Isolation of Thorium B (Lead)-binding Substance from the Erythrocytes of Rabbit Blood

THE distribution of lead in blood fractions has been investigated by several workers. Recent studies made by using lead salts labelled with thorium B [1,2], and with radium D [3], showed that the lead is mainly held by the erythrocytes. The present communication deals with the isolation of the component mainly responsible for the uptake of thorium B by the erythrocytes.

The method of activating blood with thorium B was essentially the same as that described by Alexander[2]. An oxygen stream striking the surface of radiothorium was led into 20 ml. freshly drawn rabbit blood (heparin was used as an anti-coagulant) for 30 min. After activating, the blood was shaken for 30 min., since the maximum uptake of thorium B by the erythrocytes takes place during this process[1,2].

The active blood was centrifuged and the plasma removed. The erythrocytes were washed thrice with an equal volume of cold 0·9 per cent sodium chloride. The washed erythrocytes were chilled. To the chilled mass, an equal volume of cold distilled water and an equal volume of cold ether were added and mixed well and centrifuged. The clear hæmolysate was drawn out with a capillary. When the hæmolysate was dialysed against distilled water, it was found that 61 per cent of the thorium B present in it was in a non-dialysable form and the rest was in a dialysable form. To the hæmolysate at 0° C., one-third its volume of absolute alcohol at − 10° C. was added. It was kept in the ice chest for 30 min. and centrifuged. The precipitate was washed with cold 25 per cent alcohol until the last washings were colourless. The precipitate was found to be fifteen times more active than the same weight of hæmolysate. The precipitate was insoluble in water, in mineral acids and 0·9 per cent saline, but was soluble in N/50 sodium hydroxide up to about 0·5 per cent. The biuret, Millon–Nasse, xanthoproteic and ninhydrin tests were positive. With Benedict's reagent it gave a green precipitate; but no sugars were detected when the material was subjected to paper chromatography according to the method of Partridge[4]. It had a very weak blood group B specificity when tested according to the method of Bray et al.[5].

The precipitate was dissolved in 10 ml. N/50 sodium hydroxide and the protein was denatured by shaking with Sevag's reagent. It was shaken with 2 ml. of the reagent, a mixture of chloroform and n-butanol (10 : 1), for 30 min. and centrifuged. This operation was repeated several times until the supernatant solution gave no reaction with ninhydrin. The active component was precipitated by passing carbon dioxide for 10 min. into the protein-free supernatant solution. The precipitate, separated by centrifugation, retained all the activity present in the supernatant solution. The precipitate was insoluble in water but was soluble in N/50 sodium hydroxide. It was dissolved in 10 ml. N/50 sodium hydroxide, and a few drops of ammonium hydroxide were added and then precipitated with 20 per cent lead acetate. The lead salt was washed four times with 5 ml. of distilled water each time and once with absolute alcohol. It was dried in vacuo over anhydrous calcium chloride. The analyses of the lead salt, kindly performed by Dr. Simon (Heidel-berg), were : C, 35·95 ; H, 4·88 ; N, 12·14 ; Pb, 26·08 per cent. 30 mgm. of the lead salt was suspended in 9 ml. of distilled water, acidified with 6N hydrochloric acid, hydrogen sulphide was passed to saturation and the suspension centrifuged. The supernatant solution was freed of hydrogen sulphide by bubbling nitrogen into it. It gave a positive Millon–Nasse reaction and a white precipitate with bromine water. It gave an orange colour with diazotized p-nitroaniline[6] and a blue colour with phosphotungstic–phosphomolybdic reagent[7]. These results suggest that the non-protein component is most likely a phenolic compound.

It appears from the above that the substance which is responsible for the uptake of 70 per cent of the non-dialysable thorium B present in the hæmolysate consists of a protein and a non-protein component. The non-protein component is most probably a phenolic compound.

Full details of this investigation will be published elsewhere. I wish to thank Prof. G. de Hevesy for his interest and encouragement during the course of this investigation.

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[1] Hevesy, G., Advancement of Science, 9, 57 (1952).
[2] Alexader, E., Ark. Kemi, 4, 363 (1952).
[3] Mortensen, R. A., and Kellog, K. E., J. Cell. and Comp. Physiol., 23, 11 (1944).
[4] Partridge, S. M., Biochem. J., 42, 238 (1948).
[5] Bray, H. G., Henry, H., and Stacey, M., Biochem. J., 40, 124 (1946).
[6] Theis, C. R., and Benedict, S. R., J. Biol. Chem., 61, 67 (1924).
[7] Rakestraw, N. W., J. Biol. Chem., 56, 109 (1923).

Fixation of Radioactive Colchicine by Ehrlich Ascites Carcinoma Cells

IN this communication we wish to report briefly some results on the fixation of radioactive colchicine by the cells of the ascitic form of Ehrlich carcinoma, which is one of a number of tumours used by us in our work with radioactive colchicine.

Ehrlich ascites carcinoma, known since 1932 [1], and recently described in detail by G. Klein[2], consists of a suspension of discrete tumour cells floating in ascitic fluid. This type of tumour was found by us to be well suited to study the fixation of radioactive colchicine by individual cells, as their number can easily be counted, and the use of radioactive colchicine affords a sensitive and accurate method of measuring the amount of the drug present in tumour cells.

Table 1. NUMBER OF MOLECULES OF RADIOACTIVE COLCHICINE PER CELL IN RELATION TO INJECTED DOSES

Exp.	No. of animals	Dose of colchicine given (mgm.)	Total number of ascites cells (millions)	Molecules per cell (calculated)
1	1	0·5	146	$4·6 \times 10^6$
	2	0·5	277	$7·0 \times 10^6$
	3	0·5	226	$7·2 \times 10^6$
	4	1·0	316	$5·8 \times 10^6$
	5	1·0	400	$5·1 \times 10^6$
	6	1·0	550	$5·3 \times 10^6$
	7	1·5	380	$7·3 \times 10^6$
2	1	1·24	436	$10·0 \times 10^6$
	2	1·24	102	$3·6 \times 10^6$
3	1	1·24	407	$13·5 \times 10^6$
	2	1·24	480	$17·9 \times 10^6$
	3	1·24	563	$3·4 \times 10^6$