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H. M. H. Kamel
Glasgow University

K. E. Carr
Glasgow University

S. P. Hume
Hammersmith Hospital, London

J. C. L. Marigold
Hammersmith Hospital, London

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STRUCTURAL CHANGES IN MOUSE SMALL INTESTINAL VILLI FOLLOWING LOWER BODY HYPERTHERMIA

H.M.H. Kamel, K.E. Carr*, S.P. Hume+ and J.C.L. Marigold+

Pathology Department, Royal Infirmary, Glasgow University, Scotland;
*Anatomy Department, Glasgow University, Scotland;
+MRC Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W11 0HS, U.K.

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Abstract

Heating an exteriorised loop of mouse small intestine resulted in marked changes in the shape of the villi as reported earlier. However, the exteriorisation techniques resulted in non-uniformity in both temperature and effect around circumference of intestine and, in addition, the extent to which handling contributed to the observed damage was not known. The work has therefore been extended using lower-body heating in the temperature range 37.5° - 43.0°C.

Heating in the temperature range 37.5°C to 41.0°C produced minimal to moderate structural changes, manifested as scattered, vertically collapsed villi amongst predominantly "normal" villi. No villi showed conical or rudimentary forms of collapse. Such villi were, however, seen after heating at 41.5°C and were greatly increased in number after heating at 42.0°C. The most severe damage was observed after heating at 43.0°C.

Although the lower body heating method gave information which was less complicated by technical considerations, the hyperthermic damage observed was qualitatively similar to that previously seen following local administration of hyperthermia to an exteriorised loop of intestine. Direct quantitative comparisons between the two methods of heating are difficult because of differences in equilibration time and temperature. However, using a comparable heating time, less damage was scored following the exteriorisation technique compared with in situ heating.

KEY WORDS: Scanning Electron Microscopy, light microscopy, hyperthermia, intestine.

*Address for correspondence:
K.E. Carr, Anatomy Department,
Medical Biology Center,
Queen's University, Belfast,
Northern Ireland G11 800
Phone no.: 0232-229241

Introduction

Over the past few years, many studies have investigated the clinical use of hyperthermia in the treatment of malignant tumours. However, the optimal hyperthermal treatment schedule has still to be defined in terms of temperature, duration, frequency and method of heat application such that maximal therapeutic gain is achieved. Although there is some evidence that tumours are more heat sensitive than their surrounding normal tissues and that some tumour cell lines are killed at lower temperatures than their normal counterparts (Mondovi et al, 1969 a,b; Overgaard and Overgaard 1972; Giovanella et al, 1973 and Dickson 1977) there is a marked variation in response between both normal and malignant tissues and cells (Chen & Heidelberger et al., 1969; Levine and Robins 1969; Giovanella et al., 1973 and 1976 and Song et al., 1980). In the majority of cases, it may prove most beneficial to give hyperthermia in combination with conventional radiotherapy.

Small intestine is sensitive to radiation and, since it is at risk during radiotherapy for abdominal tumours, it is an appropriate model to use in the investigation of the effects of radiation and hyperthermia on normal tissues. Changes in villous shape have frequently been used to follow small intestinal damage after ionising radiation (Carr and Toner, 1972; Anderson and Withers, 1973; Cieciura et al, 1976;; Lieb et al, 1977; and Friberg, 1980) and recently a scoring system has been developed (Carr et al, 1982) to quantify villous collapse through clearly defined stages linked to changes in villous area (Altmann, 1974).The stages and scores are as follows :-

Stage	Score
Erect (VE) or laterally collapsed villi (VL)	0
Vertically collapsed villi (VV)	1
Horizontal villi (VH)	1
Conical villi (VC)	3.5
Rudimentary villi (VR)	6
Absent villi (VA)	8.5

The terms used in describing villi are those reported by Carr (1981) as rather arbitrary substages for classification of secondary villous damage.
These are:-

i) Lateral villous collapse (VL). Villus leans or bends away from the normal erect posture.
ii) Vertical villous collapse (VV). Villi remain erect, but become broader and shorter. Prominent creasing pattern and separation of the villi may be seen as well.
iii) Conical villi (VC). The villi are conical in shape, with broad bases and narrow tips.
iv) Rudimentary villi (VR). Villi have lost almost all of their original shape.
v) Flattened mucosa (VA). Only very few mounds, reminiscent of villi, may be seen.

