The Effect of Hyperthermia Combined with Radiation on Crypts of the Mouse Jejunum

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The effect of local hyperthermia of 41 to 43 ℃ for 30 minutes on radiosensitivity of normal tissue was studied utilizing jejunal crypt microcolony assay. Hyperthermia of this range enhanced the radiation effect and the effect was mainly additive without significant effect on the slopes of cell survival curves.

At the isoeffect level of 20 microcolony formation, the thermal enhancement ratio was 1.02, 1.10 and 1.39 for 41°, 42° and 43℃, respectively.

The distribution of microcolony formation along the circumference of jejunum was not uniform, having more colonies around the mesenteric border, and this suggests the effect of uneven cooling by blood circulation.

Key Words: Hyperthermia, Radiation, Jejunal crypt microcolony

INTRODUCTION

One of current interests in radiation treatment of cancer focuses on killing of radioresistant hypoxic cells in the tumor. Of several modalities, heat is one of the most potent agent for increase of radiosensitivity of hypoxic cells. The use of hyperthermia in the treatment of cancer is not a new idea. Clinical report of cancer remission associated with febrile disease dates back to 1866. Despite some encouraging and anecdotal reports, data of earlier studies were not consistent enough to warrant a sustained effort in applying heat in cancer therapy. However, over the last 20 years, studies with cell cultures, animal experiments and a few clinical studies have provided a firm basis for hyperthermia as a potential new modality in cancer therapy. The basic biological effects of heat can be summarized as follows: 1) heat kills cells in a predictable and repeatable way, 2) the relatively radioresistant S-phase cells are radiosensitized by heat[3,23], 3) cells that are deficient in nutrients and/or at acidic condition are more sensitive to heat[18,20], 4) heat inhibits the repair of sublethal and potentially lethal damage by ionizing radiation[2,5,8,21], 5) hyperthermia has not been shown to be carcinogenic and is only weakly mutagenic, 6) but cells become less sensitive to subsequent heat by prior hyperthermia[9,12,14,15].

In many in vivo experiments, combination of hyperthermia with radiation has been proven to be effective in treatment of malignancies with improvement of therapeutic ratio. But in most of these experiments the therapeutic ratio was estimated from comparision of the thermal enhancement of radiation effect on tumor and that on skin. However, the skin reaction is of less concern compared to the tolerance of critical normal tissues such as central nervous system, lungs, kidneys, gut and liver.

In this experiment, the enhancement of radiation effect by local hyperthermia of therapeutic range was studied utilizing mouse jejunum as a normal tissue.

MATERIALS AND METHODS

Experimental animal: Male ICR mice of 30 to 35 gram body weight were used. The mice were fed with standard laboratory animal diet and water ad libitum.The animals were kept at room temperature

Radiation: A single dose of whole body irradiation was given without anesthesia. Five mice were put into a lucite chamber (Fig. 1) and were irradiated with Cs-137 animal irradiator (J.L. Shepherd and Assoc., U.S.A.), at room temperature. During irradiation, the lucite chamber was rotated 6 rounds per minute and dose rate was 380 rad per minute.

For control group of irradiation alone, a single dose of 1,100 to 1,500 rad was given with increment of 100 rad. Hyperthermia: A short segment of jejunum was immersed into water bath for local hyperthermia. Under pentothal sodium anesthesia (60 mg/kg), a loop of jejunum 3 cm distal to the duodeno-jejunal junction was exteriorized through a 1 cm abdominal incision. The portion of jejunal loop heated was marked with subserosal stitch for later identification.

A specially designed tray (Fig. 2) was used to hold anesthesized mouse and permit the exteriorized jejunal loop to immerse into water bath (Dae Yang Medical Instrument Co.) filled with Hartman's solution (Fig. 3).

