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ADVERSE EFFECTS OF POLYETHYLENE GLYCOL AND PHENYTOIN ON THE RECTAL MUCOSA OF THE RAT

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ABSTRACT

The effects of polyethylene glycol (used as suppository base), a surfactant (Polyoxyl 59 stearate - Myrj 59®) and phenytoin on the rectal mucosa of the rat were investigated. Four different types of effects were observed in the 52 rats treated with the various polyethylene glycol, surfactant and phenytoin combinations. Polyethylene glycol had a definite necrotic effect on the rectal mucosa of the rat. This adverse effect was more pronounced when phenytoin and polyoxyl 59 stearate were combined with the polyethylene glycol.

INTRODUCTION

Rectal drug delivery is used to overcome problems of first-pass metabolism, gastrointestinal irritation or patient compliance that may be associated with oral administration of drugs[1]. With rectal administration, the suppository exposes a small region of the rectal epithelium to undiluted excipients and drugs. The area exposed in the rectum and the duration of the exposure is determined by the liquefaction and spreading characteristics of the suppository base. The drug and

the suppository base will determine the nature and extent of reactions in the rectal mucosa. When the damage is extensive, the rectal mucosa has an inflammatory response which can change the absorption barrier[2]. It has been demonstrated that there is a very poor **in vitro/in vivo** correlation with suppositories as far as bioavailability is concerned. A factor that may be responsible for this is the possible interaction of the base and the base/drug complex with the rectal mucosa[1]. This study was directed to determine the influence of the suppository base alone and with phenytoin as well as in combination with a surfactant on the rectal mucosa of the rat. Since a similar study[3] indicated that phenytoin did not cause any epithelium irritation ("a very minimal oedema of the **lamina propria** of the rectal epithelium") in dogs, after high doses of phenytoin in solution, it was considered unnecessary to study the influence of phenytoin on the rectal epithelium of rats alone.

MATERIALS AND METHODS

Materials

Phenytoin raw material was provided by Warner Lambert (Cape Town, South Africa). The three PEG suppository bases, PEG 1000, 1500 and 4000 were obtained from BDH (Poole, United Kingdom), and the Myrj 59® was provided by ICI (Johannesburg, South Africa).

Preparation of Suppositories

Suppositories (200mg) with a length of 10mm and a diameter of 5mm were prepared by melting the base over a water bath and dispersing the drug into the molten mass. The homogenized molten base was then poured into a stainless steel mould used for nasal bougies.

Experimental Procedure

The trial protocol was approved by the internal review board of the university before commencement of the experiment.

Fifty-two Sprague Dawley rats were randomly divided into 4 groups, each group receiving a different treatment.

The four treatments where:

A = Control (no suppository was inserted)

B = PEG (1000:1500:4000 (1:2:1))

C = PEG (1000:1500:4000 (1:2:1)) + phenytoin(50mg)
D = PEG (1000:1500:4000 (1:2:1)) + phenytoin : Myrj 59(50mg:50mg)

The rats were fasted 24 hours before the trial day while water was allowed **ad libitum**. The animals were anesthetized with halothane (fluothane®) and kept under sedation with a ventilation bag filled with air and halothane (2%). A suppository was inserted, and the rectum was closed off with a metal clamp to prevent leakage. In each treatment group 4 rats were sacrificed at either 1, 2, 4 or 8 hours after administration of a treatment, except with the control group only four rats were used at 2 hours. The colons were removed and fixed with Serra's solution (a mixture of ethanol, formalin (10%) and glacial acetic acid in the ratio 6:3:1), for one hour, and preserved in 90% ethanol until evaluation. Each colon was evaluated individually for effects on the mucosa. The histological parameters used for the evaluation were as follows:

1 Oedema of the segment,
2. neutrophil infiltration in the segment,
3. desquamation of the epithelium, and
4. necrosis of the epithelium.

The histological effects of the different treatments on the mucosa were classified according to the intensity of the effect. Each parameter was rated, with a value of 0 to 4, according to the degree and extent of the reaction observed, if no reaction was observed it was indicated by NR (no reaction) (see table 1.).

