sium in the presence of an overall normal medullary potassium concentration would indicate that a good portion of the "extra" water of the medulla could be located outside of cells. However, additional studies, both histological and microchemical, including the determination of the magnitude of papillary fluid concentrations, are necessary before the phenomenon of the renal water content difference can be resolved.

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1. Nocenti, M. R., Cizek, L. J., Proc. Soc. Exp. Biol. & Med., 1967, v124, 767.

2. Levinsky, N. G., Davidson, D. G., Berliner, R. W., J. Clin. Invest., 1959, v38, 730.

3. Manitius, A., Levitin, H., Beck, D., Epstein, F. H., ibid., 1960, v39, 684.

4. ———, ibid., 1960, v39, 693.

5. Levitin, H. Goodman, A., Pigeon, G., Epstein, F. H., ibid., 1962, v41, 1145.

6. Kessler, E., Allen, R. L., Kirman, D., Strauss, H., Am. J. Physiol., 1964, v207, 109.

7. Ruiz-Guiñazú, A. Arrizurieta, E. E., Yelinek, L., ibid., 1964, v206, 725.

8. Malvin, R. L., Wilde, W. S., ibid., 1959, v197, 177.

9. Levinsky, N. G., Berliner, R. W., J. Clin. Invest., 1959, v38, 741.

10. Moore, F. D., Olesen, K. H., McMurrey, J. D., Parker, H. V., Ball, M. R., Boyden, C. M., The Body Cell Mass and its Supporting Environment, W. B. Saunders Co., Philadelphia, 1963.

11. Berliner, R. W., personal communication.

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Yellow Bone Marrow as Adipose Tissue. (31983)

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The yellow variety of bone marrow (YBM) resembles in composition the homologous adipose tissue of other body sites. Marrow fat reflects the fatty acid composition of the diet, is prone to loss on fasting and its respiratory quotient indicates mainly fat oxidation(1). Experiments reported here were aimed to demonstrate that YBM is capable of metabolic responses characteristic of adipose tissue.

Materials and methods. Adipose tissues were obtained from *ad libitum* fed male guinea pigs weighing 500 to 700 g and male albino rats weighing about 200 g, anesthetized by intraperitoneal injection of sodium pentothal. The tibiae of the guinea pigs were exposed and opened by longitudinal incision. The YBM section, appearing as a yellowish solid cylinder, was carefully lifted out, weighed and incubated in a metabolic shaker at 37°C, as specified in the Tables. To compare the metabolic responses of the YBM with those of other adipose tissues, pieces of epididymal fat pad of similar weight (30 to 40 mg) were also cut out from the guinea pig and from the rat. After incubation, the tissues were rinsed thoroughly in 0.9% NaCl and in 1% bovine albumin solution and extracted by grinding in Dole's heptane-isopropanol mixture(2). Separation of triglyceride (TG) and free fatty acid (FFA) components, hydrolysis of TG and titrations of FFA were done by previously applied methods(3,4), which were scaled down to fit the small amounts of tissue and medium. Radiochemicals were purchased from Amersham (Great Britain) and radioactivity was measured in a Packard Tri-Carb Scintillation Spectrometer. Fat content was estimated gravimetrically after evaporation of a washed chloroform-methanol extract of the tissue, prepared according to Folch *et al*(5). DNA and protein contents in the delipidated tissue residues were determined by the methods of Schneider(6) and Lowry *et al*(7).

Results. Table I shows the conversion of U-C^{14}-glucose to CO_2 and TG in guinea pig tissues as compared with epididymal adipose tissue of the rat. The guinea pig YBM showed uptake and distribution of glucose radioactivity similar to the homologous epididymal

TABLE I. Uptake of U-C^{14}-Glucose by Adipose Tissues of Guinea Pig and Rat.

Tissue	Total uptake*	CO_2	TG	% of total in TG	% of TG radioactivity in fatty acids
	mμmoles/100 mg 2 hr				
Guinea pig					
Bone marrow	179	104 ± 13	75 ± 6	41.9	7.4 ± 2.5
Epididymal	164	115 ± 14	49 ± 5	29.9	7.7 ± 3.3
Rat					
Epididymal	537	287 ± 52	250 ±29	46.6	27.7 ± 6.3

Values given are means ± SE for 6 experiments.

Pieces of adipose tissue were incubated in Warburg flasks of 5 ml capacity in 1 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3 mg glucose and 1 μC U-C^{14}-glucose. A folded piece of filter paper was included in the center well. At the end of 2 hr CO_2 was trapped by injecting 0.2 ml of 1 N hyamine hydroxide on the filter paper and acidification of the medium. After additional 15 min of incubation the tissue was removed, washed and extracted.

* Sum of radioactivity recovered in CO_2 and TG.

fat pad. In these tissues the incorporation into TG amounted to 1/2 to 2/3 of that found in CO_2, and only about 7% of the TG radioactivity was recovered in the fatty acid moiety. The uptake of glucose by rat adipose tissue was about 3 times higher than by guinea pig tissues with about equal distribution between CO_2 and TG. The proportion of radioactivity appearing in the fatty acid moiety of the TG was also markedly higher in rat than in the guinea pig adipose tissues.

