TOPOGRAPHICAL VARIATIONS IN CRYPT RESPONSE

AFTER CYTOCIDAL TREATMENT

a

thesis

submitted

by

KHAMZA KIBWANA YUSSUF MAUNDA

to the

Department of Medical Biophysics

of the

University of Dundee

in partial fulfilment of the requirements

for the

DEGREE OF MASTER OF SCIENCE

Paterson Laboratories
Christie Hospital and
Holt Radium Institute
Wilmslow Road
Manchester M20 9BX.

OCTOBER, 1981

CONTENTS

PAGE

Contents 1

List of Tables 3

List of Figures 4

List of Plates 5

Acknowledgements 6

Declaration 7

Abstract 8

CHAPTER ONE:

INTRODUCTION 9

CHAPTER TWO:

MATERIALS AND METHODS 13

2.1. Animals 14

2.2. Equipment 14

2.3. Drugs 14

2.4. Experimental Procedures 15

CHAPTER THREE:

RESULTS 18

3.1. Macroscopic Observation 19

3.2. Microcolony Assay 19

3.3. Cellular Kinetics 33

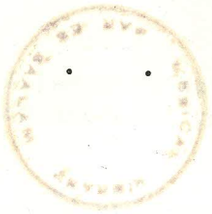
CHAPTER FOUR:

DISCUSSION 40

4.1. Microcolony Assay 41

CONTENTS

	PAGE
4.2. Cellular Kinetics	45
APPENDIX A	48
APPENDIX B	53
APPENDIX C	71
REFERENCES	74



LIST OF TABLES

TABLE NO.		PAGE
1	Parameters of crypt survival curves after gamma irradiation.	24
2	Parameters of crypt survival curves after gamma irradiation.	25
3	Parameters of crypt survival curves after intraperitoneal injection of nitrogen mustard.	32
4	Parameters of cellular kinetics in ileal crypts/villi, for patch and non-patch areas of intestine.	36
5	Parameters of ileal crypt survival in BDF_1 male mice, measured 4 days after treatment by gamma rays or nitrogen mustard, by scoring all crypts around an entire circumference.	42
6	Comparison of cellular kinetics in the mucosa of the small intestine of different mouse strains.	47
7a	Correction factors for single dose gamma irradiation experiment.	56
7b	Correction factors for split-dose gamma irradiation experiments.	57
7c	Correction factors for nitrogen mustard experiment.	58

LIST OF TABLES

TABLE NO.		PAGE
1	Parameters of crypt survival curves after gamma irradiation.	24
2	Parameters of crypt survival curves after gamma irradiation.	25
3	Parameters of crypt survival curves after intraperitoneal injection of nitrogen mustard.	32
4	Parameters of cellular kinetics in ileal crypts/villi, for patch and non-patch areas of intestine.	36
5	Parameters of ileal crypt survival in BDF_1 male mice, measured 4 days after treatment by gamma rays or nitrogen mustard, by scoring all crypts around an entire circumference.	42
6	Comparison of cellular kinetics in the mucosa of the small intestine of different mouse strains.	47
7a	Correction factors for single dose gamma irradiation experiment.	56
7b	Correction factors for split-dose gamma irradiation experiments.	57
7c	Correction factors for nitrogen mustard experiment.	58

LIST OF FIGURES

FIGURE NO.		PAGE
1	Survival fraction of ileal crypts overlying the Peyer's patches, 4 days after graded single and split doses of gamma rays.	22
2	Survival fraction of ileal crypts overlying antimesenteric non-lymphoid submucosa, 4 days after graded single and split doses of gamma rays.	23
3	Surviving fraction of ileal crypts, $3\frac{1}{4}$ days after graded doses of nitrogen mustard.	29
4	Percentage of ^{3}H-TdR-labelled mitoses (PLM) in ileal crypts of mice sacrificed at various intervals after injection of ^{3}H-TdR.	34
5	Average position of the highest ^{3}H-TdR-labelled cell at various intervals after injection of ^{3}H-TdR.	38

LIST OF PLATES

PLATE NO.		PAGE
1	Transverse section of ileum of untreated BDF_1 male mouse, showing crypts overlying normal submucosa and crypts overlying a Peyer's patch.	20/21
2	Transverse section of ileum containing a (denuded) Peyer's patch, 4 days after 12 Gy of gamma rays.	26/27
3	Transverse section of ileum from a non-patch length of gut, 4 days after 15 Gy of gamma rays.	26/27
4	Transverse section of ileum containing a Peyer's patch, $3\frac{1}{4}$ days after 3mg kg^{-1} of nitrogen mustard.	30/31
5	Transverse section of ileum from a non-patch length of gut, $3\frac{1}{4}$ days after 3mg kg^{-1} of nitrogen mustard.	30/31

ACKNOWLEDGEMENTS

I would like to thank Dr J H Hendry, Head of the Department of Radiobiology, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, for making it possible for me to undertake this study in the department and for his considerable advice and encouragement throughout the course of the research.

I am highly appreciative of the guidance and encouragement given me by Dr J V Moore, whom I have been working with very closely throughout the tenure of this research. His tolerance contributed significantly towards the accomplishment of this thesis.

I also wish to thank Mr F G Cowie and Mr D A Broadbent for doing all the injections and irradiation work required. Their contribution in the photographic work required in this thesis is appreciated. Special thanks go to Miss Elaine Mercer for typing this thesis.

Finally, I would like to express my sincere thanks to all staff of the Paterson Laboratories for their kindness during my stay here.

DECLARATION

I declare that no portion of the work described in this thesis has previously been submitted in support of an application for another degree or qualification of this or any other University or Institute of Learning.

ABSTRACT

The response of murine ileal crypts to cytotoxic treatments has been analysed, using the crypt microcolony assay, with respect to three topographic zones - crypts overlying Peyer's patches (P), non-patch crypts on the antimesenteric aspect of an intestinal circumference (NPA), non-patch crypts adjacent to the mesenteric attachment (NPM). For γ-rays, survival was highest in P crypts, intermediate in NPA crypts, lowest in NPM crypts. It could not be clearly demonstrated that this was caused by a high number of clonogenic cells in P crypts. The ratio of Do's between P and NPM crypts was 1.65:1. For nitrogen mustard, crypt survival was again highest for P crypts, but NPA crypts were more sensitive than NPM crypts. It is suggested that these differences in response reflect differences in the delivered dose of drug to the three zones, following intraperitoneal injection. The cellular kinetics of unperturbed P and NP crypts were similar.

1. INTRODUCTION

Peyer's patches, described by J.C. Peyer in 1677, (Ten Cate, 1969), are groups of subepithelial lymphoid follicles located throughout the small intestine. The mean number of patches over the length of the intestine has been found to vary in different strains of mice, 9 in male albino mice (Chin and Hudson, 1971), $8.8^{+}1.4$ in dd-mice of both sexes (Abe and Ito, 1977) and 7.7 in male mice of the A/JAX strain (Smith et al, 1980). Their size was also variable as noted by all these investigators but the patches showed a constant relationship to the intestinal wall, being present on the anti-mesenteric aspect of the small intestine.

It was observed by Altmann (1979) that following treatment with high but sublethal doses of methotrexate in adult male rats, crypt recovery over the Peyer's patches, as measured by cellular kinetics, took place earlier (day 3) and was more marked than elsewhere. He had also seen a similar effect following abdominal X-irradiation of 9 Gy. This differential regeneration of crypts over the patches after irradiation and/or cytotoxic drug treatment has also been noted in the Paterson Laboratories (unpublished).

Merino (1976) noted that when exteriorized intestine of mouse was heated, there was a heterogeneous response in terms of survival of whole intestinal crypts. The smallest effect occurred in regions adjacent to the mesenteric blood vessels. They suggested that this might be due to local cooling. A similar heterogeneity in hyperthermic killing of crypts was observed by Hume et al (1979). These authors suggested the damage to be correlated with distance from the mesentery and presumably with distance from the major blood vessels.

Whole intestinal crypts can be ablated also by high doses of cytotoxic drugs (Moore, 1979). In this case, the route of administration could influence the response of the tissue. Tuohimaa et al (1973) reported that following an intraperitoneal administration of tritiated nucleotides, visceral organs from the injected side contained consistently more silver grains than those from the opposite side. Sections of the abdominal organs showed

a decreasing concentration gradient of reduced silver grains extending from the serosal part of the organ to the deeper layers. In addition, they also noted that initial labelling was observed in sectors of organs which protruded into the peritoneal cavity. In contrast, they did not observe this concentration gradient following the administration of the tritiated nucleotides intravenously. So, they suggested that tritiated nucleotides administered intraperitoneally may penetrate the abdominal organs directly via the serosal surface without prior entry into the general circulation.

Factors such as blood flow, temperature or mode of administration of an assaying agent could be regarded as 'extrinsic' to the crypt. 'Intrinsic' factors that might lead to differences in response include (1) the absolute size of the reservoir of clonogenic cells in each crypt, (2) the kinetics of the proliferative fraction of crypt cells. Clonogenic cell number is deduced from curves of crypt survival after cytotoxic treatments, measured either by an in situ regeneration, or 'microcolony' assay (Withers and Elkind, 1970), or by incorporation of radiolabel into crypts (Hagemann et al, 1971). Potten and Hendry (1975) estimated this number to be 84 ± 31 per murine jejunal crypt, while Masuda et al (1977) obtained a value of 140, i.e a very high proportion of the total proliferative cells in crypts of murine small intestine. More recently, Moore (1979) and Moore and Broadbent (1980) have shown that estimates of clonogenic cell number may vary with the agent of assay, being lower by factors of 10 to 100 when using cytotoxic drugs rather than radiation.

As regards the cellular kinetics of crypts it is known that parameters such as cell cycle time, growth fraction and overall cell number, differ along the length of the gastrointestinal tract (e.g. Kovacs and Potten, 1973). The quantitative morphology of crypts may even vary in different regions of a single circumferential sample of gut, e.g. in guinea pig colon (Sawicki and Rowinski, 1980).

The present experiments were designed to compare intestinal crypts associated with Peyer's patches and those overlying non-lymphoid submucosa with respect to:-

(1) the response of clonogenic cells to gamma-radiation and to the alkylating agent, nitrogen mustard. This drug was chosen because the survival curve for non-patch crypts presents a marked contrast to that for radiation, having a small shoulder and a very low calculated number of clonogenic cells per crypt (Moore, 1979).

(2) the cellular kinetics of the overall crypt-villus populations. It has been suggested that the proliferative response to drugs or radiation may differ in the two zones (Altmann, 1979). Whether this reflects intrinsic differences in the unperturbed cellular kinetics is unknown.

2. MATERIALS AND METHODS

2.1. Animals

Male B6D2F$_1$ (Pat) mice, aged 9-10 weeks were used at the mean weight of 28.5 gm. B6D2F$_1$ is the F$_1$ progeny of female C57 Black-6/PAT and male DBA-2/PAT mice which were bred by brother-sister matings. The mice were weaned at 3-4 weeks and kept in boxes of 10 until they were required for the experiments. During the experiments the mice were housed, 3-8 per cage, in an Experimental Animal Room, under a 12 hour dark (1800 to 0600), 12 hour light regimen. Food (Oakes standard mouse diet) and water were supplied ad libitum.

2.2. Equipment

Cs-137 Gamma-Ray Source

The Cs-137 source located at the Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, has been designed mainly for the irradiation of small animals. It is a high energy gamma emitter (667 KeV) with a half life of 30 years.

In the Cs-137 source, eight individual sources giving a total of 13.32TBq are situated so that a volume of 3 cms diameter by 10 cms length is irradiated homogenously at a dose rate of 5 Gy per minute.

The sources remain permanently inside a shield and the mice are introduced in a cavity in a steel plug which is motored in and out of the shield. The exposure time is controlled by an electric timer. The unanaesthetized mice can be irradiated in pairs or individually in a perspex tube. The perspex tube has air holes and a forced air supply is introduced into the end of the irradiation cavity.

2.3. Drugs

2.3.1. Tritiated Thymidine - ^{3}H-TdR

Tritiated thymidine was supplied by the Radiochemical Centre, Amersham, England, in sterile vials each containing 5.0 mls of aqueous thymidine solution with an activity of 9.62TBq/mmol.

2.3.2. Mechlorethamine Hydrochloride

Mechlorethamine hydrochloride (Boots, Nottingham) is a white, or almost white, hygroscopic, vesicant, crystalline powder. It is supplied in sterile vials each containing 10 mg of the powder.

2.4. Experimental Procedures

2.4.1. Labelling Experiment

Tritiated thymidine (518K Bq in 0.3ml solution) was injected intraperitoneally stat in every animal. The animals were then sacrificed by cervical dislocation at different time intervals ranging from 1 hour to 30 hours. Three animals were used per experimental point.

The whole length of the intestine from the duodenum to the ileocaecal junction was removed together with its mesentery. The specimen was examined macroscopically and the number of Peyer's patches, their longitudinal sizes and position along the intestine were noted. The specimens were fixed for one hour in a freshly prepared 3:1 solution of 70% absolute alcohol and glacial acetic acid respectively. After fixation the specimens were rinsed in 70% absolute alcohol and retained overnight in a fresh solution of 70% absolute alcohol.

The Peyer's patches in the ileum were used. Lengths of ileum each containing a Peyer's patch were dissected out - in total five lengths per animal. Care was taken in making these preparations, so that a piece of mesentery was included in every piece of ileum. This makes the identification of mesenteric and anti-mesenteric areas easy during microscopy. To get an internally controlled sample, further five pieces without Peyer's patches were also taken - each piece made just next to a corresponding one with a Peyer's patch.

For ease of sectioning, the lengths were bound together in two groups for every animal (those containing Peyer's patches and those without) with micropore surgical tape before histological processing. Then 5-micron

transverse sections were prepared and fixed on chrome gelatin subbed slides (details of this and subsequent histological and autoradiographic procedures are given in the Appendix).

The slides were then de-waxed and stained with Schiff's Reagent (Feulgen stain). This stains cell nuclei a light pink colour, enabling the black grains of the ^{3}H-TdR labelling to stand out well in contrast. Once the sections were stained, they were covered with the stripping film emulsion (AR10 film, Kodak Ltd). The slides were then boxed and stored at 4^{o}C for six weeks before developing. They were developed in the high-contrast Kodak formula D-19 and fixed with Hypam fixer (Ilford Ltd).

2.4.2. Microcolony Assay Experiments

2.4.2.1. Irradiation Experiments

Two irradiation experiments were done, single-dose response and split-dose response. A Cs-137 gamma ray unit was used and for the single-dose response experiment, mice received graded whole body single doses of 2-17 Gy at a dose rate of 5 Gy/min at 10.00 hrs. Four animals were used per experimental point for the dose range of 2-9 Gy and eight animals per point for the dose range of 10-17 Gy. In the split-dose response experiment, a first dose of 11 Gy was given at 10.00 hrs followed 5 hrs later by graded doses of 1-11 Gy. Eight animals were used per experimental point.

The animals were sacrificed on the fourth day by cervical dislocation and the intestinal specimens were processed as detailed in the Labelling Experiment, except that in this case the sections were stained with Haematoxylin and Eosin and there was no need of chrome gelatin subbed slides.

