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STUDIES ON ELECTROPHORETIC PATTERN OF SERUM PROTEINS OF THE TOAD *BUFO ANDERSONII*

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AMPHIBIANS are cold-blooded vertebrates and are known to hibernate and aestivate during winter and summer seasons respectively. Atmospheric physical factors influence the physiology of the animals. Therefore it was proposed to study the serum protein fractions of toad by agar gel electrophoresis during winter and summer seasons.

Ten male and ten female toads were procured during winter and summer months. The animals were killed and blood was collected immediately from the heart into sterile tubes for serum separation. The serum was assayed for various protein fractions by agar gel electrophoresis¹ using

barbiturate buffer (pH 8.6). The gels were stained with amido black and scanned on a densitometer.

The serum showed four distinct fractions, viz. albumin and alpha-, beta- and gamma-globulins. The proportions of these fractions are shown in table 1.

All the four serum protein fractions of toad were cathodic (figure 1). In fish², sheep³, and buffalo⁴ gamma-globulin is anodic.

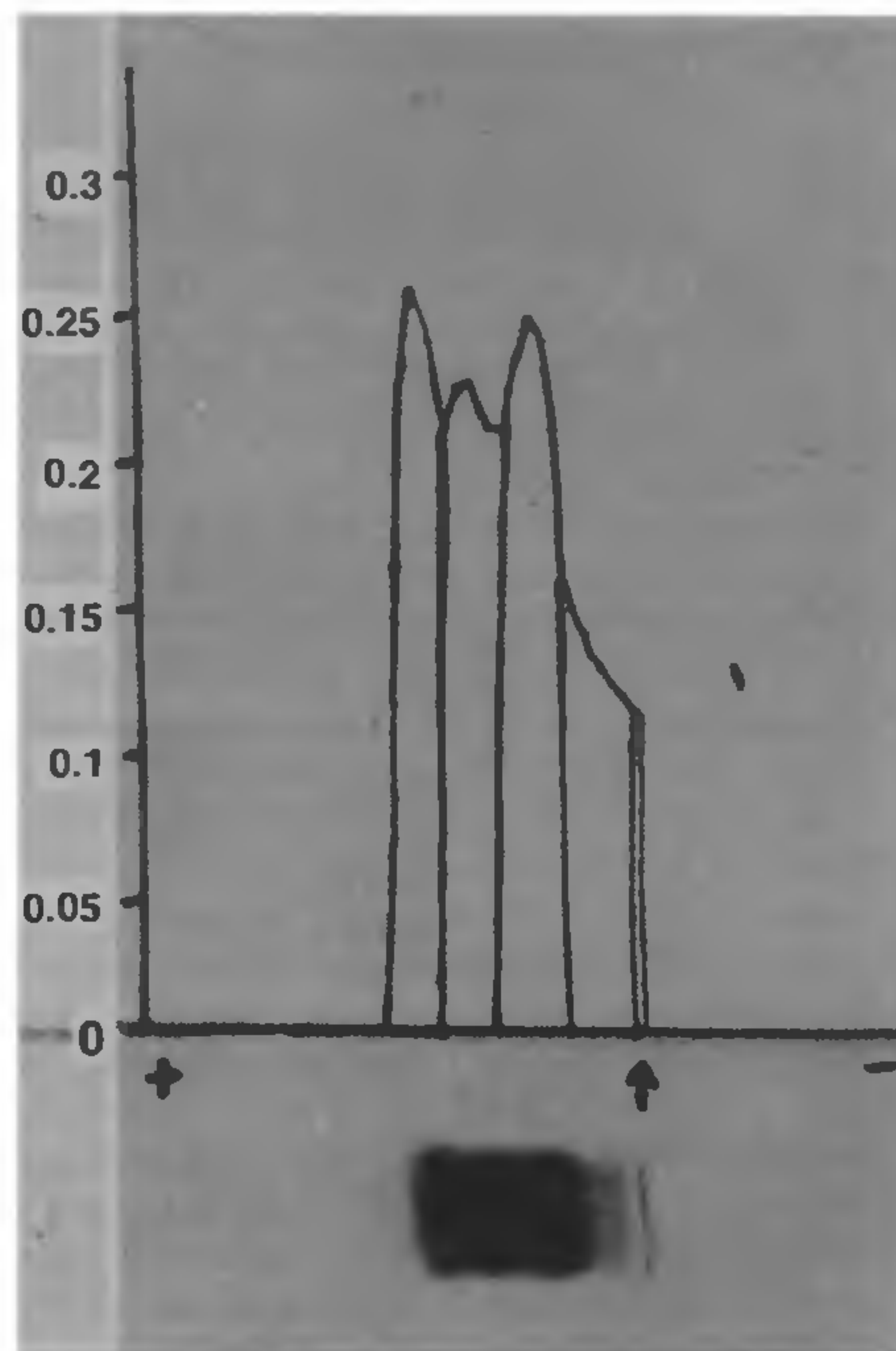


Figure 1. Agar gel electrophoretic pattern of serum of *Bufo andersonii*.

