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# **Effect of serum dilution fluids on the wear of unirradiated and high dose gamma-irradiated, vitamin E stabilized UHMWPE**

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## **Abstract**

In orthopaedic tribology, the ISO 14242-1 standard recommends that the serum lubricant is diluted with deionized water (DW). Sometimes, phosphate buffered saline (PBS) is used instead for the dilution. Noncyclic, multidirectional pin-on-disk tests showed that the dilution of alpha calf serum with PBS or phosphate buffer resulted in a 5 to 40-fold decrease in the wear factor of unirradiated and of vitamin E stabilized, highly crosslinked ultrahigh molecular weight polyethylene (UHMWPE) compared with DW dilution. It appears that the phosphates were mainly responsible for this phenomenon. PBS dilution may result in a substantial underestimation of clinical wear. With 1:1 DW dilution, the mean wear factor of highly crosslinked UHMWPE was 5.7-fold lower than that of unirradiated UHMWPE.

**Keywords:** Biomedical devices; Lubrication environment; UHMWPE; Noncyclic pin-on-disk

## 1. Introduction

In orthopaedic tribology, the most commonly used lubricants are serum-based, because their use is known to make it possible to reproduce clinical wear mechanisms in vitro, provided that the relative motion is multidirectional [1]. The wear rate of ultrahigh molecular weight polyethylene (UHMWPE) has been shown to depend on the type of serum used [2,3]. The type of serum used in wear testing varies among research groups, and no consensus of the optimum type exists. One of the commonly used types is the alpha calf fraction because it has shown good stability against protein degradation in the wear testing of orthopaedic implant materials [4]. The ISO 14242-1 standard [5] recommends that serum is diluted with deionized water (DW) so that the protein mass concentration of the resulting lubricant is not less than 17 g/l. The protein concentration of the lubricant is also known to affect the UHMWPE wear rate [6,7]. The typical concentration in the joint fluid of prosthetic joints is 30 g/l [8]. The protein concentration used in biotribology laboratories varies, and no consensus of the optimum concentration exists either [1].

Sometimes phosphate buffered saline (PBS) has been used for the dilution [9–11]. It was found in a multidirectional pin-on-disk study that the dilution of alpha calf serum with PBS resulted in a 4 to 6-fold decrease in the wear rate of 50 kGy gamma-irradiated, moderately crosslinked UHMWPE compared with DW dilution [12]. This finding is surprising because serum itself is a buffered solution with a neutral pH and salinity close to that of PBS. It was considered interesting to find out (a) if a similar decrease occurs with unirradiated and with vitamin E stabilized, 100 kGy gamma-irradiated, highly crosslinked UHMWPE, ‘VEXLPE’, and (b) whether the phosphates or sodium chloride or their combined action is responsible for the decrease. Vitamin E stabilization has been introduced in the recent years to prevent the in vivo oxidation of highly crosslinked UHMWPE without compromising mechanical properties, which is typical of heat treatments used earlier to prevent oxidation [13–15].

Crosslinking has been shown to be beneficial for the wear resistance [13–15], but it has been suggested that the presence of vitamin E hampers crosslinking and therefore the wear resistance is inferior to that of heat-treated, crosslinked materials [16]. After a few preliminary circular translation pin-on-disk (CTPOD) trials, it was hypothesized that (1) the polyethylene wear is significantly reduced by the dilution of serum lubricant with PBS, phosphate buffer, saline, or their combined action, compared with DW dilution, and that (2) the VEXLPE wear rate is between those of unirradiated UHMWPE and of highly crosslinked, heat-treated UHMWPE.

## **2. Materials and methods**

The computer-controlled, servo-electrically driven, 16-station RandomPOD wear test device has been described in earlier papers [17,18]. The pin translated biaxially and noncyclically relative to the disk. The slide track was always within a circle of 10 mm diameter. The average sliding velocity was 15.5 mm/s (range 0 to 31.4 mm/s). The acceleration varied from 0 to 300 mm/s<sup>2</sup>, and its derivative was continuous. Hence, the motion was smooth even in reversals. The direction of sliding changed continually (mean accumulated change 500°/s) relative to the pin. The proportional-pneumatic load varied noncyclically. The average load was 73 N (range 0 to 142 N).