The crypts of the ileum of the irradiated mouse are depopulated in 1-2 days, but the surviving cells of the crypt which are capable of further proliferation, regenerate the crypt during the assay time. In the technique originally described by Withers and Elkind (1970), the number of regenerating crypts for whole circumference of the intestine are counted. For each dose of the agent a surviving fraction (SF) was calculated relative to untreated

controls ($121^{+}_{-}3$ for $B6D2F_1$, Pat, mice; all errors quoted as $^{+}_{-}2$ S.E, i.e. the 95% confidence limits). Regenerating crypts often differ in size from unstimulated crypts, so a correction factor (the ratio of the diameter of untreated crypts to the diameter of treated crypts) has been applied to allow for the altered probability of encountering a crypt of larger or smaller diameter in a section of given thickness (Hendry and Potten, 1974)*.

For the purpose of these experiments where a comparison of crypt survival between crypts over the Peyer's patches and those over the non-patch areas was required, a slight modification to the scoring technique described by Withers and Elkind (1970) had to be made. An ocular microscope grid was used so that fixed lengths of 20 grid squares were scored and hence the surviving fraction of the crypts were expressed as crypts per grid square. Three separate areas of the ileal circumference were scored, the Peyer's patch (P) area from sections containing Peyer's patches, the non-patch antimesenteric (NPA) area and the non-patch mesenteric (NPM) area from sections not containing Peyer's patches. The main difficulty with this scoring technique is scoring along the ileal circumference curvatures and curvatures over the Peyer's patches. This can be overcome by using the centre of the grid as a pivot, so that the grid can be rotated to follow the curvature and the scoring can then be done in halves (12-30 sections per dose point were scored).

2.4.2.2. Cytotoxic Drug Response Experiment

Mechlorethamine hydrochloride was dissolved in sterile water and graded single doses in a range of 0.5-13.0 mg kg^{-1} (injection volumes of 0.23ml - 0.74ml) were injected intraperitoneally. Four animals were used per experimental point. The animals were sacrificed in three and a quarter days and the specimens were processed as already described for the irradiation experiments. The scoring of surviving crypts was carried out as in the irradiation experiments.

* See Appendix B

3. RESULTS

3.1. Macroscopic Observation

Peyer's patches were observed as subserosal bulges located along the entire antimesenteric aspect of the small intestine. Their size varied, but in general the duodenal end had the smallest patches and the sizes increase along the length of the intestine with the terminal ileum having the largest patches. In this strain of mice, the mean number of patches was found to be 10.35 ± 0.2 along the entire length of the small intestine. No patches were found in the colonic wall.

3.2. Microcolony Assay

3.2.1. Irradiation Experiments

Survival curves of ileal crypts were established for single doses of gamma rays and for split doses (Figures 1 and 2). Curves were fitted to the data by a computer programme (Gilbert, 1974). The following parameters were measured: reciprocal of the final slope (Do); dose required to reduce the crypt population to 63% of its initial number i.e. a measure of the 'shoulder' on crypt survival curves (LD_{37}); exponential ($LD_{37/Do}$) i.e. the extrapolation number of the crypt survival curve (N); extrapolation number for individual cells surviving a first, high dose (E), obtained from the split-dose experiments; N/E i.e. the number of clonogenic cells per crypt (A).

Data were analysed under 3 sets of conditions: (1) curves were fitted independently to each set of data for single or split doses; (2) a common Do was found for the single plus split doses in a given topographic region; (3) a common Do was obtained for all sets of data.

Under condition (1), the independently fitted Do's for regions P, NPA, NPM did not differ significantly from each other (Table 1), the estimate for region P having a large proportional error. Significant differences were observed between pairs of split-dose curves. Under condition (2), mean Do's tended to be higher in region P (2.47 Gy) and NPA (2.04 Gy) than in NPM (1.52 Gy). Under condition (3), the overall common Do was 1.92 Gy (Table 2).

PLATE 1: Transverse section of ileum of untreated BDF_1 male mouse, showing crypts overlying normal submucosa and crypts overlying a Peyer's patch (PP). The patches are always found on the antimesenteric (A) aspect of the intestine, never on the mesenteric (M) aspect. H and E stain. Magnification X65.


A
M
PP

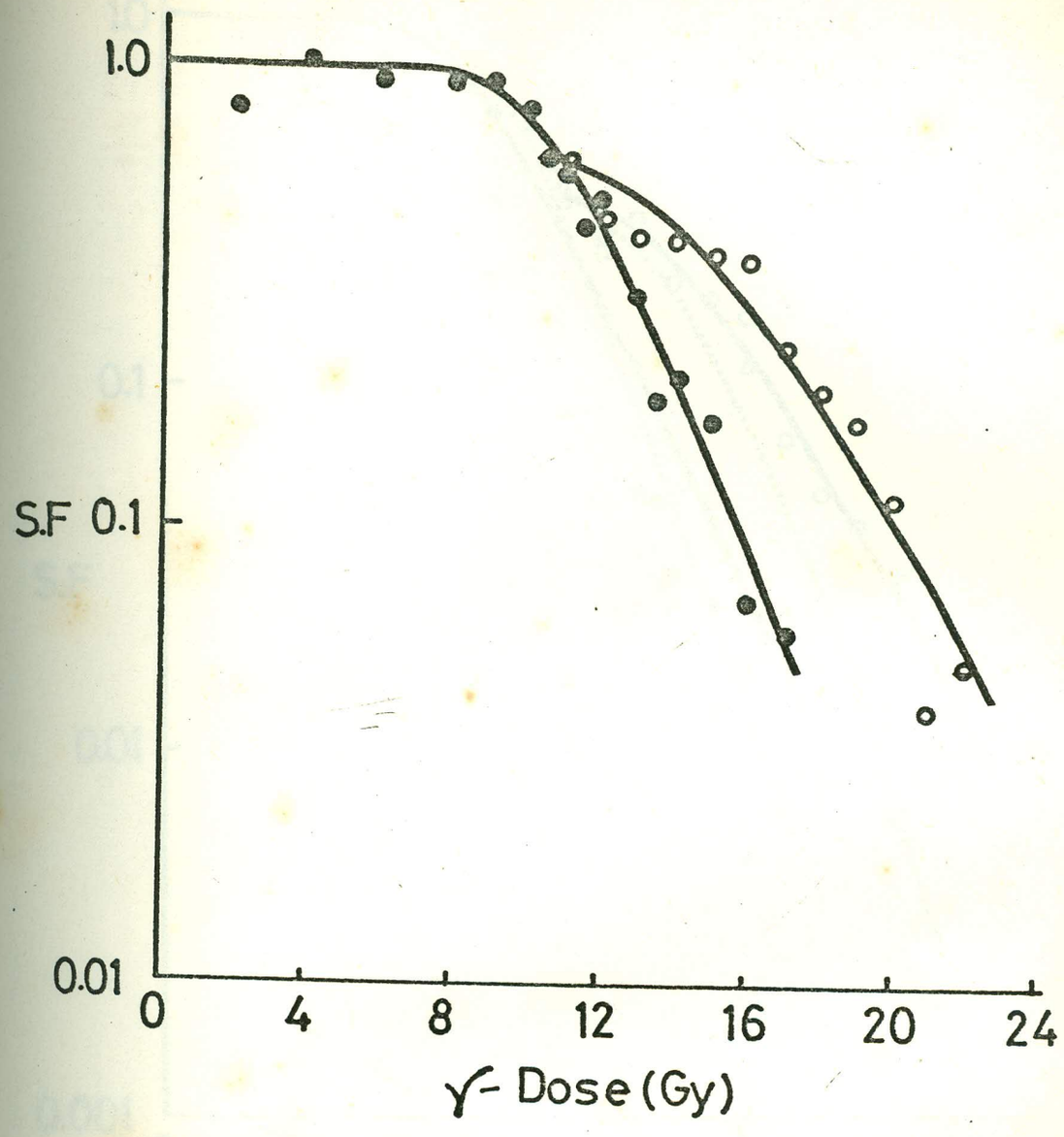


FIGURE 1: Surviving fraction (SF) of ileal crypts overlying Peyer's patches, 4 days after graded single (●) and split (O) doses of γ-rays. Best-fit lines calculated by a computer programme (Gilbert, 1974). In split dose experiments, the surviving fraction after the primary dose was 0.62. Data points for the second doses were also calculated relative to numbers of crypts in untreated controls (see Appendix B).

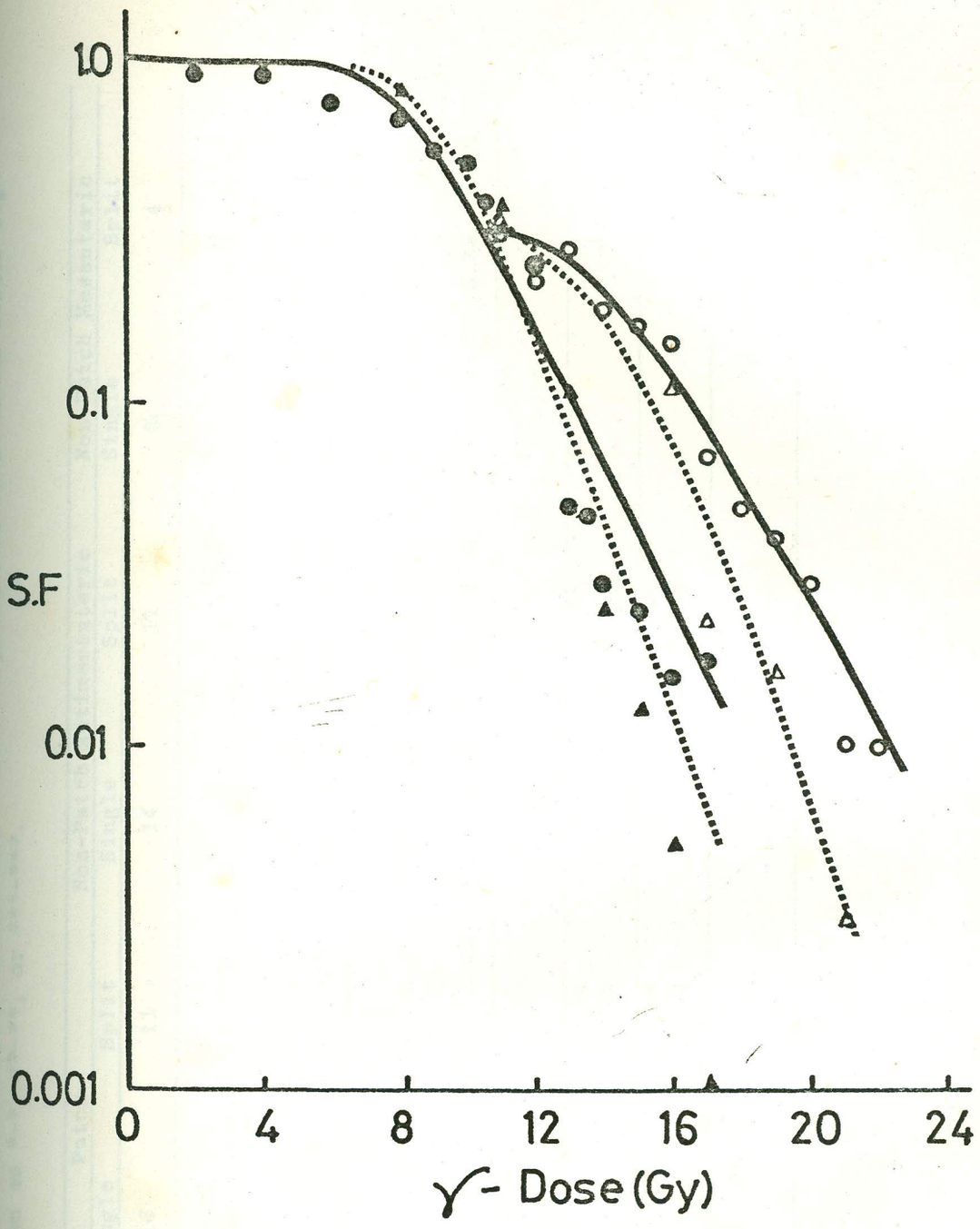


FIGURE 2: Surviving fraction of ileal crypts overlying non-lymphoid submucosa. Solid lines are for single (●) and split (○) doses of γ-rays, survival measured on the antimesenteric aspect of the gut circumference. Dashed lines are for single (▲) and split (△) doses, measured on the mesenteric aspect.

TABLE 1: Parameters of crypt survival curves after γ-irradiation. Pairs of parameters where means differ significantly ($p<0.05$) are shown as *-*, **-**, or ***-***.

	Patch				Non-Patch Antimesenteric				Non-Patch Mesenteric			
	Single		Split		Single		Split		Single		Split	
Data points per curve	16		11		14		11		8		4	
Dose range (Gy)	2 - 17		1 - 11 (2nd doses)		2 - 17		1 - 11 (2nd doses)		8 - 17		5 - 10 (2nd doses)	
Do + ISE (Gy)												
Independent	2.18	0.74	3.05**	0.30	2.02	0.40	2.32***	0.17	1.53	0.19	1.31***	0.30
Common Fit	2.47	0.56	2.47	0.56	2.04	0.28	2.04	0.28	1.52	0.15	1.52	0.15
LD_{37} + ISE (Gy)												
Independent	10.85**	0.77	3.73	0.37	8.52*	0.65	3.17	0.25	9.18**	0.38	3.60	0.55
For Common Do	10.62**	0.65	4.30	1.02	8.49*	0.46	3.47	1.14	9.19**	0.30	3.25	0.56
N (single-doses)	73.7^{+129}_{-47}				64^{+54}_{-29}				422^{+370}_{-197}			
N^1(split-doses)			5.7^{+4}_{-2}				5.5^{+5}_{-2}				8.5^{+4}_{-3}	
E(=N^1 ÷ cell multiplicity)			3.6^{+4}_{-2}				4.6^{+5}_{-2}				7.0^{+4}_{-3}	
A	20.5^{+42}_{-17}				13.9^{+19}_{-9}				60.3^{+63}_{-38}			

TABLE 2: Parameters of crypt survival curves after γ-irradiation.

	Patch				Non-Patch Antimesenteric				Non-Patch Mesenteric			
	Single		Split		Single		Split		Single		Split	
Data points per curve	16		11		14		11		8		4	
Dose range (Gy)	2 - 17		1 - 11 (2nd doses)		2 - 17		1 - 11 (2nd doses)		8 - 17		5 - 10 (2nd doses)	
Do + ISE (Gy)												
Independent	2.18	0.74	3.05	0.30	2.02	0.40	2.32	0.17	1.53	0.19	1.31	0.30
Common Fit	1.92	0.18	1.92	0.18	1.92	0.18	1.92	0.18	1.92	0.18	1.92	0.18
LD_{37} + ISE (Gy)												
Independent	10.85	0.77	3.73	0.37	8.52	0.65	3.17	0.25	9.18	0.38	3.60	0.55
For Common Do	11.03**	0.38	4.99***	0.74	8.63*	0.37	3.62	1.12	8.64**	0.52	2.55***	1.02
N (single-doses)	313^{+242}_{-136}				89.5^{+53}_{-33}				90^{+59}_{-35}			
N^1 (split-doses)			13.4^{+8}_{-5}				6.5^{+5}_{-3}				3.8^{+3}_{-2}	
$E(=N^1 \div$ cell multiplicity)			8.5^{+8}_{-5}				5.4^{+5}_{-3}				3.1^{+3}_{-2}	
A			36.8^{+45}_{-27}				16.6^{+18}_{-11}				29^{+34}_{-22}	

Analysis of variance indicated that this fit was less good than those obtained under either conditions (1) or (2). Under all 3 conditions, the LD_{37} was significantly higher for crypts overlying Peyer's patches than for those in regions NPA and NPM (Tables 1 and 2).

