Bone marrow in male and female rats

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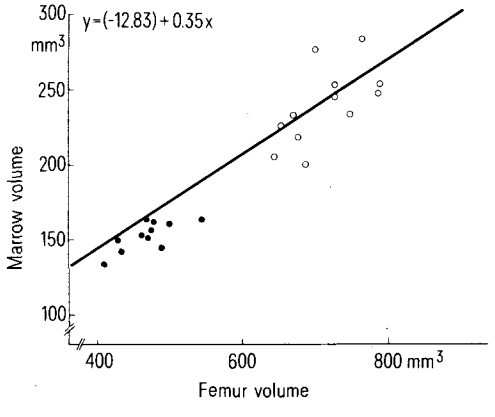
Summary. Sex differences of high significance in the marrow weight relative to femur volume, as well as to body weight, were found in adult rats. A positive significant correlation was found between the femur volume and the marrow volume in both sexes, while a negative significant correlation between femur density and the marrow content (mg/mm^3) exists only in male rats.

The marrow content in individual skeletal bones has been analyzed in some animals[1-3], but no such measurement has yet been made in relation to the sex of animals. In some conditions, especially in osteoporosis, the changes in the bone, which also involve the marrow component, occur earlier and proceed at a faster rate in females than in males[4-6]. Therefore the relationship between the bone marrow and sex seems to be very important. This paper describes the actual marrow in the femur of adult male and female rats on both volume and weight basis.

Methods and results. The analysis was performed on the right femur of 1-year-old male and female albino rats, b.wt 457±13 g and 255±9 g, respectively. A new method was used to remove available marrow substance from the bone by means of the centrifugal force, 10,000× g for 30 min[17]. The use of femur freed of marrow permits direct determination of the volume of the space which was occupied by the marrow, by the method of Robinson and Elliott[8] as modified by Mueller et al.[9]. On the other hand, it permits calculations of femur density with and without the marrow substance, by summing up the fractional volumes (g/cm^3) occupied by ash, organic matter, absorbed water and marrow. The weight of the marrow is determined from the difference in the total femur weight and the femur without marrow (it is a little higher than the actual weight of the marrow substance because of 'surface' water which is also lost by centrifugation). The results are summarized in the table. The amount of marrow tissue in the femur was significantly greater in male than in female rats both in absolute (mg) and relative terms (percent by wet weight). The volume of marrow space, although significantly greater in absolute terms (mm^3) in males than in females, was only marginally significant ($p > 0.05$) in relative term (percent of total femur volume). The ratio of marrow weight to marrow volume was significantly greater in male than in female rats. In relation to b.wt male rats also showed a significantly greater marrow content than female rats, while their ash content, as already known[10,11] was significantly lower. The density of the femur without marrow was similar in male and female animals while the density of the femur with the marrow substance was significantly higher in the males than in the females. A positive, linear regression (figure) fits the points fairly well when the marrow volume is plotted against the femur volume. The analyses show that these correlations are real ($p < 0.05$ and $p < 0.01$) and strong ($r = 0.61$ and $r = 0.83$) for both male and female rats. A negative correlation was found between the marrow content in the femur and the density of the femur with the marrow substance. However, this correlation is statistically significant in male ($r = -0.57$, $p < 0.05$) but not in female ($r = -0.37$, $p > 0.1$) rats.

Discussion. For the animals of our strain, the percent sexual dimorphism (calculated as 100 (M/F) − 1) of already well-known sexual differences in bone dimensions[10-12] (weight and volume of the femur, as well as the weight and volume of its main components, ash and organic matter) is in the range of 50–54%.

A new observation presented in the paper is that the degree of sexual dimorphism for the marrow part of the rat femur is different, i.e. 109% for the weight and 61% for the volume. A more important finding is that the density values in male femur, including the marrow substance, are not simply the reflection of the mineral content (mg/mm^3) but are also significantly influenced by its marrow component. Therefore, when comparing the density of bones with the



Marrow volume is plotted as a function of femur volume for male (○) and female (●) rats. There were no significant differences in the so-formed regressions. Therefore, the common least-squares fitted regression line was drawn through the points.

