probably, to this hormone and not to pancreozymin. Neither the route (portal *versus* systemic) nor the mode of injection (single dose *versus* constant infusion) changed the amylase or trypsin concentration of pancreatic secretion. With the experimental procedures employed, the physiological effect of cholecystokinin seems to be unmodified by intraportal injection.

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Marrow Distribution in Rat Femurs Determined by Cell Enumeration and Fe^{59} Labeling.* (28250)

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Anatomical, hematokinetic and radioisotopic procedures have been used to determine the distribution of bone marrow in man and laboratory animals(1-14). Although the rat femur is frequently the source of marrow for hematological studies, no definitive information is available on the relative and absolute distribution of marrow within the whole bone (diaphysis and epiphyses) of the animal. Bone marrow estimations, made either as cellular numbers per mg or cells per mm^3 of marrow are usually based on marrow recovered solely from femoral diaphyses. This is because technical difficulties encountered in recovering marrow from the bone spicules in the epiphyses make these areas inaccessible to routine quantitation and because of the assumption that epiphyseal marrow content is negligibly small.

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The purpose of the present investigation was to determine the marrow nucleated cellular content, weight and distribution within the entire rat femur using both direct cell enumeration as well as radioiron labeling procedures.

Methods. A. Diaphyseal marrow quantitation. Male rats (260-280 g) of a modified Long-Evans strain were used in all phases of this study. Cellular quantitation of femoral diaphyseal bone marrow was accomplished by a modification of the method of Fruhman and Gordon(5). The femur was split lengthwise and the exposed marrow aspirated by mouth into a weighed hematocrit tube (1.3-1.5 mm $\times$ 75 mm, Clay-Adams "Red Tip") with a rubber tubing assembly which was attached to one end of the tube. The amount of marrow recovered from the diaphysis was estimated by subtracting the initial empty tube weight from the final tube weight after it contained the marrow.§ The marrow was then

§ It would now seem erroneous to estimate total marrow mass, as reported earlier, by first weighing the entire intact femur and the then dried bony casing cleaned of marrow (the difference in weight representing total marrow mass). This is due to the consider-

expelled into a test tube containing 2 ml of isologous serum, uniformly suspended with a rubber-bulbed glass pipette and counted in a hemocytometer. Differential cell counts were made from Wright-stained smears of marrow suspensions. Nucleated red cells were enumerated from smears stained by a modification of Ralph's hemoglobin staining technique (15) as follows: slides were flooded with a 1% solution of benzidine in absolute methanol and allowed to stand for 1 minute. The benzidine solution was then tipped off and a Superoxol (Hydrogen peroxide 30%, Merck) mixture (70% ethanol-Superoxol, 3:1) added to the slides for another minute. The slides were rinsed in tap water and then lightly counterstained with hematoxylin to permit marrow cell nuclei to become just visible. With this procedure there was no difficulty in identifying hemoglobin-containing nucleated red cells since their cytoplasm was distinctly golden in color. This technique does not permit identification of the very early erythroid precursors which lack sufficient amounts of hemoglobin to stain adequately. These latter cells, numbering approximately 1% of the total nucleated cell population were identified in Wright-stained slides.

B. Total cell enumeration method. To determine total marrow nucleated cell content and distribution, 15 femurs (of which 12 included both femurs of 6 rats) were cleanly separated with a fine serrated knife into proximal and distal epiphyses and diaphyses. The diaphysis was split in half and the bone marrow inserted into a test tube containing 2 ml of isologous serum. Proximal and distal epiphyses were placed into separate watch-glasses containing isologous serum, cut into numerous segments and as much marrow as possible freed from the bony spicules characteristic of these areas. The suspensions of bone, spicules and serum were then agitated with a glass rubber-bulbed pipette further to dislodge marrow still adhering to small bone particulates. The marrow suspensions were transferred to test tubes and the bony spicules remaining on the watch-glasses washed 2-3 times with fresh serum. This serum was recovered and added to the test tubes to give a final volume of 2 ml. Total nucleated cell counts and marrow differentials, based on 1000 cells, were then performed. Five to 10% of the cells recovered were unidentifiable in smears due to the destruction and cytological distortion produced by this procedure.

C. Radioiron method. Femoral marrow distribution was also determined with an Fe^{59} isotopic method(8,9). Ten rats (contributing 19 femurs) were injected intraperitoneally with 1.5 μc Fe^{59} (Chloride) per 100 g body weight. The rats were sacrificd 4-6 hours later when marrow Fe^{59} uptake was near maximal(16) and blood Fe^{59} minimal (9). Femurs were amputated, cleared of adhering tissue and proximal and distal epiphyses separated from the diaphysis. All 3 parts were placed separately into equal volumes of a corrosive acid concentrate (1 perchloric acid : 1 nitric acid : 4 sulfuric acid) to diminish errors resulting from differences in counting geometry of the respective parts. Following a one-week digestion period, the radioactivity of each part was determined in a well-type gamma scintillation counter. Since 70% of the Fe^{59} is taken up by the nucleated erythroid cells(16) and the cytological studies indicated a relatively uniform distribution of myeloid and erythroid cells throughout the entire femoral marrow space (see *Results* and Table I), the % radioactivity in each part was taken to reflect the marrow distribution in the femur.