Two types of UHMWPE were included in the tests: (1) unirradiated GUR 1020 (ASTM F648, ISO 5834-1,2) that represented a virgin material, and (2) vitamin E (0.1 %) stabilized, gamma-irradiated (100 kGy) GUR 1020-E (ASTM F648, ISO 5834-1,2, ASTM F2695, ASTM F2565) that represented a highly crosslinked material, hereafter abbreviated as VEXLPE. The diameter and length of the cylindrical polyethylene pins were 9.0 mm and 12.0 mm, respectively. Hence, the nominal contact pressure varied between 0 and 2.2 MPa. The arithmetical mean surface roughness value  $S_a$  of the machined pins was c. 2  $\mu\text{m}$ . The

counterface was polished CoCr ( $S_a = 0.01 \mu\text{m}$ ). The surface roughnesses were measured with a white light interferometry profilometer. The lubricant was HyClone Alpha Calf Fraction serum SH30212.03 (GE Healthcare Lifesciences, HyClone Laboratories, Inc., South Logan, UT, USA), pH 7.8, that was used as such, or diluted with PBS, phosphate buffer (PB), saline solution or ultrapure, deionized water (DW, Milli-Q grade). The total protein concentration of undiluted serum was 40 g/l. The fluids used for the dilution were prepared with ultrapure DW, and their compositions were as follows.

PBS (HyClone SH30256.02):  $\text{KH}_2\text{PO}_4$  144 mg/l,  $\text{Na}_2\text{HPO}_4$  795 mg/l, NaCl 9g/l (pH 7.1)

PB:  $\text{KH}_2\text{PO}_4$  144 mg/l,  $\text{Na}_2\text{HPO}_4$  795 mg/l (pH 7.8)

Saline: NaCl 9g/l

The pH values were provided by the manufacturers. With unirradiated UHMWPE, the dilution ratios varied from 1:3 to 3:1. In addition, each fluid was used as a lubricant without serum. All VEXLPE tests ( $n = 16$ ) were run with a dilution ratio of 1:1 (DW, PBS, PB, and saline). The lubricant bulk temperature was maintained at  $20.0 \text{ }^\circ\text{C} \pm 0.5 \text{ }^\circ\text{C}$  to retard microbial growth, which has been shown to increase the variation of wear [19]. Hence the use of the toxic  $\text{NaN}_3$  (sodium azide) as an antimicrobial agent in the lubricant, which similarly has been shown to increase the variation of wear, was unnecessary [19]. The number of pins with each lubricant tested varied from 2 (lubricants without serum) to 3 and 4 (serum-based lubricants). Three tests of 1 000 h duration each were run (total  $n = 48$ ). The test was stopped once a week for a gravimetric wear measurement of the pins and the replacement of the used lubricant. The wear rate (mg/km) was obtained by linear regression. The first wear measurement was preceded by a running-in phase of 7 days, which was omitted in the regression. The wear factor  $k$  ( $\text{mm}^3/\text{Nm}$ ) was obtained by multiplying the wear rate by the sliding distance between the first and the last measurement points, and by dividing by the density and by the integral of the product of the instantaneous load and the incremental

sliding distance between the first and the last measurement points. The t-test with a threshold p-value of 0.05 was used to assess whether differences in mean wear factors were statistically significant.

### 3. Results

The mean wear factors  $k$  of unirradiated UHMWPE using serum diluted 1:1 with PBS and PB were 9 and 19 per cent, respectively, of that obtained with 1:1 DW dilution (Fig. 1). With 1:1 saline dilution, the mean  $k$  was close to that with 1:1 DW dilution and with undiluted serum. The wear factor showed a decreasing trend with decreasing protein concentration with PBS dilution. With VEXLPE, the mean  $k$  values using serum diluted 1:1 with PBS and PB and saline were 2.5, 11 and 56 per cent, respectively, of that obtained with 1:1 DW dilution (Fig. 2). With PBS, PB, and saline lubrication, i.e., without serum, the wear of unirradiated UHMWPE was too low to be quantified considering the accuracy of the gravimetric method,  $\pm 0.05$  mg. With all serum-based lubricants, the wear was readily quantified. No polyethylene transfer to the CoCr counterface was observed with serum-based lubricants, nor with PBS lubrication. With PB, there was subtle transfer and with saline, the transfer was mild (Fig. 3), according to the qualitative assessment used in [19]. All worn polyethylene surfaces with serum-based lubricants were burnished (Figs. 4 and 5), and their  $S_a$  values were reduced to 0.05 to 0.1  $\mu\text{m}$ .

