that aflatoxin $B_1$ affects the rate of $^{14}C$-acetate incorporation into cholesterol in rat liver and in their studies aflatoxin treatment also resulted in elevated levels of cholesterol. These investigators suggested that the inhibitory action of aflatoxin on acetate incorporation into cholesterol was specific and not the result of general hepatic injury.

In our experiments the level of sterol digitonides increased 22% with a concomitant decrease in specific activity of 42%. The increase of lipid and cholesterol content in the previous studies could, as suggested by SHANK and WOGAN, have resulted from failure of transport of these metabolites from the liver. The present in vitro studies, in which transport is not involved, indicate that the toxin affects both the rate of synthesis and turnover of lipids in the skin. Furthermore, the failure of aflatoxin to affect the respiratory rates of skin also suggests that the effects on lipid metabolism are of a more specific nature than that of general toxicity. CLIFFORD and REES[4] have reported comparable results on the respiratory rates of aflatoxin poisoned liver.

Although it is generally concluded that the toxicity of aflatoxin $B_1$ is tissue specific, the similarities of results from the present study with human skin to those of previous ones of lipid metabolism in animal liver suggest that a) at least this facet of aflatoxin action may be common to all tissues actively engaged in lipid metabolism via similar biosynthetic pathways and b) that this effect of aflatoxin on lipid metabolism is of a specific nature. The relationship of these findings to the possible occurrence of dermatoses in human skin must await further study[15].

*Zusammenfassung.* Aflatoxin $B_1$ hindert den in vitro Einbau des $^{14}C$-Azetats in die Gesamtlipide, die freien Sterine, die Phospholipide und die Neutralfette der menschlichen Haut. Die Atmungsgeschwindigkeit der toxinbehandelten Gewebe wurde nicht beeinflusst.

H. S. BLACK, J. D. SMITH,
B. J. AUSTIN and E. W. RAUSCHKOLB

*Veterans Administration Hospital and Departments of Dermatology and Biochemistry, Baylor College of Medicine, Houston (Texas 77031, USA), 8 June 1970.*

[15] Supported in part by The Robert A. Welch Foundation Grant No. Q-253, USPHS Grant No. EC00110-03, and Clinical Investigatorship Award of Veterans Administration.

## Calcium Sensitivity of Succinate and Pyruvate Dehydrogenases in the Denervated Amphibian Skeletal Muscle

The calcium-sensitive proteins, as well as acidic proteins, of amphibian gastrocnemius muscle showed cathodal migration suggesting a net positive sign of charge on them[1]. Similarly some of the dehydrogenases were also found to possess a net positive sign of charge[2–4]. Since the acidic protein environment is known to elevate the activity of dehydrogenases[2–4] and the denervated muscle has altered calcium levels, it is essential to see whether calcium-sensitive proteins or calcium sensitivity of the dehydrogenases exists in the denervated muscle.

*Material and methods.* *Rana hexadactyla* of medium size were denervated unilaterally by sciatic nerve section under aseptic conditions. The frogs were maintained in aquarium tanks and were fed with cockroaches or earthworms. After 30 days, the normal and denervated gastrocnemius muscles were isolated and used for enzymatic studies.

5% (wt/vol) homogenates of normal and denervated muscles were prepared in 0.25 *M* sucrose solution and centrifuged at 1500 *g* for 20 min. 2 ml aliquots of each supernatant was transferred into 3 centrifuge tubes. The first tube received 1 ml 0.25 *M* sucrose and this served as the control. The second and third tubes received 1 ml 10 m*M* calcium chloride and 1 ml 1.5 m*M* sodium citrate respectively. The mixtures were centrifuged at 1000 *g* for 10 min and the levels of the succinate dehydrogenase (SDH, EC 1.3.99.1) and pyruvate dehydrogenase (PDH, EC 1.3.4.1) activities were determined in the supernatant, adopting the triphenyl tetrazolium chloride reduction method of SRIKANTAN and KRISHNAMOORTHY[5] as standardised by GOVINDAPPA and SWAMI[3]. The chosen molar concentrations of calcium chloride and sodium citrate were arrived at after several preliminary experiments, where their effects on enzyme activities were found to be optimal.

