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PATHOPHYSIOLOGY OF BONE LOSS IN CASTRATED ANIMALS

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Summary—The pathophysiology of bone loss in castrated animals is reviewed. Both male and female rats rapidly lose metaphyseal trabecular bone from the tibia and the femur due to an imbalance between bone resorption and bone formation. The aetiology of sex hormone deficiency-induced bone loss is not fully understood. It seems unlikely that the bone loss is due to changes in the circulating levels of the calciotropic hormones or to an increase in the spontaneous release from peripheral blood monocytes of the bone resorption stimulating cytokine IL-1. Changes in the sensitivity of bone of castrated rats to calciotropic hormones may play a role as well as the lack of direct stimulatory effects of gonadal oestrogens and androgens on bone cells. In addition several data indicate that prostaglandins may be involved.

INTRODUCTION

Since life expectency is increasing, age-related disorders are becoming more common. These disorders do not only cause suffering for the patients, but also lead to high costs for the community [1].

Osteoporosis is such a disorder and is defined as a reduced amount of normally mineralised bony tissue per volume of bone. Osteoporosis increases the risk of fractures, when bone mass is diminished to such an extent that the skeleton has insufficient strength to withstand normal mechanical loading [2]. At present, considerable research is being done to study the aetiology of osteoporosis and to find drugs that can be used for the prevention and treatment of this disorder.

In males and females spontaneous bone loss occurs during aging. This loss is associated with a reduction in the bone-forming capacity of osteoblasts and may, later in life, result in the development of senile osteoporosis or osteoporosis type II [3]. In females, a strong acceleration of mainly trabecular bone loss occurs in the late premenopausal and early postmenopausal years [4, 5]. This bone loss, which is most likely due to oestrogen deficiency, is osteoclast-mediated [6] and may provide a basis for the development of postmenopausal osteoporosis or osteoporosis type I, which characteristically occurs in women 15–20 yr after the menopause [7].

Studies on the aetiology of osteoporosis are hampered by the fact that sex hormone deficiency-induced bone loss does not occur spontaneously in experimental animals. Such a type of bone loss can, however, easily be induced for example in the rat and dog by surgical ovariectomy or inhibition of ovarian function with LHRH agonists or antagonists [8–13].

In this paper we will review the course of ovariectomy- and orchiectomy-induced trabecular and cortical bone loss in the rat and ovariectomy-induced bone loss in dogs. In addition, we will compare what is known about the aetiology of bone loss in animals and in humans.

OVARIECTOMY-INDUCED BONE LOSS IN THE RAT

After ovariectomy trabecular bone is lost very fast from several sites from the rat skeleton, e.g. from the metaphyses of the femur and tibia and, at a much lower rate, from the vertebra. Bone-histomorphometrical studies have shown that the loss of trabecular bone from the tibia is due to a strong increase in bone remodelling [10–12]. Bone remodelling in intact rats is characterised by a balance between bone resorption and bone formation; in ovariectomised rats, however, the process of bone remodelling is in dysbalance: the increase in bone resorption is not completely compensated by an increase in bone formation. Histological studies in early postmenopausal

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females have shown that during oestrogen deficiency osteoclasts make deeper resorption lacunae while osteoblast activity is normal [14]; this provides a morphological explanation for the dysbalance during remodelling in early postmenopausal women. Such data are not available for the rat, but the fact that in the rat early after ovariectomy trabecular perforation occurs, strongly suggests that, here too, oestrogen deficiency causes an increase in the bone-resorbing capacity of osteoclasts. Only when the depth of a formed resorption lacuna exceeds trabecular plate thickness can trabecular perforation occur.

Measurements of trabecular thickness have shown that this parameter does not diminish after ovariectomy in the rat [15] which furthermore suggests that osteoblast activity is not reduced after ovariectomy. On the basis of this consideration it is most likely that in the ovariectomised rat, as in early postmenopausal women, trabecular bone loss is osteoclast-mediated.

Ovariectomy also affects cortical bone mass in rats. In a study in growing female ORGA rats, we found that ovariectomy not only increases longitudinal and periosteal growth but also reduces endosteal bone formation. This observation is in line with those of Turner *et al.* [12], who found in ovariectomised rats, using histomorphometry, that periosteal bone-forming surfaces were increased while endosteal bone-forming surfaces were decreased.