Table 2 shows that guinea pig YBM assimilated 1-C^{14}-linoleate by conversion into TG at a rate similar to the homologous epididymal fat pad. In rat epididymal fat tissue the esterification of linoleate was about twice that of guinea pig tissues. Addition of glucose to the medium approximately doubled the esterification of linoleate in all tissues.

TABLE II. Esterification of 1-C^{14}-Linoleate by Adipose Tissues of Guinea Pig and Rat. (mμmoles converted to TG/100 mg tissue per hr.)

	Guinea pig		Rat
	Yellow bone marrow	Epididymal fat pad	Epididymal fat pad
Without glucose	16 ± 2	21 ± 2	45 ± 3
With glucose (3 mg/ml)	28 ± 2	44 ± 3	91 ± 5

Values given are means ± SE for 12 experiments. Pieces of adipose tissue were incubated for 1 or 2 hr in 1 ml of 4% albumin solution, pH 7.4, containing 0.4 to 0.6 μeq of 1-C^{14}-linoleate of specific activity about 1 μC/μeq. Rate of esterification was calculated as outlined previously(3). The radioactivity found in the FFA fraction of the tissue at end of incubation was less than 1% of that converted into the TG in the case of rat tissue, and less than 3% in the case of guinea pig tissues. This was disregarded in the calculations.

To compare the activities of the adipose tissues from the various sites and species, the composition of the investigated tissues was determined (Table III). No appreciable differences were found with respect to the content of fat or DNA, but in the rat tissue the protein content was somewhat higher. On the whole these results indicate that any comparison of tissue activity related to wet weight would render essentially valid conclusions.

TABLE III. Composition of Guinea Pig and Rat Adipose Tissues.

Constituent, mg/g wet wt	Guinea pig		Rat
	Yellow bone marrow	Epididymal fat pad	Epididymal fat pad
Fat	809 ±9	825 ±14	834 ±11
Protein	9.7 ± .3	10.4 ± .4	13.2 ± .4
DNA	2.4 ± .3	2.2 ± .2	2.1 ± .2

Values in the Table are means ± SE for 8 determinations.

Table IV indicates that guinea pig YBM released FFA and that this release was enhanced by hormones, known to activate TG lipolysis in other adipose tissues. To study lipolysis in minute amounts of tissue an assay system was elaborated, in which the tissue TG were first labeled by incubating with 1-C^{14}-linoleate and then reincubated in contact with the hormones. The release of labeled FFA from the tissue during the reincubation period reflected the extent of lipolysis in the compartment of newly esterified TG. The percentage values of FFA release in the Table should not, therefore, be construed to repre-

TABLE IV. Effect of Hormones on Release of FFA from Labeled Adipose Tissues of Guinea Pig and Rat.

Hormone added:	% of tissue radioactivity released as FFA		
	Guinea pig		Rat
	Yellow bone marrow	Epididymal fat pad	Epididymal fat pad
	Release in 1 hr		
None	3.8 ± .3	3.1 ± .2	1.6 ± .1
l-epinephrine bitartrate (5 μg)	+ .5	+1.5	+2.2
dl-norepinephrine hydrochloride (5 μg)	+ .3	+ .9	+1.1
Corticotropin (10 μg)	+5.2	+4.4	+3.1
	Release in 4 hr		
None	5.4 ± .4	4.7 ± .4	2.6 ± .3
Bovine growth hormone (20 μg)	+5.7	+ 9.3	+4.4
Idem and dexamethasone (.2 μg)	+7.0	+11.5	+8.3

Adipose tissues were incubated with 1-C^{14}-linoleate as described in Table II. The tissues were then washed thoroughly and transferred for 15 min interim incubation in 3% albumin solution, to remove any traces of external radioactivity. Thereafter the tissues were again transferred for 1 or 4 hr reincubation in a fresh albumin solution with or without hormones, as indicated in Table. The labeled FFA released during this reincubation were selectively extracted and counted. The label in tissue TG was also determined. The release was calculated as follows: $\frac{C^{14}\text{-FFA released} \times 100}{C^{14} \text{ in tissue TG} + C^{14}\text{-FFA released}}$.

In control experiments, the small amount of radioactivity in tissue FFA fraction (see footnote to Table II) remained practically without appreciable change during the reincubation and was disregarded in these calculations.

Values in the Table for tissues incubated without hormone are means ± SE for 8 experiments with different animals. Hormone effects are illustrated by mean increments in 3 paired experiments, each with tissue pieces of one animal. Corticotropin was obtained from Organon (Holland) and Raben-type bovine growth hormone was kindly supplied by Dr. Z. Laron from the Beilinson Hospital.

sent a release of a fraction of total tissue fat, since as discussed elsewhere the newly esterified TG do not equilibrate rapidly and the lipolytic breakdown occurs in a pool of specific activity higher than the tissue average. However, the procedure renders conspicuous the effects of hormonal stimuli on a comparative basis.

