decreased number of lymphocytes carrying B and T markers.

The effect of biotin treatment of peripheral blood lymphocytes on EAC and RE rosette formation is shown in table 3. The RE rosettes in the blood of deficient guinea-pigs were significantly increased when the lymphocytes had been pre-treated with biotin; the number increased following the increase of biotin in the incubation medium (20 µg and 100 µg per ml of lymphocyte suspension): the values come within the range of values shown by normal guinea-pigs. No increase of the RE rosettes was observed when the peripheral blood lymphocytes from normal guinea-pigs were pre-treated with biotin. The percentage of EAC rosettes of biotin-treated lymphocytes was increased when the peripheral blood lymphocytes from either normal or biotin-deficient guinea-pigs were utilized. A slight but statistically significant increase of EAC rosettes was observed when the peripheral blood lymphocytes from normal animals were pre-treated with biotin at a concentration of 100 µg/ml of lymphocyte suspension.

On the lymphocytes of the peripheral blood from biotin-deficient animals the various concentrations of biotin had an enhancing effect on EAC rosette formation. The percentage of EAC rosettes reached the normal values when the lymphocytes were pre-treated with biotin at a concentration of 100 µg/ml of lymphocyte suspension. Such results confirm once again the importance of biotin for the defence mechanisms of the organism: this is probably related to a regulatory activity of biotin on the biochemical pathways[16].

1 A.E. Axelrod, Nat. Vitam. Found. Nutr. Symp. Ser. *5*, 27 (1952).
2 A.E. Axelrod and J. Pruzansky, Ann. N.Y. Acad. Sci. *63*, 202, 209 (1955).
3 B.B. Carter and A.E. Axelrod, Proc Soc. exp. Biol. Med. *67*, 416 (1948).
4 F. Petrelli and G. Marsili, Acta Vitam. Enzym. *23*, 86 (1969a).
5 F. Petrelli and G. Marsili, Boll. Soc. ital. Biol. sper. *46*, 71 (1969b).
6 F. Petrelli and G. Marsili, J. reticuloendoth. Soc. *9*, 86 (1971).
7 F. Petrelli and G. Marsili, Boll. Soc. ital. Biol. sper. *49*, 1103 (1973).
8 F. Petrelli, G. Marsili and G. Centioni, Boll. Soc. ital. Biol. sper. *49*, 1109 (1973).
9 M.C. Coots, A.E. Harper and C.A. Elvehjem, J. Nutr. *67*, 525 (1959).
10 A. Boyum, Scand. J. clin. Lab. Invest. *21*, suppl. 97 (1968).
11 G. Sandberg, O. Söder and V. Ernström, Int. Archs Allergy appl. Immun. *50*, 374 (1976).
12 G. Michlmayr and H. Uber, J. Immun. *105*, 670 (1970).
13 A.B. Wilson and R.R.A. Coombs, Int. Archs Allergy appl. Immun. *44*, 544 (1973).
14 F. Petrelli, F. Farabollini and G. Marsili, Boll. Soc. ital. Biol. sper. *45*, 270 (1968).
15 F. Petrelli, G. Marsili and I. Santarelli, Boll. Soc. ital. Biol. sper. *50*, 1095 (1974).
16 F. Petrelli, P. Moretti and M. Paparelli, Molec. Biol. Rep. *4*, 247 (1978).

Effect of starvation on the blood of *Ophiocephalus punctatus* (Bloch)

M.N. Raizada and C.P. Singh[1]

Department of Zoology, Agra College, Agra 282002, U.P. (India), 5 December 1980

Summary. During starvation the values of RBC, WBC counts, Hb content and packed cell volume declined. After 15, 21 and 27 days of starvation little fluctuation was noted but major fluctuation was recorded after 33 days of starvation in all the above parameters.