Apparently, endogeneous oestrogens stimulate endosteal osteoblast activity. The increase in periosteal bone formation in ovariectomised rats may be related to the significant increase in body weight gain (see Fig. 1).

The decrease of the trabecular bone mass in the metaphyses, the reduction of the amount of newly formed endosteal bone and the slight increase in bone volume results in a significant reduction in the ratio of bone weight and bone volume, i.e. in the specific mass. This parameter has frequently been used in studies in the rat as an indicator for osteoporosis [9].

AETIOLOGY OF OESTROGEN DEFICIENCY-INDUCED BONE LOSS IN THE RAT

Various studies have been performed in rats to identify the regulatory factors involved in bone loss after ovariectomy. Since 99% of total body calcium is located in the skeleton, it has been hypothesized that the calciotropic

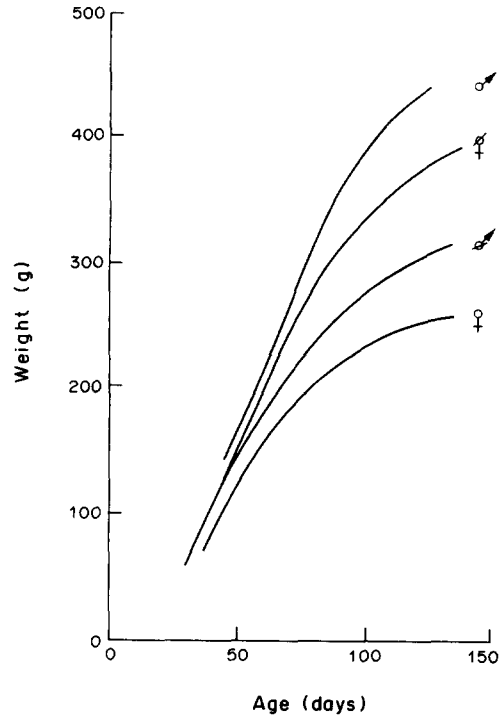

Fig. 1. Effect of gonadectomy on body weight gain in rats. (From Ref. [7].)

hormones may be involved. However, plasma levels of PTH and 1,25 $(OH)_2$vitD_3 do not change after ovariectomy [13, 16–18]. The basal level of plasma calcitonin, on the other hand, is significantly reduced in ovariectomised rats [16, 19, 20]. It is, however, unlikely that oestrogen deficiency-induced bone loss can be attributed to a reduction in plasma calcitonin levels, since no difference in bone loss was observed between ovariectomised and ovariectomised thyroidectomised rats [19]. Furthermore, plasma calcitonin was not reduced in ovariectomised rats on a low calcium intake, while they did rapidly lose trabecular bone [17]. These data indicate that bone loss is not mediated by changes in the plasma levels of the calciotropic hormones. A similar conclusion on the lack of a role in the aetiology of early postmenopausal bone loss was drawn by Falch [21].

As originally suggested by Heaney [22], it may not be the plasma levels of the calciotropic hormones which are of importance, but changes in the sensitivity of bone to these hormones. This hypothesis is sustained by: (a) the observation that bone of ovariectomised rats is more sensitive to the resorbing actions of PTH [23], and (b) by the recent *in vitro* finding that 17β-oestradiol reduces the PTH-induced

formation of cyclic AMP in osteoblasts, which mediate the bone-resorbing activity of PTH [24]. 17β-Oestradiol has stimulatory activity on the proliferation of primary cultures of osteoblasts and osteoblast-like cells suggesting that 17β-oestradiol has both anti-catabolic and anabolic effects [25–28]. In a study in ovariectomised rats, in which 17β-oestradiol was infused directly onto trabecular bone in the femur, further evidence for the anabolic activity of 17β-oestradiol was obtained [29].

