Bone Marrow Sulphydryl Levels

DURING the course of other investigations the sulphydryl levels of rat bone marrow have been measured. Unlike other tissues examined, the bone marrow was found to show a high protein-bound sulphydryl level and a relatively low acid-soluble sulphydryl level. The investigation was extended to determine whether this unusual distribution of sulphydryl was a feature of other bone marrows.

All animal bone marrows were forced out of the femora of freshly killed animals by air pressure. The human specimens were obtained by sternal puncture. Estimations of total sulphydryl were made by the method of Calcutt and Doxey[1] using *p*-chloro-mercuribenzoic acid as the reactant. Acid-soluble sulphydryl was determined by the variation of the above method devised by Calcutt, Doxey and Coates[2], using trichloroacetic acid as the protein precipitant. The difference between the total sulphydryl and the acid-soluble sulphydryl levels as determined on two samples of the same marrow was taken as representing the protein-bound sulphydryl value.

The results for the animal experiments are shown in Table 1. It was noted that the rabbit marrow was extremely fatty in comparison with the others, and this may partly explain the lower total and protein-bound values obtained. It was also found that, proportionate to their size and weight, rats of the Wistar strain have considerably larger amounts of femoral marrow than do rats of the August strain.

Findings in respect of the human cases are given in Table 2. These samples were all taken from hospital patients (diagnoses are shown in the Table), and the possibility of the results being affected by the illness cannot be excluded. Nevertheless, the same general feature of high protein-bound sulphydryl and low acid-soluble sulphydryl is apparent.

The values for acid-soluble sulphydryl found in this work were lower than those previously obtained from animal livers but rather higher than those found for muscle, kidney, spleen or a range of animal tumours[3]. The present values are, however, considerably lower than those previously recorded by Lutwak-Mann for rat marrow (150–198 mg/100 g)[4]. This may arise from the fact that these last values were obtained by the non-specific iodometric method. An earlier investigation[5] gave 0·5 mg/100 g of glutathione for rabbit marrow. This is much lower than the present figures but, as no details of method were given, comment is not possible.

The protein-bound sulphydryl levels found in the present work are much higher than any previously found for other tissues. Liver has shown the highest values for normal tissues with levels up to about 7·5 μg sulphydryl per 100 mg wet weight[6], while one tumour has given 10·65 μg sulphydryl/100 mg wet weight[7]. This raises the question as to why bone marrow should have such unusually high levels of protein-bound sulphydryl. Possible explanations are: (1) marrow cells have protein with more than the normal number of cysteine residues; (2) the marrow cell protein has a greater than usual exposure of sulphydryl groups; or (3) the marrow cells contain much more protein than do other cells. Some further evidence is available in measurements of the total sulphydryl levels in denatured specimens of August bone marrow. A 1 per cent solution of 'Teepol' (Shell Chemical Co., Ltd.) was used to denature the homogenized marrow, this method having previously[6] been found to be a very effective one. Using marrow from six male rats, a mean value of 35·8 μg sulphydryl per 100 mg wet weight of tissue and a range of 24·8–50·9 were obtained. This value is only a little above the total sulphydryl values for male August rats and suggests that in bone marrow most of the protein-bound sulphydryl is normally exposed. This contrasts with previously[6] obtained values for liver and tumours where only one-twelfth to one-thirtieth of the protein-bound sulphydryl was normally found exposed.

These findings have a particular interest in respect of the great sensitivity of bone marrow to certain cytotoxic agents. It has already been suggested by Calcutt and Connors[7] that sensitivity to alkylating agents is correlated with a high ratio of protein-bound to acid-soluble sulphydryl. It now appears that bone marrow has such a high ratio and in fact one which is higher than for any other tissue so far examined. At the same time bone marrow sensitivity to alkylating agents is a limiting factor in their use.

Table 1. LEVELS OF SULPHYDRYL IN ANIMAL BONE MARROWS

Species	Sex	No. of samples	Total sulphydryl	Acid-soluble sulphydryl	Protein-bound sulphydryl
Rat (August)	M	10	29·9 (23·2–36·0)	5·9 (4·1–9·9)	24·0 (16·7–30·1)
Rat (August)	F	10	33·5 (29·3–39·0)	5·8 (2·3–8·4)	27·6 (21·6–33·6)
Rat (Wistar)	M	6	45·7 (40·6–52·3)	9·5 (6·0–13·1)	35·0 (28·6–44·5)
Rat (Wistar)	F	6	44·8 (31·1–53·7)	10·8 (7·7–14·0)	34·0 (19·7–41·3)
Guinea-pig	M	6	35·6 (28·9–46·1)	10·2 (7·6–12·3)	26·9 (18·5–35·4)
Rabbit	M	5	14·9 (12·3–16·2)	6·8 (3·5–9·5)	8·1 (6·1–9·8)

For each value the mean and range (in parentheses) are shown. All values are expressed as μg sulphydryl/100 mg wet weight of tissue.

Table 2. LEVELS OF SULPHYDRYL IN HUMAN BONE MARROW

Sex	Age	Diagnosis	Total sulphydryl	Acid-soluble sulphydryl	Protein-bound sulphydryl
M	57	Carcinoma of stomach	12·3	1·6	10·7
M	68	Lymphosarcoma	10·2	1·8	8·4
M	39	Melanoma	17·1	1·9	16·2
M	78	Mycosis fungoides	23·7	3·5	20·2
F	62	Plasmacytoma	15·9	1·3	14·6
F	47	Thyroid enlargement	18·6	3·1	15·5
F	20	Disseminated lupus erythematosus	9·2	2·1	7·1
F	78	Carcinoma of colon	9·4	1·1	8·3

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Normal Human Serum Fluoride Concentrations

RESULTS from a new method of analysis of fluoride from serum indicate that the generally accepted value for normal humans is too high by as much as a factor of ten. The average serum fluoride concentration of sixteen non-fasting individuals, all presumably drinking fluoridated water, was found to be 0·7 μM ± 0·4 *S.D.* (0·013 p.p.m.) and the average difference in duplicates was 0·18 μM. (The very low values found here for serum fluoride make p.p.m. an awkward unit. Therefore the shift has been made to the more useful and acceptable chemical concentration terms, mμmole (millimicromole) and μM (micromolar).) The generally accepted average serum fluoride value is 8 μM (0·15 p.p.m.) as found by Singer and Armstrong, regardless of the concentration of fluoride in the drinking water up to 130 μM (2·5 p.p.m.)[1].

The method is being published in detail elsewhere, but essential features are diffusion at 25° C from 3 N hydro-