characteristics of erythrocytes of different animals. GOODWIN[2] using the PCV in the calculation of intracellular glucose has drawn important conclusions which might have been different if observed haematocrit values had been corrected for plasma trapping in accordance with the species of blood used. Similarly, failure to take account of the typical amount of plasma trapping in estimations of ionic concentrations, glycolytic rate and other characteristics of erythrocytes, when calculations involve the use of observed PCV, could lead to erroneous results. This is especially true for goat blood which was found to contain a considerable amount of plasma in the packed red cell column.

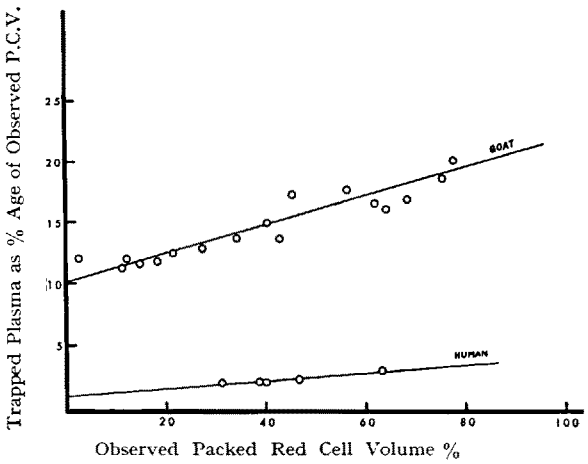

Fig. 1. – The amount of plasma in the packed red cell column of goat and of human blood expressed as a percentage of the observed packed red cell column.

Plasma trapping was estimated as described by CHAPLIN and MOLLISON[3] with slight modifications. Preliminary investigation showed that goat erythrocytes were not affected by the high concentrations of EVAN'S Blue dye used[4]. Plasma trapping in the packed red cell column of fresh heparinized goat and human blood centrifuged in Wintrobe tubes at 1500 X G for 55 min varied directly with the height of the red cell column (Fig. 1).

The regression equation in the case of human blood was:

$$Y = 0{\cdot}352\,X + 0{\cdot}839,$$

and in the case of goat blood:

$$Y = 0{\cdot}119\,X + 10{\cdot}22,$$

where Y is the percentage plasma trapping and X the observed PCV as a percentage of whole blood. At an haematocrit level of 45% trapping for human blood was 2·4%, but for goat blood it was 15·6%.

It was possible to increase the degree of packing of red cells by increasing the time and force of centrifugation. This is demonstrated in Figure 2 which shows percentage decrease in observed PCV plotted against total force or 'impulse'[5] measured in dyne-seconds. Actual determinations showed that this decrease was the result of diminishing amounts of trapped plasma and not of loss of cell contents. However, it would be impracticable to increase centrifugation to bring about the same degree of packing of goat cells as of human cells centrifuged at 1500 X G for 55 min. It is therefore important when

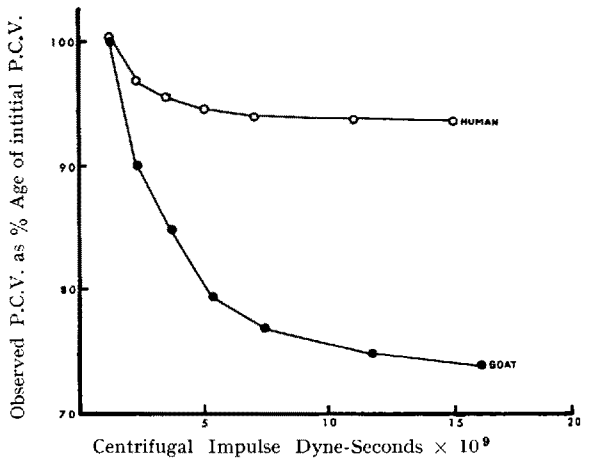

Fig. 2. – The effect of varying centrifugal force on the observed packed red cell volume of human and of goat blood.

centrifuging under usual laboratory conditions to make an accurate allowance for plasma trapping according to the species of blood under investigation.

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Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London, December 17, 1956.

Zusammenfassung

Nach Zentrifugieren unter gleichen Bedingungen enthält bei Ziegenblut die Erythrozytensäule ungefähr siebenmal soviel eingeschlossenes Plasma wie bei Menschenblut. Mit Hilfe der angegebenen Formeln können diese Plasmamengen berechnet werden; dabei werden die Resultate in Hämatokritwerten ausgedrückt. Es ist wichtig, bei der Berechnung der Eigenschaften von Erythrozyten diese Unterschiede zwischen den verschiedenen Spezies in der Menge des zurückgehaltenen Plasmas zu berücksichtigen.