The physiological response of an animal to fasting is closely related to its nutritional state, which alters from time to time. Starvation affects the normal body metabolism and if it is prolonged may even cause the death of the animal. Decline in the various body constituents of fish, after starvation, have been reported by several authors[2-4]. However, the effect of starvation on the blood parameters of fish has attracted little attention[5-7]. This paper deals with the effect of starvation on the blood of the fresh water fish *Ophiocephalus punctatus* (Bloch). The fish were starved for 33 days.

Material and methods. The fish, *Ophiocephalus punctatus* were procured from the local fish market during the month of November, 1979 and were kept in aquaria for acclimation for 7 days. After feeding daily for 7 days the fish were starved. Blood samples were collected after 15, 21, 27 and 33 days. The blood was obtained from the caudal vein and transferred into double oxalated vials. For the total RBC and WBC counts a haemocytometer was used. Haemoglobin (Hb) was estimated by Sahle's haemometer. The ultra micro-method as described by Natelson[8] was applied for the estimation of packed cell volume (PCV). Blood samples collected from normally-fed fish served as controls.

Observations. In the present study notable variations in the total RBC and WBC counts, Hb content and PCV of the starved fish were observed. The normal values of these in the control fish were: RBC, $3.31 \times 10^6/mm^3$; WBC, $13000/mm^3$; Hb, 15.0 g%, and PCV, 49.23%. A direct correlation was found between the values of the above parameters and periods of starvation, i.e. values gradually decreased with the increasing period of starvation (table). The values declined gradually in the beginning, i.e. after 15, 21 and 27 days but a sudden fall was observed after 33 days of starvation (table).

Discussion and conclusions. In the present study the values of RBC and WBC counts, Hb content and PCV decreased with an increase of the starvation period. The values showed a gradual decline of all parameters during a period of 27 days of starvation but a sudden fall was observed after 33 days of starvation (table).

Smallwood[9] observed that the red blood cell count of *Amia calva* dropped from $1640000/mm^3$ to $400000/mm^3$ after starvation for 20 months. Murachi[10] found a decrease in PCV from 50% to 30% and a corresponding reduction in Hb from 11 to 7 g% in *Cyprinus carpio.* Higginbotham and Meyer[6] also observed a fall in PCV and Hb content in *Ictalurus lacustris punctatus,* when fishes were in poor condition (emaciated). Thus, it may be suggested that with a variable period of starvation the RBC count and Hb content also decrease accordingly.

Effect of starvation on total RBC and WBC counts, Hb content and PCV of *Ophiocephalus punctatus* (Bloch) during different time periods

Constituents	Control	Starvation time 15 days	21 days	27 days	33 days
Total RBC count (10^6/mm^3)	3.31±0.05 (10)	2.88±0.16 (6)	2.47±0.10 (5)	2.40±0.14 (4)	0.68±0.07 (4)
Total WBC count (per mm^3)	13000±176.62 (10)	7900±278.95 (6)	7600±256.06 (5)	6400±147.42 (4)	5800±138.26 (4)
Haemoglobin content (g %)	15.0±0.34 (10)	13.8±0.33 (6)	10.8±0.05 (5)	10.6±0.69 (4)	8.0±0.22 (4)
Packed cell volume (%)	49.23±1.50 (10)	47.38±0.12 (6)	37.36±0.46 (5)	36.66±1.24 (4)	30.00±0.09 (4)

All values are means±SEM. Number of fish in parentheses.

Smirnova[11] reported that white blood cells (WBC) were reduced in number during starvation of a fresh water fish, *Lota lota.* A decline in red cell number was observed in the beginning, but later on, due to prolonged starvation, the number of RBC increased. This he accounted for by the decreased volume of blood, which was a result of prolonged starvation. The restoration of feeding in this case led to a further decline in red cell count, since blood volume increased on the availability of food.