To estimate total marrow nucleated cell content, 10 additional rats (contributing 19 femurs) were injected with Fe^{59} as described above. The radioactivity of the entire femur was ascertained and a serum suspension of diaphyseal marrow made from it. Both radioactivity and total number of nucleated cells in this marrow suspension were determined.¶

able water loss from bone occurring during the weighing process. This loss can be as rapid as 1 mg of water per minute during the first half hour of manipulation with losses totaling as much as 50 mg after one hour.

¶ This procedure of measuring the nucleated cellular numbers and radioactivity of the non-centrifuged marrow suspension appears valid. Thus the cells that might have been destroyed in the mechanical processes involved in preparing the marrow suspensions

TABLE I. Relative Distribution of Nucleated Red Cells and Total Bone Marrow in Rat Femurs.

	No. femurs	% in proximal epiphysis	% in diaphysis	% in distal epiphysis
A. Nucleated red cells	15	18.4 ± 1.1* (12.8–27.8)	19.5 ± 1.7 (10.9–35.7)	17.0 ± 1.3 (10.3–28.2)
B. Total nucleated cells as determined by cell counting	15	17.5 ± 1.1 (9.4–24.3)	64.4 ± 1.6 (52.0–75.5)	18.1 ± .9 (13.8–25.0)
C. Total nucleated cells as determined by radioiron uptake	19	25.5 ± 1.2 (17.2–39.0)	53.7 ± 1.3 (45.6–64.9)	20.8 ± 1.0 (13.6–29.8)

* Mean ± standard error of mean (range).

The total number of nucleated cells within the entire femur was then estimated by applying the formula:

$$\frac{\text{Counts/min (cpm) diaphyseal marrow suspension}}{\text{No. of nucleated cells in suspension}} = \frac{\text{cpm femur}}{\text{No. of nucleated cells in femur}}$$

Furthermore, since the weight of the diaphyseal marrow sample was determined before its suspension in serum, total femoral marrow mass could be calculated using the formula:

$$\frac{\text{cpm diaphyseal marrow suspension}}{\text{Marrow wt (mg) in hematocrit tube}} = \frac{\text{cpm femur}}{\text{Marrow wt (mg) in femur}}$$

In one series of experiments, Fe^{59} distribution was determined in femurs obtained from 4 rats rendered polycythemic by intraperitoneal injections of isologous washed red cells (17). Since erythropoiesis in these animals was markedly depressed (only 0.7-3.5% marrow nucleated erythrocytes), this study controlled against the possibility that the observed Fe^{59} uptake in marrow contained within femurs of normal rats used in this study (11-35% marrow nucleated red cells) was due to non-erythroid elements. Radioiron uptake in these polycythemic femurs was less than 1/20th that found in femurs of normal rats.

Bone particles cleaned of marrow were also counted and no significant isotopic activity was detected.

Results. The % nucleated red cells in femoral diaphyses and epiphyses, as determined by differential counts on marrow smears, are shown in Table I, A. It is seen that the relative numbers of nucleated erythrocytes were fairly evenly distributed among the 3 parts of the bone marrow cavity. A wide range of values was obtained when comparing different femurs. This may be attributable to one or more factors: normal differences in femoral cell numbers, disproportionate cellular destruction during mechanical extirpation of the marrow, and counting errors inherent in the technique. In most cases, however, only small differences in nucleated erythroid distribution were detected in the 3 regions of the same femur.

A relatively equal distribution of marrow reticulocytes was also noted. In 6 femurs, the mean values (± stand. errors of mean) for diaphyseal and proximal and distal epiphyseal reticulocytes were: 24.6 ± 0.7%, 24.6 ± 2.0%, and 22.5 ± 1.1%, respectively.

Estimations of the relative distribution of total nucleated cells within rat femurs are given in Table I, B and C. The data in Table I B, based on cell enumeration procedures, indicate that about 64% of the marrow was contained within the diaphyses whereas 36% was present in the epiphyseal regions. This compares to 54% and 46% for these areas, respectively, when the radioiron procedure was used (Table I, C). The lower figure for epiphyseal areas in the cell enumeration experiments was probably due to inability to remove all the marrow and to destruction incurred during mechanical removal of cells

(which have contributed radioiron to the serum) are most likely counted as cells in the hemocytometer despite the fact they are reduced to fragments or free nuclei(14).

TABLE II. Total Nucleated Marrow Cells and Corresponding Marrow Weights in Rat Femurs as Estimated by Radioiron Uptake. T.N.C., total nucleated cells; T.M. wt, total marrow weight.

Animal	Right femurs		Left femurs	
	T.N.C. (mill)	T.M. wt (mg)	T.N.C. (mill)	T.M. wt (mg)
A	270	182	250	168
B	228	131	206	139
C	213	114	266	164
D	223	113	183	111
E	368	127	368	132
F	260	100	308	106
G	323	111	324	110
H	345	117	269	110
I	295	144	326	128
J	282	162	—	—
	280.7 ± 5.8*	130.0 ± 10.8	279.7 ± 6.3	129.7 ± 7.8

* Mean ± standard error of mean.

from epiphyseal spicules.