Statistical Analysis

One way analysis of variance followed by Tukey's studentized range tests were used to determine any statistical significant differences between the observed reactions of treatments B, C and D at different times. The calculations were done with a SAS[4] GLM procedure.

RESULTS

The results of the rated effects of the 4 treatments on the mucosa of the rats are given in table 2.

Table 1

Example how results were obtained with treatment D after one hour.

	Type of effect				
Rat no	O	N.I.	D	N	Total
1	1	NR	NR	NR	1
2	3	1	3	2	9
3	2	1	2	1	6
4	1	NR	1	NR	2
Total	7	2	6	3	18*

Where:

O = Oedema
N I = Neutrophil Infiltration
D = Desquamation of the epithelium
N = Necrosis of the epithelium
NR = No Reaction
* = See table 2

Micrographs of the rectal mucosa of the rat before and after the administration of a suppository illustrates the typical reactions observed (see figures 1 - 5).

DISCUSSION

The results showed no epithelium changes with treatment A (control, no suppository).

Table 2

Cumulative, rated values of all the parameters, in the rats after administration of the four treatments at 1, 2, 4 and 8 hours.

	Time (h)				
Treatment	1	2	4	8	Total
A	0	0	0	0	0
B	4	20	11	13	48
C	5	21	11	45	82
D	18*	21	28	24	91

* See table one

Cumulative:

Judging by the cumulative effect the biggest reaction was found with treatment D (base + phenytoin + surfactant). The onset of the changes with D occurred earlier in comparison with treatments B and C. This can possibly be explained by the presence of the surfactant (Myrj 59®) which increased the dissolution rate of phenytoin and the contact with the epithelium of the rectum after necrosis from the PEG.

Statistical Analysis:

The results obtained with the statistical analysis indicate a statistical difference in the effects between treatment B and C after 8 hours, but no statistical differences between the treatments after 1,2 and 4 hours (see table 3 and Figure 6). Thus a necrotic effect was obtained with the base(B) which was more pronounced with the combination of phenytoin (C) after 8 hours. The addition of a surfactant (D) increased the necrotic effect (cumulative) but no

