PEROXIDASE ACTIVITY OF LEUCOCYTES IN DIFFERENT ANIMALS AND MAN*

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Peroxidases hold an important position in oxidation and reduction of enzymes (1,4). These are widely distributed in plant and animal kingdom but their occurrence in animal tissues is limited (4). Chemically, peroxidases contain ferri-proto-porphyrin ix (haemin) as the prosthetic group and show a characteristic absorption spectra. They act by electron transfer from the substrate H_2O_2, reducing it to water and releasing oxygen which oxidises compounds like benzidine, if present, to different coloured compounds. This fact has been exploited for evolving a very sensitive test for the presence of blood in urine, faeces etc. and also for histochemical location of peroxidases in leucocytes and thyroid etc.

Cytoplasmic granules of granulocytes exhibit strong peroxidase activity, a fact which is utilised in the histochemical identification of this series of cells (2). A good amount of work has been done in man on peroxidase activity in leucocytes but very little information is available on this test in animals (2,4) except in sheep (3). The investigation reported here is a comparative study of peroxidase activity at different developmental stages in various species of animals and man.

MATERIALS AND METHODS

Experiments were performed in rats, rabbits, pigs, pigeons, fowls, frogs and men at four different stages of thier respective development as indicated in Table I. In each species at each developmental stage, three individuals were taken. Three smears from each individual were prepared, studied and the peroxidase activity per cell for different types of leucocytes studied.

Blood smears were prepared from tail tips in rats, from ear tips in rabbits and pigs, from wing veins of pigeons and fowls, from cutaneous veins in case of frogs and from flnger tips in the humans. Samples from males and females were taken in the morning and evening to study sex and diurnal variations. The smears were immediately stained as per method given below and were then examined under oil immersion for peroxidase activity in the leucocytes. Method of the staining used in this study was similar to the one described by Kaplow (5).

* Received 22-9-1970.

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TABLE I

Selection of material for study according to the stages of development in various animals and man.

Species	*Infant*	*Young*	*Adult*	*Old*
Rat	Upto 21 days	Upto 50 days	After 60 days	After 1.5 years.
Rabbit	1 week	Upto 8 weeks	4-12 months	After 1 year
Pig	8 weeks	8 weeks—4 months	After 8 months	After 4 years
Man	2 years	8-19 years	20-40 years	Above 40 years
Pigeon	After 3 weeks	1-2 years	3-6 years	After 8 years
Fowl	8 weeks	Upto 6 months	1-2 years	After 2 years
Frog	Above 20 days	2-3 months	6 months—1 year	After 2 years

STAINING PROCEDURE :

1. Prepare a thin smear of blood on a clean slide.
2. Cover it with 0·5% copper sulphate solution and allow it to stand for 25 seconds.
3. Pour the copper sulphate solution and cover the slide with 0.1% benzidine solution and 4% hydrogen peroxide. Keep it for 5 minutes.
4. Pour the benzidine solution and wash the slide with glass distilled water.
5. Cover the slide with diluted Giemsa's stain (1:1) and allow it to stand for 30 minutes.
6. Wash the slide with glass distilled water, dry and examine under oil immersion.

RESULTS

The peroxidase activity per cell type is shown in Table II. Calculation of the peroxidase activity was based on the intensity and size of the stained spot within the cells.

There was no difference in the peroxidase activity of cells in the blood smears taken from male or female rats. Similarly no significant variation in the peroxidase activity was observed when samples were taken from the same animal at different times during the day. Fig. 1 depicts the average peroxidase activity in the eosinophils at different developmental stages in various animal species investigated in this study. Maximum activity per cell in most of the species was found to be in the eosinophils. Basophils and neutrophils showed equal activity (+2) in rat, rabbit and in pig. In man, however, the peroxidase activity was higher in basophils (+3) than in neutrophils (+2).

TABLE II

Peroxidase activity per cell type in different leucocytes at different developmental stages of various animals and man.