Table 1 Proportions of various serum protein fractions of toad

Sex	Season	Protein fraction*				Albumin/ globulin ratio
		Albumin	Alpha-globulin	Beta-globulin	Gamma-globulin	
Male (n=10)	Summer	23.89±0.715	25.51±0.598	32.44±0.674	18.14±0.493	0.31
	Winter	23.27±0.626	25.00±0.556	32.75±0.810	18.98±0.515	0.30
Female (n=10)	Summer	24.17±0.462	21.38±1.031	34.37±0.651	20.06±1.08	0.31
	Winter	24.09±0.382	28.91±0.993	31.72±0.835	15.26±1.189	0.31

*Values expressed as mean ± S.E.

Albumin/globulin ratio was nearly the same in all the cases. Beta-globulin fraction is the highest in all toads in both the seasons. This is similar to the findings reported earlier^{5,6} for other animals. Gamma-globulin was lowest in all the cases.

The levels of alpha-globulin in male and female toads were significantly different ($P > 0.01$) in both seasons. Changes in globulin fractions were seen in female toads: beta- and gamma- globulin fractions increased significantly ($P > 0.05$ and $P > 0.01$ respectively) in summer. This is similar to the results of Ashton⁷ for sheep. The alpha-globulin fraction was significantly higher during winter in female toads ($P > 0.001$). Perk and Loble⁸ had found a direct genetic control on plasma protein fractions.

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RADIOMODIFYING EFFECT OF CHAMPHOR AS REVEALED BY SISTER CHROMATID EXCHANGE

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SISTER chromatid exchange (SCE) represents reciprocal exchanges between replicated sister chromatids at homologous points and its analysis is a sensitive means of monitoring genotoxicity¹⁻³. Camphor has been reported to enhance radiation-induced damage in bacterial cells under hypoxic conditions⁴, in mouse transplantable mammary adenocarcinoma⁵, and in spermatogonia of adult mice⁶. In view of the potential utility of camphor in cancer therapy,

studies were undertaken to evaluate the radiomodifying effect of camphor on normal tissues by SCE analysis. In this paper we report the radioprotective effect of camphor in mouse bone marrow.

Three-to-four-month-old inbred Swiss albino mice were randomly assorted into 15 groups (table 1), each with four animals. Each animal, except controls, received camphor ($0.5 \mu\text{mol/g}$ body wt) dissolved in olive oil intraperitoneally. Whole-body gamma irradiation was given 30 or 45 min after administration of camphor. One day before sacrificing the animals, 9 injections of BrdU (10^{-2} M), spaced hourly, were administered intraperitoneally. Each injection contained deoxycytidine (5×10^{-3} M) to reduce the toxicity to BrdU. Seventeen hours after the last BrdU injection, colchicine (0.4 mg/ml) in distilled water was injected intraperitoneally (4 mg/100 g body wt). Two hours later, animals were sacrificed by cervical dislocation. Chromosome preparations were made and stained with Hoechst 33258 and Giemsa⁷. Animals were kept at $25 \pm 2^\circ\text{C}$ and food and water were given *ad libitum*.

Table 1 presents the frequency of SCE in bone marrow cells after various doses of gamma irradiation in the presence or absence of camphor. In the different control groups (first three rows in table 1) the frequency of SCE was within normal limits. Animals given camphor and no radiation showed enhancement in SCE frequency but that was not statistically significant. Animals exposed to increasing doses of whole body gamma radiation showed significantly higher incidence of SCE.

Animals administered camphor prior to irradiation showed a marked reduction in SCE compared to the correspondingly irradiated group that did not receive camphor (table 1 and figure 1). Compared to 30 min interval between camphor treatment and irradiation, 45 min interval resulted in a slight decrease in number of SCE. The difference between the 30 min and 45 min groups is significant only at 2.0 Gy radiation dose. Further, the reduction in SCE frequency due to camphor became more pronounced with increasing levels of radiation exposure. Maximum reduction in frequency of SCE by camphor was at 2.0 Gy. The radioprotective potential of camphor is thus evident from this study.

The bone marrow cells are well oxygenated and oxygen is an effective radiation sensitizer. The present findings agree with previous reports¹. Goel *et al.*⁶ studied radiosensitizing effects of camphor in the testicular system, which is often used as a hypoxic model for *in vivo* studies. They observed