the possible indicator role of the diazotized bilirubin.

These experiments showed that there was little difference between the results from exposure to light and total absence of light. This means that samples, once they are diazotized, may be extracted and chromatographed in a room with normal lighting. Undiazotized bilirubin should still be protected from light, as when it is put into solution or before the diazo-reagent and ethanol are added since OSTROW[16] has studied factors that affect the photo-decomposition of bilirubin and biliverdin and found that biliverdin formation is the first step during photodecay of bilirubin.

In conclusion, the effects of normal room lighting and hydrogen-ion concentration on diazotized bilirubin were investigated. The room light did not affect the spectrophotometric determination of diazotized bilirubin whereas pH had a marked influence on the optical density readings[17].

Zusammenfassung. Die Wirkung normaler Raumbelichtung (elektrisches fluoreszierendes Licht) und diejenige der Wasserstoffionen-Konzentration auf diazotiertes Bilirubin wurde untersucht. Während elektrisches Licht bei den gegebenen Versuchsbedingungen zu keiner Änderung der optischen Dichte führte, bewirkte ein Zusatz von Natronlauge eine Verminderung der optischen Dichte und zwar unbeeinflusst durch den Zusatz von HCl.

R. F. POTREPKA and J. L. SPRATT

Department of Pharmacology,
College of Medicine, University of Iowa,
Iowa City (Iowa 52240, USA), 4 August 1969.

[14] R. D. HARBISON, R. C. BOERTH and J. L. SPRATT, Biochem. J. *104*, 46C (1967).
[15] G. L. LAGE and J. L. SPRATT, Archs. Biochem. Biophys. *126*, 175 (1968).
[16] J. D. OSTROW, Fedn. Proc. *28*, 336 (1969).
[17] This study was supported by U.S. Public Health Service Research Grant No. NB-04925 and Training Grant No. 5T1-GM-141.

Regulation of Glutamate Pyruvate Transaminase in the Amphibian (*Rana hexadactyla*) Kidney and Liver Homogenates

That the levels of activities of enzymes in heterogeneous homogenate are under the influence of protein and amino acid charges has been suggested by several investigations in the amphibian tissues[1-3]. The addition of positively charged proteins and amino acids elevated the levels of activity of succinate, lactate and glutamate dehydrogenases and decreased that of proteases[2]. The addition of negatively charged proteins and amino acids induced an opposite effect. These observations indicate that the subcellular charges in the environment play an important role in the regulation of enzyme activity, either by activating or inactivating the enzyme systems. The present investigation deals with the possible regulation of glutamate pyruvate transaminase (GTP; EC 2.6.1.2) by protein and amino acid charges in the amphibian kidney and liver homogenates.

Rana hexadactyla were double pithed and the kidneys and the liver were quickly excised. They were washed in the amphibian Ringer's medium[4] for 10 min. The tissues were homogenized in 0.25 M sucrose (wt./vol.) and the supernatants were used for the GPT enzyme assay by

[1] S. GOVINDAPPA, Doctoral Dissertation, S. V. University, Tirupati (1967).
[2] P. MURALIKRISHNADASS, Doctoral Dissertation, S. V. University, Tirupati (1967).
[3] A. SUBBARAMI REDDI, Doctoral Dissertation, S. V. University, Tirupati (1969).
[4] G. M. CAVANAUGH, *Formulae and Methods IV* (Marine Biological Laboratory, Woods Hole, Mass. 1956), p. 56.

the colorimetric method given by BERGMEYER[5]. The experimental tubes contained 0.25 mg of γ-globulins (Nutritional Biochemicals Corporation, Cleveland, Ohio), bovine albumins (L. Light and Co. Ltd., Colnbrook, England), glutamic acid (B.D.H.) and lysine (B.D.H.).

The enzyme activity was higher in the homogenates of kidney than in the liver. In general, the kidney enzyme activity was elevated by the γ-globulins, albumins and glutamic acid and inhibited by lysine. But the elevation by the γ-globulins and the inhibition by lysine were not statistically significant. Albumins and glutamic acid significantly elevated the enzyme activity. The liver enzyme activity was elevated by all the proteins and amino acids added, the elevation by the γ-globulins and lysine was significant, whereas the effect of albumins and glutamic acid was non-significant. The results show that the regulation of GPT is different in both the tissues. In other words, the relatively negative charge density contributed by the albumins and glutamic acid favours the kidney enzyme, while it has no action on the liver enzyme. Conversely, the relatively positive charge density in the homogenate contributed by the γ-globulins and lysine accelerates the liver enzyme, while it has no effect on the kidney enzyme. It is therefore suggested that the differential regulation of the same enzyme by different charges might be due to organ-specificity[6].