The effect of hyperthermia on small intestinal villi of mice has been described (Carr et al, 1982) using the techniques of exteriorising an intestinal loop prior to heating and the damage following heating at 43.0°C for 20 minutes was quantified in a preliminary report (Carr et al, 1983b). Both response and recovery were very rapid; a value of 2.5 (representing the mean predominant villous score from specimens from four animals) was measured 2 hours after treatment but by 24 hours the score had dropped to 0.5, a value similar to that seen in exteriorised control animals. However, the damage after hyperthermia varied substantially from animal to animal and, in addition, damage in regions near to the mesenteric attachment was approximately 50% less than that seen in the antimesenteric region. In order to overcome the variation around the circumference seen in exteriorised samples, an alternative heating technique has been utilised by applying hyperthermia to the lower body of the mouse. The current paper describes the effects of lower-body heating at temperatures ranging from 37.5°C to 43.0°C on mouse small intestinal villi. The villous changes have been examined using both SEM and light microscopy and the villous scoring system has been used to quantify the damage.

MATERIALS AND METHODS

Female HC:CFLP mice (3 months old) were subjected to hyperthermia. The following schedules and procedures were used.

Heating Techniques

The mice were anaesthetised with an intraperitoneal injection of sodium pentobarbitone at 0.07 g/kg body weight and the lower part of the body to the xiphisternum was immersed in a thermostatically controlled heated water bath. Full details of the heating technique have been published previously (Hume and Field, 1978). The duration of heating was either 1 hour (using a water temperature of 37.5°C, 40.0°C, 41.0°C, 41.5°C, 42.0°C) or 30 minutes (using a temperature of 43.0°C). Using lower-body heating, the temperatures of both the lumen of the intestine and the peritoneal cavity (measured using fine copper-constantan thermocouples in sample mice) stabilised at approximately 0.2°C above the water bath temperature for each treatment temperature used. However, equilibrium temperature was not attained until approximately 10 minutes after immersion of the animal. In the text, water bath temperatures and times of immersion are given. Six animals were studied for each temperature point. Mice were killed by cervical dislocation two hours after the heating was complete.

Control Tissue

Samples of small intestines were taken from mice which (a) had no treatment or (b) were anaesthetised but received no further treatment, in which case the sample was taken 3 hours after the anaesthetic was administered.

Four animals were studied for each type of control.

Sampling

From each animal, 2 pieces of small intestine about 2-3 cm long, were taken from the segment beginning 15 cm from the pylorus. One was fixed with buffered formaldehyde for H & E sections from paraffin blocks for light microscopy, and the other with 5% glutaraldehyde (buffered with Millonig's phosphate buffer) at 4°C and pH 7.4. The specimens were fixed for periods of 1 to 3 weeks. Glutaraldehyde fixed specimens were prepared for SEM by post fixation in 1% osmium tetroxide,

dehydration through ethanol and critical point drying from amyl acetate. They were then mounted with conductive adhesive and coated with gold/palladium in a sputter coater. The specimens were examined in a JEOL T300 scanning electron microscope. The whole area of each specimen (1.5cm x 1 cm) was examined, and scored according to the system described by Carr et al (1982). The score was given as a mean of the score values of the dominant different villous patterns observed.

RESULTS

Control Specimens

Small intestine from control animals showed characteristic features of normal erect villi, (Figure 1).

Villi from anaesthetised control animals, (Figure 2), showed slight swelling throughout their length, although they looked erect. They were of normal height apart from scattered villi which showed vertical collapse. Creases when present, were shallow, while polygonal outlines of enterocytes were difficult to identify. Goblet cell extrusion was clearly seen. The stroma of some villi showed slight oedema and a mild increase in cellular infiltrate, which was often lymphocytic, (Figure 3). Intraepithelial lymphocytes were identifiable. The crypts and pericryptal stromal compartments looked normal.