### 4. Discussion

The mean wear factor  $k$  of unirradiated UHMWPE was 11-fold lower when the serum was diluted 1:1 with PBS compared with DW ( $p < 0.001$ ). For VEXLPE the corresponding decrease was as large as 40-fold ( $p < 0.001$ ). The differences were larger than those, 4 to 6-fold, obtained for 50 kGy gamma-irradiated UHMWPE [12]. The decrease appears to be due

to the phosphates ( $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$ ) used for buffering since a similar, although not quite as large, decrease was observed with PB dilution. This indicates that there was a small combined effect of phosphate buffer and saline in the reduction of wear of both polyethylene materials. The difference in wear between 1:1 PBS and PB dilution was 2-fold for unirradiated UHMWPE ( $p = 0.05$ ) and 4-fold for VEXLPE ( $p < 0.001$ ). Hence, hypothesis (1) was supported by the test results. For unirradiated UHMWPE the 1:1 dilution with saline led to a slight increase in the mean  $k$  compared with DW dilution ( $p = 0.47$ ). In contrast, for XLPE the 1:1 dilution with saline led to a 44 per cent decrease of  $k$  compared with DW dilution ( $p = 0.002$ ). There was a large difference between PBS and DW not only as a fluid used for the dilution but also as a lubricant without serum (Fig. 1). PBS eliminated polyethylene transfer and wear, whereas substantial transfer and wear, although erratic, occurred with DW [19]. The difference may be attributable to the known ‘antiwear’ properties of phosphates so that they formed a boundary layer on the metal surface, which reduced the adhesive wear of the polyethylene counterface [20]. In serum, the phosphates may have interacted with proteins in the boundary lubrication. Regarding the friction however, PBS and DW are close to each other [19]. Serum itself is a bicarbonate buffered solution with a neutral pH. Hence, its dilution with a buffer appears unnecessary because the dilution with DW does not change the pH of the lubricant. The dilution with saline on the other hand may be considered logical in the sense that the osmolality of the lubricant remains unchanged [12].

Regarding the wear, the differences between the fluids used for the dilution observed in the present study were much larger than the differences between the two main types of serum used in orthopaedic tribology, bovine vs. alpha calf [6]. This difference was 20 per cent at most in a multidirectional hip simulator test with gamma-sterilized (25 kGy) UHMWPE cups against polished CoCr heads [6]. The corresponding difference in another hip simulator study



was 30 per cent [2], and in a knee simulator study it was 27 per cent [3].

With 1:1 DW dilution, the mean  $k$  of VEXLPE was 5.7-fold lower than that of unirradiated UHMWPE ( $p < 0.001$ ). With 1:1 PBS dilution, the difference was 20-fold ( $p < 0.001$ ). In three hip simulator studies against CoCr heads, the following reductions in wear rate were obtained with vitamin E stabilized, highly crosslinked UHMWPE: (1) 10-fold, gamma-sterilized vs. 85 kGy gamma-irradiated [13], (2) 5-fold, unirradiated vs. 90 kGy electron beam irradiated [14], and (3) 20-fold, gamma-sterilized vs. 100 kGy electron beam irradiated [15]. The variation of reduction is wide but the observed wear resistance of the high dose irradiated, vitamin E stabilized material is always superior, as in the present study. Clinical wear factors are scarce in literature. A value of  $2.1 \times 10^{-6} \text{ mm}^3/\text{Nm}$  can be found for the acetabular cups of the Charnley design [21]. This is substantially lower than the values obtained for unirradiated UHMWPE in the present study using DW and saline dilution and undiluted serum (Fig. 1). The difference may be partly attributable to the fact that the Charnley cups were sterilized by gamma-irradiation. Hence, they were moderately crosslinked.

