LXXIV. THE METABOLISM OF BONE MARROW

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IN the course of work on histological changes in the bone marrow of rabbits, it became of interest for various reasons to know to what metabolic type this tissue belongs. Search of the literature revealed that very little is known on this subject. The metabolism of leucocytes from blood (both normal and leukaemic) has been considerably studied [Fleischmann & Kubowitz, 1927; Barron & Harrop, 1929; Glover *et al.*, 1930; Soffer & Wintrobe, 1932], but the cells in marrow from which some of these are derived are not necessarily identical in their behaviour. The presence of the nucleated precursors of the erythrocytes may also be expected to affect the metabolism of marrow.

With regard to whole bone marrow the only measurements we have found are those of v. Bréza [1926], who, using teased preparations, found that in the rabbit $Q_{O_2} = 3.5-5.0$ and in the guinea-pig $Q_{O_2} = 4.0-6.0$, reckoned on the fat-free dry wt.

In addition Fujita [1928] used cells from the bone marrow of the rat, selecting those cells which escaped into the medium when the marrow was gently stirred with serum or Ringer. In serum he found that such cells have a metabolism roughly of the type characteristic of embryonic tissues ($Q_{O_2} = -9.2$, $Q_{CO_2}^{O_2} = +3.7$, $Q_{CO_2}^{N_2} = +20.1$), but their behaviour in Ringer was quite different. Here the same relationship was true initially ($Q_{O_2} = -12.9$, $Q_{CO_2}^{O_2} = +3.8$, $Q_{CO_2}^{N_2} = +25.8$), but in the course of 3 hr. the respiration decreased while the aerobic glycolysis increased (final values $Q_{O_2} = 0.0$, $Q_{CO_2}^{O_2} = +17.5$, $Q_{CO_2}^{N_2} = +18.9$). This was interpreted by Warburg [1929] as an inhibition of the respiration by the Ringer's solution.

EXPERIMENTAL

Preparation of material. The whole of the marrow from six bones (femora, tibiae and humeri) of a rabbit (except for a small portion taken for histological examination) was removed as rapidly as possible, placed in about 20 ml. of Ringer in a 50 ml. flask, and shaken gently for a few minutes. The marrow rapidly broke up into pieces small enough to give fairly accurate sampling and to give reliable measurements of metabolism, according to the criteria of Warburg [1923]. v. Bréza [1926] states that suspensions of marrow should not be used for measurements of respiration, on account of the instability of the cells. We have seen no sign of metabolic instability in our experiments, and in most cases found the metabolic reactions to proceed linearly (see below).

Calculation of results. The usual method of expressing these results, viz. in terms of dry wt. of tissue, cannot be satisfactorily applied to marrow. This tissue, in the rabbit, consists predominately of adipose tissue in which the fat is metabolically inactive, and moreover the proportion of fat in different normal marrows varies very widely. To overcome this difficulty, it was decided to estimate the amount of cellular material in the marrow samples by determination of

the total N content. To establish the relationship between fat-free dry wt. and N content, marrow samples from 10 rabbits were analysed, with results given in Table I.

Table I

Sample	Fat % of dry wt.	Nitrogen % of fat-free dry wt.
1	71	14.6
2	70	13.9
3	71	14.5
4	88	15.2
5	71	15.0
6	90	14.6
7	89	14.2
8	84	14.6
9	91	15.2
10	88	13.9
	Average	14.6

While the wt. of the non-fatty residue varied between 9 and 30 % of the dry wt. of the sample, the N content of this residue varied only between 13.9 and 15.2 %, with an average of 14.6 %.

At the end of every experiment the contents of the manometer vessels were washed out into Kjeldahl flasks and the total N determined in the usual way. The figure for fat-free dry wt. of tissue was obtained by multiplying the N by 6.85. In experiments where the lactic acid formed was estimated chemically, the solution was deproteinized and the N determination made on the protein precipitate. Trichloroacetic acid was found to be unsuitable as precipitant, the chemical estimations in such filtrates being invariably too low. This was attributed to solution of a part of the lactic acid in the fat, for when a neutral precipitant was used, so that the lactic acid remained dissociated, satisfactory results were obtained. The procedure used was to wash out the contents of the Barcroft vessels with a mixture of 15 ml. 0.75 % ZnSO_4 , 7H₂O and .5 ml. 0.1 N NaOH and heat the solution in a boiling water bath for 5 min. In these filtrates the lactic acid was determined by the method of Friedemann *et al.* [1927], and the results showed satisfactory agreement with the manometric figures.