Several investigations have drawn attention to a possible role of *local* factors as mediators of ovariectomy-induced bone loss. It is already known for a long time that the prostaglandin E_2 (PGE_2) is a potent stimulator of bone resorption *in vitro* [30]. Measurements of the production of PGE_2 by calvaria of neonatal rats show that PGE_2 production is increased in ovariectomised rats [31]. *In vivo* data further indicate a possible role for prostaglandins, since trabecular loss from the metaphyses of ovariectomised rats was partly prevented by treatment with the non-steroidal anti-inflammatory drug Naprosyn® (naproxen) [32]. Another local factor that has been implicated in oestrogen deficiency-induced bone loss is interleukin-1 (IL-1). This cytokine is synthesized by osteoblasts upon stimulation with tumour necrosis factor α or lipopolysaccharide [33, 34] and strongly stimulates bone resorption *in vitro* and *in vivo* [35–37]. Peripheral blood monocytes of early postmenopausal women spontaneously release increased amounts of IL-1 and following treatment with 17β-oestradiol, a reduction of this IL-1 release was observed [38, 39].

In view of the close anatomical contact between monocytes and bone cells one may assume that increased levels of locally released IL-1 stimulates bone resorption after the induction of oestrogen deficiency. Preliminary studies in rats, however, failed to show an increase in the release of IL-1 from monocytes after ovariectomy (Pacifici, personal communication; Schot, unpublished data). Obviously, additional studies are necessary to further investigate the possible role of IL-1 in oestrogen deficiency-induced bone loss.

OVARIECTOMY-INDUCED BONE LOSS IN THE DOG

Bone loss also occurs in dogs following ovariectomy, however, the mechanism of bone loss is not completely clear. Faugere *et al.* [40] found, initially after ovariectomy, a strong increase in osteoclast activity and in bone remodelling. The increase in bone resorption was temporary and 4 months after ovariectomy bone resorption was normal. Osteoblast numbers were, however, significantly increased, suggesting that the maintenance of bone loss in the dog is due to impaired osteoblast function [41]. These data are in line with those of Dannucci *et al.* [42].

In another study, however, no increase in bone remodelling was found after ovariectomy; it was concluded that bone is lost due to a change in the balance between bone resorption and bone formation [43].

EFFECTS OF ORCHIECTOMY IN MALE RATS

Studies in humans have revealed that hypogonadism is associated with a low bone mass and an increased risk of the development of osteoporosis [44, 45]. It was also found that castration of young males causes a significant increase in bone remodelling and loss of trabecular from the spine [46]. Apparently, a reduction in sex hormone levels causes similar effects in males and females.

Little is known on the aetiology of androgen deficiency-induced bone loss but, since androgens have a positive effect on the calcium balance by increasing the calcium uptake from the intestine and reducing the urinary excretion of calcium, calciotropic hormones may be involved. In addition, it has been reported that monocytes of hypogonadal males release increased amounts of IL-1 [47].

Only a limited number of studies have been performed on the effect of orchiectomy in the rat. As in females, castration results in a change in body weight gain. In contrast to ovariectomised female rats, castrated male rats gain less weight than intact animals (see Fig. 1). In addition, skeletal growth is impaired [8, 48, 49]. Both longitudinal and periosteal growth are reduced by orchiectomy, indicating that the male sex hormone stimulates both the proliferation of the epiphyseal plane chondrocytes and the bone-forming capacity of the periosteal osteoblasts. Castration of 1-yr-old male rats, which did not show measurable longitudinal growth, resulted in a significant reduction of bone volume [50]. Since length did not change, the reduction in volume was most likely due to periosteal bone resorption.

In addition to effects on cortical bone mass, effects of orchiectomy on trabecular bone mass

have also been reported. Verhas *et al.* [50] found a significant increase in activity of osteoclasts located on trabeculae near the epiphyseal plate and a strong increase in bone turnover rate at those sites in the femur and tibia where high amounts of trabecular bone occur. Apparently, during the increase in bone turnover there is a greater increase in bone resorption than in bone formation, since trabecular bone is lost from the methaphyses of the femur of young castrated male rats [51–53]. From these observations it may be concluded that orchiectomised rats lose trabecular bone by an imbalance in bone remodelling, as was also observed in castrated males [46].

With regard to the aetiology of bone loss in male rats, almost nothing is known. It seems unlikely, however, that such loss is related to changes in the levels of the calciotropic hormones, since in young castrated males no changes in plasma calcium, phosphate, $25(OH)vitD_3$ and $1,25(OH)_2vitD_3$ were found [53]. It seems more likely that androgen deficiency changes the sensitivity of the bone to the activity of the calciotropic hormones, as testosterone has been found to increase the biological activity of calcitonin in thyroidectomised rats [54].