The Biosynthesis of 6-Azauracil Riboside by *Escherichia coli* Growing in the Presence of 6-Azauracil

Recently we have found that 6-azauracil (3, 5-dioxo-2, 3, 4, 5-tetrahydro-1, 2, 4-triazine) inhibits the growth of a number of microorganisms (ŠORM and ŠKODA[1]; cf. also HANDSCHUMACHER and WELCH[2]). We further found that the inhibitory effect on the growth of *E. coli* can be completely removed by uracil, cytosine, or uridine, and that the inhibition is strictly competitive in character[3]. We

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Dependence of 6-azauracil riboside production and culture growth on the concentration of 6-azauracil

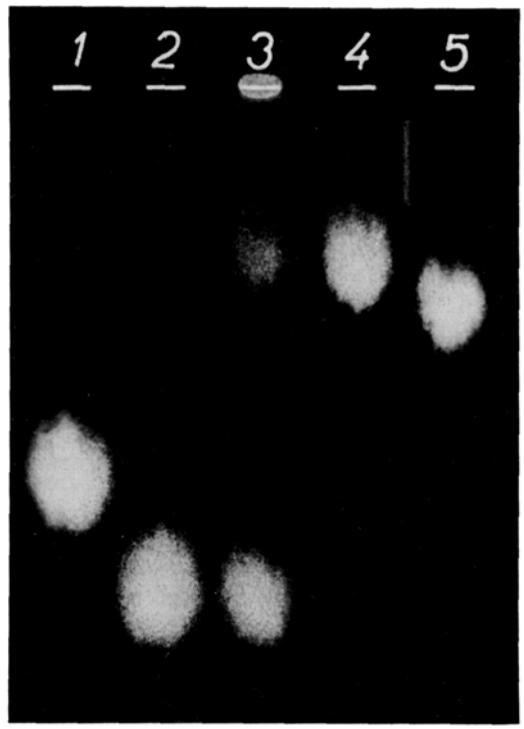
Molarity of 6-azauracil	Growth (as percentage of maximum growth)	6-Azauracil riboside (as percentage of maximum production)
0	100	0
2×10^{-4}	58	29
4×10^{-4}	50	56
6×10^{-4}	44	85
8×10^{-4}	39	88
10^{-3}	37	100

have further been able to show that 6-azauracil has a cancerostatic effect on a number of experimental tumours (ŠORM, JAKUBOVIČ, and ŠLECHTA[4]; cf. also HAKALA, LAW, and WELCH[5], SABLÍK and ŠORM[6]).

Some 'unnatural' purine and pyrimidine bases are known to be incorporated into nucleic acids by various biological systems (MATTHEWS[7], ZAMENHOF and GRIBOFF[8]) evidently by way of the corresponding ribosides or desoxyribosides. Nucleosides of such 'unnatural' analogues have hitherto been isolated from nucleic acids only in the case of 5-bromouracil[9]; from 6-azathymine, a desoxyriboside was obtained by a transdesoxyribosidation reaction[10]. HANDSCHUMACHER and WELCH further found[2] that resting cells of *Streptococcus faecalis* incubated with 6-azauracil and thymidine accumulated a compound which had the properties expected of 6-azauracil desoxyriboside.

We have now found that cultures of *E. coli* B growing on media containing glucose and inorganic salts only, with the addition of sub-bacteriostatic concentrations of 6-azauracil, accumulate 6-azauracil riboside in the medium. The gradual disappearance of 6-azauracil from the medium, and the accumulation of its riboside, were followed by paper chromatography. The amount of the riboside formed at various concentrations of 6-azauracil, and the corresponding intensities of growth of the cultures, are recorded in the table.

With a view to isolating the 6-azauracil riboside, we carried out the stationary cultivation of *E. coli* in 10 l of medium containing a 8×10^{-4} *M* concentration of 6-azauracil, from a large inoculum. After removal of the cells, the azauracil riboside together with unchanged azauracil was adsorbed on active charcoal, eluted with 50% ethanol containing 1% ammonia and the residue obtained on evaporation of the eluate chromatographed on a column of cellulose powder with *n*-butanol saturated with water as the eluent. By this procedure, a sharp separation of 6-azauracil from its riboside was achieved. The 6-azauracil riboside was purified by a second chromatography in the same system and analyzed (Analysis, Calculated for $C_8H_{11}N_3O_6$: C 39·18%; H 4·52%; N 17·13%; Found: C 38·81%; H 4·82%; N 16·84%). Phosphorus was absent. In paper electrophoresis, the isolated product behaved as a homogeneous substance resembling uridine in its electrophoretic mobility. The



Chromatogram of a hydrolysate of 6-azauracil riboside (photographed by UV-light). Whatman No. 1 paper, developed with *n*-butanol saturated with water. *1* uracil, *2* 6-azauracil, *3* hydrolysate of 6-azauracil riboside (4 N–HCl, 15 h, 105°C), *4* 6-azauracil riboside, *5* uridine.

ultraviolet spectrum of 6-azauracil riboside shows $\lambda_{max.}$ 262 mμ, ε $6{\cdot}1 \times 10^{-3}$ (in water, while 6-azauracil has $\lambda_{max.}$ 258 mμ, ε $5{\cdot}4 \times 10^{-3}$, in water). After hydrolysis with 4 N–HCl and chromatography a single base, identical with 6-azauracil, was detected (figure); chromatographic analysis after hydrolysis with N–H_4SO_4 revealed ribose as the sole sugar component.

Note added in proof: 6-azauracil riboside has now been obtained crystalline, m.p. 160–161°C.

This work and further experiments will be described in detail in the Collection of Czechoslovak Chemical Communications.

J. ŠKODA, V. F. HESS, and F. ŠORM

Department of Biochemistry, Institute of Chemistry, Czechoslovak Academy of Science, Prague, December 13, 1956.

Zusammenfassung

Bei der Kultivierung von *E. coli* in 6-azaurazilhaltigem Medium wird ein neuer Stoff akkumuliert, der als 6-Azaurazilribosid identifiziert wurde.

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