In earlier RandomPOD studies with alpha calf serum diluted 1:1 with DW at 20 °C and polished CoCr counterfaces the mean  $k$  of gamma-sterilized (25 kGy) UHMWPE ('Sulene-PE') was  $3.92 \times 10^{-6} \text{ mm}^3/\text{Nm}$  [18]. The wear of electron beam irradiated (95 kGy), heat-treated, highly crosslinked UHMWPE ('Durasul') was so low that it could not be quantified with a balance of 0.01 mg resolution [22]. The former value is 3.3-fold larger compared with the mean  $k$  obtained for VEXLPE in the present study,  $1.19 \times 10^{-6} \text{ mm}^3/\text{Nm}$  ( $p < 0.001$ ). Hence, the higher gamma dose (100 kGy vs. 25 kGy) appears to be beneficial for the wear resistance, presumably by increased crosslinking. The VEXLPE wear was readily quantified, however, and it was of substantial magnitude. The total wear of the four VEXLPE pins of the 1:1 DW test after 55 km of sliding was  $4.22 \text{ mg} \pm 0.40 \text{ mg}$ . Therefore, the electron beam

irradiation appears to be a more efficient method to improve the wear resistance compared with a high dose of gamma-irradiation. On the other hand, it has been suggested that vitamin E hampers crosslinking and therefore reduces the beneficial effect of irradiation on the wear resistance [16]. In either case, hypothesis (2) was supported by the results. Heat-treated, highly crosslinked UHMWPE materials have shown oxidation in vivo, which may deteriorate the wear resistance [23]. A more durable protection against oxidation particularly is expected from the vitamin E stabilization [24].

Since the mean wear rate of unirradiated UHMWPE in serum diluted 1:1 with DW was close to that in undiluted serum,  $6.84 \pm 0.68 \times 10^{-6} \text{ mm}^3/\text{Nm}$  vs.  $6.53 \pm 0.30 \times 10^{-6} \text{ mm}^3/\text{Nm}$  ( $p = 0.37$ , Fig. 1), it can be stated that the protein concentration of 20 g/l is sufficient with the alpha calf serum based lubricant. This finding was similar to that obtained for gamma-sterilized UHMWPE [19]. The presence of serum in the lubricant was always necessary to produce measurable and repeatable wear. The reason for this well-known fact is still poorly understood, but an electrochemical effect due to albumin has been suggested [25]. The obvious benefit of the DW dilution is that the possibility of underestimating the in vivo wear rate is minimized. Since clinical wear factors are not available for contemporary types of UHMWPE, it is logical in a lubricant study to make the wear rate with undiluted serum the benchmark to which wear with diluted serum is compared. This supports the standard recommendation of the dilution with DW.

The principal limitation of the study was that the specimens were a pin and a disk with a flat-on-flat contact, instead of a ball-in-socket contact of a real hip prosthesis that can be tested in a full-scale hip simulator [26]. The difference may somewhat affect the lubricant ingress into the contact, but since all lubricants were ‘watery’, viscosity was not likely to affect the wear rates. The wear mechanisms produced have however been validated in earlier papers [17–19,22]. The burnished appearance that dominated in all tests with a protein-

containing lubricant (Figs. 4 and 5) is typical of retrieved polyethylene acetabular cups [27–29]. A multistation pin-on-disk device is probably at its best in simultaneous large capacity wear screening of several, say, 20 different bearing couples with adequate n values [30]. After this, only the most promising couples will proceed to more expensive joint simulator testing. Another limitation was that wear particles were not analysed. Clinically, the majority of polyethylene particles are in the 0.1  $\mu\text{m}$  to 1  $\mu\text{m}$  size range [31], and therefore they are biologically active [15]. In general, multidirectional, serum lubricated wear tests have been shown to produce wear particles that fit well into this range [32]. VEXLPE wear particles have been shown to be similar to conventional UHMWPE particles [15].