The temperature of the jejunum was measured

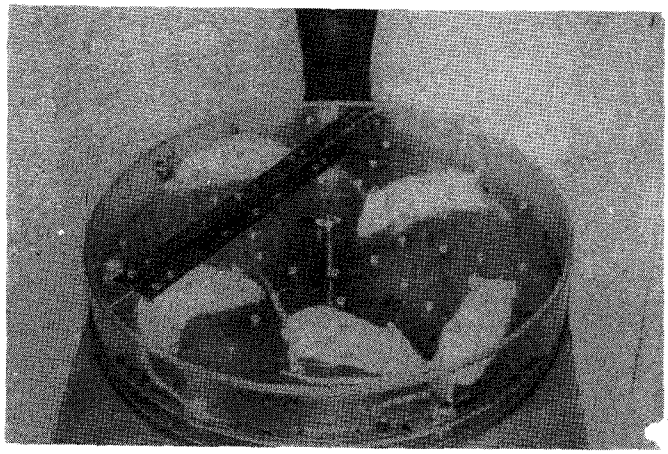

Fig. 1. A specially designed vessel that can hold three V-shaped devices with vertical slit.

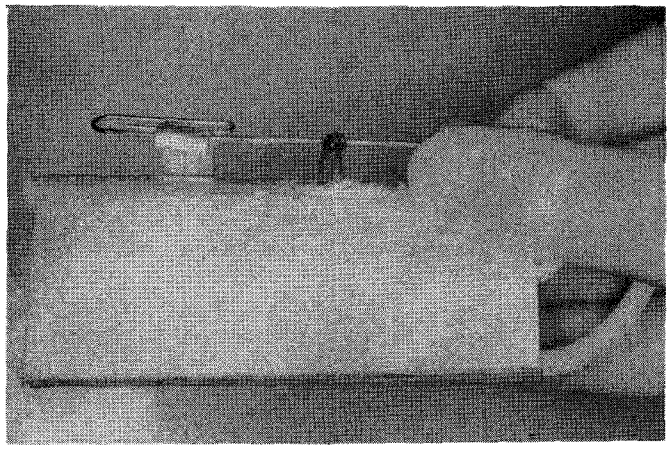

Fig. 2. Each anesthetized mouse was positioned in a specially designed device. A exteriorized jejunal loop was fixed by a struct which projected down into the Hartman's solution.

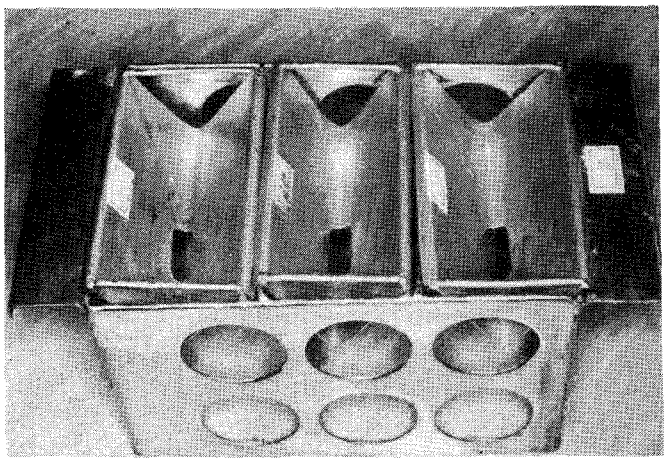

Fig. 3. Five mice in the rotating lucite box were irradiated simultaneously by the Cs-137.

Table 1. Experimental Design

Group	Radiation (rad)	Temperature (°C)	No. of mice
Control	–	–	10
Radiation alone	1,100		10
	1,200		10
	1,300		10
	1,400		10
	1,500		10
Hyperthermia alone		41	10
		42	10
		43	10
		44	10
Radiation + Hyperthermia *	1,100	41	10
	1,200	41	10
	1,300	41	10
	1,500	41	10
	1,600	41	10
	1,000	42	10
	1,100	42	10
	1,200	42	10
	1,300	42	10
	1,400	42	10
	600	43	10
	700	43	10
	800	43	10
	900	43	10
	1,000	43	10

* Hyperthermia for 30 min, after irradiation

with thermocouple (Bailey Instrument Inc.), and the temperature of control group was kept at 37.5 ℃ and those for experimental group were kept at from 41 to 44 ℃ with increment of 1 ℃.

Hyperthermia was applied for 30 minutes as soon as possible after radiation. Actually it took about 15 minutes for preparation of mice.

