Effects of catecholamines on free fatty acid release from bone marrow adipose tissue

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Abstract We have studied the effect of epinephrine and isoproterenol on free fatty acid (FFA) mobilization from bone marrow adipose tissue in dog tibia after constant-flow autoperfusion of the nutrient artery by ipsilateral femoral arterial blood. The perfusions of epinephrine (0.025 μg/min) or isoproterenol (0.005 μg/min) significantly increased the FFA level in the nutrient vein of the tibia. Moreover, our data demonstrate that in vitro the bone marrow adipose tissue was less responsive to catecholamines than omental adipose tissue. It can be concluded that bone marrow adipose tissue is able to release FFA after administration of catecholamines but to a lesser extent than in other adipose tissue (omental adipose tissue). These results support the hypothesis that the bone marrow adipose tissue is involved in local nutrition rather than in the total energy supply of the animal.—**Tran, M-A., L. Dang Tran, and M. Berlan.** Effects of catecholamines on free fatty acid release from bone marrow adipose tissue. *J. Lipid Res.* 1981. **22:** 1271–1276.

Supplementary key words epinephrine · isoproterenol · omental adipose tissue

Hormonal control of lipolysis in adipose tissue varies according to the species of animal (1). In the dog, control seems to be due mainly to catecholamines which are very efficient lipolytic agents both in vivo (2, 3) and in vitro (4).

In the dog, the most widely studied adipose tissues are those of the omental, subcutaneous, and mesenteric regions. From many investigations it appears that catecholamine sensitivity differs according to the tissues. In omental and subcutaneous tissues, catecholamine administration or sympathetic stimulation induces lipid mobilization. On the other hand, mesenteric tissue remains insensitive to sympathetic stimulation and releases fatty acids only with levels of norepinephrine that are ten times greater than those required to produce the same effect in the subcutaneous tissue (5–7). Thus, in the same species, there are quantitative differences in the control of lipid metabolism from different localities.

The adipose tissue of the bone marrow constitutes a significant lipid reserve, the physiological role of which is, as yet, unclear. The most widely accepted hypothesis suggests that the adipocytes of the bone make up a filling tissue for the bone marrow enclosed in the rigid walled cavity of the bone. Thus, the adipose cell in the bone would have a purely structural role (8, 9). On the other hand, Doan, Cunningham, and Sabin (10) and Krause (11) propose that the adipose tissue of the bone marrow plays a metabolic role participating in hematopoietic processes. Finally, Bathija, Davis, and Trubowitz (12) consider that the adipose tissue of the bone marrow does not participate in the energy requirements brought about by fasting; thus this adipose tissue would not be an energy storage tissue and therefore not involved in the energy balance of the organism.

The present report concerns an investigation of the effect of epinephrine and isoproterenol on the adipose marrow of the dog tibia in vivo, using a bone perfusion technique, and in vitro. In the in vitro experiments, we compared the mobilization of fatty acids of the tibia bone marrow with those of the omental adipose tissue.

MATERIAL AND METHODS

In vivo studies

Adult dogs (15–25 kg), fasted for 24 hr, were anesthetized with chloralose (80 mg/kg i.v.), curarized with gallamine (2 mg/kg i.v.), and artificially respired with an Ideal-Palmer pump. Small doses of pentobarbital (10–15 mg) were injected every hour for the maintenance of anesthesia. The nutrient artery and vein of the tibia were dissected out by Tran and Geral's technique. (13) (**Fig. 1**). The femoral artery on the same side was dissected and the deep femoral artery was ligated so that the tibia was only irrigated

Abbreviation: FFA, free fatty acid.

