of 3,4-benzpyrene (that is, 7·2 (ref. 8) to 4·9 = 2·3 eV), then its value will be approximately 8·1 (ref. 8) − 2·3 = 5·8 eV. The energy available at the absorption cut-off, 3·38 + 2·88 = 6·26 eV, still exceeds this value.

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BIOCHEMISTRY

Effect of Glucose on Fatty Acid Uptake and Oxidation by Bone Marrow Cells from Guinea-pigs

In their investigations of the fat composition and *in vitro* oxygen consumption of marrow from fed and fasted rabbits, Evans *et al.*[1] observed a respiratory quotient of 0·85 for marrow cell suspensions incubated in the absence of glucose but in the presence of all the fatty material of whole marrow. The authors were unable to detect any uptake of fatty acid by the marrow cells and concluded that saturated fats were probably not degraded in the marrow for the production of local energy. In the work recorded here we have re-examined the question of *in vitro* uptake and oxidation of fatty acid by bone marrow cells. Our results indicate that fatty acid is taken up and oxidized by washed bone marrow cells suspended in a medium containing 5 per cent albumin as a carrier for fatty acid. Furthermore, we have found that glucose exerts a considerable influence on the rate of uptake and oxidation of fatty acid.

Male guinea-pigs of *NIH* strain, weighing 350–500 g each, were used in this work. Bone marrows from the femora and tibiae were trimmed of the fatty portion, collected in a glass homogenizing tube containing ice cold Krebs–Ringer phosphate solution, *p*H 7·4, and gently dispersed by hand with six passes of a loosely fitting 'Teflon' pestle. The suspension was allowed to stand for 2–3 min so that any bone fragments present could sediment to the bottom of the tube. The upper portion of the suspension was then transferred to a glass centrifuge tube and centrifuged for 5 min at 20–30 g. The supernatant fraction containing most of the mature erythrocytes and marrow fat was discarded. The remaining pellet was washed once and resuspended in a suitable volume of the Krebs–Ringer medium. A mean (±*S.E.*) of 89 ± 2 per cent of the nucleated cells was found to be viable using the trypan blue method. Differential counts of the nucleated cells in five different marrow suspensions showed that 34 ± 1 per cent of the cells were myeloid leucocytes at the band stage or older, 32 ± 2 per cent were immature myeloid leucocytes, 29 ± 2 per cent were nucleated erythroid cells and 5 ± 1 per cent were lymphocytes. The marrow cells were incubated in 3·0 ml. of Krebs–Ringer phosphate medium, *p*H 7·4, containing 5 per cent human serum albumin (American Red Cross, Cohn Fraction *V*, without heat treatment or stabilizers) extracted essentially free of fatty acid according to Goodman's method[2]. Sodium palmitate (Fisher Scientific Co.) when added was dissolved in the albumin solution as described previously[3]. The methods for measuring uptake of oxygen and respiratory quotient and for isolating and determining free fatty acids and glucose in the medium have also been described previously[3].

Table 1. Effect of Glucose on Fatty Acid Uptake and Oxidation by Bone Marrow Cells from Guinea-pigs[1]

Substrate added	Oxygen uptake μl./h/mg dry wt.	Respiratory quotient	Palmitate uptake μmol./h/10 mg dry wt.	Glucose uptake
None	4·1 ± 0·1	1·12	—	—
Glucose	3·8 ± 0·2*	1·28	—	3·0 ± 0·2
Palmitate	4·0 ± 0·1	0·86	0·45 ± 0·08	—
Glucose + palmitate	4·8 ± 0·1†	1·00	0·69 ± 0·08‡	3·7 ± 0·5

* $P < 0·05$, P value versus no substrate added.
† $P < 0·01$, P value versus no substrate added.
‡ $P < 0·02$, P value versus palmitate added.
[1] Each value represents the mean (±*S.E.M.*) of five determinations. The cells were incubated in the presence of 3·0 ml. Krebs–Ringer phosphate medium, *p*H 7·4, containing 5 per cent serum albumin. Initially 3·1 μmoles palmitate and 5·6 μmoles glucose per ml. medium were present.

Our results, summarized in Table 1, show that in the absence of glucose, palmitate was taken up by the bone marrow cells and was presumably oxidized to carbon dioxide since the respiratory quotient fell from 1·12 to 0·86. The latter respiratory quotient is in good agreement with the value obtained by Evans *et al.*[1]. It is possible that the failure of these workers to detect fatty acid uptake by rabbit marrow suspensions might be due to the fact that they did not add albumin to the incubation medium as a carrier for fatty acid. In the absence of albumin, the concentration of fatty acid would be extremely low since long chain fatty acids are only slightly soluble in aqueous media.

Table 1 further shows that palmitate uptake was greatly enhanced (56 per cent) by the addition of glucose, but apparently glucose rather than palmitate was the preferred substrate for oxidation since the respiratory quotient was 1·0. Palmitate had no significant effect on glucose uptake. It should be noted here that glucose also has been reported to stimulate fatty acid uptake by rat skeletal muscle *in vitro*, but, unlike the results obtained with bone marrow, no sparing effect of glucose on fat oxidation was observed with skeletal muscle[4].

Oxygen uptake was not affected by adding palmitate alone and only a small increase (17 per cent) was noted in the presence of glucose plus palmitate. These findings are in marked contrast to our previous observations with guinea-pig exudate leucocytes[3]. These cells showed a 2–3 fold increase in oxygen uptake on addition of palmitate to the medium. The respiratory quotient of such cells in the presence of glucose plus palmitate was 0·82, suggesting that fatty acid rather than glucose was the preferred substrate for oxidation. Future investigations will be aimed at identifying the factors (for example, cell maturity) which are responsible for the observed metabolic differences between bone marrow and exudate cells.

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Interaction of Bilirubin with Albumin

Recent electrophoretic investigations using isotopically-labelled pigment[1] have confirmed earlier findings that in plasma, unconjugated bilirubin is bound only to albumin. By dialysis with semi-permeable membranes, 1 mole of albumin was found to bind tightly 2 moles of the pigment[1]. However, equilibration of the two phases could not be achieved in these dialysis experiments because of rapid