After hyperthermia, the exteriorized jejunal loop was replaced in the peritoneal cavity and abdominal wound was closed. Mice showed normal activity after recovery from anesthesia. However, there were some loss of mice after hyperthermia of high temperature, reaching 80% after heating at 44 ℃. Jejunal microcrypt assay: Standard method of jejunal microcolony survival assay was used. Ninety hours after radiation with or without hyperthermia, the mice were sacrificed by cervical disarticulation and the heated jejunal segments were taken out. The jejunal segments were fixed in neutral formalin for 24 hours and 3 pieces of cross section were sampled 0.5 cm apart. The entire circumference of jejunal loop were examined under microscope after Hematoxilin & Eosin stain, and crypts consisting of more than 10 epithelial cells with prominent nuclei were regarded as regenerating crypts from one or more surviving stem cells. The number of regenerating crypts were converted into cell surviving fraction by Poisson correction method. Statistical analysis: Survival curve was fitted to data points by weighted least square regression analysis. Slopes of cell survival curve were compared by analysis of

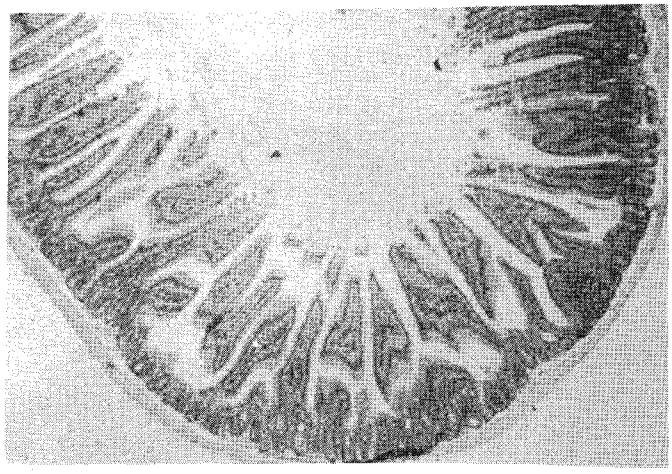

Fig. 4. H-E stained transverse section of jejunum of normal control mouse. Mean of 144 crypts were observed per entire circumference of the jejunum.

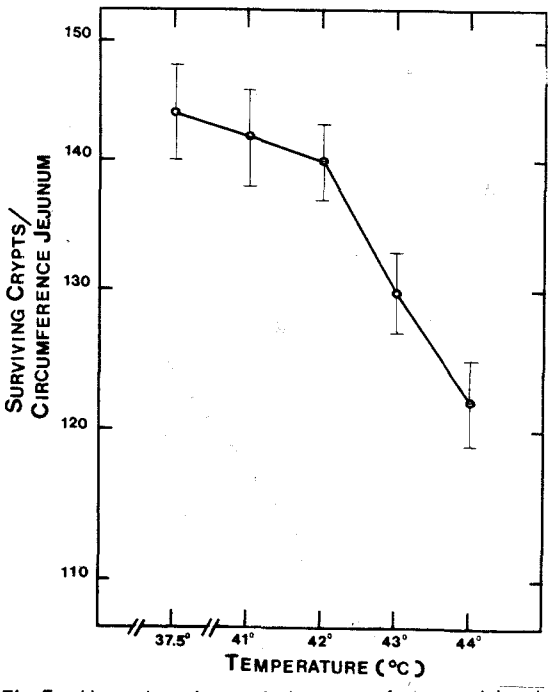

Fig. 5. Hyperthermia survival curve of mouse jejunal crypts with exteriorization and immersion in Hartman solution at 37.5°C to 144°C for 30 min.

covariance and thermal enhancement ration (TER) was estimated comparing the radiation dose needed for survival of 20 crypt stem cells.

RESULTS

An interesting feature in crypt cell survival was noticed. The regenerating microcolonies were distributed asymmetrically along the circumference of the jejunum. More microcolonies were present along the side of mesenteric attachment compared to other portion. Since there was no temperature gradient in the water bath, this pattern of asymmetry across the jejunal loop is considered to be due to the effect of more blood flow hence lower temperature along the mesenteric border and less radiosensitization (Fig. 4 & 5).

Number of regenerating jejunal crypts was 142±4 (mean±standard deviation) after hyperthermia of 30 minutes at 41 ℃, 140±3 at 42 ℃, 130± 3 at 43 ℃, and 122±3 at 44 ℃, compared to 144± 4, number of crypts of normal jejunum. The jejunal crypt cells were rarely destroyed by hyperthermia of 30 minutes at 42 ℃ or lower (Table 2., Fig 6).