Estimates of clonogenic cell number per crypt (A) in the 3 regions were made under conditions (2) and (3). The errors on such estimates are large and the three values were not significantly different. However the mean value for NPM (60) under condition (2) was 3 to 4 times higher than for P or NPA (Table 1).

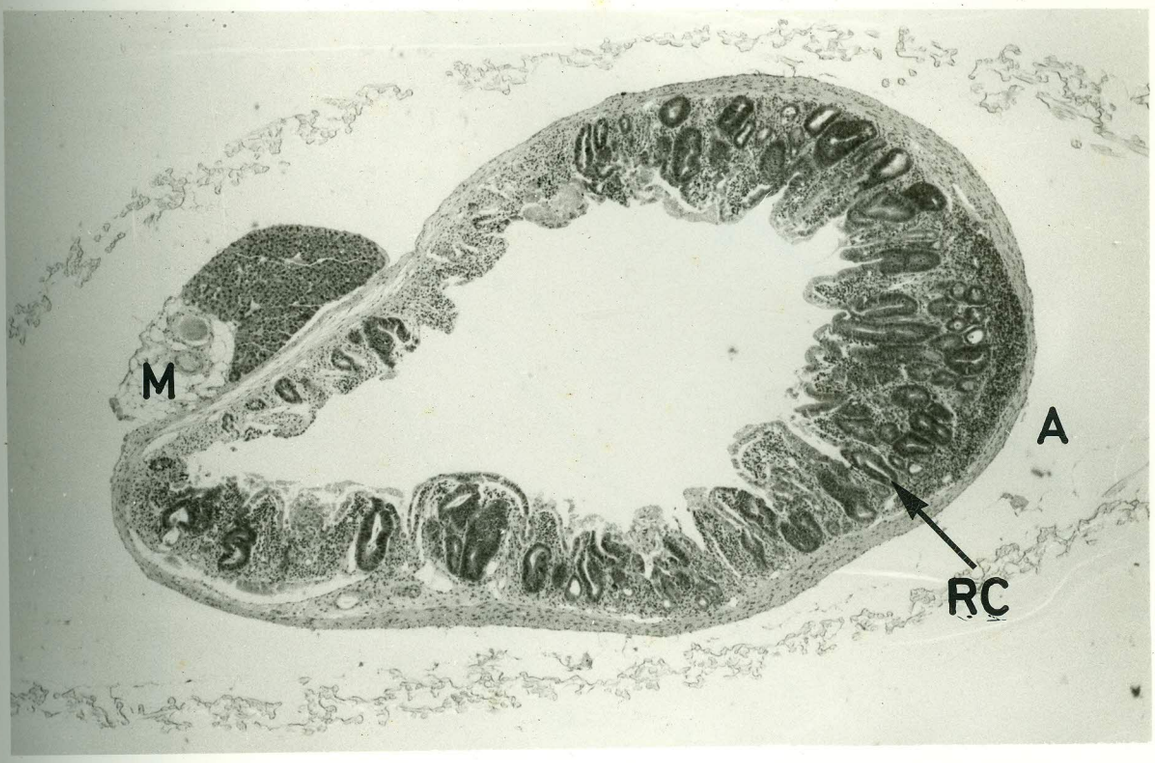
3.2.2. Cytotoxic-Drug Response Experiment

Survival of crypts over Peyer's patches were significantly higher than in either NPA or NPM (Figure 3). This was caused by (a) a high threshold dose (LD_{37} = 5.5 mg kg^{-1}), in marked contrast to the absence of any shoulder in survival curves from the other 2 regions (Table 3) and (b) a significantly higher Do in P compared to NPA, but not NPM. Note that the relative sensitivities in NPA and NPM are the reverse of those for radiation (cf. Figures 2 and 3; Plates 3 and 5). The nitrogen mustard data for NPM region were poorly fitted by any of the computer models available (Gilbert, 1969; 1974) and may represent a curve with a large initial 'shoulder' of finite slope, followed by a steeper slope beginning at 5 mg kg^{-1} (Figure 3).

A small, split dose experiment was attempted, using a first dose of 4 mg kg^{-1} and three graded second doses, given 5 hours later. For region NPA, the lowest of the second doses (3 mg kg^{-1}) ablated all crypts in all specimens. A point has been included in Figure 3 to indicate the level of survival, had 1 crypt survived out of the 500 'at risk' in the specimens scored. This point appears to fall on the single dose curve. There was some evidence of 'sparing' by splitting a dose of nitrogen mustard for both zones NPM and P. However, as 4 mg kg^{-1} is on the 'shoulder' of the former

PLATE 2: Transverse section of ileum containing a (denuded) Peyer's patch, 4 days after 12 Gy of γ-rays. Regenerating crypts (RC) are found most commonly over the patch and on the antimesenteric aspect of the circumference. H and E stain. Magnification X65.

PLATE 3: Transverse section of ileum from a non-patch length of gut, 4 days after 15 Gy of γ-rays. Three regenerating crypts are seen on the anti-mesenteric aspect. H and E stain. Magnification X65.


M
A
RC


M
A
RC

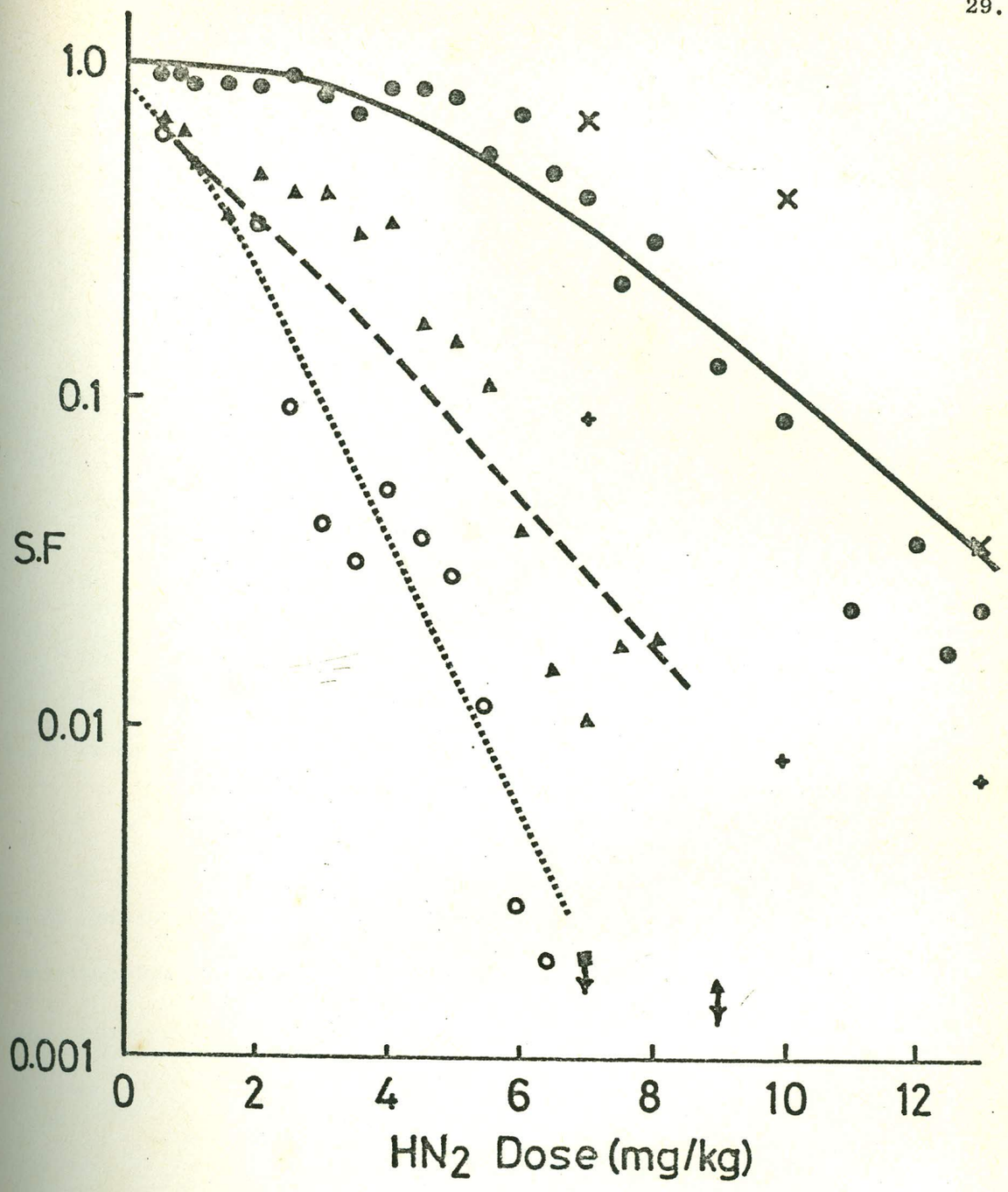


FIGURE 3: Surviving fraction of ileal crypts, $3\frac{1}{4}$ days after graded doses of nitrogen mustard (HN_2). Solid line is for single (●) doses of HN_2, in crypts overlying Peyer's patches; (X) split doses in Peyer's patch crypts. Fine dashed line is for single (O) doses measured from crypts on the antimesenteric aspect of non-patch circumferences; (↕) shows the theoretical upper limit of survival after a split dose of 4 + 3 mg kg^{-1}. Broad dashed line is for single (▲) doses measured from crypts on the mesenteric aspect of non-patch circumferences; (↕) theoretical upper limit of survival after a single dose of 9 mg kg^{-1}; (+) split doses in mesenteric non-patch crypts.

PLATE 4: Transverse section of ileum containing a Peyer's patch, $3\frac{1}{4}$ days after 3 mg kg^{-1} of nitrogen mustard. Regenerating crypts are concentrated over the patch and on the mesenteric aspect. H and E stain. Magnification X75.

PLATE 5: Transverse section of ileum from a non-patch length of gut, $3\frac{1}{4}$ days after 3 mg kg^{-1} of nitrogen mustard. Regenerating crypts are concentrated on the mesenteric aspect. H and E stain. Magnification X65.


RC
A
RC
M

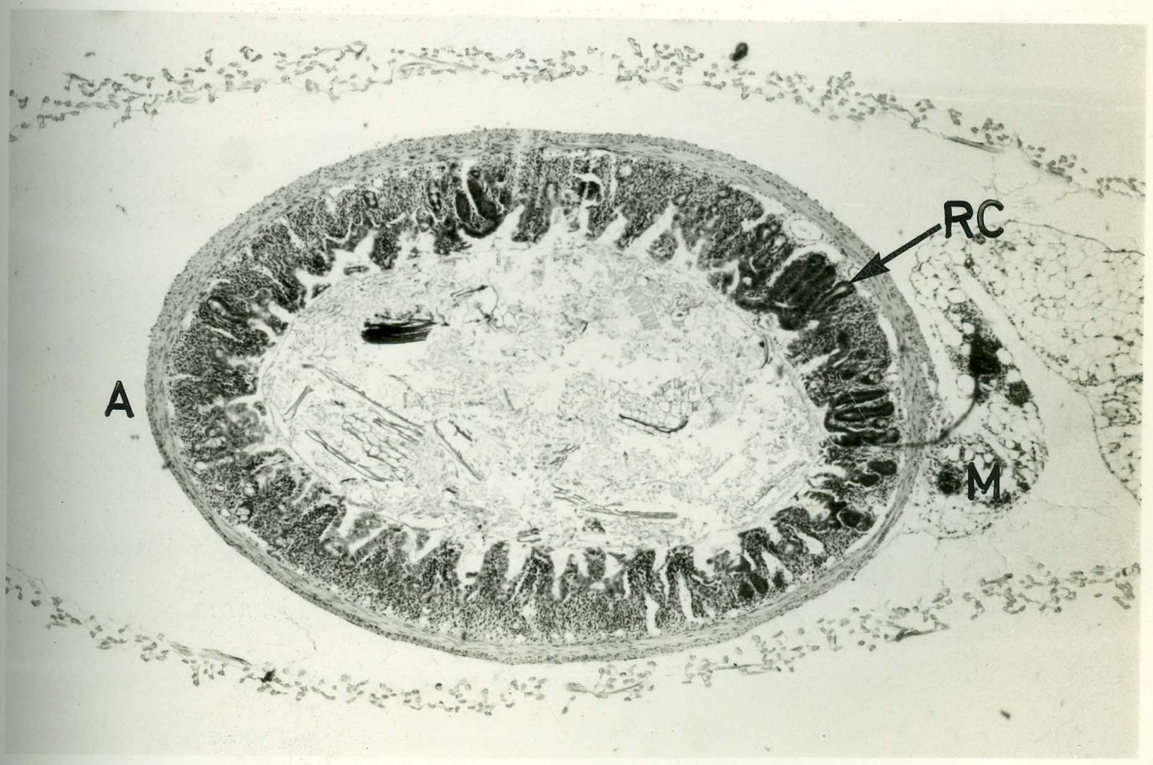

RC
A
M

TABLE 3: Parameters of crypt survival curves after intraperitoneal injection of nitrogen mustard.

	Patch		Non-Patch Antimesenteric		Non-Patch Mesenteric	
Data points per curve	12		14		17	
Dose range (mg kg^{-1})	5.5 - 13.0		0.5 - 6.5		0.5 - 0.8	
Do + ISE (mg kg^{-1})						
Independent	1.87^{*}	0.15	1.04^{**}	0.09	1.89^{**}	0.32
Common Fit	1.38	0.12	1.38	0.12	-	
LD_{37} + ISE (mg kg^{-1})						
Independent	5.56^{*}	0.23	0.54^{*}	0.15	0.33	0.53
For Common Do	6.01^{**}	0.24	0.06^{**}	0.26	-	
N (for Common Do)	77.9^{+40}_{-27}		$1.04^{+0.22}_{-0.18}$			
R.F (=E) From Moore, 1979	4.7	0.9	4.7	0.9		
A	16.6^{+9}_{-7}		$0.22^{+0.06}_{-0.06}$			

curve and the threshold region of the latter, no accurate estimate can be made of D_2-D_1 (Figure 3). A measurement of the split-dose recovery factor (=E) for nitrogen mustard-treated crypts was made by Moore (1979) and found to be 4.7 ± 0.9. Inserting this value into the present results an estimate of A can be made (Table 3). In region P the value was either 4 (independent Do) or 17 (common Do with NPA). In region NPA the value of N was only 1.0 to 1.8, so that the application of E in this case yielded the biologically meaningless answer of 0.22 ± 0.06.

3.3. Cellular Kinetics

Scoring was done at a total magnification of X1000 using an oil immersion objective lens. Only well defined crypts that were clearly bisected along their longitudinal axis, with their villi, were scored and the crypt top was taken as the point where the crypt epithelium forms a genu before it continues into the villus epithelium. Crypts over the Peyer's patches were scored separately to those over the non-patch areas; there was no further division, in the latter case, into NPA and NPM areas.