## 5. Conclusions

In order to find out whether the dilution of serum lubricant with PBS, PB or saline instead of DW results in a significant change of the wear rate of UHMWPE and VEXLPE, a series of pin-on-disk tests, intended to reproduce clinical wear mechanisms of total hip prostheses, were performed. The UHMWPE and VEXLPE wear rates proved to be highly sensitive to the serum diluent. The dilution of serum with PBS or PB caused a 5 to 40-fold decrease in the wear factor of unirradiated and highly crosslinked UHMWPE compared with DW dilution. The dilution with saline did not cause a similar decrease. Hence, it appeared that the phosphates were mainly responsible for the phenomenon. Of the diluents studied, the use of DW, as recommended in the ISO 14242-1 standard, appears to be the least likely to lead to an underestimation of the clinical wear rate. Although PBS may appear a justified diluent from a biochemistry point of view, it cannot be recommended for orthopaedic tribology tests. PB and saline dilution may also result in a significant reduction of the wear rate, and they showed no advantage compared with DW. Alpha calf serum diluted 1:1 with DW appeared to be the most appropriate lubricant for orthopaedic tribology tests. With 1:1 DW dilution,

VEXLPE gamma-irradiated by 100 kGy showed a 5.7-fold lower wear factor compared with unirradiated UHMWPE. Still the VEXLPE wear was substantial and readily measurable, in contrast with earlier tests with electron beam irradiated, heat-treated UHMWPE.

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## **Declarations of interest**

None.

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## Figure captions

Figure 1. Variation of wear factor of unirradiated UHMWPE with protein concentration of alpha calf serum diluted with PBS, PB, or saline (S). Reference value, DW 1:1, is from [17]. 0 mg/ml represents fluid without serum (DW value from [19]), and 40 mg/ml represents undiluted serum.

Figure 2. Wear factor of VEXLPE in alpha calf serum diluted 1:1 with DW, PBS, PB, or saline. Protein concentration was 20 mg/ml.

Figure 3. Optical micrographs from polished CoCr disks after tests against unirradiated UHMWPE lubricated by (a) PB and (b) saline. Both showed polyethylene transfer, in contrast with tests lubricated by serum-based fluids and by PBS.

Figure 4. Optical micrographs from worn VEXLPE pin with two different magnifications. Lubricant was alpha calf serum diluted 1:1 with DW. There were no protuberances typical of conventional UHMWPE and DW dilution [17–19], nor was there any orientation of surface features, such as scratches or grooves, because of random motion.

Figure 5. VEXLPE test pin of 9.0 mm diameter worn for 1000 h (55 km) against polished CoCr disk in alpha calf serum diluted 1:1 with DW. Worn surface was burnished. Total wear was 4.8 mg.

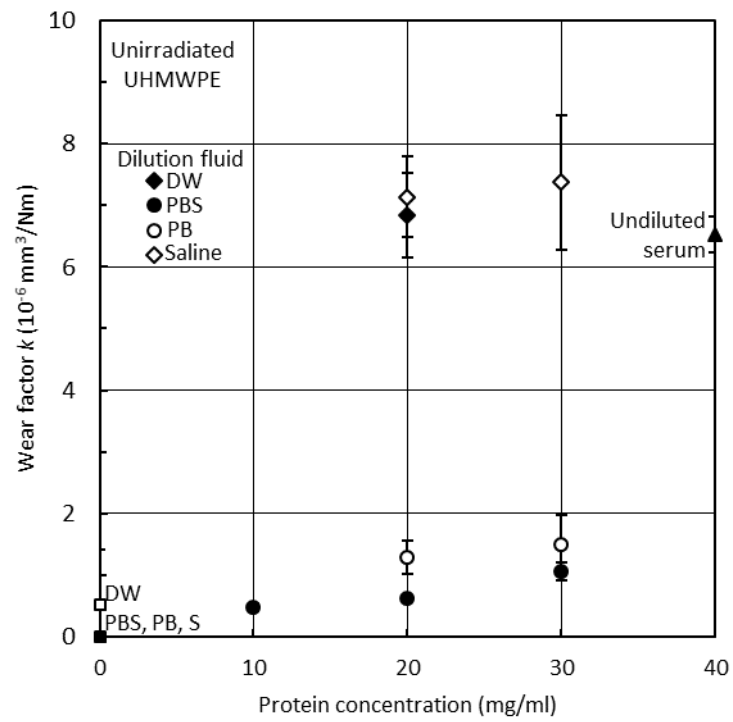


Figure 1.