It may be seen in Table IV that in the YBM and epididymal fat pad of guinea pig the increment in FFA release upon addition of l-epinephrine or norepinephrine was only marginal, in contrast to the marked stimulation of release seen in rat tissue. On the other hand, with corticotropin or growth hormone, the adipose tissues of guinea pig released larger percentages of radioactivity than the rat tissue.

While the effect of corticotropin was clearly evident upon 1 hour contact with the tissue, 4 hours were required to demonstrate the stimulation of FFA release in the presence of growth hormone. The effect of the latter was potentiated by addition of traces of dexamethasone, especially in the case of rat tissue.

Discussion. The experiments demonstrate the capacity of YBM to synthesize fatty acids and glycerol from glucose, to take up and esterify long chain FFA from an external medium and to release FFA under hormonal stimulation. All these activities are typical of adipose tissue function. Thus, the YBM seems to represent a metabolically active variety of fat store, similar to depots in other anatomical sites, but presumably with a specialized local importance.

From the quantitative point of view the activities in YBM resemble those of the epididymal fat pad of the same animal. However, both these tissues from the guinea pig exhibited lower activity than rat tissue. These differences appear related to species specific enzyme endowment rather than to the proportions of major tissue constituents or to tissue structure. Comparative experiments with isolated fat cells of guinea pig and rat adipose tissues (unpublished) yielded differences of

similar magnitude and seem to confirm this assumption.

Further species differences are apparent in the responses to hormonal stimuli. The pituitary hormones of peptide structure, corticotropin or growth hormone elicited larger FFA release from the YBM and epididymal fat pad of the guinea pig than from the rat adipose tissue. With catecholamine hormones the converse was true. These species differences are in agreement with those noted by Rudman *et al*(8). The observations on growth hormone effects in guinea pigs, extend those of Fain *et al*(9), who reported that prominent *in vitro* activation of lipolysis by growth hormone in rats may be observed only after a lag period and supplementation with glucocorticoids.

Summary. Yellow bone marrow of guinea pig is capable of triglyceride synthesis from glucose or from free fatty acids and contains a lipolytic system susceptible to activation, particularly upon contact with pituitary hormones. Its metabolic activity is similar to homologous epididymal adipose tissue, although both guinea pig tissues are appreciably less active than the rat epididymal fat pad. The composition and the metabolic characteristics indicate that the yellow bone marrow represents a typical adipose tissue.

1. Shafrir, E., Wertheimer, E., in Adipose Tissue, Handbook of Physiology, A. E. Renold, G. F., Cahill, Jr., ed., Am. Physiol. Soc. publishers, 1965, Chap. 43, Sec. 5, p427.

2. Dole, V. P., J. Clin. Invest., 1956, v35, 150.

3. Kerpel, S., Shafirir, E., Shapiro, B., Biochim. Biophys. Acta, 1961, v46, 495.

4. Gorin, E., Shafrir, E., ibid., 1963, v70, 109.

5. Folch, J., Lees, M., Sloane-Stanley, G. H., J. Randall, R. J., J. Biol. Chem., 1951, v193, 265.

6. Schneider, W. C., Methods in Enzymology, Colowick, S. P., Kaplan, N. O., ed., Academic Press, Inc., publishers, New York, 1957, v3, 680.

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Biol. Chem., 1957, v226, 497.

8. Rudman, D., Brown, S. J., Malkin, M. F., Endocrinology, 1963, v72, 527.

9. Fain, J. N., Kovacev, V. P., Scow, R. O., J. Biol. Chem., 1965, v240, 3522.

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The Binding of Calcium in Mixtures of Phospholipids.* (31984)

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Recently there has been renewed interest in the interaction of small cations with various phospholipids(1-4). This interest has no doubt been generated to a large extent by certain findings concerning the role of phospholipids in membrane structure and function, by the effects of small cations on membrane permeability, and by new techniques for the separation of the various types of phospholipids in relatively purified form. This recent work on ion-binding indicates that phospholipids bind small cations in a reversible, electrostatic combination, and that even in aqueous micellar suspensions of phospholipids, all of the ionizable groups are readily available. In a study of the binding of calcium in aqueous suspensions of phospholipids, we have confirmed these findings and in addition demonstrated a marked effect of pH on binding. This pH dependence has been shown to be directly related to the nature of the dissociable groups in the various phospholipids.

Methods and materials. The binding measurements were made by using the technique of equilibrium dialysis(5,6). A given amount of desalted phospholipid was dispersed in water either by stirring or evaporation of an organic solution in the presence of water. A known volume of the resulting suspension (usually 10 ml) contained inside a cellophane membrane was placed in an equal volume of 10 m*M* calcium chloride solution and the system ad-

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