Number of regenerating microcolonies decreased constantly with increase of radiation dose from 109±4 after 1100 rad to 19±4 after 1500 rad (Table 2). The number of surviving crypt cell decreased from 203 to 20. The D_0 calculated from this data was 147 rad (Fig. 7).

Number of regenerating microcolonies decreased with hyperthermia and as the temperature increased, the number of microcolonies decreased. The effect of hyperthermia of 30 minutes at 41 ℃ was not siginficant, but hyperthermia at 42

Table 2. Number of Surviving Crypts per Circumference of Jejunum : Radiation or Hyperthermia Alone

Group	Radiation (rad)	Temperature (°C)	No. of evaluable mice/tested mice	No. of Crypts
Control			10/10	144 ± 4 (S.D.)
Radiation alone	1,100		10/10	109 ± 4
	1,200		9/10	90 ± 3
	1,300		9/10	50 ± 5
	1,400		8/10	36 ± 4
	1,500		7/10	19 ± 4
Hyperthermia alone		41	8/10	142 ± 4
		42	7/10	140 ± 3
		43	5/10	130 ± 3
		44	2/10	122 ± 3

* Mean of 2 evaluable mice

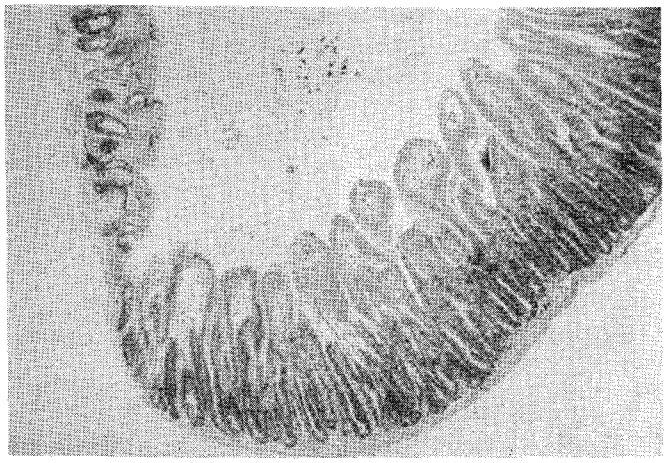

Fig. 6. H-E stained transverse section of mouse jejumun heated to 43°C for 30 min following irradiation with 800 rads. It shows the asymmetric distribution of surviving crypts around the circumference, with maximum survival adacent to the mesenteric attachment.

or 43 ℃ decreased the number significantly (Table 3). The slope of cell survival curve was a little less steep with 42 or 43 ℃ hyperthermia, the D_0 being 175 rad (Fig. 8). The main feature in change of cell survival curve by hyperthermia was left shift. Radiation dose needed for the isosurvival level of 20 cell survival per circumference decreased with hyperthermia, being 1520 rad for radiation alone, 1490 rad with hyperthermia at 41 ℃, 1380 rad at 42 ℃, and 1090 rad at 43 ℃. The estimated thermal enhancement ratio was 1.02 at 41 ℃, 1.10 at 42 ℃ and 1.39 at 42 ℃.

DISCUSSION

To apply hyperthermia in treatment of cancer patient in clinical practice, it should be proven that hyperthermia is effective and, at the same time, safe. Most of studies on effect of hyperthermia combined with radiation were performed using skin as normal tissue. But skin of mice is regarded as having some hypoxic cells, and so, the enhancement of radiation effect on skin by hyperthermia is expected to be greater than that for other tissue or

organ that have no hypoxic cells. Moreover, the skin is usually not a tissue of concern in modern radiotherapy.

The jejunum is one of the best substitute for skin in study of normal tissue, because the epithelial cells of jejunum are all well oxygenated, the small bowel can be included in the area of hyperthermia and it is a radiation dose limiting organ.