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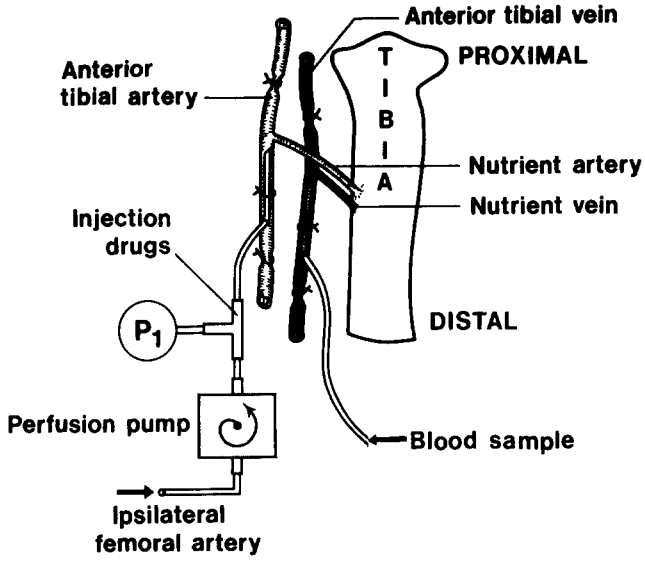

Fig. 1. Cannulation technique for nutrient vein and artery. P_1 denotes the electromanometer for measuring the perfusion pressure.

by the nutrient artery. The animals were heparinized (550 IU/kg every 2 hr) and controlled flow perfusion of the nutrient artery was then started with femoral arterial blood. The perfusion rate (0.5–0.8 ml/min) was different for each experiment so as to obtain a perfusion pressure approximately equal to carotid arterial pressure; it was then kept constant. The pump was associated with a debit-meter in order to check constant blood flow continuously. The anterior tibial vein was ligated above the junction with the nutrient vein and a catheter was introduced into the anterior tibial vein up to the junction with the nutrient vein in order to obtain samples of bone blood. The nutrient artery perfusion pressure and the carotid arterial pressure were measured with Beckman pressure transducers linked to a chart recorder. The following drugs were introduced into the perfusion line: epinephrine hydrochloride (Aguettant) and isoproterenol hydrochloride (Winthrop). The drugs were dissolved in saline. The injection volume was 0.02/ml for 30 min. Blood samples from the nutrient vein were taken at 0, 15, 35, 55, 75, and 95 min. Simultaneously, samples from the carotid artery were taken at 0, 35, 55, 75, and 95 min in order to assay free fatty acids by the procedure of Dole and Meinertz (14) and to record the pH with a Metrohm pH meter.

The epinephrine and isoproterenol perfusions were carried out between the 20th and the 50th minute.

In vitro studies

Incubation procedure. Immediately after removal, omental adipose tissue (3–5 g) or bone marrow adipose tissue was cut into several small pieces and an amount equivalent to 70–90 mg of lipid was incubated in plastic vials for 60 min at 37°C in 2 ml of incubation medium (Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose and 35 mg/ml of defatted albumin). The pH of the medium was previously adjusted to 7.4 with NaOH after the buffer had been equilibrated with 95% O_2–5% CO_2. Adipokinetic substances were added in portions of 20 μl, after suitable dilution, just before starting the incubation procedure. At the end of the incubation, the vials were quickly packed in an ice bath to stop the tissue metabolic activity.

Analytical techniques. At the end of incubation, FFA released into the incubation medium was measured using the method of Dole and Meinertz (14). Some blood was present in bone adipose tissue samples and glycerol determination was not possible using an enzymatic method. To express metabolic activity, the total lipid of adipose tissue in each incubation vial was extracted according to the method of Dole and Meinertz and the mass was estimated gravimetrically after complete evaporation of the solvent.

The data were calculated on a per 100 mg total lipid basis. Each assay was carried out in duplicate and the titration values were averaged.

The pharmacological agents used were the same as for in vivo assays.

Expression of the results. The mean values are given with standard error of the mean (S.E.M.). The significance of the differences within an experiment was estimated using Student's paired *t*-test.

RESULTS

Effect of epinephrine and isoproterenol on the mobilization of free fatty acids from the bone marrow of autoperfused tibia

Epinephrine. The effect of two doses of epinephrine, 0.005 μg/min and 0.025 μg/min, were studied, and the changes in the levels of FFA in the nutrient vein and carotid arterial blood were followed over 95 min. In five dogs, perfusion of epinephrine at 0.005 μg/min for 30 min into the nutrient artery of the tibia did not significantly increase the level of FFA in the nutrient vein (**Fig. 2**). During the experiment, the average percent increases in the level of FFA did not rise more than 15% above the starting values. Simultaneously, in the carotid artery blood, the level of FFA showed slight but insignificant fluctuations. The maximal change did not exceed 15% (Fig. 2). At this dose (0.005 μg/min) epinephrine caused a strong increase in the perfusion pressure of the nutrient artery by stimulation of the intraosseous vaso-