Specimens after heating at 37.0°C to 41.0°C

At 2 hours after heating for 1 hour at 37.5°C or 40.0°C, (Figures 4, 5 respectively), both the morphology and internal features of the villi were very similar to those seen after anaesthesia alone. The majority of the villi were either normal in shape or were slightly swollen. In most cases, the swelling was evident over the lower two thirds where the creasing pattern was not prominent. The upper third was tapering and wrinkled.

After heating at 41.0°C the damage was more severe: the villi were swollen in all cases, being classified as vertical and scattered conical villi. In some cases there were scattered prominent extrusion zones. Dilated capillaries were seen in the stroma of the villi (Figure 6).

Specimens after heating at 41.5°C to 43.0°C.

Two hours after heating for one hour at 41.5°C. structural changes were observed in all specimens. Villi showed either vertical or conical collapse and had distorted contours (Figure 7). They looked markedly deformed with their tops studded with masses of badly damaged swollen epithelial cells. In one mouse, sloughing of the epithelial covering of the villous tips with exposure of the underlying lamina propria was observed. The extruded cells were rounded or oval in shape. Microvilli were either lost or looked stunted, swollen and widely separated, (Figure 8). Sometimes protruding blebs sprouted from the villous surface, while lack of intercellular boundaries between some of them gave an impression of cell fusion. The epithelial covering over the lower part of the villus looked more normal. There was a cellular infiltrate of the lamina propria with polymorphonuclear leukocytes, mast cells, eosinophils and lymphocytes. Capillaries were prominent and dilated.

After heating at 42.0°C, villi were vertical, conical or, more commonly, rudimentary. Extrusion zones were prominent with clusters of severely swollen epithelial cells. Areas covered with rudimentary villi had extruded cells lying free on the surface and between villi. The extruded cells were rounded or globular and lacked a microvillous covering. Villous stroma was markedly retracted, sometimes with clear spaces separating the villous core from the badly damaged and degenerating epithelial cells. The covering epithelial cells, particularly near the villous tips, showed swelling, hydropic degeneration or pyknotic darkly stained nuclei. The lamina propria contained moderate infiltration by polymorphonuclear leukocytes, mast cells and scattered lymphocytes. The latter were also detected between the covering epithelial cells. Goblet cells were sometimes seen near the villous base. In most specimens the crypts looked normal.

The severest degree of damage was seen after heating at 43.0°C for 30 minutes. Most specimens had a few conical villi but the majority of villi were rudimentary in shape and some areas had an absence of villi, (Figure 9). Distended swollen desquamated epithelial cells were seen almost covering the severely damaged and distorted mucosal surface. The covering enterocytes showed swelling, hydropic degeneration and pyknotic nuclei and were often cuboidal in shape. The cellular infiltrate of the retracted lamina propria was not prominent, (Figure 10). Some of the changes in response with temperature are listed in Table 1. For each treatment, all the animals within the same group showed similar changes with only moderate individual variations. Within the same animal no marked variation was observed around the circumference of the intestinal loop.

TABLE 1

VILLOUS FEATURES SEEN AFTER HYPERTHERMIA

Temperature	Main Villous pattern	Cap. Dilatn.	Infilt.	Shed. Strip.
37.5°C (60m)	VE	-	-	-
40.0°C (60m)	VE/VV	-	-	-
41.0°C (60m)	VV/VE	+	-	-
41.5°C (60m)	VC	+	+	+
42.0°C (60m)	VC/VR	+	+	+
43.0°C (30m)	VR	±	-	+

VE = Erect villi VV = Vertical villi

VC = Conical villi VR = Rudimentary villi

Table 2

Highest, lowest and mean score of the different groups of animals at different temperature points.

Treatment	Highest Score	Lowest Score	Mean Score
C.C.	0.5	0	0.1
A.C.	0.5	0	0.1
37.5°C (60m)	0.5	0.5	0.5
40.0°C (60m)	1.5	0.5	1.0
41.0°C (60m)	2.3	1.5	1.6
41.5°C (60m)	3.5	2.3	3.1
42.0°C (60m)	6.0	4.8	5.1
43.0°C (30m)	7.3	6.0	6.4

C.C. = Control control
A.C. = Anaesthetised control.