*Results and discussion.* Since the binding of calcium to proteins is known to be pH dependent[6], the effects of calcium and citrate on SDH and PDH activities were studied at the pH 5.8, 6.8 and 7.8 (Tables I and II). The pH value of 6.8 represents the muscle homogenate pH and this condition will provide a native environment to the muscle homogenate. The pH values 5.8 and 7.8 were selected so that a relatively acidic and alkaline environment respectively was induced on the enzyme system.

At 6.8 pH both the enzymes are activated by calcium and inactivated by citrate. Thus, it appears that both the enzymes require an increase in the positive charge density in the environmental protein for their activation, since calcium is known for induction of positive charges on protein by neutralizing negative charges[7]. At pH 5.8, the SDH activity of normal and denervated muscles was significantly elevated by calcium and suppressed by citrate. At pH 7.8, the SDH was inhibited both by calcium and citrate, but the inhibition by calcium was more pronounced and statistically significant while the inhibition by citrate was not significant. Increase in the acidity (i.e., pH 5.8) of the medium normally involves the depletion of negative charges contributed by carboxyl and phosphate groups of proteins as some of the groups approach the pKa values in the direction of acidity. Addition of calcium at this pH might have further decreased the negative charge density

[1] R. V. KRISHNAMOORTHY, Ph. D. thesis, Sri Venkateswara University, Tirupati (A.P.) (1963).
[2] K. S. SWAMI and R. V. KRISHNAMOORTHY, Archs int. Physiol. Biochim. *72*, 373 (1964).
[3] S. GOVINDAPPA and K. S. SWAMI, Indian J. exp. Biol. *3*, 209 (1965).
[4] Y. SURENDRANATH REDDY, Curr. Sci. *36*, 127 (1967).
[5] T. N. SRIKANTAN and C. R. KRISHNAMOORTHY, J. scient. ind. Res. *14*, 206 (1955).
[6] V. WEIMER, Physiol. Zool. *26*, 231 (1953).
[7] A. MARTANOSI, C. M. MOLINO and J. GERGELY, J. biol. Chem. *239*, 1057 (1964).

Table I. Effect of calcium and citrate on succinate dehydrogenase activity of normal and denervated muscle homogenates

| Muscle | pH 5.8 | | | pH 6.8 | | | pH 7.8 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | $CaCl_2$ added | Sodium citrate added | Control | $CaCl_2$ added | Sodium citrate added | Control | $CaCl_2$ added | Sodium citrate added |
| Normal[a] | 319.9±22.6[b] | 405.6±63.1 | 250.1±36.7 | 168.9±19.7 | 252.3±14.3 | 130.9±10.6 | 66.7±13.2 | 46.9±7.8 | 64.8±15.8 |
| Difference[c] from control (%) | | +26.8 | −21.8 | | +49.4 | −22.5 | | −31.2 | −2.8 |
| Significance | | $P<0.02$ | $P<0.01$ | | $P<0.001$ | $P<0.001$ | | $P<0.05$ | N.S. |
| Denervated | 143.9±25.5[b] | 206.9±42.9 | 112.1±11.5 | 77.8±20.1 | 138.4±36.9 | 46.6±8.9 | 29.6±4.9 | 22.3±4.3 | 26.4±10.2 |
| Difference[c] from control (%) | | +43.8 | −22.1 | | +77.8 | −40.1 | | −24.9 | −10.9 |
| Significance | | $P<0.05$ | $P<0.05$ | | $P<0.02$ | $P<0.02$ | | $P<0.05$ | N.S. |

Enzyme activity expressed as μg of formazan/g wet wt./h. [a] Each value is mean of 6 individual observations. [b] ± S.D. [c] The values indicate % increase (+) or decrease (−) in activity in relation to the control. N.S., not significant.