Note added in proof: In a recent publication Coble [54] demonstrated that naproxen has no lasting ability to halt estrogen depletion bone loss in aged ovariectomised rats. This may indicate that prostaglandins are only involved in the early phase of oestrogen deficiency-induced bone loss in the rat.

REFERENCES

1. Melton L. J. III: Epidemiology of fractures. In *Osteoporosis Etiology, Diagnosis, and Management* (Edited by B. L. Riggs and L. J. Melton III). Raven Press, New York (1988) pp. 133–154.
2. Nordin B. E. C.: *Metabolic Bone and Stone Disease*. Churchill Livingstone, London (1984).
3. Melton L. J. III. and Riggs B. L.: Clinical spectrum. In *Osteoporosis Etiology, Diagnosis and Management* (Edited by B. L. Riggs and Melton L. J. III). Raven Press, New York (1988) pp. 155–181.
4. Slemenda C., Hui S. L., Longcope C. and Johnston C. C.: Sex steroids and bone mass. A study of changes about the time of menopause. *J. Clin. Invest.* **80** (1987) 1261–1269.
5. Elders P. J. M., Netelenbos J. C., Lips P., Ginkel F. C. van and Stelt P. F. van der: Accelerated vertebral bone loss in relation to the menopause: a cross-sectional study on lumbar bone density in 286 women of 46 to 55 years of age. *Bone Min.* **5** (1988) 11–19.
6. Parfitt A. M.: Bone remodeling: relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. In *Osteoporosis Etiology, Diagnosis, and Management* (Edited by B. L. Riggs and Melton L. J. III). Raven Press, New York (1988) pp. 45–94.
7. Riggs B. L. and Melton L. J. III: Evidence for two distinct syndromes of involutional osteoporosis. *Am. J. Med.* **75,** (1983) 889–901.
8. Saville P.: Changes in skeletal mass and fragility with castration in the rat: a model of osteoporosis. *Am. Geriathr. Soc.* **17** (1969) 155–166.
9. Hodgkinson A., Aaron J. E., Horsman A., McLachlan M. S. F. and Nordin B. E. C.: Effect of oophorectomy and calcium deprivation on bone mass in the rat. *Clin. Sci. Molec. Med.* **54** (1978) 439–446.
10. Wronski T. J., Lowry P. L., Walsh C. C. and Ignaszewski L. A.: Skeletal alterations in ovariectomized rats. *Calc. Tiss. Int.* **37** (1985)324–328.
11. Wronski T. J., Dann L. M., Scott K. S. and Crooke L. R.: Endocrine and pharmacological suppressors of bone turnover protect against osteopenia in ovariectomized rats. *Endocrinology* **125** (1989) 810–816.
12. Turner R. T., Vandersteenhove J. J. and Bell N. H.: The effect of ovariectomy and 17β-estradiol on bone histomorphometry in growing rats. *J. Bone Min. Res.* **2** (1987) 115–122.
13. Goulding A. and Gold E.: a new way to induce oestrogen-deficiency osteopenia in the rat: comparison of the effects of surgical ovariectomy and administration of the LHRH agonist on bone resorption and composition. *J. Endocr.* **121** (1989) 293–298.
14. Eriksen E. F., Melsen F. and Mosekilde L.: Reconstruction of the resorptive site in iliac trabecular bone: a kinetic model for bone resorption in 20 normal individuals. *Metab. Bone Dis. Rel. Res.* **5** (1984) 235–242.
15. Faugere M. C., Okamoto S., De Luca H. and Malluche H. H.: Calcitriol corrects bone loss induced by oophorectomy in rats. *Am. J. Physiol.* **250** (1986) E35–E38.
16. Kalu D. N., Hardin R. R. and Cockerham R.: Evaluation of the pathogenesis of skeletal changes in ovariectomized rats. *Endocrinology* **115** (1984) 507–512.
17. Kalu D. N., Liu C. C., Hardin R. R. and Hollis B. W.: The aged rat model of ovarian hormone deficiency bone loss. *Endocrinology* **124** (1989) 7–16.
18. Ismail F., Epstein S., Fallon M. D., Thomas S. B. and Reinhardt T. A.: Serum bone gla protein and the vitamin D endocrine system in the oophorectomized rat. *Endocrinology* **122** (1988) 624–630.
19. Klein P., Raue F., Sterz. H. and Ziegler R.: Calcitonin deficiency is not the mediator of osteopenia in ovariectomized rats. *Acta Endocr.* **287** (Suppl. 117) (1988) 168–169.
20. Yamazaki I. and Yamaguchi H.: Characteristics of an ovariectomized osteopenic rat model. *J. Bone Min. Res.* **4** (1989) 12–22.
21. Falch J. A.: Postmenopausal bone loss-causes and consequences. With special reference to the significance of Vitamin D metabolites. Thesis, University of Oslo (1987).
22. Heaney R. P.: A unified concept of osteoporosis. *Am. J. Med.* **39** (1965) 877–880.
23. Orimo H., Fujita T. and Yoshikawa M.: Increased sensitivity of bone to parathyroid hormone. *Endocrinology* **90** (1972) 760–763.
24. Fukayama S. and Tashjian A. H.: Direct modulation by estradiol of the response of human bone cells (SaOS-2) to human parathyroid hormone (PTH) and PTH-related protein. *Endocrinology* **124** (1989) 397–401.
25. Gray T. K., Flynn T. C., Gray K. M. and Nabell L. M.: 17-beta-estradiol acts directly on the clonal osteoblastic cell line UMR106. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 6267–6271.
26. Gray T. K., Mohan S., Linkhart T. A. and Baylink D. J.: Estradiol stimulates *in vitro* the secretion of insulin-like growth factors by the clonal osteoblastic cell