Quantitation of responses

Villous structure in the groups of animals examined at different temperature was scored according to the system described earlier (Carr et al, 1983a). The results are shown in Table 2. It can be seen that the severity of damage increased with increasing treatment temperature. A rise in temperature from 37.5°C to 41.0°C resulted in a villous score of 1.3, reflecting only slight damage.

Figure 1

SEM of untreated control small intestinal villi, showing normal erect villi. Bar = 100µm

Figure 2

SEM of anaesthetised control small intestinal villi, showing normal erect villi. Bar = 100 µm.

Figure 3

Light microscope view of anaesthetised control small intestinal villi, showing moderate cellular, stromal infiltrates and slightly swollen villi. H & E stain. Bar = 100 µm.

Figure 4

SEM of small intestinal villi 2 hours after heating at 37.5°C for 1 hour. Apart from the prominent creases villi look normal. Bar = 100 µm

Figure 5

SEM of small intestinal villi 2 hours after heating at 40.0°C for 1 hour. Villi are swollen, and their tips taper gently and are wrinkled. Bar = 100 µm.

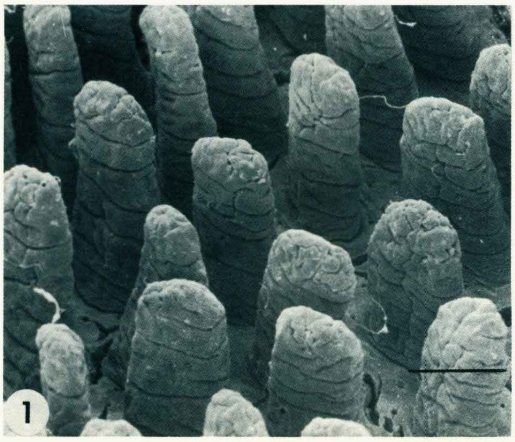
Figure 6

Light micrograph of small intestinal villi 2 hours after heating for 1 hour at 41.0°C. Villi show active extrusions at their tips and prominent capillaries in their stroma. H & E stain. Bar = 100 µm.

However, 2 hours after a heat treatment of 41.5°C for one hour the villous score was more than doubled to a value of 3.1. As the temperature was increased further, from 41.5°C to 43.0°C, the mean score was doubled again, to 6.4.