3.3.1. Percentage Labelled Mitosis (PLM) (Table 4A)

Labelled and unlabelled mitoses, which were confined to the crypts, were scored to give the PLM. A minimum of 70 mitoses were scored for every animal, giving a minimum total of 210 mitoses per experimental point in each of the two areas. The cell cycle parameters were derived from the PLM curves (Figure 4) by the well-known method of Quastler and Sherman (1959) and smooth curves were fitted to the data by a computer programme (Gilbert, 1972)*. The values of the cell cycle parameters (Table 4), do not show significant difference between the P region and the NP region (average Tc for patch crypts = 15.1h, for non-patch crypts = 14.7h).

3.3.2. Labelling Index and Growth Fraction (Table 4B)

Labelling indices (LI; i.e. the number of ^{3}H-TdR-labelled interphase cells divided by the total number of labelled plus unlabelled interphase

* See Appendix C

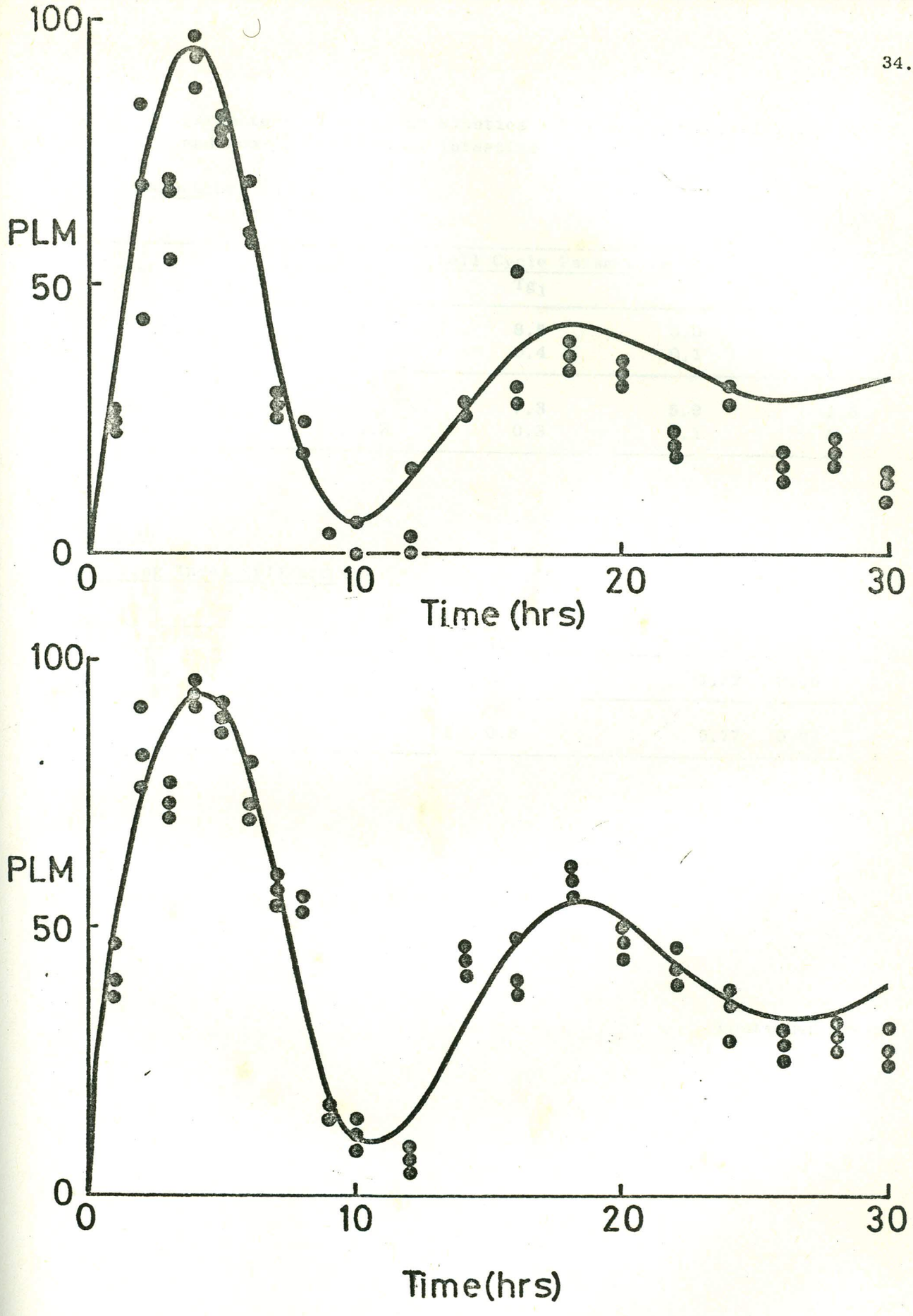


FIGURE 4: Percentage of ^{3}H-TdR-labelled mitoses (PLM) in ileal crypts of mice sacrificed at various intervals after injection of ^{3}H-TdR. Data points are the PLM values for individual animals. Smooth line fitted by a computer programme (Gilbert, 1972). Upper panel-crypts overlying Peyer's patches. Lower panel-crypts in non-patch areas.

TABLE 4A Parameters of cellular kinetics in ileal crypts/villi, for patch and non-patch areas of intestine.

Percentage Labelled Mitosis

Regions	Cell Cycle Parameters (hr ± 1 SE)			
	Tc	Tg_1	Ts	Tg_2
Patch	15.1	8.5	5.0	1.6
	0.4	0.4	0.1	0.1
Non-Patch	14.7	7.3	5.9	1.5
	0.2	0.3	0.1	0.1

TABLE 4B

Labelling Index (LI) and Growth Fraction (GF)

Regions	LI ± 1SE		G.F ± 1SE	
Patch	37	1.7	1.12	0.06
Non-Patch	31	0.8	0.77	0.03

Continued Over/...

TABLE 4C Parameters of cellular kinetics in ileal crypts/villi, for patch and non-patch areas of intestine.

Region	Interval					
	1 to 10h (crypt)			12 to 30h (villus)		
	Correlation coefficient (r^2)	Slope (cell positions/h)	Intercept (cell position)	r^2	Slope	Intercept
Patch (label front)	0.67	1.00* 1SD 0.39	12.0	0.95	1.78** 0.20	0.5
Patch (50% peak)	0.95	1.26 0.15	7.9			
Non-patch (label front)	0.89	1.17 0.20	8.9	0.87	1.22** 0.23	8.7
Non-patch (50% peak)	0.89	1.51 0.26	7.6			
Patch plus non-patch (50% peak)	0.90	1.40 0.16	7.7			

cells) were measured 1 hour after injection of ^{3}H-TdR. Cells were scored up to position 20 in each crypt and a total of 2000 cells were scored per animal. The LI for patch crypts was significantly higher than in the non-patch crypts (Table 4).

An estimate of the growth fraction (the proportion of cells in cell cycle) may be obtained from the LI and the duration of phases of the cell cycle (from the PLM curve). Assuming that the crypt represents a steady-state population:

$$\frac{\text{Duration of S-phase}}{\text{Duration of cell cycle}} = \frac{\text{Number of cycling cells in S-phase}}{\text{Total number of cycling cells}} = LI_p$$

LI_p for the 'pure' population of cycling cells measured by the PLM method.

but the measured LI_m may include both cycling and non-cycling cells, so that:

$$\%\ LI_m/\%LI_p = \frac{\%\text{Number of cells in S-phase}}{\text{Total Number of Cells}} \times \frac{\text{Total No. of (cycling) cells}}{\text{No. of cycling cells in S-phase}}\% = G.F$$

The growth fractions for patch and non-patch crypts are high (Table 4). For patch crypts, it is inferred that all cells up to position 20, are proliferative.

3.3.3. Cell Migration (Table 4C)

Movement of cells up the crypt and villus was measured by scoring the cell positions in which ^{3}H-TdR label was observed, for intervals between 1 and 30h after injection of ^{3}H-TdR. The highest cell position in the crypt containing label was noted. An average of 20 crypts were scored per animal. Data from the three animals per point were pooled and the mean cell position corresponding to the label front, was calculated. By approximately 10h, the label front had reached the villus. After this time two parameters were measured (a) cell position of the front on the villus, and (b) the position of the labelled cell regarded as being nearest the crypt/villus junction. Mean values for patch- and non-patch crypts were plotted for each time interval (Figure 5). Straight lines were fitted to the data by linear regression,

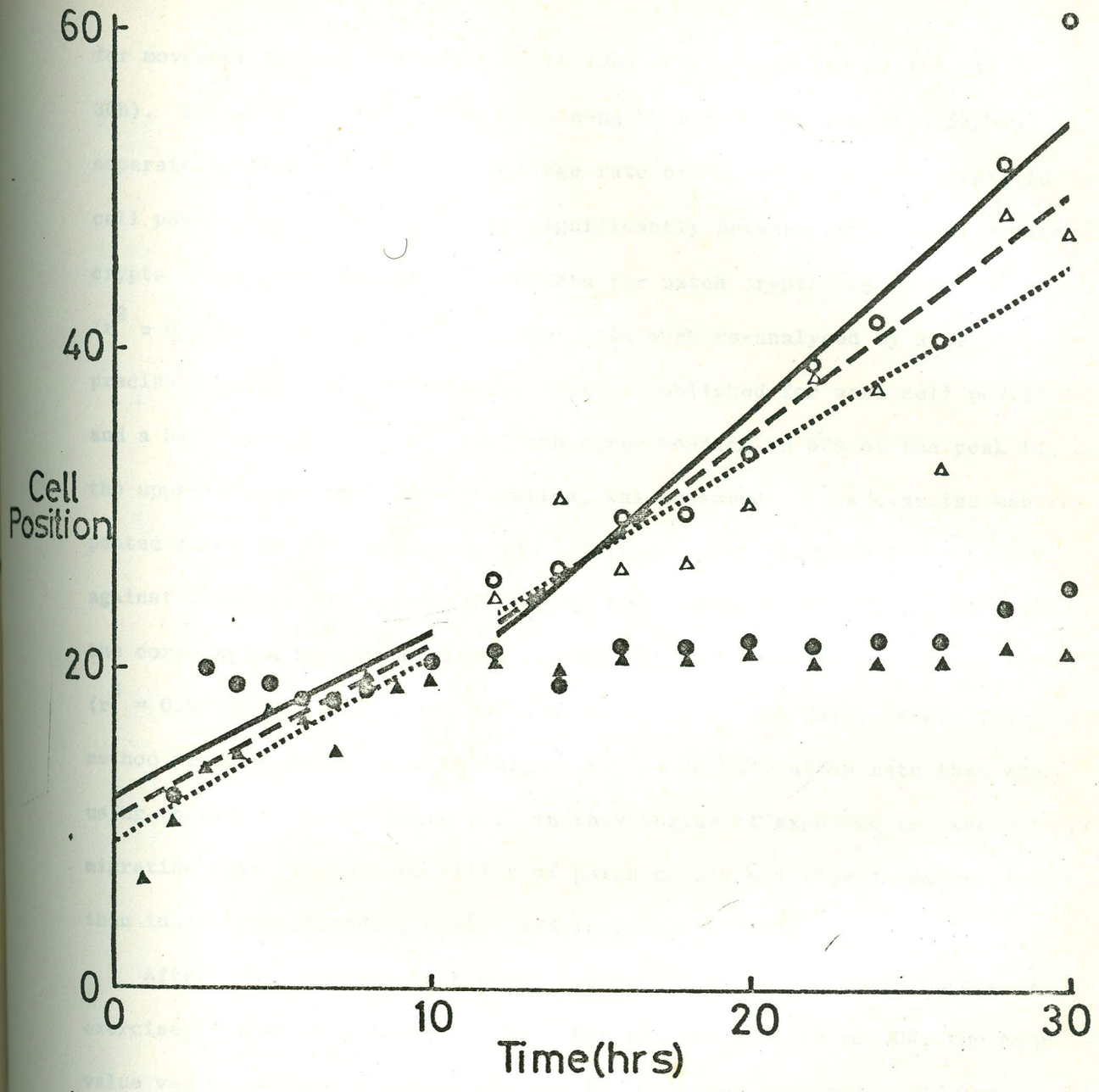


FIGURE 5: Average position of the highest ^{3}H-TdR-labelled cell at various intervals after injection of ^{3}H-TdR. Peyer's patch areas:label in crypt (●), label on villus (○). Non-patch areas:label in crypt (▲), on villus (△). Data points are the mean values from measurements in 20 crypts/villi. Solid lines obtained by separate linear regression calculations for crypts (1 to 10h) and villus (12 to 30h) overlying Peyer's patches. Fine dashed lines are the regressions for non-patch areas. Broad dashed lines are the regressions with both sets of data pooled.

for movement through the crypt (1 to 10h) and through the villus (12 to 30h). The data for the patch and non-patch zones were analysed first separately, then pooled. The average rate of migration up the crypt (in cell positions/h) did not differ significantly between patch and non-patch crypts (Table 4). The fit to the data for patch crypts was rather poor (r^2 = 0.67). Accordingly, the crypt data were re-analysed by a second, more precise method. Labelling indices were established for each cell position and a histogram drawn.* The position corresponding to 50% of the peak LI, on the upper edge of the LI distribution, was measured. This exercise was repeated for each interval up to 10h. Cell position (50% peak) was plotted against interval and a curve fitted by linear regression.** Using this method, the correlation between position and interval was greater for patch crypts (r^2 = 0.95; Table 4) and the residual variance of the data, less. This method yielded insignificantly higher values for migration rate than when using the label front (Table 4). In this series of experiments, the average migration rate through the villus of patch crypts was significantly higher than in the corresponding crypts and in non-patch villi.

After 12h, scoring the highest labelled-cell in the crypt becomes an exercise in measuring crypt height. For intervals of 12 to 30h, the mean value varied between positions 20 and 21 for non-patch areas, and between 19 and 25 for patch areas.

* Figures 6A and 6B, upper.

