extent of sarcomere fluctuation may be one of the main factors which determines the energy requirements of the muscle for maintaining isometric tension and also possibly the rate at which the muscle fatigues. Presumably sarcomere fluctuation is related to the length of time that each cross link on the myosin filament is engaged with the actin filament. The fluctuations probably only occur with the random release or engagement of many cross links. In the fast muscle there is presumably a greater probability of sarcomere movement as the length of time that each cross link is engaged is probably far less than in the slow muscle[3].

Zusammenfassung. Es wird gezeigt, dass die Schwankung der Sarkomerenlänge bei Benützung der «laser beam» – Methode während der isometrischen Kontraktion der M. latissimi dorsi beim Hühnchen verschieden war. Der Schwankungsumfang war in den vorderen und hinteren Muskeln ungefähr gleich (900 Å), hingegen war die Frequenz in den hinteren phasischen Muskeln dreimal höher als in den vorderen tonischen Muskeln.

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Effect of Light and pH on Diazotized Bilirubin

While studying the enzyme, bilirubin glucuronyl transferase, variable results had been obtained on the determination of diazotized non-conjugated bilirubin. Previous workers have shown that undiazotized bilirubin is very sensitive to light and pH[1–10] and that extinction and absorption maximum values of azo dyes depend on such factors as pH, alcohol, and albumin[11]. It was therefore desirable to note whether light and pH had significant effects on the spectrophotometric assay of diazotized non-conjugated bilirubin under the conditions of the aforementioned experiments. As noted below, normal room light did not significantly affect the spectrophotometric determination of diazotized bilirubin whereas pH had a marked influence on the optical density readings. These observations will undoubtedly be of interest to other laboratories doing bilirubin determinations.

Effect of pH. To study the effect of pH on spectrophotometric properties of bilirubin, a bilirubin solution was made as follows: 4.5 ml ethanol, 95%; 0.6 ml of 0.25*M Tris* buffer, pH 8.0 at 25 °C; 0.5 ml bilirubin solution (19 mg bilirubin (Sigma) rapidly dissolved in 10 ml of 0.2*N* NaOH and 28.5 ml distilled water); and 1.95 ml of distilled water. 3 ml of concentrated diazo reagent[12] was then added. After shaking for 30 min, the solution was diluted to 100 ml with methanol. Series of samples were set up, using NaOH and HCl solutions, to give various hydrogen-ion concentrations. Equal volumes of diazotized bilirubin solution were then added. Optical density was read at 535 nm (Spectronic 20) and the pH was determined (Beckman Expanded Scale Model 76). Water was used in each series as a control replacing the acid or base. Conditions were reversed by adding HCl solution to the more alkaline samples (compared to the control at pH 2.3). Each sample was brought to the same volume so that the concentration of bilirubin was the same in each cuvette. Optical density and pH were determined again.

The effects of acid and base on diazotized bilirubin colorimetry are shown in the Figure. Upon addition of NaOH, optical density readings decreased as the pH was increased from the control sample pH value. Acid lowered the pH but did not produce changes in the optical density readings compared to the control. To determine whether the diazotized bilirubin was acting as an indicator[13], HCl was added to the samples whose pH values had been increased. It was presumed that changing the pH back to the control level might give optical density readings similar to that of the control samples. This was not the case. The addition of HCl resulted in changing the pH to a value around 2 but produced only a minimal increase in the optical density readings.

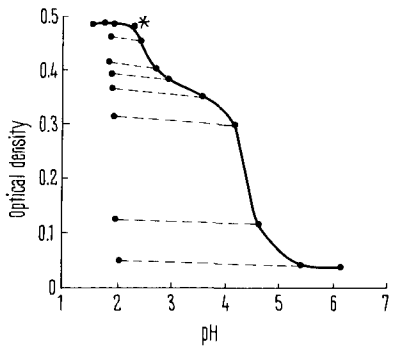
Diazotized bilirubin was therefore markedly affected by the hydrogen-ion concentration of the solution. Different optical density readings were obtained with the same concentration of bilirubin just by changing the pH through the addition of NaOH. It is therefore important to keep a pH of 2 or less throughout the procedure after the addition of concentrated diazo reagent to obtain consistant results.

Effect of light. For the study on light, diazotized conjugated and non-conjugated bilirubin samples were obtained by the extraction and chromatographic tech-

[1] R. J. CREMER, P. W. PERRYMAN, D. H. RICHARDS and B. HOLBROOK, Biochem. J. *66*, 60P (1957).
[2] J. E. O'HAGAN, T. HAMILTON, E. G. LE BRETON and A. E. SHAW, Clin. Chem. *3*, 609 (1957).
[3] J. BRUCKNER, Am. J. clin. Path. *32*, 513 (1959).
[4] R. C. BURNSTINE and R. SCHMID, Proc. Soc. exp. Biol. Med. *109*, 356 (1962).
[5] J. FOG and B. BUGGE-ASPERHEIM, Nature *203*, 756 (1964).
[6] S. H. BLONDHEIM and N. A. KAUFMANN, J. Lab. clin. Med. *65*, 659 (1965).
[7] R. BRODERSEN, Acta chem. scand. *20*, 2895 (1966).
[8] I. W. DE EWENSON, F. A. GIANTURCO and P. GRAMACCIONI, Experientia *22*, 14 (1966).
[9] T. K. WITH, in *Bile Pigments – Chemical, Biological, and Clinical Aspects* (Academic Press, New York 1968), pp. 11 and 351.
[10] L. HEILMEYER, in *Spectrophotometry in Medicine* (Adam Hilger Ltd., London 1943), p. 155.
[11] M. JIRSA and V. JIRSOVA, Clin. Chem. *5*, 532 (1959).
[12] A. PH. WEBER and L. SCHALM, Clin. chim. Acta *7*, 805 (1962).
[13] R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience Publishers, New York 1949), p. 154.

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.

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[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.