DISCUSSION

After lower body heating, small intestinal villi changed in shape from erect structures to conical or rudimentary structures in a manner qualitatively similar to that seen after an exteriorised loop is heated (Carr et al, 1982). Several points, however, merit further consideration. These include (a) the dependence of response on temperature, (b) the extent to which the damage is variable, (c) the applications of the villous scoring system to compare lower body heating effects with those produced by heating an exteriorised loop (Carr et al, 1983b) or by irradiation (Carr et al, 1983a). These three topics will be dealt with under separate headings.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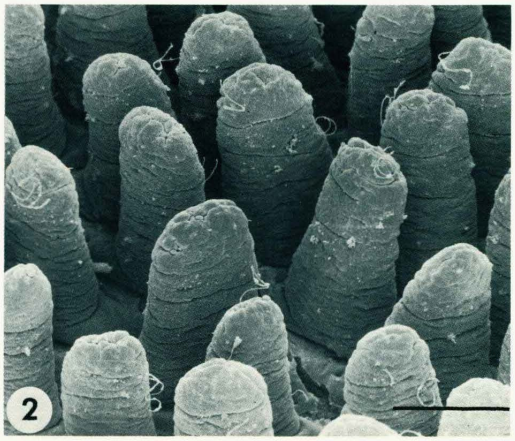
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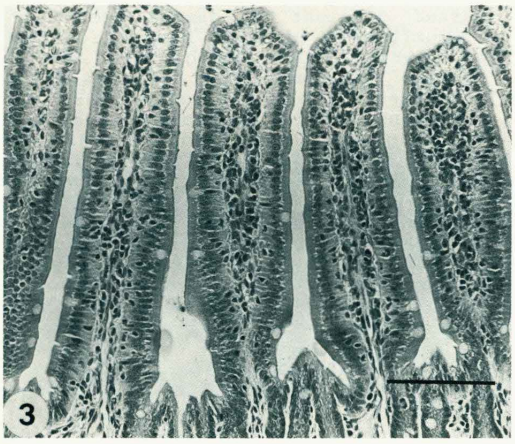
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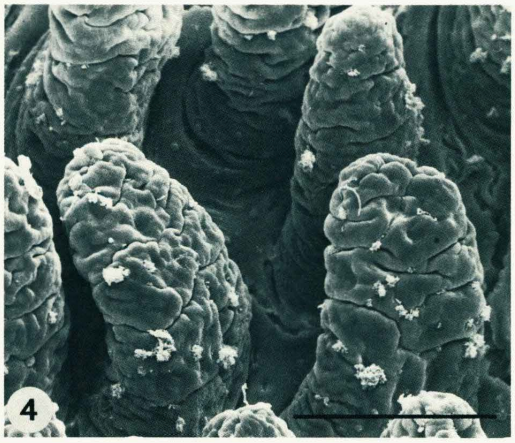
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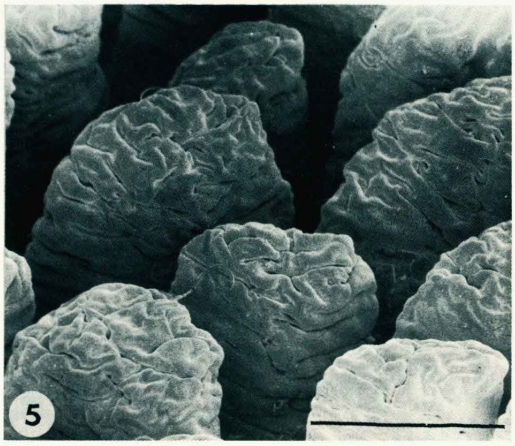
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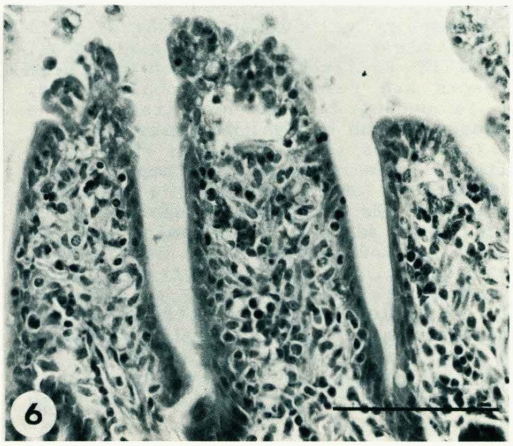
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Dependence of villous damage on hyperthermic temperature

Following local hyperthermia given to an exteriorised loop of intestine, thermal damage to villi was found to be maximal 2 hours after treatment (Carr et al, 1982, 1983b). The work of Breipohl (personal communication), based on light microscope examination of small intestine exposed to lower body heating at 41.0°C described a similar pattern of expression of damage and recovery. For these reasons, the specimens exposed to lower-body hyperthermia were taken 2 hours after treatment.

After heating at 37.5°C or 40.0°C, the villi showed only slight changes in both contour and structure; their appearance was not substantially different from villi in animals which were only subjected to anaesthesia. It is of interest, however, that dilated capillaries were seen following heating at 41.0°C, (Table 1), and it may be that this capillary dilatation is the active forerunner of the onset of the villous collapse produced by heating at higher temperatures. Vascular damage has been suggested as being important in the expression of thermal injury in vivo (Reinhold et al, 1978; Marmor et al, 1979; Song et al, 1980; von Ardenne and Reitnauer,. 1980 and Emami et al, 1981), and the "threshold" temperature for villous collapse reported here is very similar to the temperature required to cause disturbance in villous vasculature, assessed histologically in the same strain of mice, using thick sections and a benzidine stain for haemoglobin, (Falk, 1983).

Damage produced by heating at temperatures above 41.0°C increased in severity as the temperature was raised. The main features were epithelial cell extrusion and progressive villous collapse up to the stage where villi were absent in some mucosal areas. Since it is likely that both of these effects are related to stromal damage, it is useful to comment first on the changes in the mucosal contours.