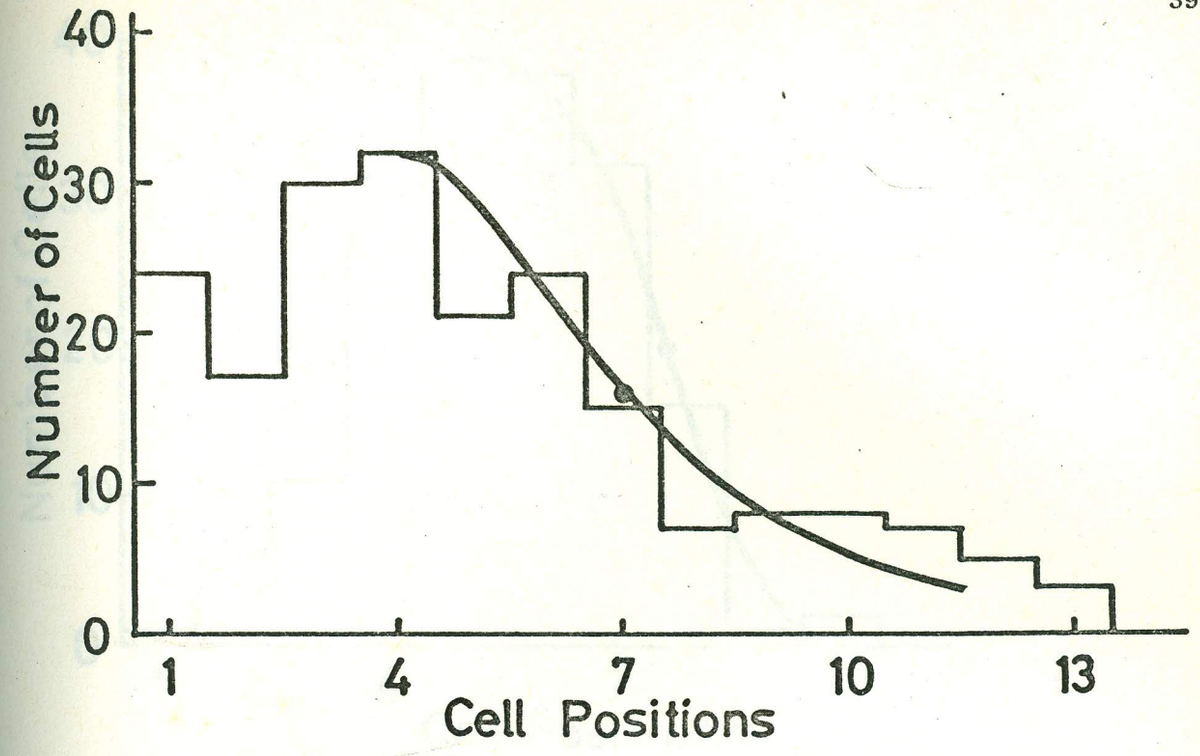
** Figures 6A and 6B, lower.

for movement through the crypt (1 to 10h) and through the villus (12 to 30h). The data for the patch and non-patch zones were analysed first separately, then pooled. The average rate of migration up the crypt (in cell positions/h) did not differ significantly between patch and non-patch crypts (Table 4). The fit to the data for patch crypts was rather poor (r^2 = 0.67). Accordingly, the crypt data were re-analysed by a second, more precise method. Labelling indices were established for each cell position and a histogram drawn.* The position corresponding to 50% of the peak LI, on the upper edge of the LI distribution, was measured. This exercise was repeated for each interval up to 10h. Cell position (50% peak) was plotted against interval and a curve fitted by linear regression.** Using this method, the correlation between position and interval was greater for patch crypts (r^2 = 0.95; Table 4) and the residual variance of the data, less. This method yielded insignificantly higher values for migration rate than when using the label front (Table 4). In this series of experiments, the average migration rate through the villus of patch crypts was significantly higher than in the corresponding crypts and in non-patch villi.

After 12h, scoring the highest labelled-cell in the crypt becomes an exercise in measuring crypt height. For intervals of 12 to 30h, the mean value varied between positions 20 and 21 for non-patch areas, and between 19 and 25 for patch areas.

* Figures 6A and 6B, upper.

** Figures 6A and 6B, lower.



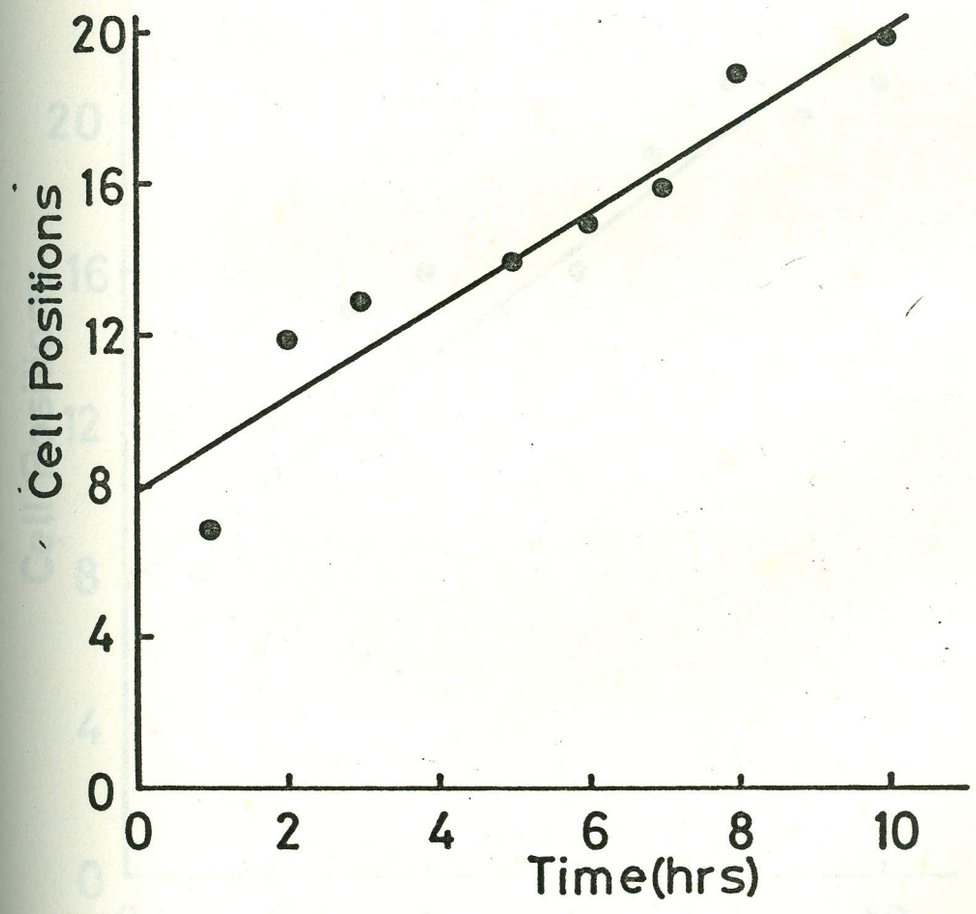
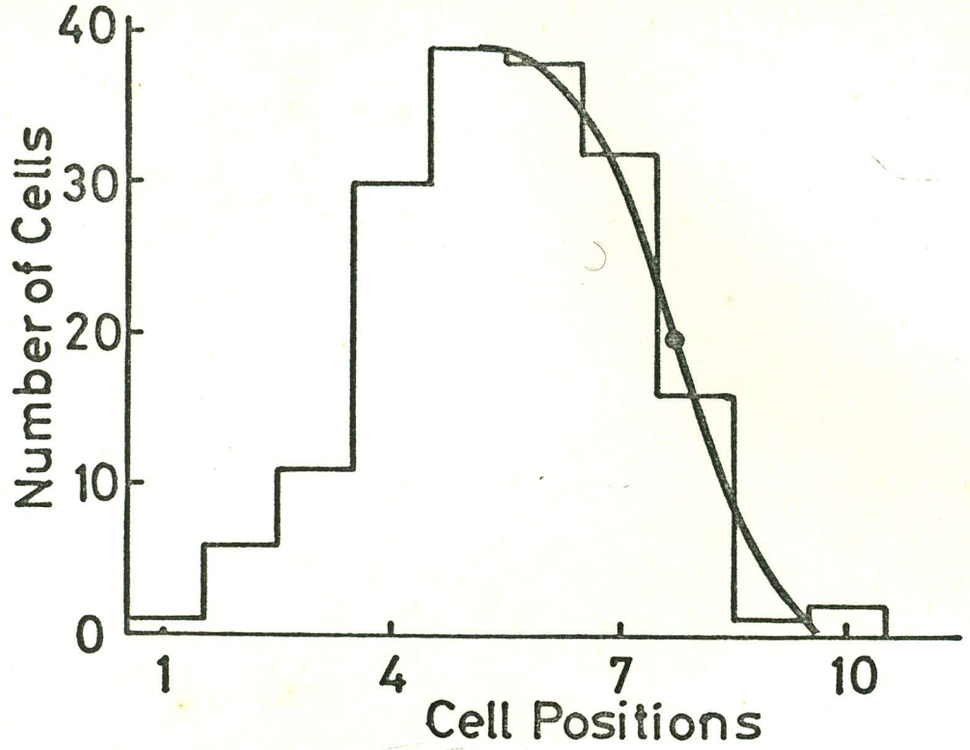


FIGURE 6A: Upper Section: Number of labelled cells distribution curve for the mouse ileal crypt over the patch area 1 hr after intraperitoneal ^{3}H-TdR, (●) 50% peak value.
Lower Section: Movement of the 50% peak value on the labelling index distribution curve with time after ^{3}H-TdR over the patch area.



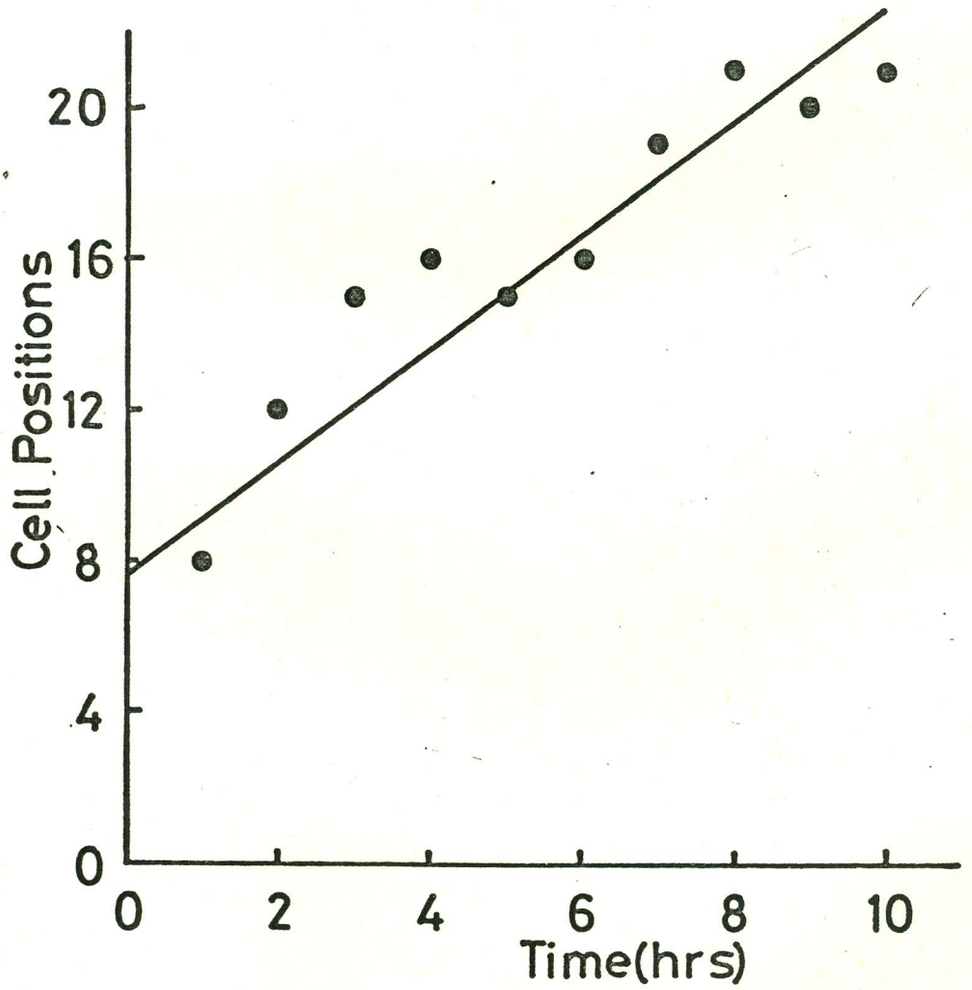


FIGURE 6B: Upper Section: Number of labelled cells distribution curve for the mouse ileal crypt over the non-patch area 1 hr after intraperitoneal ^{3}H-TdR, (●) 50% peak value.
Lower Section: Movement of the 50% peak value on the labelling index distribution curve with time after ^{3}H-TdR over the non-patch area.

4. DISCUSSION

4.1. Microcolony Assay

4.1.1. Radiation

The intestinal crypt microcolony assay (Withers and Elkind, 1970) consists of scoring the numbers of regenerating crypts in the intestine of animals treated 3 to 4 days earlier by cytocidal agents. Conventionally, this scoring is done in transverse sections of gut, and by counting all regenerating crypts around an entire section circumference. Results obtained by this method for $B6D2F_1$ (Pat) male mice treated by γ-rays or nitrogen mustard, are given in Table 5 (Moore, personal communication).

In the present work, it has been demonstrated that the values of parameters of crypt survival obtained by this method are likely to be composite, as local variations exist in the sensitivity of crypts to treatment. Notably in these mice, crypt survival after a given dose of radiation was always higher for crypts overlying Peyer's patches (P crypts) than in non-patch antimesenteric (NPA) crypts or non-patch mesenteric (NPM) crypts. This is in accordance with an earlier, qualitative observation of Altmann (1979). The enhanced survival of P crypts was caused both by an enlarged 'shoulder' on the crypt survival curve (LD_{37} = 10.62 Gy) and by a shallower final slope (Do = 2.47 Gy; Table 1 cf. Table 5). We have observed that Peyer's patches are confined to the antimesenteric aspect of the gut circumference. It is possible therefore, that the resistance of P crypts was caused by their position rather than by the patches *per se*. The comparison of NPA with NPM crypts revealed that sensitivity to radiation was indeed less on the antimesenteric aspect of the circumference (Do = 2.04 Gy; 1.52 Gy for NPM crypts). The value obtained from the entire circumference was 1.68 Gy, so that a (linear?) gradient of sensitivity may exist across an intestinal circumference. Merino et al (1978) and Hume et al (1979) observed asymmetries in the survival of intestinal crypts subjected to heat, with survival least among antimesenteric crypts. This was attributed to inferior rates of cooling in zones

TABLE 5: Parameters of ileal crypt survival in BDF_1 male mice, measured 4 days after treatment by γ-rays or nitrogen mustard, by scoring all crypts around an entire gut circumference.

	Radiation		Nitrogen Mustard
	Single	Split	Single
Data points per curve	29	22	23
Dose range	2 - 17 Gy	1 - 14 Gy (2nd doses)	0.5 - 13.0 mg kg^{-1}
$Do \pm 1SE$			
Independent	1.68 0.09 Gy	1.98 0.27 Gy	1.69 ± 0.07 mg kg^{-1}
Common Fit	1.70 0.09	1.70 0.09	
$LD_{37} \pm 1SE$			
Independent	9.26 0.12 Gy	3.02 0.48 Gy	2.48 0.12 mg kg^{-1}
For Common Do	9.23 0.11	3.45 0.31	
N (single)	228^{+78}_{-58}		$4.34^{+0.43}_{-0.39}$
N^1 (split)		$7.60^{+1.8}_{-1.5}$	
E		$5.55^{+1.30}_{-1.11}$	(Recovery factor) $4.70^{+0.90}_{-0.81}$
A	$41.1^{+17.0}_{-13.2}$		$0.92^{+0.20}_{-0.18}$

TABLE 5: Parameters of ileal crypt survival in BDF_1 male mice, measured 4 days after treatment by γ-rays or nitrogen mustard, by scoring all crypts around an entire gut circumference.