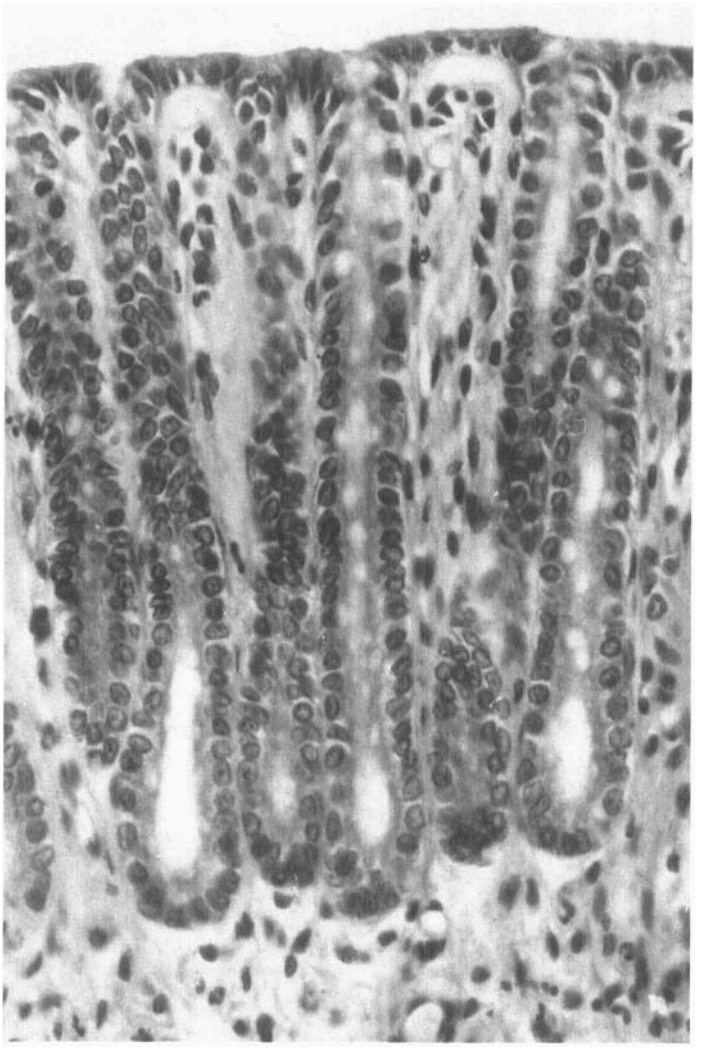

FIGURE 1

Normal rectal mucosa of the rat (x 700).

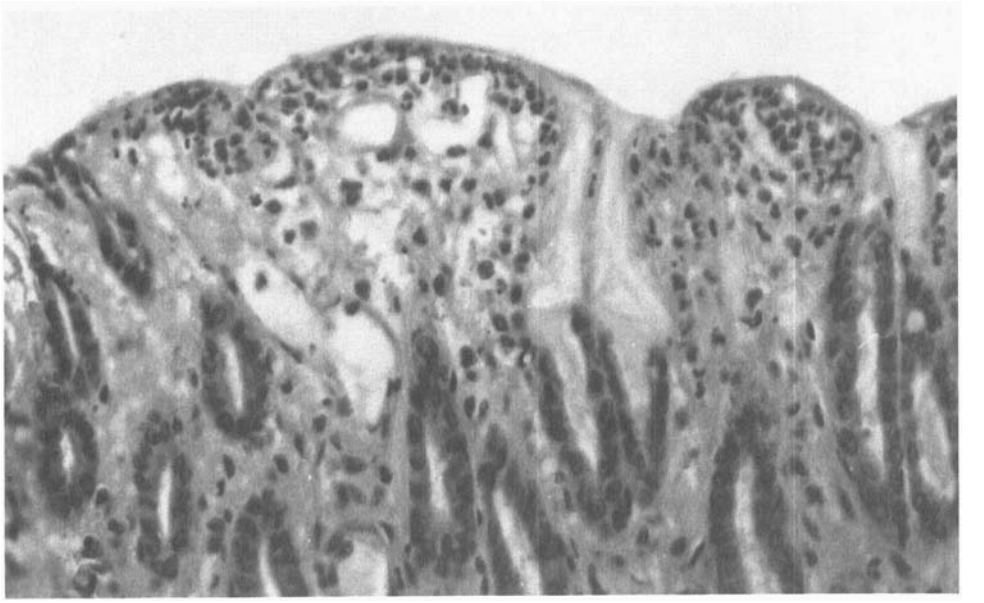

FIGURE 2

Necrosis of the **lamina propria** and an inflammatory reaction. This is characterized by oedema and neutrophil infiltration of the mucus membranes (x 700).