Within the stromal compartments, dilatation of capillaries described above was also a prominent feature after heating at 41.5°C. The lamina propria was also infiltrated with mast cells, eosinophils, granulocytes and lymphocytes, perhaps partly as a result of the leakiness of the dilated capillaries. The infiltrate continued to be a feature after heating to 42.0°C. This may well lead to the increase in extrusion at the villous tips as also described by Breipohl (personal communication), because of pressure on the epithelial/stromal boundary due to the stromal oedema. After heating to 43.0°C, neither of these stromal changes was seen. Collapse of the villi is probably not linked directly with the disruption of the epithelial sheet. It is, however, perhaps due to a disturbance of the supporting stromal framework of the villus. This disturbance may also be caused by stromal oedema resulting from the damaged capillaries. In order to explain this link further, information is needed on the histological and ultrastructural change in the fibroblast, pericyte and smooth muscle components of the villous and pericryptal connective tissue.

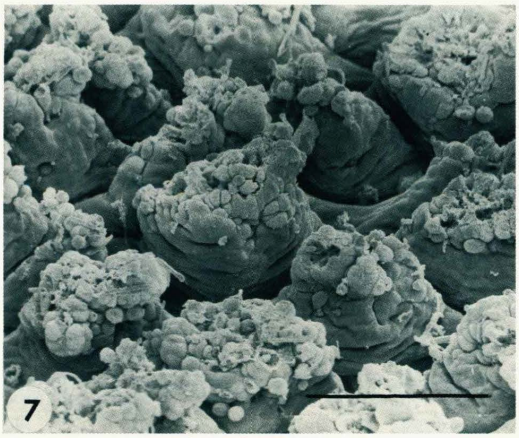
Variation of response

When hyperthermia was applied to an exteriorised loop of intestine, there was a variation in severity of response within each group of animals and also a marked gradient of damage around the circumference of each sample (Carr et al, 1982); least damage was seen in regions adjacent to the mesenteric attachment (Carr et al, 1983b 9). Following lower body hyperthermia there was a greater consistency within both groups and animals, the former probably due to the fact that the intestine is no longer being handled during exteriorisation, and the latter due to a more even heating pattern. Although a detailed comparison of the damage produced by the two techniques must be delayed until larger groups of animals have been studied after heating an exteriorised loop, the preliminary data show that while patches of mucosa scored as high as 8.5 2 hours after heating a gut loop at 43.0°C for 20 minutes, there is a lot of variation and the mean score is only 2.5.

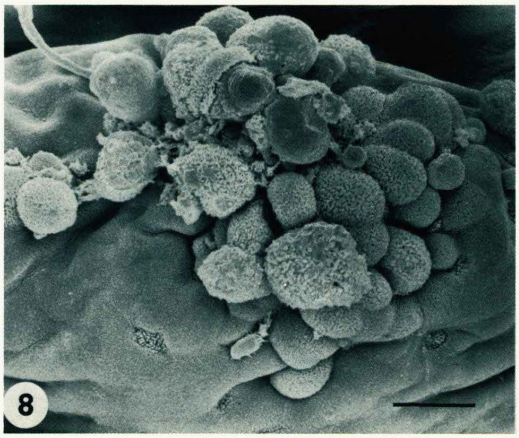
Villous scoring

The villous scoring method is particularly suitable for plotting the results from lower body heating, since the results are so consistent within each group, (Table 2). The mean scores progress from 1 after heating at 40.0°C (corresponding to vertical villi, score 1) through 3.1 after heating at 41.5°C (approximately to conical villi, score 3.5) to 6.4 after heating at 43.0°C (worse than rudimentary villi, score 6.0).

