8 S. Caccamese, K.L. Rinehart, Jr, P.D. Shaw and R.D. Johnson, 170th nat. Meet. Am. chem. Soc. Chicago 1975, Abstracts, ORGN 19.
9 A Varian MAT mass spectrometer, Model 112, equipped with a chemical ionization ion source and interfaced to a Varian SS 100 computer system, was coupled to a Varian gas chromatograph, Model 1740.
10 T.G. Halsall and I.R. Hills, Chem. Commun. *1971*, 448.
11 R.F. Lee, J.C. Nevenzel, G.A. Paffenhöfer, A.A. Benson, S. Patton and T.E. Kavanaugh, Biochim. biophys. Acta *202*, 386 (1970).
12 M. Blumer, M.M. Mullin and R.R.L. Guillard, Mar. Biol. *6*, 226 (1970).
13 R.F. Lee and A.R. Loeblich, III, Phytochemistry *10*, 593 (1971).
14 J.S. Warner, Analyt. Chem. *48*, 578 (1976).
15 The presence of $C_3H_7^+$ ions in the reactant gas is shown by the weak peaks at m/e 329 for component C and m/e 327 for component D.
16 F.H. Field, J. Am. chem. Soc. *90*, 5649 (1968).

Effect of seasonal variations and cold acclimation on serum transaminase activity of common Indian frog *Rana tigrina*[1]

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Summary. A definite seasonal change is observed in 2 transaminases, SGOT and SGPT of *R. tigrina*. Cold acclimation significantly depresses transaminase activity of serum.

Although transamination reactions have been well-documented and a number of studies have been made on the kinetics and properties of transaminases in mammalian[2-4] and nonmammalian tissues[5-9], very little information is available on the effect of cold acclimation and seasonal variations on the transaminase activity. Almost nothing is known about these factors in amphibians. The present work is an attempt in this direction to fill the void.

Materials and methods. The frogs of both the sexes ranging from 200 to 500 g in weight were obtained from the local suppliers and kept in a vivarium maintained outside the laboratory in perfectly natural conditions. The blood was always obtained by aortic puncture from pithed and dissected frogs. Transaminase activities were recorded for each month during 1976 to 1977. The influence of cold acclimation was studied by keeping the frogs at 13 °C (inside the refrigerator), whereas the room temperature was 38 °C at the same time. The frogs were taken out at regular intervals and their transaminase activity was determined. Appropriate controls were run simultaneously. Transaminase activities were determined according to Reitman and Frankel[10] using Spectronic-20 Spectrophotometer, at a temperature of 37 °C and pH 7.5. The results are expressed in RF-units. 1 RF-unit is equivalent to the formation of 4.82×10^{-4} µM glutamate/min. The points in the figures represent an average of 5-6 readings±SEM. 2 transaminases aspartate aminotransferase (EC 2.6, 1.1) and L-alanine aminotransferase (EC 2.6, 1.2) would be referred here after as SGOT and SGPT respectively.

Results. The values of SGOT and SGPT observed throughout the year are shown in figure 1. SGOT has been found much more active than SGPT. The serum transaminase activity is lowest in January (SGOT 45.7±1.0 and SGPT 12.5±0.99), rise gradually till the peak values are observed in August (SGOT 133.6±2.0 and SGPT 28.8±1.35). Thereafter the activity again shows a gradual fall till almost the same level is reached as in January. The effect of cold acclimation of the frogs kept at 13 °C for various periods has been shown in figure 2. A steep fall was recorded in transaminase activity from 2nd day onwards.

Discussion. It is well-known that transaminases are widely distributed in plant and animal tissues[11] and establish a link between the metabolism of amino acids, carbohydrates and fats. The normal values of SGOT and SGPT recorded in *R. tigrina* compare favourably with the values recorded for other vertebrates[12,13] and invertebrates[6-9], where GOT activity has been observed to be higher than GPT.

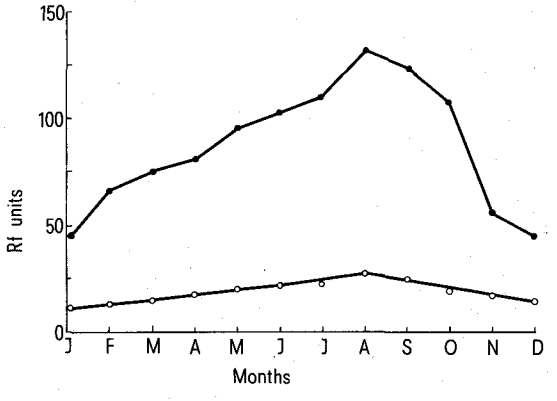

Fig. 1. Monthly average SGOT (●——●) and SGPT (○——○) activity showing seasonal change in *Rana tigrina*. (Average values of the whole year: SGOT 87.6±6.0; SGPT 20.0±2.0 RF-units.)

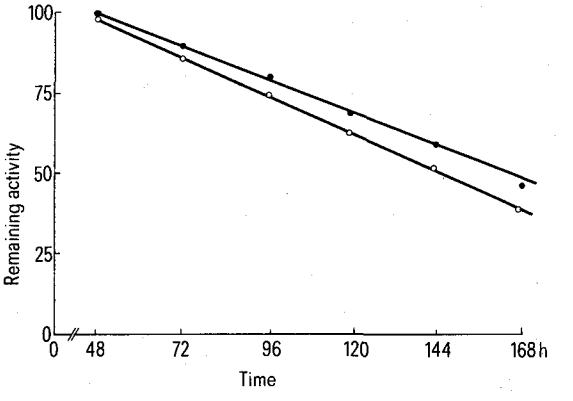

Fig. 2. Effect of cold acclimation on SGOT (●——●) and SGPT (○——○) activity in *Rana tigrina*.