Changes in red, white and immature blood cell numbers during starvation have also been reported in *Salmo gairdnerii* by Kawatsu[12]. His results also reveal that the decline in red cell count was preceded by an increase. So it may also be suggested that with prolonged fasting the volume of the blood decreases resulting in the increase of the number of blood cells in a unit area. But after prolonged fasting when food is given it again increases the blood volume which makes the number of blood cells less in a unit area. Constant availability of normal food probably accelerates the division of blood cells in the bone marrow, which results in the increase of blood cells to the normal level. This normal condition is not possible during the starved (ill health) condition of the fish.

1 Thanks are due to CSIR, New Delhi, for financial assistance.
2 Y. Creach and A. Serfaty, C. r. Séanc. Soc. Biol. *159*, 483 (1965).
3 I. Robertson, R.M. Love and W.P. Cowie, J. Sci. Fd Agric. *18*, 563 (1967).
4 Y. Creach, D. Sc. thesis, University Paul Sabatier, Toulouse/France.
5 S.H. Khan, Indian J. Zool. *2*, 59 (1974).
6 A.C. Higginbotham and D.K. Meyer, Q.J. Fla Acad. Sci. *11*, 119 (1948).
7 L.I. Smirnova, Vop. Ikhtiol. *4*, 677 (1962).
8 S. Natelson, Am. J. clin. Path. *21*, 1153 (1951).
9 W.M. Smallwood, Biol. Bull. mar. Biol. Lab., Woods Hole *31*, 453 (1916).
10 S. Murachi, J. Fac. Fish. Anim. Husb. Hiroshima Univ. *2*, 241 (1959).
11 L.I. Smirnova, Dokl. Proc. Acad. Sci,USSR, biol. Sci,*160*, 107 (1965).
12 H. Kawatsu, Fish. Res. Lab., Tokyo *15*, 167 (1965).

Altered circadian rhythm of catecholamines in patients with apallic syndrome[1]

D. Ratge, E. Knoll, U. Diener, A. Hadjidimos[2] and H. Wisser

Department of Clinical Chemistry, Robert-Bosch-Krankenhaus, Auerbachstrasse 110, D-7000 Stuttgart 50 (Federal Republic of Germany), and Department of Neurosurgery, Katharinen-Hospital, D-7000 Stuttgart (Federal Republic of Germany),
27 November 1980

Summary. Circadian rhythms of catecholamines were investigated in 4 healthy subjects and in 6 patients suffering from an apallic syndrome. The clinical picture of this syndrome is characterized by disturbed consciousness (coma vigile), by suspension of the sleeping and waking rhythm, by lack of emotional reactions and by appearance of primitive motor patterns. 5 of the 6 apallic patients showed an abolished rhythmicity compared with the control group. These results were interpreted as an indication that endogenous, centrally controlled processes are the cause of circadian rhythms.

Circadian rhythms are the expression of endogenous processes, which have the characteristics of self-sustained oscillations. They are influenced by exogenous periodic factors (=Zeitgeber) such as light-dark cycles, environmental temperature and social cues[3-5]. These factors cause the period of the biological rhythm - which is about 24 h (=circadian) after the elimination of all Zeitgebers - to adapt to the period of the environmental periodicity which is exactly 24 h[6]. In patients suffering from an apallic syndrome, the functions of the brain stem are retained. Traumatic, hypoxic or inflammatory processes lead to a disintegration of the cerebrum functions to the level of the mesencephalon[7]. The clinical symptoms of the apallic syndrome are: disturbed consciousness, suspension of the sleeping and waking rhythm with irregularly distributed waking and sleeping phases over the 24-h day, lack of emotional reactions, stretched position of torso and extremities and appearance of primitive motor patterns. An investigation of such patients with regard to circadian rhythms is of interest in view of the possibilities that either the influence of exogenous factors has been abolished or that the center responsible for the rhythms has been damaged. Another possibility might be the impairment of the afferent tracts, which lead to the suprachiasmatic nuclei as putative circadian oscillators[8].

4 healthy male subjects (24-29 years of age) and 6 patients