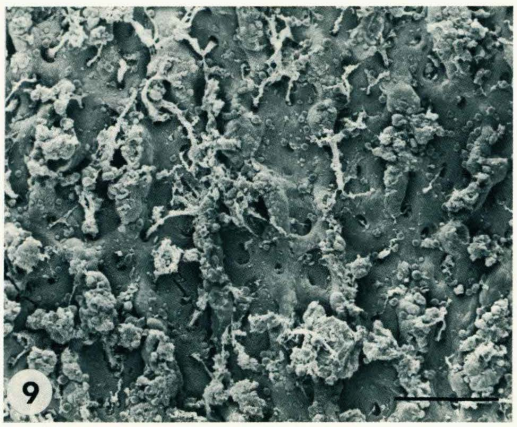
Following localised hyperthermia to intestine, oedema, villus fragility and paralytic ileus have been suggested as possible causes of death occurring within the first day post treatment (Merino et al, 1978; Hume et al, 1979; Milligan et al, 1984). When combined



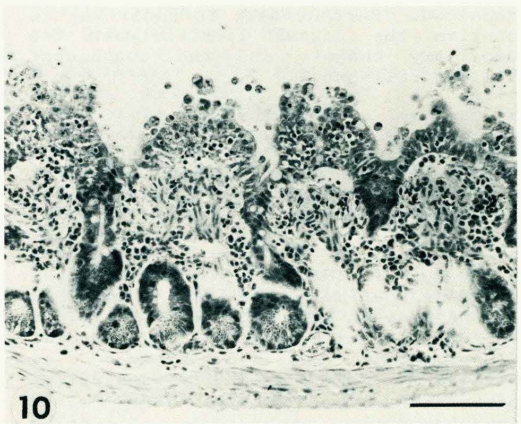
SEM of small intestinal villi 2 hours after heating at 41.5°C for 1 hour. Villi are severely damaged with prominent extrusion zones at their tips. Some of the villi show sloughing. Bar = 100μm.



SEM of villus 2 hours after heating at 41.5°C for 1 hour showing a prominent extrusion zone studded with badly damaged epithelial cells. These are swollen, while their covering microvilli are separated or completely lacking. Bar = 10 μm.



SEM of small intestinal mucosa 2 hours after heating at 43.0°C for 30 minutes. Villi are almost absent, while scattered rudimentary forms are seen. Bar = 100μm.



Light micrograph of mouse small intestinal mucosa 2 hours after heating at 43.0°C for 30 minutes. Villi show atrophy, while their tips are covered with severely damaged and necrotic extruded epithelial cells. H & E stain. Bar = 100μm.

with studies of hyperthermal damage to crypts, quantitation of villous collapse offers a means of comparing hyperthermal injury within different compartments of the mucosa. The results reported above, together with those of Hume et al, (1983) suggest that, in contrast to ionising radiation, hyperthermia has a primary effect on villus structure. In addition, if temperatures for threshold response are considered, it appears that the enterocytes on the villi are more susceptible to thermal injury than the epithelial cells in the crypts. Villous scores following x-irradiation of the small intestine of C_3H/He mice have been published previously (Carr et al, 1983a). Three and a half days after treatment with 20 Gy X-rays (single

dose, whole body) the mean villous score, was 1.85. This is substantially lower than that for lower body heating to 44.0°C (6.4). By comparison with Table 2, the effect of 20 Gy X-rays is similar to the effect of heating to just over 41.0°C (score for 41.0°C = 1.6). Although the mouse strains used have not been identical, it is of interest that the temperature corresponding to 20 Gy X-rays should be at the point where the hyperthermic damage begins to be substantial. Any such comparison of course between villous damage after irradiation and heating relies on only one expression of villous integrity. Note must also be taken of the fact that substantial damage is seen 3 days after irradiation and 2 hours after hyperthermia. Whatever the time factor, it seems that the temperature range 41.0°C to 41.5°C is a vital one in assessing the likely damage to normal small intestinal tissues.

The lower body heating experiments therefore pinpoint the temperature range at which damage begins to be severe, while the exteriorisation experiments underline the extent to which such localised damage can be rapidly repaired. Further work is under way to confirm the causal link between the capillary changes and the subsequent villous collapse.

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DISCUSSION WITH REVIEWERS

W C Dewey Is the phenomenon a true collapse of villi implying loss of underpinnings, or is it a mere shortening due to loss from the tip?