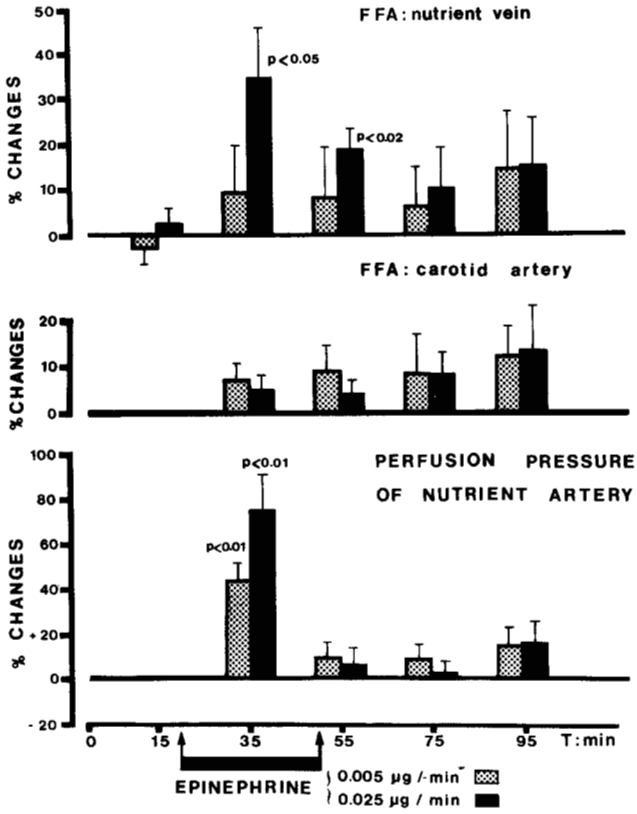

Fig. 2. Mean percentage changes induced by epinephrine (0.005 μg/min and 0.025 μg/min) on the FFA level of nutrient vein and on perfusion pressure of nutrient artery. The mean percentage changes in FFA of carotid artery also are given. Vertical lines represent standard errors. Only the dose of 0.025 μg/min induced a significant increase in the FFA level in nutrient vein ($P < 0.05$ and $P < 0.02$). The two doses significantly enhanced the perfusion pressure of the nutrient artery ($P < 0.01$).

constrictor alpha receptors. During perfusion the average increase in the perfusion pressure was 48 ± 6% (Fig. 2).

The action of epinephrine administered at 0.025 μg/min for 30 min was clearly different (Fig. 2). In six dogs, this dose brought about a significant response at 35 and 55 min with maximal FFA release during perfusion. The FFA levels measured in the nutrient vein before and at mid-perfusion are depicted in **Table 1.** The average increase in the level of FFA in the nutrient vein was 35 ± 11% with respect to the starting value. Simultaneously, the change of levels of FFA in the carotid arterial blood was approximately identical to that observed for the 0.005 μg/min epinephrine perfusion. The average increases in the levels of FFA were 5 ± 3.5 and 4 ± 3% at 35 and 55 min, respectively. These values are not significantly different from the initial value. At 0.025 μg epinephrine, the nutrient artery perfusion pressure was considerably increased (78.5 ± 13% at 35 min) and

TABLE 1. Effect of epinephrine and isoproterenol on FFA output in nutrient vein of the tibia

	FFA Output (μmol/ml)[a]	
	Epinephrine 0.025 μg/min (6)[b]	Isoproterenol 0.005 μg/min (5)[b]
Basal values	0.883 ± 0.13	0.945 ± 0.12
Mid-perfusion values	1.197 ± 0.11	1.403 ± 0.13

[a] Results are expressed as mean ± S.E.M.
[b] ( ), Number of animals.
Mid-perfusion values indicate the FFA concentration measured 35 min after the beginning of the experiment (see Figs. 2 and 3).

then it returned to the initial value in the 25 min following the end of perfusion.

In the carotid artery, the pressure did not show any noticeable modifications and the pH of the carotid arterial blood remained constant around 7.40 until 95 min when it started to decrease (−0.02 to −0.05 depending on the experiment).