Measurement of respiration, aerobic glycolysis and anaerobic glycolysis. The procedure was exactly that described by Berenblum *et al.* [1936], respiration being measured in phosphate Ringer and glycolysis in bicarbonate Ringer, all in a total volume of 5 ml. in Barcroft manometers. The marrow suspension was measured from a special wide-tipped 2 ml. pipette.

Histological methods. Small portions of marrow were fixed in 4 % formaldehyde saline, embedded in paraffin, and sections were stained by Harris's haematoxylin and aqueous eosin, and by Turnbull's modification for paraffin sections of Jenner's stain. The relative proportions of the various cells were estimated independently in the sections stained by each method, and in most cases the results were found to agree closely. Previous work has shown that it is unusual for the relative numbers of the cell types to vary significantly between different parts of the marrow of the same animal, so we feel justified in basing the histological classification of these marrows on a small sample, reserving most of the marrow for the metabolism experiments.

"Normal" and "abnormal" marrow. Like most tissues bone marrow contains a large number of different kinds of cells, which may vary greatly in their metabolism, but unlike many other tissues the proportion of these different cells may vary considerably in different normal animals, and may be made to vary

even more by various treatments. We have used marrow from animals treated in the following ways.

(1) Intravenous injection of nucleic acid or the nucleotides derived therefrom by alkaline hydrolysis. This causes a blood leucocytosis, the marrow being depleted of granulocytes. During the recovery phase haemocytoblasts are increased.

(2) Injection of saponin, which appears to have a direct toxic effect on the marrow, both granulocytes and normoblasts being liberated into the circulation.

(3) Injection of benzene, which progressively destroys the marrow cells. The leucopoietic tissue is more vulnerable than the erythropoietic. The main effect appears to be on the myelocytes and their precursors.

(4) Exposure to an atmosphere of CO calculated to produce half saturation of the haemoglobin, which leads to hyperplasia of the erythropoietic cells.

(5) Grafting of the Brown-Pearce carcinoma. When dissemination of the growth has taken place, the marrow is found to be hyperplastic [Orr, 1936]. The hyperplasia is more often leucopoietic than erythropoietic.

The normal distribution of the various cell types in rabbit bone marrow is fairly well defined, the ratio of erythropoietic to granulopoietic tissue averaging $\frac{1}{2}$ to $\frac{1}{3}$, and animals in which this ratio was outside these limits have been judged "abnormal" for the purposes of this investigation. Usually the differentiated cells constituted all but a very small fraction of the haemopoietic tissue, and when haemocytoblasts were numerous the marrow was "abnormal".

RESULTS

Normal marrow. Results for "normal" marrow were obtained not necessarily from normal (that is, untreated) rabbits, but from any rabbits which, whether treated or untreated, showed a "normal" histological picture in their bone marrow. Of the 29 rabbits studied, 8 gave marrow which satisfied the criteria of "normality", and the metabolism of these is given in Table II.

Table II

Rabbit	Treatment	Q_{O_2}	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
26. ii. 35	—	-4.5	+4.8	+9.0
582	Nucleic acid + benzene	-3.7	+1.9	+4.2
580	Nucleic acid + benzene	-4.1	+2.8	+6.7
27. ii. 35	—	-5.0	+3.9	+6.6
542	Saponin	-3.7	+4.8	+8.2
12. iv. 35	—	-3.4	+3.5	+8.0
547	—	-2.9	+3.4	+6.3
586	Brown-Pearce carcinoma	-3.5	+5.0	+8.2
Average		-3.9	+3.8	+7.1

It will be seen that the values of Q_{O_2} with one exception fall within the range of values given by v. Bréza, and that the ratios $Q_{O_2} : Q_{CO_2}^{O_2} : Q_{CO_2}^{N_2}$ are of the order 1:1:2. The phenomenon reported by Fujita [1928] has never been observed. The metabolic rates were commonly linear for the 1st hr. and respiration, aerobic glycolysis and anaerobic glycolysis gradually decreased coincidentally if the experiment was continued longer (see Fig. 1, where results up to 4 hr. are given). On occasion the falling off in velocity started earlier, but though in some cases the respiration seemed to be more unstable than the glycolysis an associated increase in the aerobic glycolysis was never found.