The authors: Quantitative measurements of stroma in the villous and pericryptal compartments would be needed to differentiate between these two possibilities : such studies have not yet been carried out.

M Porvaznik: Is the damage from the injury reversible below a given temperature and is there a temperature condition which results in irreversible damage leading to the death of the animal? If death results, is the time frame similar to death following a lethal dose of irradiation (G.I.Syndrome)?

The authors: The degree of damage to both villi and crypts is dependent on the duration of treatment as well as the temperature; for isoeffect, a 1°C change in temperature is approximately equivalent to a factor of 2 in heating time. Following the most severe treatment used in the present experiments (43°C for 30 minutes), localised heating has shown that only 40% of crypts are lost and intact villi are seen at 1 day after treatment, (Hume et al, 1979). Death rarely occurs after heating, but is thought to result from paralytic ileus.

Death following lower body or systemic hyperthermia is more common but is again dependent on temperature and duration. For a 60 minutes heating period, mice do not tolerate core temperatures above approximately 42°C. Death is very rapid (6 hours) and is probably the result of loss of body fluids and heat stroke (Hume & Field, 1978).

M Porvaznik: Do you plan to freeze-fracture the intestinal tissue lesion following hyperthermia? Freeze-fracture will reveal ultrastructural information about the changes in both the vascular and enterocyte cell-to-cell junctions.

The authors: We agree that freeze-fracture studies would provide useful extra ultrastructural information and we hope to carry out such studies in the future.

R.L. Owen: Your control villi in figure 2 appear to be conical in shape. Why do you describe mouse villi as finger shaped ? Finger shaped villi are seen in carnivores, but villi in mice are broader in their circumferential dimension than in their longitudinal dimension. Control mouse villi are thus spade or wedge shaped and not finger shaped to most observers.

The authors: In our view the villi are not exactly finger-shaped nor spade or wedge shaped. However, we feel that the control villi are as close to finger shaped as they are to any other model.

R L Owen: Do you know if there are any clinical examples of intestinal changes in humans following prolonged fever or heat stroke which correspond to your observations?

The authors: We are not aware of any reports of such clinical examples of villous damage following fever or heat stroke. Icterus and hyperthermia-induced liver changes have been reported following both fever and clinical whole-body hyperthermia, and it has been suggested that, in a minority of patients, liver damage contributes to fatal heat stroke.

T M Seed: The study compares the morphological effects on intestinal tissue of exterior vs lower body heating over a range of temperatures. Are the authors confident that the intra-intestinal tissue temperatures are identical to external bath temperatures?

The authors: As mentioned in the material and methods, the temperatures of both the lumen of the intestine and the peritoneal cavity were measured using fine copper-constantan thermo-couples in sample mice. In the lower body heating experiment, the temperature stabilised at approximately 0.2°C above the water bath temperature for each treatment temperature used. However, equilibration temperature was not attained until approximately 10 minutes after immersion of the animal. When the intestine is heated by immersion of an exteriorised loop the equilibration half-time of the exteriorised intestine is approximately 6 seconds but even at equilibrium a temperature gradient exists around the circumference; only in the region opposite to the mesenteric attachment can the temperature be guaranteed to be within 0.1°C of that of the bath. In regions nearest to the major blood vessels, the temperature can be up to 0.6°C below the bath temperature (Hume S P; Robinson J E; Hand J W, 1979. The influence of blood flow on temperature distribution in the exteriorized mouse intestine. British J Radiology, 52: 219-222).

T M Seed: The cellular infiltration seen at 41.5°C and 42.0°C but not at 43.0°C is puzzling. Do the authors have any idea why this occurs?

The authors: The cellular infiltrate encountered at that early time after heat was attributed in part to possible leakage of the affected dilated capillaries. Although it is completely speculative, it may be that at 43.0°C hyperthermia, the heat was so severe that it caused circulatory changes which resulted in an altered morphology from those seen at lower temperatures. Also in the small intestinal villi, the stage of villous collapse seemed to progress with the increase in temperature. The result is that the higher the temperature, the greater the degree of villous collapse, and so the smaller the apparent volume of stroma and the less the apparent cellularity in the lamina propria. Cell counting would answer this question.