Animals	*Stages of development*	*Cells*				
		Eosinophil	*Basophil*	*Neutrophil*	*Lymphocyte*	*Monocyte*
Rat	Infant	2.3	2.0	2.0	—	1.0
	Young	3.0	2.0	2.0	—	1.0
	Adult	3.0	2.0	2.0	—	1.0
	Old	2.3	2.0	2.0	—	1.0
	Average	2.66	2.0	2.0	—	1.0
Man	Infant	3.0	2.3	2.0	—	1.0
	Young	3.6	3.0	2.0	—	1.0
	Adult	4.0	3.0	2.0	—	1.0
	Old	4.0	3.0	2.0	—	1.0
	Average	3.66	2.8	2.0	—	1.0
Rabbit	Infant	2.3	2.0	2.0	—	1.0
	Young	3.0	2.0	2.0	—	1.0
	Adult	4.0	2.0	2.0	—	1.0
	Old	4.0	2.0	2.0	—	1.0
	Average	3.33	2.0	2.0	—	1.0
Pig	Infant	2.3	2.0	2.0	—	1.0
	Young	4.0	2.0	2.0	—	1.0
	Adult	3.3	2.0	2.0	—	1.0
	Old	3.0	1.3	1.3	—	1.0
	Average	3.16	1.8	1.8	—	1.0
Pigeon	Infant	1.0	—	—	—	—
	Young	1.0	—	—	—	—
	Adult	1.0	—	—	—	—
	Old	1.0	—	—	—	—
	Average	1.0	—	—	—	—
Fowl	Infant	0.6	—	—	—	—
	Young	1.0	—	—	—	—
	Adult	1.0	—	—	—	—
	Old	0.3	—	—	—	—
	Average	0.75	—	—	—	—
Frog	Infant	0.3	—	—	—	—
	Young	1.0	—	—	—	—
	Adult	1.0	—	—	—	—
	Old	1.0	—	—	—	—
	Average	0.83	—	—	—	—

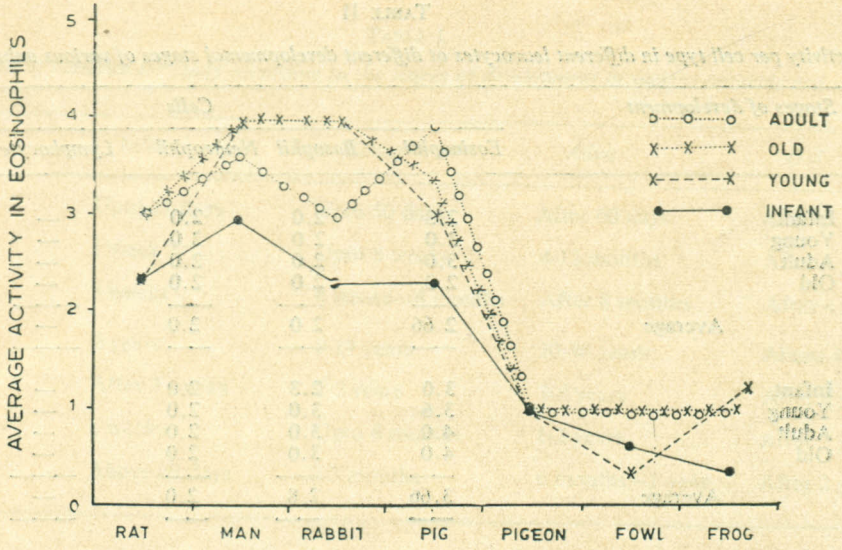

Fig. 1

Average peroxidase activity per eosinophil in the stained blood films taken from various animals and man at four different stages of development.