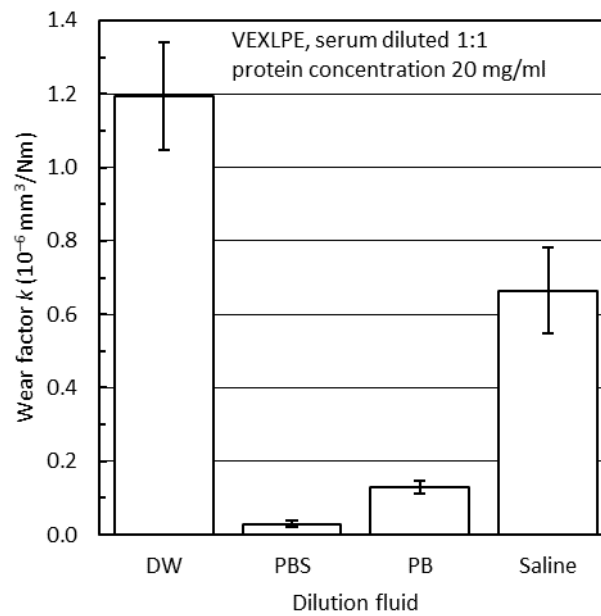
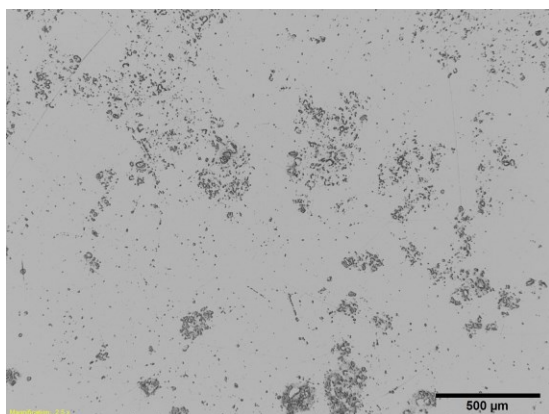
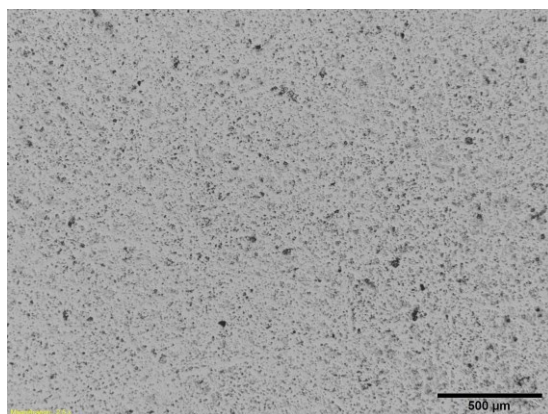


Figure 2.

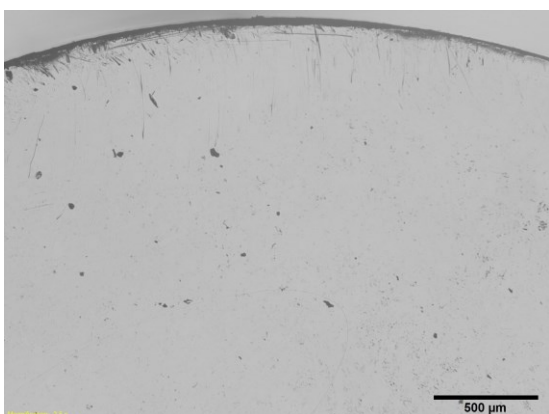


(a)

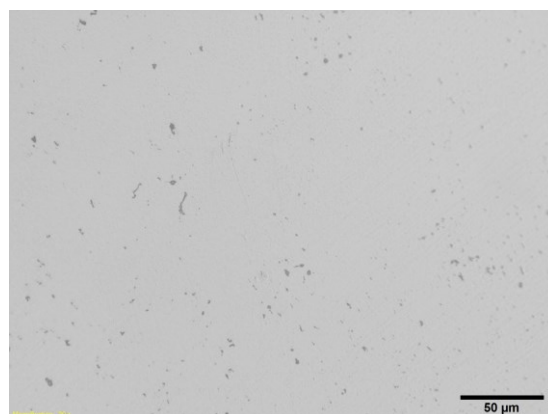


(b)

Figure 3.



(a)



(b)

Figure 4.



Figure 5.