Isoproterenol. In the five dogs, perfusion of isoproterenol into the nutrient artery at 0.005 μg/min for 30 min caused a significant increase in the FFA level in the nutrient vein lasting much longer than that observed for a dose of epinephrine five times greater (0.025 μg/min for 30 min). Table 1 indicates the FFA concentration in the nutrient vein before and at mid-perfusion of isoproterenol. We observed the response after 35 min (mid-perfusion) and FFA release continued until 75 min (**Fig. 3**). Simultaneously, in the blood of the carotid artery, the fluctuations in the level of FFA were low and not significant. Thus at 35, 55, and 75 min, the average percentage increase in the levels of FFA in the blood of the nutrient vein were significantly different ($P < 0.02$) from those observed in the carotid arterial blood at the corresponding times. At this dose (0.005 μg/min) isoproterenol did not bring about significant variations of the perfusion pressure of the nutrient artery. In three experiments it decreased appreciably during perfusion but for all five experiments the average decrease at 35 min (mid-perfusion) oscillated around 8 ± 3.5% (hypotension only appeared at higher doses, at least 0.02 μg/min). The arterial pressure of the carotid remained unchanged and the pH was constant around 7.40 until 95 min when it fell by 0.02–0.05 units.

Effect of epinephrine and isoproterenol on the adipose tissue of the tibia bone marrow and on omental tissue in vitro

In **Table 2** we compared in vitro the spontaneous FFA release and adrenergic response of bone marrow adipose tissue and omental adipose tissue, which is

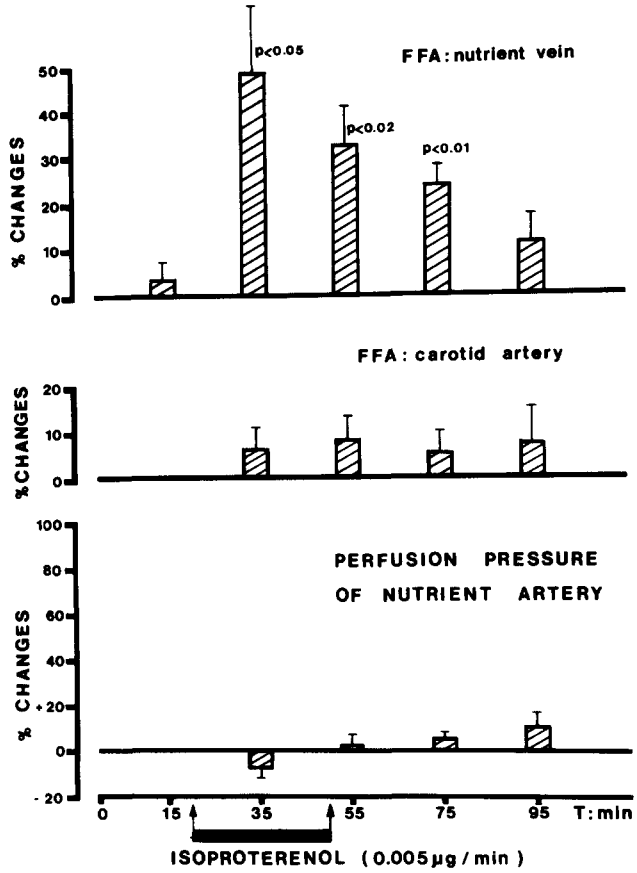

Fig. 3. Mean percentage changes induced by isoproterenol (0.005 µg/min) on the FFA level of the nutrient vein and on perfusion pressure of the nutrient artery. The mean percentage changes in FFA of carotid artery are also given. Vertical lines represent standard errors. This dose significantly induced an increase of FFA levels in the nutrient vein. ($P < 0.05$, $P < 0.02$, and $P < 0.01$) and did not modify the perfusion pressure of the nutrient artery.

known to be very sensitive to catecholamines. Basal FFA production was less in omental adipose tissue than in bone marrow adipose tissue, probably reflecting a strong turnover of FFA (i.e., a high degree of release and reesterification in a medium containing glucose).