Monocytes in the blood smears of these animals exhibited only a uniformly small peroxidase activity (+1). No activity was detectable in the lymphocytes of any species including man. It is also worth noting that in frog, pigeon and fowl only the eosinophils showed the peroxidase reaction. In adult and old rats eosinophils showed an average activity of (+3) per cell. In the infant and young rat, however, the eosinophil peroxidase activity was low. Peroxidase activity was maximum (+4) in young and old men. It was lower in infants and adults (+3 to +3.5) Similar was the case with respect to rabbits where the activity varied between +4 in old rabbits and +2 in infant rabbits. Similarly, age dependent variation was also found in the peroxidase activity of eosinophils in the blood smears taken from pigs. It may also be noted from Figure 1 that the peroxidase activity in the pigeons, fowls and frogs of any age was the lowest and barely detectable.

DISCUSSION

It is known that the peroxidase reaction is positive in the presence of tyrosine (4) or histidine (1). This technique is therefore used to see the relative concentration of these substances in different tissues e.g. tyrosine in sections of thyroid gland, formation of thyroxine from diiodotyrosine *in vitro* and for histidine to histamine in case of different leucocytes. In this study peroxidase reaction was found to be positive in the eosinophils but negative in the lymphocytes of all the species investigated. It indicates the importance of histamine dynamics in the development of biological functions of the eosinophils.

In clinical cases of allergy, there is a frequent rise of eosinophil count in blood. Maximum concentration of histamine is found in eosinophils. It is possible therefore that increased

quantity of eosinophils aims at storing the histamine in its intrinsic form during these conditions. This may be a defensive mechanism employed by the body to keep a check on sudden increase in the extrinsic histamine.

During stress or emergency the eosinophils disappear from the blood to be trapped in the reticuloendothelial system. According to Selye (6) a large concentration of histamine in these cells suggests their role of releasing histamine in the General Adaptation Syndrome. Thus the other function of eosinophils may therefore be to release histamine suddenly at the time of need at other places in the body. In this study it was found that the peroxidase activity is lowest in the eosinophils of amphibians and avians indicating perhaps the inadequacy of intrinsic mechanisms or comparative lack of capacity for adaptation to stress in external or internal environments in these species. On the other hand it is also known that these species have in their blood the RBCs which are nucleated and which show peroxidase reaction. The property of reacting favourably to stresss and during allergic conditions if these occur, therefore, may be shared by the nucleated RBCs and the leucocytes in these species.

Considering the immense amount of peroxidase reactivity in the leucocytes of human beings and their equally great capacity to withstand stress, the peroxidase activity may be considered yet another scale to evaluate the evolution of biological processes.

SUMMARY

The peroxidase activity of leucocytes was studied in various species of animals at different stages of their development. Lymphocytes showed no peroxidase activity in any species. Eosinophils showed this activity in all the animals. In human beings this activity was also present in basophils, neutrophils and monocytes. In frogs and birds the peroxidase activity of leucocytes was the lowest and was present only in eosinophils. In general it was seen that the peroxidase activity was lower in the infants of rat, man, rabbit and pig than the adults of these species.

REFERENCES

1. Bell, G.H. and J.N. Davidson. *A Text Book of Physiology and Biochemistry*, London., E. & S. Livingstone Ltd., 1961, pp. 82.
2. Cantaraw, A. and B.S. Chepartz. *Biochemistry*, Philadelphia., W.B. Saunders Co., Maruzen Asian Edition, 1962, pp. 388, 804.
3. Duale, P.V. Peroxidase reaction of sheep leucocytes. *Biol. Int. Conf. Col. Vet. Est.*, 7 : 821, 1960.
4. Everson Pearse, A.G. *Histochemistry*, London. J. and A. Churchill, Ltd., 1960, pp. 516.
5. Kaplow, L.S. Simplified myeloperoxidase stain using benzidine dihydrochloride. *Blood*, 26 : 215, 1965.
6. Selye, H. The general adaptation syndrome and the diseases of adaptation. *J. Clin. Endocr.*, **6** : 117, 1946.