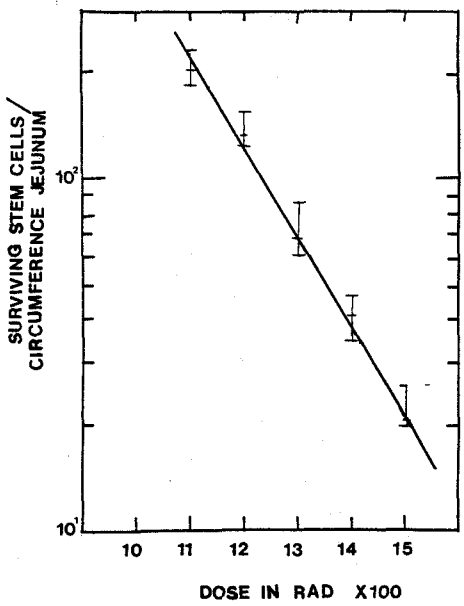

Fig. 7. Radiation survival curve of mouse jejunal crypt stem cells.

In studying the biological effect of hyperthermia, there are many a variables such as temperature, time length of hyperthermia, sequence of the two modalities, and time interval between the two.

In this experiment, the length was limited to 30 minutes and radiation was given first. These experiments show that the predominent effect of combined heat and radiation was to displace the radiation survival curves to the left without significant change in slope. Similar results have been obtained for crypt survival at four days following lower body hyperthermia[6] and for local heating of exteriorized intestine.[16] The largest displacement occured when the heating temperature was increased from 42℃ to 43℃. After mild hyperthermia the tissue response after combined treatment is normally qualititively identical with the radiation response. It is therefore possible to compute a thermal enhancement ratio (TER), as the ratio of doses of radiation alone to that with hyperthermia to produce a given level of damage. TER was 1.02 at 41℃, 1.10 at 42℃ and 1.39 at 43℃. A survival curve displacement to the left is compatible both with independent cell killing by heat and irradiation, and with interactive effect in which heat reduces the size of the survival curves shoulder.

The effect of the sequence of heating and irradiation on thermal enhancement has been re-

Table 3. Number of Surviving Crypts per Circumference of Jejunum (Radiation plus Hyperthermia)

Group	Radiation (rad)	Temperature (°C)	No. of evaluable mice/tested mice	No. of Crypts
Radiation + Hyperthermia	1,100	41	10/10	98 ± 6
	1,200	41	9/10	79 ± 5
	1,300	41	8/10	47 ± 2
	1,500	41	7/10	17 ± 3
	1,600	41	6/10	9 ± 2
	1,000	42	9/10	82 ± 4
	1,100	42	7/10	65 ± 4
	1,200	42	7/10	45 ± 4
	1,300	42	6/10	25 ± 4
	1,400	42	5/10	16 ± 4
	600	43	6/10	114 ± 5
	700	43	5/10	90 ± 6
	800	43	5/10	61 ± 5
	900	43	5/10	45 ± 4
	1,000	43	5/10	29 ± 6

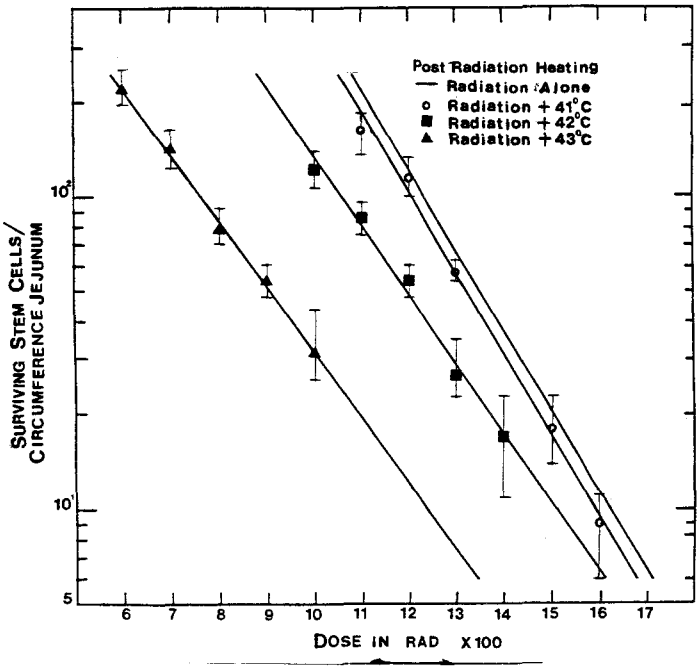

Fig. 8. Effect of postirradiation heating of mouse jejunum on survival of crypt stem cells. A loop of jejunum was exteriorized and heated for 30 min at 41°C to 43°C after irradiation in situ. The mean number of surviving stem cells per circumference is plotted as a function of the radiation dose on semilogarithmic coordinates.