The effect of increasing the concentration of epinephrine and isoproterenol on FFA release was also studied. In omental adipose tissue, epinephrine and isoproterenol induced a strong stimulation. A significant effect appeared with 0.06×10^{-5} M. Isoproterenol elicited appreciable FFA release at 0.06×10^{-5} M and 0.6×10^{-5} M. The maximal effect of the two catecholamines was similar at 6×10^{-5} M.

In bone marrow adipose tissue, epinephrine and isoproterenol promoted a weaker FFA release than in omental adipose tissue. The lowest dose of both catecholamines was ineffective. A significant stimulating effect only appeared with 0.6 and 6×10^{-5} M and the level of FFA production was increased only about 60%. Moreover, there was no significant difference between the two catecholamines.

DISCUSSION

The hormone-sensitive metabolism of the adipose tissue has been widely studied in vitro; experiments carried out in vivo, however, are much rarer due to technical difficulties. Rosell (15) and Ballard and Rosell (5, 6) carried out perfusions of subcutaneous and mesenteric adipose tissue in dogs. On the other hand, no investigations concerning the effect of hormonal substances on the adipose tissue of perfused bone marrow are reported in the literature.

Our in vivo and in vitro experiments showed that the administration of epinephrine and isoproterenol induced FFA mobilization from the adipose tissue of the tibia bone marrow. The in vivo results however showed a clear difference in the intensity of the response of the bone adipose tissue to the administration of these two substances. At a dose of 0.005 µg/min, only isoproterenol, induced a very significant FFA output. The effect of epinephrine did not appear until the dose was five times stronger and even then the effect was lower than that of isoproterenol (Fig. 3). Under the same conditions the vascular effects of the two catecholamines were completely different. Epinephrine always increased the perfusion pressure in the nutrient artery, and isoproterenol had no vascular effect. The vasodilator effect of the latter on bone vascularization appeared only at doses that were equal to or higher than 0.02 µg/min (13).

There are two possible explanations for the dif-

TABLE 2. In vitro effects of epinephrine and isoproterenol on FFA release in dog bone marrow adipose tissue and omental adipose tissue

	FFA Release (µmol/100 mg lipid/90 min)[a]	
Addition to Medium	Bone Marrow Adipose Tissue (7)[b]	Omental Adipose Tissue (6)[b]
Basal lipolysis	0.29 ± 0.08	0.09 ± 0.03[c]
Epinephrine		
0.06×10^{-5} M	0.23 ± 0.08	0.61 ± 0.24[d]
0.6×10^{-5} M	0.40 ± 0.09[d]	1.6 ± 0.23[e]
6×10^{-5} M	0.45 ± 0.08[d]	2.53 ± 0.46[e]
Isoproterenol		
0.06×10^{-5} M	0.29 ± 0.06	1.66 ± 0.17[e]
0.6×10^{-5} M	0.48 ± 0.10[d]	2.2 ± 0.36[e]
6×10^{-5} M	0.44 ± 0.07[d]	2.61 ± 0.46[e]

[a] Results are expressed as mean ± S.E.M.
[b] ( ), Number of animals.
[c] $P < 0.02$ as compared with bone marrow basal values.
[d] $P < 0.05$ compared to basal value.
[e] $P < 0.01$ compared to basal value.

ference observed in vivo. On one hand epinephrine, a mixed adrenergic agonist, is able to stimulate both types of adrenergic receptors; the inhibitory alpha-adrenergic effect counteracts the stimulatory effect linked to the stimulation of beta-receptors. The alpha-receptors have been clearly demonstrated in vitro in dog adipocytes (16, 17) and human fat cells (17–19). On the other hand, the difference could be linked to vascular phenomena. The intraosseous vasoconstriction brought about by epinephrine could counteract the FFA output from the bone marrow adipose tissue as proposed by Fredholm and Rosell (7) in the subcutaneous adipose tissue of the dog. However, our in vitro results do not show any noticeable differences in the FFA release promoted by the administration of epinephrine and isoproterenol. These results favor the hypothesis of involvement of vascular factors.