line, UMR106. *Biochem. Biophys. Res. Commun.* **158,** (1989) 407–412.
27. Ernst M., Schmid C. and Froesch E. R.: Enhanced osteoblast proliferation and collagen gene expression by estradiol. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 2307–2310.
28. Ernst M., Heath J. H. and Rodan G. A.: Estradiol enhances insulin-like growth factor I mRNA and has anabolic effects in osteoblastic cells from rat calvariae and long bones. *J. Bone Min. Res.* **4** (Suppl. 1) (1989) S256.
29. Takano-Yamamoto T. and Rodan G. A.: Direct effects of 17β-estradiol on trabecular bone in ovariectomized rats. *J. Bone Min. Res.* **4** (Suppl. 1) (1989) S235.
30. Klein D. C. and Raisz L. G.: Prostaglandin stimulation of bone resorption in tissue culture. *Endocrinology* **86** (1970) 1436–1440.
31. Feyen J. H. M. and Raisz L. G.: Prostaglandin production by calvariae from sham operated and oophorectomised rats: Effect of 17β-estradiol *in vivo*. *Endocrinology* **121** (1987) 819–821.
32. Lane N., Kimmel D. B. and Coble T. Effect of naprosyn on bone volume and formation in ovariectomized rats. *J. Bone Min. Res.* **4** (Suppl. 1) (1989) S193.
33. Peck W. A. and Woods W.: The cells of bone. In *Osteoporosis Etiology, Diagnosis and Management* (Edited by B. L. Riggs and L. J. Melton III). Raven Press, New York (1988) pp. 1–44.
34. Keeting P. E., Rifas L., Peck W. A. and Riggs B. L.: Production of interleukin-1β by stimulated normal osteoblast-like cells. *J. Bone Min. Res.* **4** (Suppl. 1) (1989) S256.
35. Gowen M., Wood D. D., Ihrie E. J., McGuire M. K. B. and Russell R. G. G.: An interleukin 1 like factor stimulates bone resorption *in vitro*. *Nature* **306** (1983) 378–380.
36. Heath J. K., Saklatvala J., Meikle M. C., Atkinson S. J. and Reynolds J. J.: Pig interleukin 1 (catabolin) is a potent stimulator of bone resorption *in vitro*. *Calc. Tiss. Int.* **37** (1985) 95–97.
37. Sabatini M., Boyce B., Aufdemorte T., Bonewald L. and Murphy G. R.: Infusions of recombinant human interleukins 1α and 1β cause hypercalcemia in normal mice. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 5235–5239.
38. Pacifici R., Rifas L., Vered I., McMurtry C., McCracken R., Avioli L. V. and Peck W. A.: Interleukin-1 secretion from human blood monocytes in normal and osteoporotic women: Effect of menopause and estrogen/progesterone treatment. *J. Bone Min. Res.* **3** (Suppl. 1) (1988) S204.
39. Stock J. L., Coderre J. A., McDonald B. and Rosenwasser J. L.: Effects of estrogen *in vivo* and *in vitro* on spontaneous interleukin-1 release by monocytes from postmenopausal women. *J. Clin. Endocr. Metab.* **68** (1989) 364–368.