The total numbers of nucleated cells determined by actual cell counts in 15 femurs ranged from 140-325 million cells per femur. However, counts from 2 femurs of the same rat never differed by more than 10%. Estimates, derived from radioiron uptake, of the total numbers of nucleated cells and corresponding marrow weights of 19 femurs are shown in Table II. It may be seen that with this technique, approximately 180-370 million cells per femur were obtained; differences in total cellularity between 2 femurs from the same rat seldom exceeded 25%.‖ Total marrow weights ranged from 100-182 mg per femur.

Discussion. From the data presented it is obvious that much of the femoral marrow is concentrated in 2 small regions (the epiphyses) which are not normally included in the usual quantitation procedures. It seems essential therefore, that investigations utilizing quantitative cellular changes in rat femoral marrow as an index of hemopoiesis should consider the epiphyses as well as the diaphysis. In this connection, it is conceivable that effects exerted, in some cases, may not be imparted equally to all parts of the bone marrow since epiphyses and shaft appear to differ in their vascular patterns, the cellular ecology they provide, and their relative accessibility to experimental agents. Unfortunately, removal of epiphyseal marrow is technically difficult due to the presence of numerous bony spicules. In studies of total marrow volume(9), it has been estimated that approximately 50% of the marrow is unavailable for quantitation as a result of cell destruction, filtering procedures and trapping of cells within the bony shell.

In the present study, 140-325 million cells could be mechanically removed from single rat femurs. In view of the inaccessibility of some of the femoral marrow, this figure may be considered to be in fair agreement with the 180-370 million cells estimated by our radioiron labeling procedures.

The present investigation also indicates that, for our 260-280 g rats, average weight of marrow per femur is about 130 mg. This figure is low when compared to values obtained by other workers. Lamerton(6) using radioiron determined that a single femur contained 8% of the rat's total marrow. Donohue, Gabrio and Finch(9), also employing Fe^{59}, estimated that 200-300 g rats average 17 billion nucleated marrow cells/kg (range: 13-22 billion). Thus a 275 g rat should possess a total of approximately 4.6 billion nucleated marrow cells, with 370 million (8%) in each femur. If we assume 2 million cells per mg of marrow (the mean for our modified Long-Evans rat), an estimate of 185 mg is obtained from these data as the total marrow weight per femur. An even higher figure results if Kindred's(3) estimate of total rat marrow (1-3% body weight) is used. In this case assuming total marrow mass is only 1% body weight, an average of 220 mg of marrow per femur is calculated. The discrepancy between the present findings and those of others might be due to differences in the methods and strains of rats employed.

Summary. A radioactive iron method was used in conjunction with a cell enumeration procedure to determine total marrow distribution in rat femurs. With radioiron, approximately 180-370 million marrow cells and

‖ In one case not tabulated, a 100% difference was observed. In this exception, radioiron incorporation in one femur was double the maximum observed in all other experiments.

100-182 mg of marrow were found to be contained within femurs of 260-280 g rats; 55% of this marrow was located within the diaphysis whereas the remainder was in epiphyseal regions. Using the cell enumeration procedure, approximately 140-325 million marrow cells were found within femurs of 260-280 g rats with 65% being located within the diaphyses.

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Inhibition of Aldosterone-Stimulating Activity of Hog and Dog Renin by the Plasma of Dogs Immunized with Hog Renin.* (28251)

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The pressor activity of hog renin, and also that of dog renin, is inhibited if these renins are incubated with the plasma of dogs that have received multiple injections of renin. The inhibition is believed to be due to anti-hog renin antibodies which also neutralize dog renin(1,2). Recently, it has been shown that hog and dog renin stimulate the secretion of aldosterone in dogs(3,4,5). The present experiments demonstrate that the aldosterone-stimulating as well as the pressor activity of hog and dog renin is inhibited by the plasma of dogs immunized with hog renin.

Methods. Male mongrel dogs weighing approximately 10 kg were treated with hog renin prepared by the technique of Haas and Goldblatt(6). The amount of this renin preparation which raised the systolic blood pressure of pentobarbital anesthetized normal dogs 30 mm Hg was designated as one "unit." The injected dogs received approximately 25 "units" per day subcutaneously 5 times a week for 5 to 36 weeks. The plasma of the dogs was assayed for its effect on the pressor and adrenal stimulating activity of renin. As a control, frozen plasma which had been obtained from the dogs before they were immunized was also assayed. Two "units" of hog or dog renin were mixed with 2 ml of plasma. The mixture was incubated at room temperature for one-half hour, then injected intravenously into nephrectomized hypophysectomized assay dogs. The assay dogs were prepared as described previously(5) with adrenal venous cannulae, and with arterial cannulae for measuring blood pressure continuously, using a Statham strain gauge and Grass model 5 polygraph. Two control adrenal venous blood samples were collected before the first renin-plasma mixture was injected. Each as-

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