Glutamate pyruvate transaminase activity in the sucrose homogenates of the amphibian kidney and liver in relation to protein and amino acid charges

Tissue	No. of observations	Control	γ-globulins	Albumins	Glutamic acid	Lysine
Kidney	6	78.00 ± 10.05	82.00 ± 9.13 NS	98.00 ± 13.05 S	92.00 ± 8.16 S	69.00 ± 10.21 NS
Liver	5	27.00 ± 4.00	45.00 ± 10.81 S	30.00 ± 7.45 NS	33.00 ± 8.89 NS	42.00 ± 12.21 S

Activity is expressed as GPT units/mg protein. S, values are significant; NS, values are non-significant.

Zusammenfassung. Die Wirkung der Protein- und Aminosäurebelastung auf die Glutamat-Pyruvat-Transaminase in Niere und Leber von *Rana hexadactyla* wurde untersucht. Die Enzymaktivität war in der Niere höher als in der Leber. γ-Globulin und Lysin erhöhte die Leberenzyme und war ohne Einfluss auf die Nieren-Transaminase.

A. SUBBARAMI REDDI and K. S. SWAMI

Department of Zoology,
Sri Venkateswara University,
Tirupati (Andhra Pradesh, India), 29 July 1969.

[5] H. U. BERGMEYER, *Methods of Enzymatic Analysis* (Academic Press, New York 1965), p. 846.

[6] Acknowledgement. One of the authors (A.S.R.) acknowledges with thanks the C.S.I.R., New Delhi, for financial assistance during the tenure of which the present work has been carried out.

The Binding of Cardiac Glycosides to Bull Spermatozoa

Investigations of net changes in alkali cation content[1-3] and of Na^+-K^+-activated ATPase activity[4,5] have provided some evidence for active transport of Na^+ and K^+ in mammalian spermatozoa. Recently the technique of specifically binding ^3H-labelled inhibitors of cation transport (ouabain and digoxin) has been used to demonstrate active transport sites on cell membranes and to estimate the number of such sites[6-9]. The present report describes the application of this technique to bull spermatozoa.

Materials and methods. Semen samples collected from 2 normal Jersey bulls were cooled slowly to 20 °C, and spermatozoa separated from seminal plasma by centrifugation (1200 *g*, 10 min at 20 °C). The spermatozoa were washed once and resuspended in an artificial bull seminal plasma (ABSP)[3]. Washed cells were inspected microscopically for motility, then counted and monitored electronically[10].

Naturally-decapitate spermatozoa from a Guernsey bull were separated into fractions of heads and motile midpiece-tails by rate-sedimentation on an ABSP:sucrose density gradient in a zonal centrifuge rotor (A type, M.S.E. Ltd.).

Cell concentration was adjusted to 5×10^8 cells/ml and cells preincubated at 37 °C for 15 min in ABSP. Tritiated digoxin or ouabain was then added usually to a final concentration of $5 \times 10^{-6} M$. Following a further 30 min incubation, the binding reaction was stopped by the addition of a large excess of $10^{-3} M$ ouabain in ABSP, and the cells washed 3 times in 50 vol. of ABSP by centrifugation (2000 *g*, 10 min). The final pellet was dissolved in 2 ml NCS solubilizer (Nuclear-Chicago), scintillator added, and counted in a Packard Tricarb Spectrometer. Labelled ouabain was obtained from New England Nuclear Corp. and digoxin from Dr. A. RUTSCHMANN, Sandoz AG, Basle (Switzerland).

Results and discussion. The time-course of binding at an ouabain concentration of $5 \times 10^{-6} M$ was determined, from which the reaction appeared to be complete after 15 min. The concentration of glycoside chosen in these experiments, $5 \times 10^{-6} M$, represents a concentration high enough to saturate most of the transport sites. Using ^3H-digoxin instead of ^3H-ouabain nearly equivalent numbers of bound molecules per cell were obtained. The glycoside bound to the spermatozoa was not removed to any signifi-

[1] P. J. QUINN and I. G. WHITE, Biochem. J. *104*, 328 (1967).
[2] P. J. QUINN and I. G. WHITE, Aust. J. biol. Sci. *21*, 781 (1968).
[3] J. M. O'DONNELL, J. Reprod. Fert. *19*, 207 (1969).
[4] S. UESUGI and S. YAMAZOE, Nature *209*, 403 (1966).
[5] P. J. QUINN and I. G. WHITE, J. Reprod. Fert. *15*, 449 (1968).
[6] H. MATSUI and A. SCHWARTZ, Biochim. biophys. Acta *151*, 655 (1968).
[7] R. W. ALBERS, G. J. KOVAL and G. J. SIEGAL, Mol. Pharmac. *4*, 324 (1968).
[8] J. F. HOFFMAN and L. J. INGRAM, Proc. First Intern. Symp. Metabolism and Permeability of Erythrocytes and Thrombocytes (Thieme, Stuttgart 1969).
[9] J. C. ELLORY and R. D. KEYNES, Nature *221*, 776 (1969).
[10] J. M. O'DONNELL, J. Reprod. Fert. *19*, 263 (1969).