40. Faugere M. C., Friedler R. M., Fanti P. and Malluche H. H.: Induction and maintenance of bone loss after cessation of ovarian function. *J. Bone Min. Res.* **4** (Suppl. 1) (1989) S176.
41. Malluche H. H., Faugere M. C., Rush M. and Friedler R. M.: Osteoblastic insufficiency is responsible for maintenance of osteopenia after loss of ovarian function in experimental beagle dogs. *Endocrinology* **119** (1986) 2649–2654.
42. Dannucci G. A., Martin R. B. and Patterson-Buckendahl P.: Ovariectomy and trabecular bone remodeling in the dog. *Calc. Tiss. Int.* **40** (1987) 194–199.
43. Snow G. R. and Anderson C.: The effects of 17β-estradiol and progestagen on trabecular bone remodeling in oophorectomized dogs. *Calc. Tiss. Int.* **39** (1986) 198–205.
44. Seeman E., Melton L. J. III, O'Fallon W. M. and Riggs B. L.: Risk factors for spinal osteoporosis in men. *Am. J. Med.* **75** (1983) 977–983.
45. Lindsay R.: Sex steroids in the pathogenesis and prevention of osteoporosis. In *Osteoporosis Etiology, Diagnosis and Management* (Edited by Riggs B. L. and Melton L. J. III). Raven Press, New York (1988) pp. 333–358.
46. Stepán J. J., Lachman M., Zverina J., Pacavky V. and Baylink D. J.: Castrated men exhibit bone loss: Effect of calcitonin treatment on biochemical indices of bone remodeling. *J. Clin. Endocr. Metab.* **69** (1989) 523–527.
47. Axelrod D. W., Lachman L. B., Judge D., Mallette L. E., and Gagel R. F.: Resorptive hypercalciuria and increased interleukin 1 in a young man with hypogonadism and osteoporosis: reversal with androgen treatment. *Clin. Res.* **37** (1989) 355A.
48. Schoutens A., Verhas M., L'Hermite-Baleriaux, L'Hermite M., Verschaeren A., Dourov N., Mone M., Heilporn A. and Tricot A. *Acta Endocr.* **107** (1984) 428–432.
49. Hock J. M., Gera I., Fonseca J. and Raisz L. G.: Human parathyroid hormone-(1-34) increases bone mass in ovariectomized and orchidectomized rats. *Endocrinology* **122** (1988) 2899–2904.
50. Verhas M., Schoutens A., L'Hermite-Baleriaux M., Dourov N., Verschaeren A., Mone M. and Heilporn A.: The effect of orchidectomy on bone metabolism in aging rats. *Calc. Tiss. Int.* **39** (1986) 74–77.
51. Wink G. and Felts W.: Effect of castration on the bone structure of male rats: a model of osteoporosis. *Calc. Tiss. Int.* **32** (1980) 77–82.
52. Turner R., Wakley G., Hannon K. and Bell N.: Evidence that androgens are necessary for normal development and maintenance of bone. *J. Bone Min. Res.* **3** (Suppl. 1) (1988) S222.
53. Ogata E., Shimarawa E., Suzuki H., Yoshitoshi Y., Asano H. and Ando H.: Androgens and enhancement of hypocalcemic responses to thyrocalcitonin in rats. *Endocrinology* **87** (1970) 421–426.
54. Coble T., Lane N. and Kimmel D. B.: *J. Bone Min. Res.* **5** (Suppl. 2) (1990) S118.