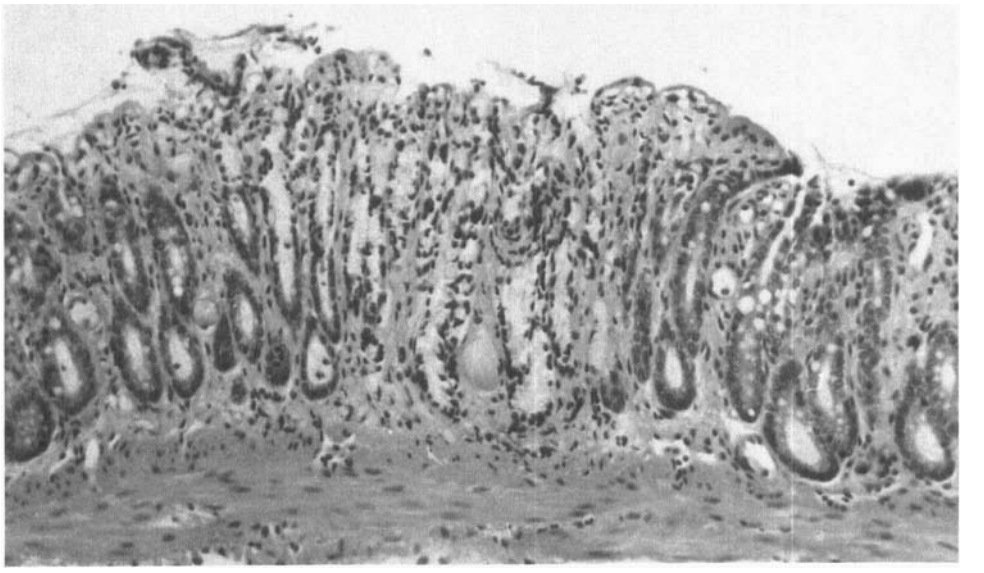

FIGURE 3

Desquamation of the epithelium and focal necrosis of the mucus membrane down to the **muscularis mucosa** (x 350).

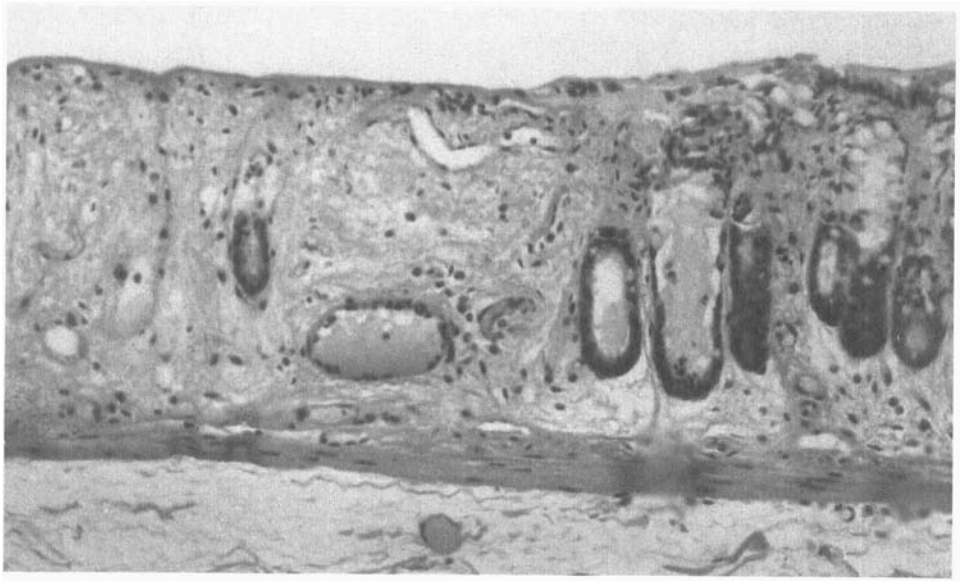

FIGURE 4

Segment of the rectal mucosa with a clear distinction between necrotic and non necrotic mucosa (x 350).