ported for several systems, both in vivo and in vitro. There seems to be little agreement, however, as to which of these is more effective. This is, at least partly, the result of variations in the test systems used, the interval between application of heat and radiation. With in vitro systems, temperature of the cells during irradiation, and between heating and irradiation are additional variables. In the in vivo experimental tumor studies reported, Crile,[4] Overgaard and Overgaard,[19] and Alfieri et al[1] and Hofer et al[10] observed a greater tumor effect when heating followed irradiation. Conversely, Thrall et al[22] found preirradiation heating to be more damaging to mouse skin when irradiation was given under normal ambient conditions.

There is general agreement among different authors and systems that the interactive effects decay seems to be more rapid when radiation precedes heating. However, fluctuations in the degree of interaction during the decay period may occur. By four hours between the two treatments the heat has no potentiating effect on the response of the intestine to radiation.[11]

In any event, the conceptual basis of comparing TER's for different assays is questionable. When heat combined with irradiation modifies the survival curve shoulder. The TER becomes a function of radiation dose, or more precisely, of the level of cellular survival chosen for the endpoint. Since different assay endpoints reflect different levels of cell survival, simple direct comparisons of TER's obtained with different assays are unjustified. However, in all cases, where the radiation survival curve shoulder is reduced by heat, it should be noted that the TER will increase as the radiation dose decreases; thus, the TER's obtained in clinical fractionated radiotherapy may greatly exceed those for single doses, in much the same ways as relative biological effectiveness (RBE) for high linear energy transfer (LEFT) irradiation increases with fractionated exposures. This effect could be offeset to some extent by development of thermal tolerance in repeatedly heated cells.

In summary, these data show that exposures of 43℃ for 30 min induce cytocidal effect and predominent thermal enhancement is observed in

43℃ of hyperthermia combined with irradiation. However, for the safety of combined treatment in clinical practice specific information should be obtained on the response of all major dose limiting organs and tissues which may be intentionally or crease in TER with fractionated exposures to heat and irradiation should also be appreciated.

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국문초록 =

마우스공장 소낭선의 방사선 효과에 온열요법의 병용이 미치는 영향에 관한 실험적 연구

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마우스의 공장에 국소 온열요법을 시행하여 온열요법 단독에 의한 공장 소낭선의 수를 측정하고 마우스에 전신 방사선조사후 마우스 공장에 국소 온열요법을 시행하여 소낭선의 수를 측정하여 다음과 같은 결론을 얻었다.

1. 온열요법 단독 시행시에 관찰된 마우스 공장의 생존 소낭선의 수는 41℃에서 142±4 (Mean±SD) 개, 42℃에서 140±3 개로 정상 대조군의 144±4 개에 비해 큰 차이는 없었으나 43℃에서는 130±3 개로 뚜렷한 감소를 보여 온열요법 단독시행시 43℃군에서 가온에 의한 세포유해효과가 나타났다.

2. 43℃의 온열요법을 시행한 군에서 생존 소낭선의 비대칭적으로 분포하였으며 장간막에 근접할수록 생존 소낭선의 수가 많아 혈류에 의한 열방출효과는 장간막에 근접할수록 더 현저함을 알 수 있었다.

3. 방사선조사후 온열요법을 시행하였을때 생존 소낭선의 근간세포의 생존곡선은 방사선조사만을 시행한 대조군에 비해 경사도의 변화는 거의 없이 좌측으로 이동하여 생존곡선의 견부가 감소됨을 알 수 있었고 이동정도는 온도가 증가할수록 심하였다. TER은 41℃때 1.02, 42℃때 1.10, 그리고 43℃때 1.39이었다.

이상의 결과로 보아 온열요법과 방사선조사의 병용방법은 가온온도가 43℃ 이상이어야 열증강효과가 뚜렷함을 보여주고 있으나 임상에서 온열요법과 방사선 조사의 병용시 두가지 방법의 시행순서, 시간간격, 가온시간 등은 앞으로 연구해야 할 과제이다.