There does not seem to be any work done on the variations in transaminase activity at different times of the year. A definite change exists in the transaminase activity of *R. tigrina*. The average values of SGOT and SGPT recorded for the whole year can be divided into 3 phases. In the first phase, from November to March, the values are relatively low (SGOT 50.0–80.0 and SGPT 15.0–19.0). The 2nd phase is close to average values, and in the 3rd phase the values are higher with the peak in August. The high values of transaminase activity (3rd phase) seem to be in direct correlation with spawning period. The high level of this enzyme may be indicative of active phase and higher metabolic activities in frogs in this period. This may also be related to rise in serum glucose[14] and protein[15] reported in this animal, during the same period.

Low values of SGOT and SGPT observed in December and January appear to be related with fall in temperature (40 °C in summer to 15 °C in winter). This is also corroborated by the results of the experiments on cold acclimation of the frog. Low values of SGOT and SGPT are observed when the frogs are acclimatized at low temperatures. Rat tissues[16] exposed to cold for 72 h also show a rapid fall in transaminase activity at early stages, but they regain the normal level after some time, but no such change was observed in frog serum upto 168 h. This may be due to the poikilothermic nature of these animals.

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2 J. Awapara and B. Seale, J. biol. Chem. *194*, 497 (1952).
3 B. Bulos and H. Philip, J. biol. Chem. *240*, 3283 (1965).
4 W.T. Jenkins, D.A. Yphantis and I.W. Sizer, J. biol. Chem. *234*, 51 (1959).
5 B.A. Kilby and E. Neville, J. exp. Biol. *34*, 276 (1957).
6 J.W. Daugherty, Exp. Parasit. *1*, 331 (1952).
7 D.V. Aldrich, A.C. Chandler and J.W. Daugherty, Exp. Parasit. *3*, 173 (1954).
8 W.B. Foster and J.W. Daugherty, Exp. Parasit. *8*, 413 (1959).
9 R.K. Sharma and K. Singh, Z. ParasitKde *54*, 251 (1977).
10 S. Reitman and S. Frankel, Am. J. clin. Path. *28*, 56 (1957).
11 P.P. Cohen, The enzyme, vol. I, part 2. Academic Press, N.Y. 1951.
12 D. Marjanovic, L. Kozic, M. Petrovic, T. Palic and V. Litricin, Vet. Glasn. *28*, 793 (1974).
13 E. Shimizu and Y. Naito, J. Fac. Agric. Iwate Univ. *11*, 37 (1952).
14 S. Kumar and S.S. Khanna, Zool. Jb. Physiol. *76*, 80 (1971).
15 K. Singh, Zool. Anz. *197*, 39 (1976).
16 J.R. Puchol and A. Carballido, Med. exp. *6*, 158 (1962).

On the modification of sulfhydryl groups by 4-cyclopentene-1,3-dione[1]

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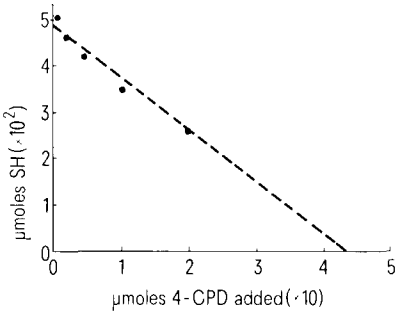
Summary. 4-Cyclopentene-1,3-dione reacts quantitatively with sulfhydryl groups of both cysteine and the sulfhydryl containing protein pinguinain. A 10fold excess of the reagent totally modifies cysteine after 15 min at room temperature and the single sulfhydryl group in native pinguinain is totally modified by a 48–86fold excess of reagent.

Recent work from this institution[2,3] has shown that the sulfhydryl reagent NEM induces electrical excitability in the ventroabdominal flexor muscles of the crustacean *Atyas occidentalis*, a naturally nonexcitable tissue. As a result of this work it has been proposed that the effects of NEM in these tissues depend on the induction of new properties in the membrane proteins, likely to arise from the conversion by NEM of $-CH_2-SH$ side chains to polar thioethers. Neither the nitrogen nor the chains attached to it appear to be essential for this effect, as 4-CPD[4], a compound similar to NEM but containing a methylene group instead of a tertiary nitrogen also induces excitability[5]. Although this compound has not been regarded as an SH reagent we decided to study whether 4-CPD does in fact react with sulfhydryl groups. The modification of a protein with 4-CPD may have interesting effects on its tertiary structure because of the presence of 2 symmetrical carbonyl groups in the reagent molecule which will enable it to form hydrogen bonds with neighboring NH_2 groups in the protein.

Materials and methods. Chemicals. 4-CPD was from Aldrich; cysteine from Sigma; DTNB from Calbiochem; BAPNA from Merck; precoated silica gel plates from Kontes/Quantum; Sephadex gels from Pharmacia.

Reaction of 4-CPD with cysteine: TLC. 1-mM solutions of cysteine and 10-mM solutions of 4-CPD were prepared in 5 mM phosphate buffer at pH 6.0. 0.5 ml of each solution was mixed for 15 min at room temperature and 30 µl of the reaction mixture was spotted in 20×4 cm precoated silica gel plates and chromatographed using butanol: acetic acid: H_2O (4:1:1) as solvent. Equal volumes of the cysteine and 4-CPD solutions were spotted as references. Cysteine and its thioether were detected by staining with ninhydrin spray and 4-CPD with iodine vapors. An identical experiment was performed with the same solutions at pH 7.6.



Modification of SH groups in the protease pinguinain by 4-CPD. The ordinate shows the DTNB titratable groups (µmoles) left after addition of varying amounts of 4-CPD. Modification performed in 5 mM phosphate at pH 7.6. The reaction was allowed to proceed for 1 h.