Table II. Effect of calcium and citrate on pyruvate dehydrogenase activity of normal and denervated muscle homogenates

| Muscle | pH 5.8 | | | pH 6.8 | | | pH 7.8 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | $CaCl_2$ added | Sodium citrate added | Control | $CaCl_2$ added | Sodium citrate added | Control | $CaCl_2$ added | Sodium citrate added |
| Normal[a] | 592.6±35.9[b] | 425.6±54.4 | 561.9±34.6 | 286.6±32.4 | 429.2±96.1 | 220.3±23.9 | 45.4±10.9 | 70.8±6.2 | 37.5±11.9 |
| Difference[c] from control (%) | | −28.3 | −5.2 | | +51.4 | −23.1 | | +56.1 | −17.4 |
| Significance | | $P<0.001$ | N.S. | | $P<0.02$ | $P<0.01$ | | $P<0.01$ | N.S. |
| Denervated | 460.6±65.2[b] | 336.6±47.8 | 384.3±56.1 | 174.6±20.8 | 269.1±33.5 | 132.6±11.0 | 31.8±9.6 | 55.6±2.8 | 24.4±6.7 |
| Difference[c] from control (%) | | −26.9 | −16.6 | | +54.2 | −24.0 | | +74.7 | −23.1 |
| Significance | | $P<0.01$ | N.S. | | $P<0.01$ | $P<0.01$ | | $P<0.01$ | N.S. |

Enzyme activity expressed as μg formazan/g wet wt./h. [a] Each value is mean of 6 observations. [b] ± S.D. [c] The values indicate % increase (+) or decrease (−) in activity in relation to the control. N.S., not significant.

in the environmental proteins and this condition might have elevated the SDH activity. Sodium citrate treatment eliminates the effect of calcium, as the citrate is a potent chelating agent of calcium, thus inducing negativity on carboxyl groups and subsequently the SDH activity has been suppressed at pH 5.8 by citrate. The increase of pH to 7.8 induces greater ionization of carboxyl and phosphate groups which in their ionized state will contribute to negative charge density in the medium. Further, the shift towards the alkalinity of the medium indicates an increase in $OH^-$ groups in the medium. In addition, other ionizable groups of proteins having pKa values in the alkaline range get involved in development of charges. Thus, the addition of calcium at pH 7.8 might have neutralized at least some of the negative charges in the medium and thus the SDH activity should have been elevated at this pH. However, in the present study both calcium and citrate had identical pattern of inactivation suggesting that they are involved in secondary reactions influencing a common factor. Thus, it may be inferred that carboxyl and phosphate groups of the environmental proteins are involved in the regulation of SDH activity, as has been suggested previously[8,9].

The PDH had a sensitivity pattern different from that of SDH. At pH 5.8, the calcium suppressed the PDH activity more than the citrate, showing a trend opposite to that observed for SDH. At pH 7.8 calcium elevated the PDH activity, a situation which is contrary to that of SDH. Probably carboxyl and phosphate groups had minor influence in the regulation of PDH activity. Thus, the elevation of PDH activity by calcium in the alkaline medium suggests that this enzyme depends on other groups such as guanidine and amino groups of proteins for its activation.

The dystrophic muscle, which exhibits more or less similar biochemical changes as the denervated muscle, showed an increased calcium content[10,11]. Further, the denervated muscle showed increased $Ca^{++}$ flux[12], and this increase is thought to be partly due to increased free $Ca^{++}$ levels in that muscle[13]. Thus the increased free calcium levels in the denervated muscle should have elevated the activities of dehydrogenases. Contrary to this, there is a decrease in the activities of SDH and PDH in the denervated muscle, suggesting that the calcium sensitivity of these dehydrogenases was altered in the denervated muscle.

*Zusammenfassung.* Es wird nachgewiesen, dass die Aktivität von Succinat Dehydrogenase und von Pyruvat Dehydrogenase in Extrakten normaler und denervierter Muskulatur durch $Ca^{++}$ im pH-Bereich von 5,8 bis 7,8 in verschiedenem Ausmass erhöht bzw. erniedrigt wird.

N. BOJJI REDDY and K. S. SWAMI

*Department of Zoology, Sri Venkateswara University, Tirupati (A.P., India), 19 June 1970.*

[8] P. INDIRA and K. S. SWAMI, Indian J. exp. Biol. *3*, 5 (1965).
[9] S. GOVINDAPPA, Ph. D. thesis, Sri Venkateswara University, Tirupati (A.P.) (1967).
[10] W. O. FENN and M. GOETTSCH, J. biol. Chem. *120*, 41 (1937).
[11] S. MORGULIS and W. OSHEROFF, J. biol. Chem. *124*, 767 (1938).
[12] E. GUTMANN and A. SANDOW, Life. Sci. *4*, 1149 (1965).
[13] A. ISSACSON and A. SANDOW, J. Pharmac. exp. Ther. *155*, 376 (1967).