	Radiation		Nitrogen Mustard
	Single	Split	Single
Data points per curve	29	22	23
Dose range	2 - 17 Gy	1 - 14 Gy (2nd doses)	0.5 - 13.0 mg kg^{-1}
$D_o \pm$ 1SE			
Independent	1.68 0.09 Gy	1.98 0.27 Gy	1.69 ± 0.07 mg kg^{-1}
Common Fit	1.70 0.09	1.70 0.09	
$LD_{37} \pm$ 1SE			
Independent	9.26 0.12 Gy	3.02 0.48 Gy	2.48 0.12 mg kg^{-1}
For Common D_o	9.23 0.11	3.45 0.31	
N (single)	228^{+78}_{-58}		$4.34^{+0.43}_{-0.39}$
N^1 (split)		$7.60^{+1.8}_{-1.5}$	
E		$5.55^{+1.30}_{-1.11}$	(Recovery factor) $4.70^{+0.90}_{-0.81}$
A	$41.1^{+17.0}_{-13.2}$		$0.92^{+0.20}_{-0.18}$

remote from major mesenteric vessels, but Merino et al (1978) also noted the possibility that antimesenteric crypts might be more sensitive to heat, because of inadequate oxygenation. Our results for irradiated crypts revealed ratios of Do's of 1.34:1 between NPA and NPM crypts, and 1.65:1 between P and NPM crypts. The exact contribution of oxygen status to these differences could be established by experiments combining radiation with an hypoxic cell sensitiser.

Survival curves for NPA crypts do not have a wider shoulder than those for NPM crypts or from whole circumferences (Table 1, Table 5). The large threshold region on P crypts remains to be explained. There was no evidence for a significantly larger Dq on the cell survival curves obtained by split doses. However, the P crypts might contain a greater average number of clonogenic cells. It is believed that the ratio of N/E, from crypt survival curves, represents this number (A). It is a peculiarity of crypt survival curves that shifts in Do occur, not around an axis at zero dose i.e. N, but instead around a point corresponding very approximately to 9 Gy. The calculation of A is therefore very sensitive to shifts in Do and is usually made on the basis of a common Do for single and split doses. Adopting this assumption, it is clear from Tables 1,2 and 5 that, in practice, the estimate of A tends to vary inversely with the average Do. Therefore, we could not demonstrate by the present experiments that P crypts contained larger numbers of clonogenic cells. It can be noted that if the minimum Do attained in these experiments (1.52 Gy, for NPM crypts) is applied to the calculation of A for P crypts, using an LD_{37} of 10.85 Gy, then the value of A would be approximately 180 per crypt.

It is of interest that our results for NPA and NPM crypts predict a resistant tail on crypt survival curves, occurring at a level of survival that would depend on the relative numbers of sensitive and resistant crypts. In the present experiments, 20 NPA crypts/121 all crypts, = 0.17, had an average Do of 2.04 Gy.

radiation (Table 1, Table 5). The non-patch crypts respond to HN_2 as though they contain only one or very few clonogenic cells, in contrast to radiation (A = 14 to 60; Table 1). The reason for this important discrepancy remains unclear. Either each crypt contains a fixed high number of microcolony-forming cells, constituting a discrete functional compartment, but whose number is not necessarily derivable directly from the N on the survival curve for whole crypts, or the value of N does permit an estimate of A, but the effective target population varies in size for different agents.

4.2. Cellular Kinetics

Altmann (1979) noted that after methotrexate, recovery as measured by mitotic rates and ^{3}H-TdR labelling commenced earlier and was more marked in crypts overlying Peyer's patches, than elsewhere. We have been unable to detect large differences in the kinetic organisation of unperturbed P and NP crypts. The labelling indices and derived growth fraction were somewhat higher in P crypts (Table 4). The high values of GF occurred because measurement was confined to the first 20 cell positions, i.e. within the proliferative zone (Al-Dewachi et al, 1975).

Table 6 shows a comparison of cellular kinetic parameters obtained in this work with those of other investigators. It can be seen that there are no major differences between the values both for the P region and NP region, with those of others. The low cell crypt height given in this paper is due to the method used in establishing the crypt height. Only ^{3}H-TdR labelled interphase cells were scored along the crypt cell column and as these cells move up the crypt in 'blocks', label-free crypt tops were encountered. When the data were pooled and a mean calculated, the label-free crypt tops pulled down the mean value. The results of Smith et al (1980) are of particular interest in that they were obtained from Peyer's patch-associated epithelium. The 2h LI for P crypts in A/JAX mice was 25% compared to a 1h LI of 38% in

the present study. The cell migration rate in the villus of A/JAX mice was 1.18 cell positions/h (read from a graph), somewhat slower than that for BDF_1 mice (1.78; Table 6).

APPENDIX A

1. Tritiated Thymidine - ^{3}H-TdR (518K Bq in 0.3 ml)

(i).	Aqueous tritiated thymidine solution	0.93 ml
(ii).	Cold thymidine solution	0.35 ml
(iii).	Sterile physiological saline solution	18.72 ml

2. Chrome-Gelatin Subbing Medium

(i).	Gelatin powder	5 gm
(ii).	Chrome alum (chromic potassium sulphate)	0.5 gm
(iii).	Distilled water	1 litre

The slides were cleaned in absolute alcohol, dried, dipped in the subbing medium, stood, and allowed to drain and dry on a slide 'rack'.

3. Preparation of Schiff's Reagent

(i). Warm two litres of distilled water. Add carefully (in the sink), 10 gm of basic fuchsin (feulgen grade) or pararosaniline. Bring to the boil, mixing with a magnetic stirrer, and when everything was dissolved, allow to cool.

(ii). Place 200 ml of 1N HCl in a similar flask and when the stain cools to 50^{o}C, filter through a medium/fine filter into the flask. Allow to cool to 25^{o}C.

(iii). Weigh 10 gm of sodium or potassium metabisulphate and pour into the flask. Stopper the flask immediately (and tightly) and shake the mixture periodically. Place in a dark cupboard overnight at room temperature.

(iv). Add 8-10 gm (or less if remaining colour is not strong) of de-colourising charcoal. Filter through a medium filter, then a very fine filter.

(v). Store in an amber bottle at 0-4^{o}C, but use at room temperature.

4. Stock Sucrose Solution

(i).	Sucrose	200 gm
(ii).	Potassium bromide (or 10% solution)	0.1 gm (1.0 ml)
(iii).	Water to make	1 litre

For use, dilute 1 volume with 9 volumes of water and mix thoroughly. Use at room-temperature - preferably between 16^{o}-23^{o}C.

5. Preparation and Staining of Slides

The slides are loaded in stainless steel slide racks and passed through the following solutions:

(i).	Xylene De-wax	10 min
(ii).	Xylene 1	10 min
(iii).	Xylene 2	10 min
(iv).	Absolute ethanol 1	10 min
(v).	Absolute ethanol 2	10 min
(vi).	90% ethanol	5-10 min
(vii).	70% ethanol	rinse through
(viii).	50% ethanol	rinse through
(ix).	30% ethanol	rinse through
(x).	Tap water	5 min
(xi).	Distilled water	2-3 min

The slides are now transferred to glass dishes.

(xii).	5N HCl (cold hydrolysis)	1 hr 10 min
(xiii).	Tap water	good rinse)
(xiv).	Distilled water	2-3 min) wash off acid
(xv).	Schiff's Reagent	1 hr
(xvi).	Sulphurous acid (x2)	5 min each
(xvii).	Tap water (x3)	5 min each
(xviii).	Distilled water (x2)	5 min each

4. Stock Sucrose Solution

(i).	Sucrose	200 gm
(ii).	Potassium bromide (or 10% solution)	0.1 gm (1.0 ml)
(iii).	Water to make	1 litre

For use, dilute 1 volume with 9 volumes of water and mix thoroughly. Use at room-temperature - preferably between 16^{o}-23^{o}C.

5. Preparation and Staining of Slides

The slides are loaded in stainless steel slide racks and passed through the following solutions:

(i).	Xylene De-wax	10 min
(ii).	Xylene 1	10 min
(iii).	Xylene 2	10 min
(iv).	Absolute ethanol 1	10 min
(v).	Absolute ethanol 2	10 min
(vi).	90% ethanol	5-10 min
(vii).	70% ethanol	rinse through
(viii).	50% ethanol	rinse through
(ix).	30% ethanol	rinse through
(x).	Tap water	5 min
(xi).	Distilled water	2-3 min

The slides are now transferred to glass dishes.

(xii).	5N HCl (cold hydrolysis)	1 hr 10 min
(xiii).	Tap water	good rinse)
(xiv).	Distilled water	2-3 min) wash off acid
(xv).	Schiff's Reagent	1 hr
(xvi).	Sulphurous acid (x2)	5 min each
(xvii).	Tap water (x3)	5 min each
(xviii).	Distilled water (x2)	5 min each

Transfer the slides to the dark room. It is important to make sure that slides are kept one way round in the dishes as it is difficult to orientate the slides in the dark room, if they are mixed.

6. Preparation for Exposure: Stripping Film Emulsion - AR.10 Film

(i). Make up to 2 litres 200 ml of stock sucrose solution with clean distilled water and pour in the clean tank.

(ii). Take the AR.10 plates, one at a time, and with a sharp blade cut through the stripping layer around an area sufficient to cover the whole of the sections, with a margin of at least 6 mm wide all round.

(iii). With the tip of the blade, peel off the emulsion layer and place it on the surface of the water, so that the emulsion side is facing downwards.

(iv). It will swell and as it does so it first crumples and then stretches out tightly and flat - leave it for 2 minutes.

(v). Gently slip the slide in underneath the emulsion and raise it out so that the emulsion covers the sections and wraps around the slide. Tap down with a damp brush and make sure the emulsion is wrapped around the underside of the slide.

(vi). Most water will drain off as the slide is gradually lifted clear of the water, but to complete the process drain off on to a filter paper. Dry on the rack for approximately 2 hours.

(vii). Box the slides with silica gel. Tape the slide box with black tape and place it inside a black bag and/or wrapping with tin foil.

(viii). Expose for 6 weeks.

7. Developing of Slides

This is undertaken with the emulsion layer in permanent contact with the sections, so that the final autoradiograph is in perfect register with the sections.

(i). Develop in high contrast Kodak formula D19 neat at 20°C for 5 min.

(ii). Immerse in stop bath at 18^{o}-21^{o}C for not less than 30 secs.

(iii). Fix in Hypam fixer (Ilford Ltd) at 18^{o}-21^{o}C for 5-10 min.

(iv). Wash in running water at 18^{o}-21^{o}C for 15 min and then stand on a rack to drip and dry.

APPENDIX B

A Double Minus Log Transformation of Mortality Probabilities (Gilbert, 1974).

A biological model of the action of radiation on an organism supposes that it contains A sensitive cells and that the fate of the organism depends only on the number of viable cells remaining. This suggests a method of plotting the mortality probability of the organism versus dose so that the characteristics of the sensitive cells may be readily deduced.

We suppose that q, the probability that a sensitive cell survives a dose D, has the asymptotic form:

$$q = E \exp(-D/Do)$$

where E is the extrapolation number and Do the measure of cell resistance. This is the shape of the multi-target curve at high doses, and in this application the doses used are not in the shoulder region. If we also suppose that α is the chance that a single cell will repopulate the organism then the average number of effective cells surviving a dose D is $A\alpha q$ and the mortality probability (Pm) that an organism contains no effective cell and so dies, is given by the first term of the Poisson distribution and is:

$$Pm = \exp(-A\alpha q)$$

$$= \exp(-\exp(-(D/Do - \ln(A\alpha E))))$$

Applying a double minus log transformation this becomes:

$$-\ln(-\ln(Pm)) = D/Do - \ln(A\alpha E)$$

Hence the double minus log transform of the mortality probability will be linear with dose, Do being the inverse slope and $\ln(A\alpha E) = LD_{37}/Do$ being the intercept where LD_{37} is the dose corresponding to Pm = 1/e.

The double minus log function $y = -\ln(-\ln(Pm))$, may be tabulated or it may be read from a simple graph that is constructed by plotting $Pm = \exp(-\exp(-y))$ on probability paper. An objective best fitting line can be fitted to the double minus log transformation of a set of experimental data by a computer programme. This has been achieved by incorporating a few alterations only into a programme for fitting probits (Gilbert, 1969, see overleaf).

The intestinal crypt is a good example of such an 'organism' described in this model, and the crypt microcolony assay (Withers and Elkind, 1970) makes the important assumptions which are in agreement with this model.

(1) one surviving cell within a crypt is sufficient for the regeneration of that crypt - therefore an inactivated crypt represent survival of none, and a surviving crypt the survival of one or more of its constituent stem cells.

(2) cells survive independently of one another.

Due to differences in size of regenerating crypts from unstimulated crypts, the experimental values of surviving fraction of crypts in our experiments include a correction factor for crypt diameter (Table 7).

Examples of computer inputs for the double minus log transformation programme and the outputs showing the calculated survival curves and best fit parameters, for gamma radiation and nitrogen mustard are shown at the end of this appendix.

TABLE 7A: Correction factors for single dose gamma irradiation experiment

	PATCH		NON-PATCH ANTIMESENTERIC		NON-PATCH MESENTERIC	
DOSE (Gy)	CRYPT DIAMETER	CORR. FACTOR	CRYPT DIAMETER	CORR. FACTOR	CRYPT DIAMETER	CORR. FACTOR
0	3.6	-	2.9	-	2.8	-
2	3.8	0.95	3.0	0.97		
4	3.6	1.00	3.0	0.97		
6	3.7	0.97	2.9	1.00		
8	3.8	0.95	3.2	0.91	3.1	0.90
9	3.6	1.00	3.3	0.88		
10	4.0	0.90	3.7	0.78	3.8	0.74
10.5	4.5	0.80	3.6	0.81	3.5	
11	4.7	0.78	3.8	0.76	3.8	0.80
11.5	4.8	0.75	4.2	0.69	4.0	0.70
12	4.1	0.89	4.1	0.71		
13	5.5	0.65	4.8	0.60	4.9	0.57
13.5	5.2	0.69	4.5	0.64	4.5	0.62
14	4.9	0.73	4.3	0.67	4.3	0.65
15	4.5	0.80	3.7	0.78	3.5	0.80
16	4.8	0.75	3.3	0.88	3.4	0.82
17	6.5	0.55	6.1	0.48	5.9	0.47

$$\text{Corrected Surviving Fraction} = \frac{\text{No. of surviving crypt}}{\text{No. of crypts at risk}} \times \text{correction factor.}$$

TABLE 7B: Correction factors for split-dose gamma irradiation experiment.