In one experiment marrow from two rats was used, and again all three metabolic rates were constant for the 1st hr., and showed approximately equal rates of decrease during 3 hr. (initial $Q_{O_2} = -5.8$, $Q_{CO_2}^{O_2} = +8.6$, $Q_{CO_2}^{N_2} = +10.8$).

Fig. 1. The metabolism of bone marrow: *A*, respiration, *B*, aerobic glycolysis, *C*, anaerobic glycolysis.

“*Abnormal*” marrows. Our object was to decide what part of the total metabolism of marrow was contributed by the various types of cells present in it, and the method used was to vary the proportions of these types by applying to the rabbits the stimuli described. It was found, however, that the amount of

Table III. *Metabolism of “abnormal” marrows*

Type of marrow	No. of rabbits	Average		
		Q_{O_2}	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$
Rich in granulocytes*	5	-4.5	+5.7	+9.5
“Normal” in granulocytes	8	-3.9	+3.8	+7.1
Deficient in granulocytes	16	-3.5	+1.9	+4.5
Rich in nucleated red cells†	12	-3.7	+2.2	+5.1
“Normal” in nucleated red cells†	9	-3.8	+3.4	+6.4
Deficient in nucleated red cells	8	-3.8	+4.1	+7.4
Rich in other cells‡	14	-3.5	+2.0	+4.5
“Normal” in other cells	14	-3.9	+3.8	+7.3
Deficient in other cells	1	-4.7	+6.7	+11.4

* Myelocytes and polymorphonuclear leucocytes in varying proportions.

† Erythroblasts and normoblasts in varying proportions.

‡ Mostly haemocytoblasts (primitive cells not differentiated into either erythroblasts or granulocytes).

variation that could be achieved was insufficient to provide really conclusive results, without using very large numbers of experiments so that statistical methods could be applied. The results from 29 rabbits do nevertheless show some rough indications, and we give them for what they are worth.

In each case the condition of the marrow was judged by histological examination of a small sample, and the classification was carried out by one of us (J. W. O.) without knowledge of the results of the metabolic experiments.

DISCUSSION

Our results show that the bone marrow of rabbits falls into the same class, as regards its metabolism, as tumour tissue, retina and kidney medulla, i.e. the ratios $Q_{O_2} : Q_{CO_2}^{O_2} : Q_{CO_2}^{N_2}$ are roughly 1:1:2. The absolute value of the metabolism is small, which is rather surprising seeing that it is an actively growing tissue. During phases of active growth (e.g. in rabbits bearing the Brown-Pearce carcinoma) the metabolism of the whole marrow is raised in virtue of the increased cell content, but the results on the basis of N content are only slightly higher than normal.

The effect on this metabolism of changes in the distribution of the different types of cells in the marrow is not very clear, but the figures justify the generalization that, while all the cells contribute to the respiration of the tissue, the greater part of the glycolysis is to be attributed to the granulocytes. In one experiment, where the glycolysis was nil, histological examination of the marrow showed it to be practically free from granulocytes; the conditions could not be repeated, however, so it would be unwise to attach undue importance to this observation.

We can explain the results of Fujita [1928] only by supposing that the cells washed out from bone marrow by stirring with serum or Ringer's solution are only a small part of the total cells of the tissue, and in their metabolic instability are far from typical of the tissue as a whole.

SUMMARY

The metabolism of the bone marrow of rabbits is found to be of the same type as that of tumour tissue, retina and kidney medulla. The average values for normal marrow are:

$$Q_{O_2} = -3.9, \quad Q_{CO_2}^{O_2} = +3.8 \quad \text{and} \quad Q_{CO_2}^{N_2} = +7.1.$$

Indirect measurements indicate that probably most of the glycolysis is due to the granulocytes, while these cells and the nucleated red cells are about equally active in their respiration.

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