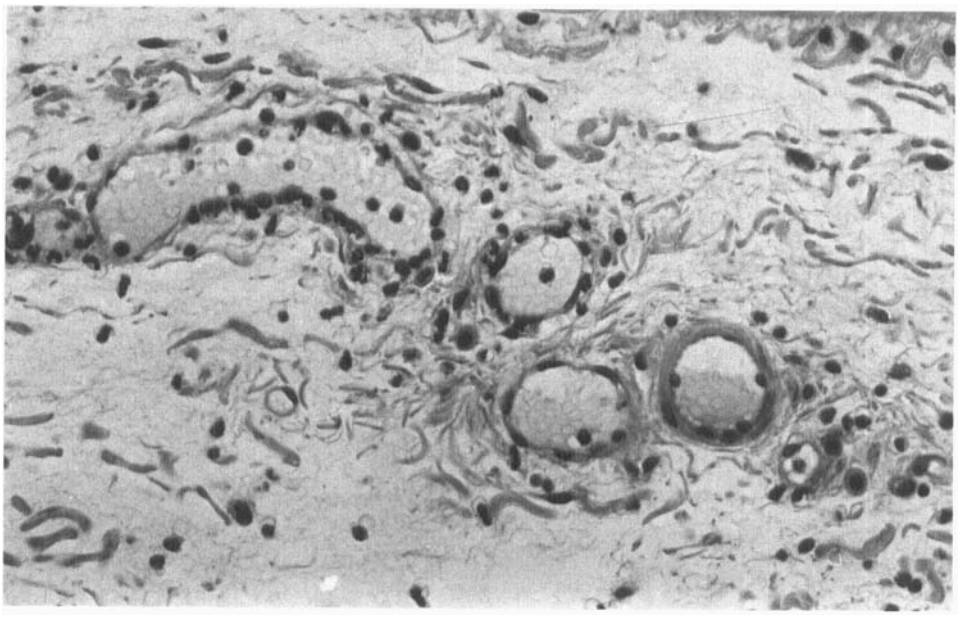

FIGURE 5

Rectal **submucosa** of the rat. Acute inflammatory reaction characterized by oedema and neutrophil infiltration especially in the blood vessels (x 700).

Table 3

P values of one way analysis of variance followed by Tukey's studentized range tests indicating significant differences between treatments at different times.

Time (h)	P value	Sig Diff*
1	0.1403	None
2	0.9659	None
4	0.0789	None
8	0.0083	B & C

* Sig Diff = Significant difference on a 5% level.

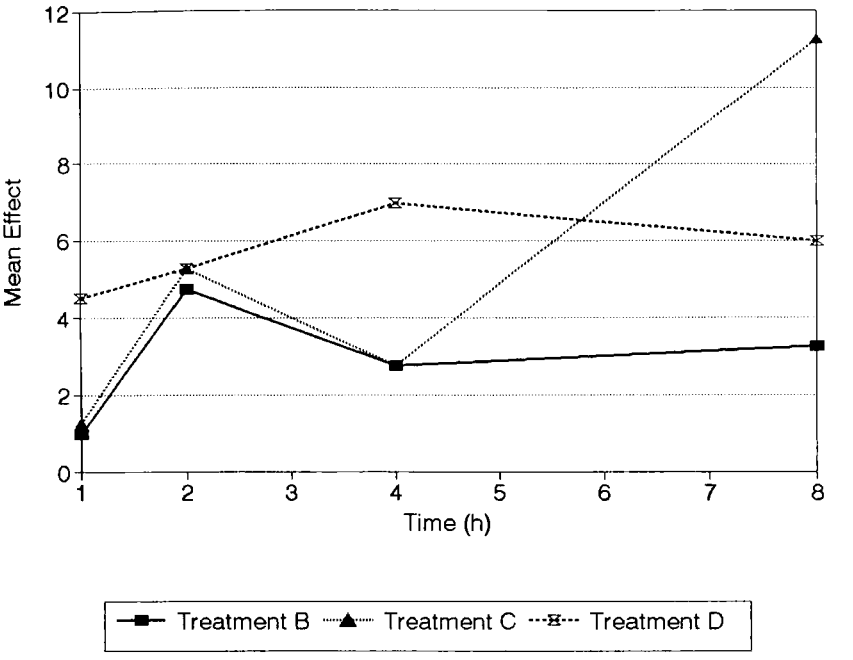

FIGURE 6

Mean effect of four rats at different times with treatment B,C and D.

statistical significant differences existed between these effects and those obtained without the surfactant (B + C).

CONCLUSION

PEG suppository bases caused necrotic effects on the rectal mucosa of rats, while it has been reported that apparently phenytoin on its own does not cause such an effect[3]. However, in our study phenytoin in combination with PEG, had an increased necrotic effect on the rectal mucosa. Although this results in rats can not be extrapolated to humans it is recommended that such studies should be done. The results of this study therefore imply that before using phenytoin with PEG bases in humans both the drug alone , and the base with the drug should be screened histologically for sensitivity reactions on the rectal mucosa of humans.

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