The data of the femur marrow in male and female rats

	Male	Female	p-values[b]
Marrow weight[a] (mg)	176.24±7.95	84.12±2.71	<0.001
Marrow volume (mm^3)	239.08±7.55	148.68±4.11	<0.01
Percent marrow weight of w.wt of femur	16.42±0.69	12.43±0.35	<0.001
Percent marrow volume of femur volume	33.55±0.86	32.22±0.51	>0.05
Marrow (mg)/g b.wt	0.39±0.02	0.33±0.01	<0.02
Ratio marrow weight to marrow volume	0.73±0.01	0.57±0.01	<0.001
Density of femur without marrow (mg/mm^3)	1.25±0.02	1.28±0.01	>0.1
Density of femur with marrow (mg/mm^3)	1.51±0.01	1.46±0.01	<0.01
Marrow content in femur (mg/mm^3)	0.25±0.01	0.18±0.01	<0.001

[a] Each figure represents the mean of 14 animals ±SE; [b] differences in arithmetic means tested by Student's t-test.

marrow material, one should keep in mind that sometimes differences between bones may be due only to the different marrow content. The exact physiological meaning of the differences presented in the marrow component of the bone in male and female rats is yet to be ascertained. However, these data may be very useful in assessing skeletal changes induced by osteoporosis, since rat and particularly its femoral bone are among the most often studied objects in this field.

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Can the binding of GABA, glycine and β-alanine to synaptic receptors be determined in the presence of a physiological concentration of Na^+?

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Summary. Bicuculline- and strychnine-sensitive components of the binding of GABA, glycine and β-alanine, which can be demonstrated in the presence of a physiological concentration of Na^+, might be related to synaptic receptors.

Transmitter-candidate amino acids are taken up by CNS fractions in vitro by potent Na^+-dependent binding and transport mechanisms which involve pre-synaptic elements and glia. Thus, one may believe that the binding of these amino acids to their post-synaptic receptors cannot be determined in the presence of physiological concentrations of Na^+. However, recent studies have revealed that such interactions can be determined under physiological conditions if studies are conducted at 0 °C (to suppress active transport mechanisms), if accurate corrections are applied to the data, if the particles are sufficiently depleted of their contents of endogenous amino acids, and if low concentrations (10^{-10}–10^{-7} M) of the labelled active ligands are employed.

Representative data from our recent publications on the binding of GABA[1], glycine[2,3] and β-alanine[4] will be used here to show components which are sensitive to in vivo antagonists of the depressant actions of these amino acids and which might be related to synaptic receptors. However, it should be realized that the antagonists used, strychnine and bicuculline-methiodide (BMI), while being the most reliable ones available today, possess many nonspecific actions[5,6].

The methods used in these experiments have been discussed in detail[1–6]. In all cases, 'synaptosomal-mitochondrial' (P_2) fractions of rat CNS regions, which are known to contain post-synaptic thickenings and post-synaptic membranes[7], were incubated with labelled ligands for 10–15 min at 0 °C. Many studies have indicated that essentially maximal values for binding of these amino acids to cerebral subcellular particles are obtained under these conditions[8–14]. All operations were performed at 0 °C, rather than at higher temperatures, to prevent active transport and catabolism of the amino acids and tissue autolysis.

The table provides values for the compartmentation of GABA in cerebral cortex, glycine in spinal cord, and β-alanine in spinal cord-plus-brain stem, in terms of total tissue content, total binding in the presence of a physiological concentration of Na^+ (i.e., Na^+-dependent binding which occurred mainly to carrier-transport sites) and antagonist-sensitive binding in the presence of Na^+ (i.e., binding to presumed synaptic receptor sites). It is evident that synaptic receptor compartments for these 3 inhibitory amino acids are quite similar at about 45–160 pmoles amino acid/g original wet wt of tissue. Also, Na^+-dependent binding and BMI-displaceable binding of GABA, as percentages of the total GABA present in cerebral cortex (at 11% and 0.003%, respectively) are quite similar to corresponding values calculated for glycine in spinal cord (4.8% and 0.004%, respectively). However, the compartmentation of β-alanine in rat brain stem-spinal cord differed markedly, its Na^+-dependent binding compartment being equal to its total tissue content and its strychnine-sensitive binding accounting for about 0.06% of its total tissue content. Hence, greater proportions of tissue β-alanine than of GABA and glycine may be involved in its Na^+-dependent binding ('inactivation') and antagonist-sensitive binding ('receptor-interaction'). It is also noteworthy that all 3 amino acids had similar ratios of antagonist-sensitive/Na^+-dependent binding in the CNS regions studied.

These results strengthen the notion that the possible 'receptor-binding' of these inhibitory amino acids can be studied in the presence of physiological concentrations of Na^+. (Taurine has not yet been studied in detail). The value for BMI-sensitive GABA binding (60 pmoles/g cerebral cortex) agrees well with values obtained for the Na^+-independent binding of GABA to CNS membrane fractions[11,15,16]. The value for strychnine binding sites of 39 pmoles/g rat spinal cord[17] is lower than that determined for strychnine-sensitive glycine binding sites (160 pmoles/g spinal cord[4]), as was expected, since strychnine and glycine probably bind to distinct CNS sites[2,4,17]. 'High-affinity', strychnine-sensitive binding of β-alanine to a rat brain stem-spinal cord