	PATCH		NON-PATCH ANTIMESENTERIC		NON-PATCH MESENTERIC	
2nd DOSES (Gy)	CRYPT DIAMETER	CORR. FACTOR	CRYPT DIAMETER	CORR. FACTOR	CRYPT DIAMETER	CORR. FACTOR
1	5.6	0.64	6.0	0.48		
2	5.7	0.63	4.6	0.63		
3	5.2	0.69	5.0	0.58		
4	5.5	0.65	5.7	0.51		
5	5.2	0.69	5.1	0.57	5.2	0.54
6	5.4	0.67	5.9	0.49	5.7	0.49
7	5.3	0.68	5.4	0.54		
8	5.0	0.72	6.3	0.46	6.3	0.44
9	6.0	0.60	5.3	0.55	5.2	0.54
10	4.7	0.77	5.3	0.55	5.3	0.53
11	4.6	0.78	6.5	0.45		

TABLE 7C: Correction factors for nitrogen mustard experiment

DOSE ($mgkg^{-1}$)	PATCH		NON-PATCH ANTIMESENTERIC		NON-PATCH MESENTERIC	
	CRYPT DIAMETER	CORR. FACTOR	CRYPT DIAMETER	CORR. FACTOR	CRYPT DIAMETER	CORR. FACTOR
0	3.6	0	2.9	0	2.9	
0.5	3.4	1.06	3.3	0.88	3.4	0
0.75	3.4	1.06	3.5	0.83	3.5	0.85
1.0	3.6	1.00	3.7	0.78	3.8	0.83
1.5	3.5	1.03	3.6	0.81	3.6	0.76
2.0	3.5	1.03	3.4	0.85	3.3	0.81
2.5	3.4	1.06	3.1	0.94	3.1	0.88
3.0	3.5	1.03	3.4	0.85	3.3	0.94
3.5	3.9	0.93	3.2	0.91	3.3	0.88
4.0	3.5	1.03	3.3	0.88	3.4	0.88
4.5	3.5	1.03	3.4	0.85	3.4	0.85
5.0	3.8	0.95	3.5	0.83	3.6	0.85
5.5	3.8	0.95	2.7	1.07	2.7	0.81
6.0	3.7	0.97	2.8	1.04	3.0	1.07
6.5	3.8	0.95	3.0	0.97	2.8	0.97
7.0	3.9	0.93			3.6	1.04
7.5	3.9	0.93			3.4	0.81
8.0	3.6	1.00			3.6	0.85
9.0	3.6	1.00				0.81
10	3.6	1.00				
11	2.9	1.24				
12	3.4	1.06				
12.5	3.1	1.16				
13	3.1	1.16				

137CS CRYPTS JM29:F8,1

	EXPT	DOSE	NUMBER	LIVE	WEIGHT
1	1PATCHSINGLE	0.000E+00	5.920E+02	5.920E+02	0.000E+00
2	2	2.000E+02	1.720E+02	1.440E+02	1.500E+01
3	3	4.000E+02	1.890E+02	1.980E+02	5.000E+00
4	4	6.000E+02	1.400E+02	1.330E+02	4.000E+00
5	5	8.000E+02	1.940E+02	1.840E+02	5.000E+00
6	6	9.000E+02	1.260E+02	1.200E+02	3.000E+00
7	7	1.000E+03	2.990E+02	2.510E+02	2.600E+01
8	8	1.050E+03	3.080E+02	2.000E+02	6.500E+01
9	9	1.100E+03	5.930E+02	3.680E+02	1.390E+02
10	10	1.150E+03	2.690E+02	1.240E+02	1.000E+02
11	11	1.200E+03	3.940E+02	2.090E+02	1.210E+02
12	12	1.300E+03	4.200E+02	1.340E+02	2.160E+0
13	13	1.350E+03	2.880E+02	5.470E+01	1.960E+
14	14	1.400E+03	4.190E+02	8.790E+01	2.730E+
15	15	1.500E+03	3.670E+02	6.240E+01	2.600E
16	16	1.600E+03	1.350E+02	9.410E+00	1.170E
17	17	1.700E+03	7.690E+01	4.610E+00	6.800E
18	18PATCHSPLIT	0.000E+00	5.930E+02	3.680E+02	1.390E+0
19	19	1.000E+02	1.770E+02	8.490E+01	6.200E+01
20	20	2.000E+02	1.770E+02	7.780E+01	6.900E+01
21	21	3.000E+02	1.770E+02	7.780E+01	6.900E+01
22	22	4.000E+02	1.770E+02	7.070E+01	7.600E+01
23	23	5.000E+02	1.770E+02	6.890E+01	7.800E+01
24	24	6.000E+02	1.770E+02	4.420E+01	1.060E+02
25	25	7.000E+02	1.620E+02	3.240E+01	1.080E+02
26	26	8.000E+02	1.620E+02	2.760E+01	1.150E+02
27	27	9.000E+02	1.770E+02	2.120E+01	1.390E+02
28	28	1.000E+03	2.650E+02	1.060E+01	2.450E+02
29	29	1.100E+03	2.060E+02	1.030E+01	1.870E+02
30	30NONPATCHSINGLE	0.000E+00	7.020E+02	7.020E+02	0.000E+00
31	31 ANTIMES-	2.000E+02	2.680E+02	2.470E+02	1.100E+01
32	32 ENTERIC	4.000E+02	3.880E+02	3.570E+02	1.600E+01
33	33	6.000E+02	3.520E+02	2.640E+02	5.000E+01
34	34	8.000E+02	4.130E+02	2.770E+02	8.200E+01
35	35	9.000E+02	4.380E+02	2.420E+02	1.610E+02
36	36	1.000E+03	6.050E+02	3.030E+02	2.020E+02
37	37	1.050E+03	4.410E+02	1.760E+02	1.890E+02
38	38	1.100E+03	1.036E+03	3.340E+02	5.320E+02
39	39	1.200E+03	6.990E+02	1.750E+02	4.200E+02
40	40	1.300E+03	7.500E+02	3.750E+01	6.780E+02
41	41	1.350E+03	4.180E+02	2.000E+01	3.790E+02
42	42	1.400E+03	7.820E+02	2.350E+01	7.360E+02
43	43	1.500E+03	6.730E+02	1.680E+01	6.400E+02
44	44	1.700E+03	1.860E+02	3.340E+00	1.790E+02
45	45NONPATCHSPLIT	0.000E+00	1.036E+03	3.340E+02	5.320E+02
46	46 ANTIMES-	1.000E+02	1.390E+02	3.330E+01	8.500E+01
47	47 ENTERIC	2.000E+02	1.280E+02	3.590E+01	7.200E+01
48	48	3.000E+02	1.280E+02	2.440E+01	8.200E+01
49	49	4.000E+02	1.280E+02	2.180E+01	9.100E+01
50	50	5.000E+02	1.280E+02	1.920E+01	9.500E+01
51	51	6.000E+02	1.280E+02	8.970E+00	1.110E+02
52	52	7.000E+02	1.390E+02	6.940E+00	1.260E+02
53	53	8.000E+02	1.710E+02	6.840E+00	1.580E+02
54	54	9.000E+02	1.820E+02	5.450E+00	1.710E+02
55	55	1.000E+03	1.280E+02	1.280E+00	1.260E+02
56	56	1.100E+03	1.390E+02	1.390E+00	1.360E+02
57	57NONPATCHMESE	8.000E+02	5.150E+02	3.760E+02	8.030E+01
58	58 -ENTERIC	1.000E+03	5.150E+02	2.930E+02	1.720E+02
59	59 SINGLE	1.100E+03	4.680E+02	1.780E+02	2.100E+02
60	60	1.300E+03	4.910E+02	5.410E+01	3.940E+02
61	61	1.400E+03	6.790E+02	1.700E+01	6.450E+02
62	62	1.500E+03	5.150E+02	6.690E+00	5.020E+02
63	63	1.600E+03	6.320E+02	3.160E+00	6.260E+02
64	64	1.700E+03	9.830E+02	9.830E-01	9.810E+02
65	65NONPATCHMES-	0.000E+00	1.036E+03	3.340E+02	5.320E+02
66	66 ENTERICSPLIT	5.000E+02	4.910E+02	5.410E+01	3.940E+02
67	67	6.000E+02	5.380E+02	1.610E+01	5.070E+02
68	68	8.000E+02	5.150E+02	8.240E+00	4.990E+02
69	69	1.000E+03	4.680E+02	1.400E+00	4.650E+02

```
   1    17   1.00E+03   2.40E+02  -1.00E+00

 1  4.62E+02
 1.00E+03  2.40E+02
 2  4.33E+02
 1.09E+03  2.22E+02
 3  4.33E+02
 1.08E+03  2.18E+02
 4  4.33E+02
 1.08E+03  2.18E+02
SURVIVAL DOUBLE LOG

LD37          1.085E+03    +_     7.742E+01
1/SLOPE       2.182E+02    +_     7.425E+01
NAT SURVIV    1.000E+00    +_     0.000E+00

EXPT                    DOSE          SURVIVAL     CAL SURV     ERROR
1PATCHSINGLE             0.000E+00    1.000E+00    1.000E+00    0.000E+00
2                        2.000E+02    8.372E-01    1.000E+00   -1.994E+01
3                        4.000E+02    1.048E+00    1.000E+00    3.367E+00
4                        6.000E+02    9.500E-01    9.999E-01   -3.156E+00
5                        8.000E+02    9.485E-01    9.749E-01   -3.779E-01
6                        9.000E+02    9.524E-01    9.027E-01    2.903E-01
7                        1.000E+03    8.395E-01    7.709E-01    8.323E-01
8                        1.050E+03    6.494E-01    6.902E-01   -7.114E-01
9                        1.100E+03    6.206E-01    6.061E-01    3.485E-01
10                       1.150E+03    4.610E-01    5.233E-01   -1.249E+00
11                       1.200E+03    5.305E-01    4.452E-01    1.886E+00
12                       1.300E+03    3.190E-01    3.110E-01    2.540E-01
13                       1.350E+03    1.899E-01    2.564E-01   -2.132E+00
14                       1.400E+03    2.098E-01    2.099E-01   -4.957E-03
15                       1.500E+03    1.700E-01    1.384E-01    1.476E+00
16                       1.600E+03    6.970E-02    8.991E-02   -7.641E-01
17                       1.700E+03    5.995E-02    5.784E-02    7.459E-02



Covariance matrix
 5.993E+03
-4.090E+03   5.514E+03
```

30 44 9.00E+02 1.60E+02 -1.00E+00

1 2.38E+03
9.00E+02 1.60E+02
2 2.83E+02
8.18E+02 2.23E+02
3 3.46E+02
8.50E+02 2.04E+02
4 3.50E+02
8.52E+02 2.02E+02
SURVIVAL DOUBLE LOG

LD37	8.516E+02	+_	6.458E+01
1/SLOPE	2.021E+02	+_	4.014E+01
NAT SURVIV	1.000E+00	+_	0.000E+00

EXPT	DOSE	SURVIVAL	CAL SURV	ERROR
30NONPATCHSINGLE	0.000E+00	1.000E+00	1.000E+00	0.000E+00
31 ANTIMES-	2.000E+02	9.216E-01	1.000E+00	-8.218E+00
32 ENTERIC	4.000E+02	9.201E-01	9.999E-01	-1.010E+01
33	6.000E+02	7.500E-01	9.689E-01	-8.925E+00
34	8.000E+02	6.707E-01	7.249E-01	-1.099E+00
35	9.000E+02	5.525E-01	5.447E-01	1.981E-01
36	1.000E+03	5.008E-01	3.811E-01	3.505E+00
37	1.050E+03	3.991E-01	3.124E-01	2.570E+00
38	1.100E+03	3.224E-01	2.536E-01	3.647E+00
39	1.200E+03	2.504E-01	1.633E-01	4.824E+00
40	1.300E+03	5.000E-02	1.030E-01	-4.542E+00
41	1.350E+03	4.785E-02	8.140E-02	-2.389E+00
42	1.400E+03	3.005E-02	6.414E-02	-3.[illegible]5E+00
43	1.500E+03	2.496E-02	3.961E-02	-1.[illegible]0E+00
44	1.700E+03	1.796E-02	1.491E-02	3.361E-01

Covariance matrix
4.170E+03
-2.008E+03 1.612E+03

64 1.12E+03 1.60E+02 1.00E+00

1 6.07E+03
1.12E+03 1.60E+02
2 1.08E+02
8.80E+02 2.23E+02
3 5.73E+01
1.27E+02 1.52E+02
4 5.38E+01
9.16E+02 1.54E+02
5 5.45E+01
9.18E+02 1.53E+02
SURVIVAL DOUBLE LOG

LD37	9.178E+02	+_	3.765E+01
1/SLOPE	1.531E+02	+_	1.872E+01
NAT SURVIV	1.000E+00	+_	0.000E+00

EXPT	DOSE	SURVIVAL	CAL SURV	ERROR
57NONPATCHMESE	8.000E+02	7.[illegible]1E-01	8.846E-01	-4.334E+00
58 -ENTERIC	1.000E+03	5.010E-01	4.427E-01	1.538E+00
59 SINGLE	1.100E+03	3.803E-01	2.623E-01	3.889E+00
60	1.300E+03	1.102E-01	7.907E-02	2.289E+00
61	1.400E+03	2.504E-02	4.195E-02	-2.143E+00
62	1.500E+03	1.299E-02	2.205E-02	-1.383E+00
63	1.600E+03	5.000E-03	1.154E-02	-1.531E+00
64	1.700E+03	1.000E-03	6.020E-03	-2.032E+00

Covariance matrix
1.418E+03
4.545E+02 3.505E+02

18 29 4.00E+02 2.80E+02 -6.30E-01

1 1.48E+01
4.00E+02 2.80E+02
2 1.36E+01
3.79E+02 3.00E+02
3 1.35E+01
3.74E+02 3.04E+02
4 1.35E+01
3.7[illegible]E+02 3.[illegible]E+02
SURVIVAL DOUBLE LOG

LD37	3.734E+02	+_	3.707E+01
1/SLOPE	3.045E+02	+_	2.976E+01
NAT SURVIV	6.300E-01	+_	0.000E+00

EXPT	DOSE	SURVIVAL	CAL SURV	ERROR
18PATCHSPLIT	0.000E+00	6.206E-01	6.091E-01	2.763E-01
19	1.000E+02	4.797E-01	5.758E-01	-1.533E+00
20	2.000E+02	4.395E-01	5.224E-01	-1.377E+00
21	3.000E+02	4.395E-01	4.535E-01	-2.329E-01
22	4.000E+02	3.994E-01	3.780E-01	3.854E-01
23	5.000E+02	3.893E-01	3.043E-01	1.631E+00
24	6.000E+02	2.497E-01	2.383E-01	2.769E-01
25	7.000E+02	2.000E-01	1.835E-01	4.698E-01
26	8.000E+02	1.704E-01	1.376E-01	1.021E+00
27	9.000E+02	1.198E-01	1.024E-01	6.752E-01
28	1.000E+03	4.000E-02	7.555E-02	-2.106E+00
29	1.100E+03	5.000E-02	5.537E-02	-3.209E-01

Covariance matrix
1.374E+03
-7.183E+02 8.857E+02

45 56 3.00E+02 2.20E+02 -3.22E-01

1 5.12E+0
3.00E+02 2.20E+02
2 3.67E+00
3.15E+02 2.34E+02
3 3.67E+00
3.17E+02 2.32E+02
4 3.67E+00
3.17E+02 2.32E+02
SURVIVAL DOUBLE LOG

LD37	3.167E+02	+_	2.473E+01
1/SLOPE	2.324E+02	+_	1.686E+01
NAT SURVIV	3.220E-01	+_	0.000E+00

EXPT	DOSE	SURVIVAL	CAL SURV	ERROR
45NONPATCHSPLIT	0.000E+00	3.224E-01	3.155E-01	3.410E-01
46 ANTIMES-	1.000E+02	2.396E-01	2.966E-01	-1.151E+00
47 ENTERIC	2.000E+02	2.805E-01	2.603E-01	3.903E-01
48	3.000E+02	1.906E-01	2.[illegible]0E-01	-4.743E-01
49	4.000E+02	1.703E-01	1.619E-01	2.181E-01
50	5.000E+02	1.500E-01	1.176E-01	9.809E-01
51	6.000E+02	7.008E-[illegible]	8.238E-02	-4.715E-01
52	7.000E+02	4.993E-02	5.630E-02	-3.102E-01
53	8.000E+02	4.000E-02	3.783E-02	1.430E-01
54	9.000E+02	2.995E-02	2.514E-02	4.017E-01
55	1.000E+03	1.000E-02	1.[illegible]E-02	-5.783E-01
56	1.100E+03	1.000E-02	1.088E-02	-9.899E-02

