THE DISTRIBUTION OF ANEURINPYROPHOSPHATE BETWEEN THE NUCLEUS AND THE CYTOPLASM OF CHICKEN ERYTHROCYTES*

by

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In an earlier report[1] we described experiments which proved that the nucleated red cells occurring in the peripheral blood of patients suffering from erythroblastosis foetalis contain about 100 times as much aneurinpyrophosphate (APP) as the erythrocytes of normal human blood. Normal nucleated leucocytes contain about 200 times as much APP as the normal non-nucleated erythrocytes. We further found 2.6 γ APP per 10^{11} red cells and 35 γ APP per 10^{11} white cells in chicken blood, which contains nucleated red cells. Hence the ratio of the contents of a white cell and a red cell was only about 13, while in normal human blood this is 200 and in normal rat blood 160.

The fact that the difference in APP content between a nucleated red cell and a nucleated white cell is much smaller than that between a non-nucleated red cell and a nucleated white cell points to some relation between the presence of a nucleus in a blood cell and the APP content of this cell.

In order to determine the contribution of the nucleus of the chicken erythrocyte to the APP content of the whole cell it is necessary to separate the nuclei from the cytoplasm. During haemolysis most of the cytoplasm leaves the cells but a larger or smaller part of the stroma and the cytoplasm, depending upon the method employed, remains bound to the nuclei and cannot be separated from them by centrifuging, not even at high rates.

According to DOUNCE AND LAN[2] the nuclei of chicken erythrocytes can be obtained by adding 1/20th volume of a 6% solution of saponin in 0.11 mol phosphate buffer, p_H 6.9, to a suspension of washed erythrocytes in 0.4% NaCl solution and centrifuging the haemolysate at high speed. We have tried this procedure. The sediment which we obtained was stained according to the MAY GRÜNWALD-GIEMSA method. The nuclei appeared to be exclusively present in a swollen state and were clumped together to form a compact mass.

When whole blood was treated in the same way, instead of washed erythrocytes,

* This work forms part of the investigations on aneurin metabolism by H. G. K. WESTENBRINK and collaborators.

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TABLE I

APP CONTENT OF HAEMOLYSED RAT BLOOD AND OF THE FRACTIONS OBTAINED BY CENTRIFUGING

	γ APP per 100 ml	
	I	II
Haemolysate	22.5	(18.2)*
Supernatant fluid	21.8	15.0
Sediment	23.4	15.6
Washed sediment	—	0.4

TABLE II

APP CONTENT AND VOLUME OF HAEMOLYSED CHICKEN BLOOD AND OF THE FRACTIONS OBTAINED BY CENTRIFUGING (CHICKENS I TO IV)

A. EXPERIMENTAL DATA

	Volume in ml				γ APP per 100 ml			
	I	II	III	IV	I	II	III	IV
Haemolysate	14.55	8.25	10.7	9.4	(4.7)	(7.1)	(6.4)	5.0 (6.2)*
Supernatant fluid	9.55	5.9	8.2	7.2	1.3	2.0	1.35	1.65
Sediment	5.0	2.35	2.5	2.2	8.8	15.4	14.6	16.7
Washed sediment	—	—	—	2.0	—	—	—	12.4

B. CALCULATIONS (ALL FIGURES ARE RELATED TO 100 ml BLOOD)

	I	II	III	IV
a. γ APP in haemolysate	(6.8)	(5.8)	(6.8)	4.8 (5.8)*
b. γ APP in supernatant fluid	1.24	1.18	1.11	1.18
c. γ APP in sediment	4.40	3.62	3.65	3.60
d. Volume of the non-nuclear part of the sediment, in ml	45	20	21	18
e. γ APP in the non-nuclear part of the sediment	0.58	0.40	0.28	0.29
f. γ APP in the nuclei (c—e)	3.82	3.22	3.37	3.31
f_1 γ APP in the washed sediment	—	—	—	2.50
g. γ APP in the cytoplasm (b + e)	1.82	1.58	1.39	1.47
h. Part of the total cell APP, localized in the nucleus	68%	67%	71%	69%

* The APP is gradually decomposed after haemolysis. When the haemolysate was deproteinized immediately after haemolysis was complete, a higher APP content (figures between brackets) was observed than was calculated from the APP contents of supernatant fluid and sediment of the centrifuged haemolysed blood. When part of the haemolysed blood was not centrifuged and deproteinized simultaneously with supernatant and sediment of the centrifuged part of the haemolysate, the determined and calculated values of the APP content of the total haemolysate were equal to each other. Obviously the difference in the first instance is caused by decomposition of the APP during centrifuging.

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we obtained beautiful nuclei of natural size. When they were washed with 0.9% NaCl solution, as recommended by DOUNCE AND LAN they swelled again.

Independent of the method used the volume of the sediment which contained the nuclei was about 20 to 30% of the volume of the blood, a much larger volume than could be occupied by the nuclei alone. When we subjected rat blood to the same treatment, a sediment was obtained with about the same volume, but which appeared to be optically empty on microscopical examination. So undamaged nuclei, completely separated from other material, could not be obtained. The only possibility appeared to be to make a reliable estimate of the APP content of the non-nuclear part of the sediment and to substract this from the total APP content of the sediment.

Therefore we first studied the distribution of APP between supernatant fluid and sediment of centrifuged haemolysed rat blood. Haemolysis was effected as described above. The haemolysate was centrifuged at about 18 000 r.p.m. (diameter of conical centrifuge 6 cm). The APP contents of the whole haemolysed blood, the sediment and the supernatant were estimated by the method of WESTENBRINK *et al.*[3], slightly modified for this special purpose. The results are assembled in Table I. We see that before washing the sediment the APP is equally distributed over sediment and supernatant. These data suggest that the sediment consists of swollen stromata imbibed with diluted cytoplasm. The latter can be removed by washing. The remaining material only contains a negligible amount of APP. We have presumed that the great bulk of the sediment obtained by similar treatment of chicken blood also consists of swollen stromata imbibed with diluted cytoplasm, in which the nuclei are imbedded. Then it is possible to calculate the APP content of the nuclei from the difference between the APP contents of the non-washed sediment and the supernatant. The results of the determinations in 4 samples of chicken blood and the pertaining calculations are given in Table II.

It appeared that about 70% of the APP of the chicken erythrocyte is concentrated in the nucleus, which according to PONDER[4] only occupies about 10% of the cell volume. The concentration of APP in the nucleus thus appears to be about 20 times as high as in the cytoplasm.

SUMMARY

The nuclei of chicken erythrocytes contain about 20 times as much aneurinpyrophosphate per unit volume as the cytoplasm.

RÉSUMÉ

Les noyaux des érythrocytes de Poulet contiennent environ 20 fois plus de pyrophosphate d'aneurine par unité de volume que le cytoplasme.

ZUSAMMENFASSUNG

Die Kerne der roten Blutkörperchen vom Huhn enthalten ungefähr 20 Mal so viel Aneurinpyrophosphat pro Volumeinheit als das Cytoplasma.

REFERENCES

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