The in vitro experiments clearly show that both epinephrine and isoproterenol provide a weaker FFA production in bone marrow adipose tissue than in omental adipose tissue. Thus, in the dog, we observed, as did Ballard and Rosell (5, 6) and Fredholm and Rosell (7) in omental, mesenteric, and subcutaneous tissues, that catecholamine sensitivity differs according to the site of the tissue. Similar results were also obtained in other species. In man, in vitro noradrenaline brings about a lipolytic response that is more intense than in the omental tissue than in the subcutaneous tissue (20). In the rat, in vitro, the mesenteric adipose tissue is more sensitive to epinephrine than the perirenal or epididimal tissues (21). In the cat, the omental adipose tissue is catecholamine-sensitive, whereas the fatty eye-socket tissue remains unaffected (22).

Even though the adipose tissue generally behaves as an energy reserve, various observations have led numerous authors to propose hypotheses concerning the physiological role of the adipose tissue in the different locations. For Aronowsky et al. (22) the adipose tissue in some regions may have a support function rather than serving as an energy depot. Furthermore, they found that adipose tissue with a structural function did not exert any adipokinetic effects compared to tissues whose chief function is fat storage. Similarly for Renold and Cahill (23) and Wertheimer (24), it was clear that the fatty reserves in various localities respond differently to lipogenic or lipolytic stimuli, and that some areas assume purely structural, essentially non-metabolic roles such as insulation against the cold or as a buffering and lubrication system in joints or the socket of the eye. Regarding these different hypotheses, the physiological role of the bone adipose tissue is, as yet, poorly defined. The most widely accepted hypothesis proposes that the bone adipocytes make up the filling tissue of the bone marrow (8, 9). Doan et al. (10) however, consider that the fatty bone marrow plays a metabolic role, participating in hematopoietic processes. Finally, the majority of authors (12, 25, 26) who have studied the role of fasting in lipid mobilization of the adipose bone tissue finds that this tissue does not constitute an energy reserve for the organism.

Our in vivo and in vitro results show that the adipose tissue of the bone is capable of FFA mobilization. This fact is not incompatible with the hypothesis of Tavassoli (27), according to which the fatty marrow of the bone must become adapted, through a decrease in volume, to any expansion of the hematopoietic tissue in order to maintain constant the volume of the bone marrow enclosed in the rigid walled bone cavity.

Our in vitro experiments show that the capacity for FFA mobilization of this tissue is reduced compared to that of the omental tissue. This supports the hypothesis that the fatty marrow of the bone is not an energy reserve and is also in agreement with the previously mentioned authors (12, 25, 26). The bone marrow adipose tissue may be an important source of free fatty acids for red cell membranes and oxidative metabolism for marrow cells. In conclusion, the adipose tissue of the bone marrow seems to be more important for local nutrition rather than for total energy supplies of the animal.

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REFERENCES

1. Rudman, D., S. J. Brown, and F. Malkin. 1963. Adipokinetic action of adrencorticotropin, thyroid-stimulating hormone, vasopressin, α and β melanocyte-stimulating hormones, fraction H, epinephrine and norepinephrine in the rabbit, guinea pig, hamster, rat, pig, and dog. *Endocrinology.* **72:** 527–543.
2. Kennedy, B. L., and S. Ellis. 1969. Dissociation of catecholamine-induced calorigenesis from lipolysis and glycogenolysis in intact animals. *J. Pharmacol. Exp. Ther.* **168:** 137–145.
3. Winkler, B., I. Rathgeb, C. Bjerknes, R. Steele, and N. Altszuler. 1973. Effect of norepinephrine on glycerol and glucose metabolism in the normal dog. *Am. J. Physiol.* **225:** 81–84.
4. Berlan, M. 1979. La lipolyse hormono-dependante, effet alpha et beta adrenergique sur les adipocytes du chien et de l'homme. Thèse Doctorates Science n° 876 Toulouse.
5. Ballard, K., and S. Rosell. 1969. Unresponsiveness of lipid metabolism in canine mesenteric adipose tissue to biogenic amines and to sympathetic nerve stimulation. *Acta Physiol. Scand.* **77:** 442–448.
6. Ballard, K., and S. Rosell. 1971. Adrenergic neuro-