Covariance matrix
6.115E+02
-2.731E+02 2.843E+02

65 69 3.80E+02 1.65E+02 -3.22E-01

1 1.80E+01
3.80E+02 1.65E+02
2 6.11E+00
3.43E+02 1.44E+02
3 5.97E+00
3.61E+02 1.31E+02
4 5.98E+00
3.60E+02 1.31E+02
SURVIVAL DOUBLE LOG

LD37	3.603E+02	+_	5.526E+01
1/SLOPE	1.310E+02	+_	3.003E+01
NAT SURVIV	3.220E-01	+_	0.000E+00

EXPT	DOSE	SURVIVAL	CAL SURV	ERROR
65NONPATCHMES-	0.000E+00	3.224E-01	3.220E-01	1.944E-02
S ENTERICSPLIT	5.000E+02	1.102E-01	9.37[illegible]E-02	1.120E+00
67	6.000E+02	2.993E-02	4.7[illegible]E-02	-1.878E+00
68	8.000E+02	1.600E-02	1.102E-02	1.066E+00
69	1.000E+03	2.991E-03	2.426E-03	2.481E-01

Covariance matrix
3.[illegible]54E+03
-1.487E+03 9.017E+02

```
  1   24   6.00E+00   1.80E+00  -1.00E+00

 1  1.06E+03
 6.00E+00  1.80E+00
 2  9.18E+01
 4.80E+00  2.48E+00
 3  7.67E+01
 4.64E+00  2.49E+00
 4  8.05E+01
 4.67E+00  2.47E+00
SURVIVAL DOUBLE LOG

LD37          4.666E+00   +_    3.293E-01
1/SLOPE       2.467E+00   +_    2.278E-01
NAT SURVIV    1.000E+00   +_    0.000E+00

EXPT                DOSE         SURVIVAL     CAL SURV     ERROR
1 PATCH             0.000E+00    1.000E+00    9.987E-01    0.000E+00
2 SINGLE            5.000E-01    9.048E-01    9.955E-01   -4.593E+00
3                   7.500E-01    9.307E-01    9.925E-01   -2.003E+00
4                   1.000E+00    8.961E-01    9.879E-01   -2.950E+00
5                   1.500E+00    8.918E-01    9.72[illegible]E-01   -1.793E+00
6                   2.000E+00    8.788E-01    9.474E-01   -1.179E+00
7                   2.500E+00    9.091E-01    9.098E-01   -7.701E-03
8                   3.000E+00    8.182E-01    8.597E-01   -5.699E-01
9                   3.500E+00    7.100E-01    7.989E-01   -1.378E+00
10                  4.000E+00    6.600E-01    7.301E-01    8.686E-01
11                  4.500E+00    8.745E-01    6.568E-01    1.799E+00
12                  5.000E+00    8.052E-01    5.824E-01    2.254E+00
13 PATCH            5.500E+00    5.368E-01    5.099E-01    4.491E-01
14 SINGLE           6.000E+00    7.135E-01    4.414E-01    3.115E+00
15                  6.500E+00    4.848E-01    3.784E-01    1.961E+00
16                  7.000E+00    4.070E-01    3.218E-01    1.273E+00
17                  7.500E+00    2.227E-01    2.717E-01   -8.613E-01
18                  8.000E+00    3.065E-01    2.281E-01    2.064E+00
19                  8.000E+00    1.294E-01    1.585E-01   -2.019E+00
20                  1.000E+01    8.626E-02    1.087E-01   -7.111E-01
21                  1.100E+01    2.304E-02    7.388E-02   -2.038E+00
22                  1.200E+01    3.693E-02    4.989E-02   -8.709E-01
23                  1.250E+01    1.701E-02    4.093E-02   -1.724E+00
24                  1.300E+01    2.295E-02    3.355E-02   -1.009E+00

Covariance matrix
 [illegible]084E-01
-4.182E-02   5.189E-02
```

```
 13   24   6.10E+00   1.81E+00  -1.00E+
```

```
 1  3.31E+01
6.10E+00  1.81E+00
 2  1.59E+01
5.57E+00  1.89E+00
 3  1.59E+01
5.55E+00  1.87E+00
 4  1.59E+01
5.56E+00  1.87E+00
SURVIVAL DOUBLE LOG
```

```
LD37          5.555E+00   +_    2.332E-01
1/SLOPE       1.872E+00   +_    1.518E-01
NAT SURVIV    1.000E+00   +_    0.000E+00
```

EXPT	DOSE	SURVIVAL	CAL SURV	ERROR
13 PATCH	5.500E+00	5.368E-01	6.430E-01	-1.848E+00
14 SINGLE	6.000E+00	7.135E-01	5.455E-01	1.918E+00
15	6.500E+00	4.848E-01	4.533E-01	5.667E-01
16	7.000E+00	4.070E-01	3.702E-01	5.317E-01
17	7.500E+00	2.227E-01	2.981E-01	-1.289E+00
18	8.000E+00	3.065E-01	2.374E-01	1.794E+00
19	9.000E+00	1.294E-01	1.459E-01	-6.316E-01
20	1.000E+01	8.626E-02	8.893E-02	-9.247E-02
21	1.100E+01	2.304E-02	5.314E-02	-1.407E+00
22	1.200E+01	3.693E-02	3.150E-02	4.544E-01
23	1.250E+01	1.701E-02	2.421E-02	-6.686E-01
24	1.300E+01	2.295E-02	1.859E-02	5.542E-01

```
Covariance matrix
 5.440E-02
-2.548E-02   2.306E-02
```

```
 40   56   1.00E+00   1.70E+00  -1.00E+00

 1  2.06E+02
1.00E+00  1.70E+00
 2  1.91E+02
4.27E-01  1.87E+00
 3  1.93E+02
3.36E-01  1.89E+00
 4  1.93E+02
3.33E-01  1.88E+00
SURVIVAL DOUBLE LOG

LD37          3.327E-01   +_    5.306E-01
1 SLOPE       1.891E+00   +_    3.163E-01
NAT SURVIV    1.000E+00   +_    0.000E+00

EXPT                  DOSE         SURVIVAL     CAL SURV     ERROR
40 NONPATCH           5.000E-01    6.619E-01    5.996E-01    9.609E-01
41 MESENTERIC         7.500E-01    6.157E-01    5.515E-01    1.053E+00
42                    1.000E+00    5.053E-01    5.047E-01    1.196E-02
43                    1.500E+00    3.498E-01    4.169E-01   -1.580E+00
44                    2.000E+00    4.733E-01    3.390E-01    2.851E+00
45                    2.500E+00    4.093E-01    2.723E-01    3.343E+00
46                    3.000E+00    4.093E-02    2.165E-01   -6.860E+00
47                    3.500E+00    3.199E-02    1.708E-01   -5.981E+00
48                    4.000E+00    3.349E-01    1.339E-01    6.983E+00
49                    4.500E+00    1.690E-01    1.045E-01    2.985E+00
50                    5.000E+00    1.470E-01    8.121E-02    3.480E+00
51                    5.500E+00    1.100E-01    6.295E-02    3.091E+00
52                    6.000E+00    3.986E-02    4.869E-02   -6.601E-01
53                    6.500E+00    1.498E-02    3.759E-02   -1.964E+00
54                    7.000E+00    1.199E-02    2.898E-02   -1.676E+00
55                    7.500E+00    1.799E-02    2.232E-02   -5.747E-01
56                    8.000E+00    1.801E-02    1.718E-02    1.170E-01


Covariance matrix
 2.815E-01
-1.340E-01   1.001E-01
```

APPENDIX C

FRACTION OF LABELLED MITOSES

Input for a computer program that fits curves to FLM data and calculates the values of the best-fitting parameters of the cell cycle (Tc, T_{G1}, Ts, T_{G2}), together with their standard errors (Gilbert, 1972).

1. CRYPTS OVERLYING PEYER'S PATCHES

```
JOB CW.GJM1,:MDELS,CP19
((((
INPUT CW.GDATA,1////

'TRUE'   PATCH GR.TOTAL 8.9.81
16    1    6    19    20    21          <<<<  Initial estimates of Tc, TG2 and
18    .098    1.2    .039                    Ts + TG2, and their coefficients
30    1                                      of variation.

0
.25    .73    .88    .93    .83        <<<<  FLM points of 1h intervals, from
.62    .34    .19    .05    .02              a smooth curve fitted by eye to
.03    .04    .15    .29    .31              the experimental data.
.35    .36    .37    .35    .34
.31    .29    .26    .25    .23
.21    .19    .18    .16    .14

.50    .0024    1.2    .039
32    1

1    .25
2    .73                                <<<<  Experimental FLM data points for
3    .65                                      various intervals between 1 and 30h.
4    .93
5    .79
6    .62
7    .29
8    .21
9    .04
10    .02
12    .04
14    .28
16    .34
18    .36
20    .33
22    .21
24    .29
26    .17
28    .20
30    .14

50    0

'FALSE'

////
))))
CW.GALG4 CW.GDATA,AFLM,,JOBCW.GJM1,1100,F2000
****
DG
```

FRACTION OF LABELLED MITOSES

2. CRYPTS OVERLYING NON-PATCH AREAS

```
JOB CWGJM2,:MDHLS,CH19
((((
INPUT CWGDATA,1////

'TRUE'    NONPATCHGR.10115981

15    1    7    19    20    21
18    .084    1.2    .042
30    1

0
.41    .70    .90    .95    .90
.77    .57    .35    .16    .07
.06    .08    .17    .29    .40
.48    .52    .54    .52    .49
.46    .42    .38    .34    .31
.30    .30    .30    .30    .30

50    .0021    1.2    .042
32    1

1    .41
2    .83
3    .74
4    .94
5    .90
6    .75
7    .56
8    .56
9    .16
10    .13
12    .07
14    .44
16    .42
18    .60
20    .49
22    .43
24    .34
26    .29
28    .30
30    .28

50    0

'FALSE'

////
))))
CWGALG4 CWGDATA,AFLM,,JOBCWGJM2,T100,P2000
****
DG
```

REFERENCES

ABE, K. and ITO, T. (1977)
A qualitative and quantitative morphologic study of Peyer's patches of the mouse.
Arch. Histol. Jap., 40: 407-420.

AL-DEWACHI, H.S., WRIGHT, N.A., APPLETON, D.R. and WATSON, A.J. (1975)
Cell population kinetics in the mouse jejunal crypt.
Virchows Arch. B Cell Path., 18: 225-242.

ALTMANN, G.G. (1979)
Growth regulating factors in intestinal mucosa during recovery from methotrexate and radiation.
Anat. Rec., 193: 469 (Meeting Abstract).

CHENG, H. and LEBLOND, C.P. (1974)
Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine.
The American J. Anatomy, 141: 461-480.

CHIN, K.N. and HUDSON, G. (1971)
Ultrastructure of Peyer's patches in the normal mouse.
Acta Anat., 78: 306-318.

GILBERT, C.W. (1969)
Computer programmes for fitting Puck and probit survival curves.
Int. J. Radiat. Biol., 16: 323-332.

GILBERT, C.W. (1972)
The labelled mitoses curve and the estimation of the parameters of the cell cycle.
Cell Tissue Kinet., 5: 53-63.

GILBERT, C.W. (1974)
A double minus log transformation of mortality probabilities.
Int. J. Radiat. Biol., 25: 633-634.

HAGEMANN, R.F., SIGDESTRAD, C.P. and LESHER, S. (1971)
Intestinal crypt survival and total and per crypt levels of proliferative cellularity following irradiation: Single X-ray exposures.
Radiation Research, 46: 533-546.

HAGEMANN, R.F. and STRAGAND, J.J. (1977)
Fasting and refeeding: Cell kinetic response of jejunum, ileum and colon.
Cell Tissue Kinet., 10: 3-14.

HENDRY, J.H. and POTTEN, C.S. (1974)
Cryptogenic cells and proliferative cells in intestinal epithelium.
Int. J. Radiat. Biol., 25: 583-588.

HUME, S.P., ROBINSON, J.E. and HAND, J.H. (1979)
The influence of blood flow on temperature distribution in the exteriorized mouse intestine during treatment by hyperthermia.
Br. J. Radiat., 52: 219-222.

KOVACS, L. and POTTEN, C.S. (1973)
An estimation of proliferative population size in stomach, jejunum and colon of DBA-2 mice.
Cell Tissue Kinet., 6: 125-134.

MASUDA, V., WITHERS, H.R., MASON, K.A. and CHEN, K.Y. (1977)
Single dose-response curves of murine gastrointestinal crypt stem cells.
Radiation Research, 69: 65-75.

MERINO, O.R., PETERS, L.J., MASON, K.A. and WITHERS, H.R. (1978)
Effect of hyperthermia on the radiation response of the mouse jejunum.
Int. J. Radiation Oncology Biol. Phys., 4: 407-414.

MOORE, J.V. (1979)
Ablation of murine jejunal crypts by alkylating agents.
Br. J. Cancer, 39: 175-181.

MOORE, J.V. and BROADBENT, D.A. (1980)
Survival of intestinal crypts after treatment by adriamycin alone or with radiation.
Br. J. Cancer, 42: 692-696.

NADKARNI, M.V., TRAMS, E.G. and SMITH, P.K. (1956)
Observations on the rapid disappearance of radioactivity from blood after intravenous triethylenemelamine-C^{14}.
Proc. Am. Assoc. Cancer Res., 2, 136 (Abstract).

POTTEN, C.S. and HENDRY, J.H. (1975).
Differential regeneration of intestinal proliferative cells and cryptogenic cells after irradiation.
Int. J. Radiat. Biol., 27: 413-424.

QUASTLER, H. and SHERMAN, F.G. (1959)
Cell population kinetics in the intestinal epithelium of the mouse.
Exp. Cell Res., 17: 420-438.

COTE
RS
L
ET
14 7HY
496

SAWICKI, W. and ROWINSKI, J. (1980)
Spatial distribution of proliferating and specialised cells and cell cycle analysis of the Guinea pig colonic crypts. In; "Cell Proliferation in the Gastrointestinal Tract". Eds. Appleton, D.R., Sunter, J.P. and Watson, A.J. Pitmann Medical, Tunbridge Wells, Kent pp.27-39.

SMITH, M.W., JARVIS, L.G. and KING, I.S. (1980)
Cell proliferation in follicle-associated epithelium of mouse Peyer's patch.
Am. J. Anat., 159: 157-166.

TEN CATE, C.L. (1969)
Casings with 'patches of Peyer'. ΠΕΡΙ ΑΔΕΝΩΝ :- From sausage to Hippokrates.
Janus, 56: 22-45.

TUOHIMAA, P., SEGAL, S.J. and KOIDS, S.S. (1973)
Routes of administration and uptake of tritiated nucleotides.
Histochemie, 34: 39-47.

WITHERS, H.R. and ELKIND, M.M. (1970)
Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation.
Internation. J. Radiation Biology, 17: 261-267.

WRIGHT, N.A. (1980)
Cell proliferation in the normal gastrointestinal tract. Implications for proliferative response.
In: "Cell Proliferation in the Gastrointestinal Tract". Eds. Appleton, D.R., Sunter, J.P. and Watson, A.J. Pitmann Medical, Tunbridge Wells, Kent, pp.3-21.

MED.THS ✓
QH 543
.5
.M386