humoral influences on circulation and lipolysis in canine omental adipose tissue. *Circ. Res.* **28:** 389–396.
7. Fredholm, B., and S. Rosell. 1968. Effect of adrenergic blocking agents on lipid mobilization from canine subcutaneous adipose tissue after sympathetic nerve stimulation. *J. Pharmacol. Exp. Ther.* **159:** 1–7.
8. Erslev, A. J. 1967. Medullary and extramedullary blood formation. *Clin. Orthop.* **52:** 25–36.
9. Ascenzi, A. 1976. Physiological relationships and pathological inferences between bone tissue and marrow. *In* Biochemistry and Physiology of Bone. Vol. 4. Calcification and Physiology. G. Bourne, editor. New York Academic Press. 403–444.
10. Doan, C. A., R. S. Cunningham, and F. R. Sabin. 1925. Experimental studies on the origin and maturation of avian and mammalian red blood cells. *Contrib. Embryol. Carnegie Inst. Washington, DC.* **16:** 163–226.
11. Krause, R. F. 1943. Changes induced by anemia in bone marrow lipids of cats. *J. Biol. Chem.* **149:** 395–404.
12. Bathija, A., S. Davis, and S. Trubowitz. 1979. Bone marrow adipose tissue: response to acute starvation. *Am. J. Hematol.* **6:** 191–198.
13. Tran, M-A., and J. P. Geral. 1978. The influence of some vasoactive drugs on bone circulation. *Eur. J. Pharmacol.* **52:** 109–115.
14. Dole, V. P., and H. Meinertz. 1960. Microdetermination of long chain fatty acids in plasma and tissues. *J. Biol. Chem.* **235:** 2595–2599.
15. Rosell, S. 1966. Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation. *Acta Physiol. Scand.* **67:** 343–351.
16. Berlan, M., and L. Dang Tran. 1978. Intervention de recepteurs adrenergiques alpha et beta dans l'effet des catacholamines sur la lipolyse d'adipocytes de chien. *J. Physiol. Paris.* **74:** 601–608.
17. Milavec-Krizman, M., and H. Wagner. 1978. Effects of the adrenergic agonists isoprenaline and noradrenaline and the alpha-blocking agents dihydroergotamine and phentolamine on the lipolysis in isolated fat cells of the rat, guinea pig, dog, and man. *Biochem. Pharmacol.* **27:** 2305–2310.
18. Lafontan, M., L. Dang Tran, and M. Berlan. 1979. Alpha-adrenergic antilipolytic effect of adrenaline in human fat cells of the thigh: comparison with adrenaline responsiveness of different fat deposits. *Eur. J. Clin. Invest.* **9:** 261–266.
19. Berlan, M., M. Lafontan, and L. Dang Tran. (1980). La lipolyse adrénergique du tissu adipeux humain: propriétés et rôle physiologique des alpha récepteurs. *J. Physiol. Paris.* **76:** 133–146.
20. Carlson, L., and D. Hallberg. 1965. Basal lipolysis and effects of norepinephrine and prostaglandin E_1 on lipolysis in human subcutaneous and omental adipose tissue. *J. Lab. Clin. Med.* **71:** 368–377.
21. Wertheimer, E., M. Hamosh, and E. Shafwi. 1960. Factors affecting fat mobilization from adipose tissue. *Am. J. Nutr.* **8:** 705–711.
22. Aronowsky, E., R. Levari, W. Kornlueth, and E. Wertheimer. 1963. Comparison of metabolic activities of orbital fat with those of other tissues. *Invest. Ophthalmol.* **2:** 259–264.
23. Renold, A. B., and G. F. Cahill, Jr. 1965. *In* Handbook of Physiology. Section 5: Adipose Tissue. American Physiological Society, Washington DC. 1–4.
24. Wertheimer, H. E. 1965. *In* Handbook of Physiology. Section 5: Adipose Tissue. American Physiological Society, Washington, DC. 5–11.
25. Dietz, A. A., and B. Steinberg, 1953. Chemistry of bone marrow. VII. Composition of rabbit bone marrow inanition. *Arch. Biochem.* **45:** 10–20.
26. Tavassoli, M. D. 1974. Differential response of bone marrow and extramedullary adipose cells to starvation. *Experientia.* **30:** 424–425.
27. Tavassoli, M. D. 1976. Marrow adipose cells. *Arch. Pathol. Lab. Med.* **100:** 16–18.