

Such a technique is not possible for human tissue and only limited cross-reactivity exists between elastins from different mammalian sources^{7,10,11}. In contrast to the immunofluorescent method for demonstration of elastin antigens in human tissue, the peroxidase-antiperoxidase method has several positive advantages. It is possible to use formalin-fixed, paraffin-embedded tissue which is available stored in most histopathology laboratories. In this way the method obviates the need to prospectively collect and store in deep-frozen state tissues that are likely to be of interest. The enhanced autofluorescence of

elastic fibers in such fixed tissue¹² precludes use of immunofluorescent method for its critical examination: the peroxidase-antiperoxidase method obviates this difficulty. Furthermore the peroxidase-antiperoxidase method allows examinations at intervals with relatively easy comparison between different tissue preparations.

We believe we have clearly demonstrated an immunological stain for human elastin in fixed tissue and believe this will be of great value in the study of diseases of human elastic tissue in a variety of different tissues¹³.

1 G.C.W. Humberstone and F.D. Humberstone, J. med. Lab. Technol. 26, 99 (1969).

2 K.S. Bhangoo, J.K. Quinlivan and J.R. Conelly, Plast. Reconstr. Surg. 57, 308 (1976).

3 V.V. Damiano, A. Tsang, J. Christner, J. Rosenbloom and G. Weinbaum, Am. J. Path. 96, 439 (1979).

4 V. Damiano, A. Tsang, U. Kuchich, G. Weinbaum and J. Rosenbloom, Conn. Tissue Res. 8, 185 (1981).

5 F.W. Keeley, Conn. Tissue Res. 8, 193 (1981).

6 K.G. McCullagh, K. Barnard, J.D. Davies and S.M. Partridge, Experientia 36, 1315 (1980).

7 K. Barnard, S.M. Partridge, A.H. Whiting, V. Fanth and K.G. McCullagh, Conn. Tissue Res., in press (1982).

8 R.C. Curran and J. Gregory, Experientia 33, 1400 (1977).

9 R.A. Daynes, M. Thomas, V.L. Alvarez and L.B. Sandberg, Conn. Tissue Res. 5, 75 (1977).

10 R.P. Mecham and G. Lange, Conn. Tissue Res. 7, 247 (1980).

11 U. Kucich, P. Christner, J. Rosenbloom and G. Weinbaum, Conn. Tissue Res. 8, 121 (1981).

12 J.D. Davies, J. Path. 114, 205 (1974).

13 J.D. Davies, K. Barnard and E.W. Young, J. Path. 137, 60 (1982).

Quantitation of proliferative mucosal cells of the Mongolian gerbil on a weight basis¹

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Summary. Quantitation of proliferative activity of the intestine of the gerbil shows a substantial decrease in proliferative activity as compared to the mouse. The use of dry vs wet weights proved to have no bearing on calculations of proliferative activity. The gerbil may prove a useful model for drug and radiation experimentation.

Use of the Mongolian gerbil, *Meriones unguiculatus*, as a research animal is expanding. Studies on the induction of tumors have been documented²⁻⁵; histocompatibility factors⁶ have been investigated, and others have done preliminary population kinetic studies⁷⁻⁹. The purpose of this study is to examine the gerbil's ileal cell population. Specifically we will report on quantitation of proliferative mucosal cells on a weight basis. **Materials and methods.** Adult male gerbils (12-18 weeks old and weighing 52-66 g) were raised from 12 original mating pairs. The colony was derived from Tumblebrook Farms, West Brookfield Mass. and inbred for 2 years before animals were used for analysis. Animals were injected i.p. with tritiated thymidine (³HTdr 0.8 µCi/g b.wt, sp.act. 7.0 Ci/mM) 45 min before sacrifice. A small piece of ileum (3 cm) approximately 2.5 cm from the ileocecal junction was removed for analysis. The mucosa was scraped free from the muscle layer, weighed and used for scintillation counting using the Biological Material Oxydizer (Oxymat Teledyne). The techniques of Hageman

et al.¹⁰ were used to determine the number of crypts and the number of proliferative cells per mg of ileum. A determination of cpm/mg was made for both dry and wet cellular weights. Quantitation used the following equations. Number of crypts per mg intestine = A/B
Number of S cells per mg = A × C/B (where S represents the population of cells in the synthetic phase of the cell cycle)
Number of proliferative cells per mg = $\frac{A \times C/B}{D}$
Where
A = cpm/mg intestine
B = cpm/crypt
C = number of labeled cells/crypt
D = fraction of proliferative cells in S(T_S/T_C) (where T_S represents the time of the S phase; T_C the time of the cell cycle)

Quantitation of proliferative activity of Mongolian gerbil mucosal cells on a weight basis

	cpm/crypt (B) ^a	cpm/mg (A) ^b	crypts/mg (A/B)	S cells/mg	$\left(\frac{A \times C/B}{D}\right)$	Proliferative cells/mg $\left(\frac{A \times C}{B}\right)$
Dry wt		$1.0 \times 10^4 \pm 1.5 \times 10^3$	3.8×10^3	8.5×10^4		1.7×10^5
Wet wt	2.67 ± 2.26	$1.6 \times 10^3 \pm 1.9 \times 10^2$	6.0×10^2	1.3×10^4		2.7×10^4

Cpm/crypt + cpm/mg obtained from liquid scintillation counting. Other figures were derived from equations. ^aSE based on 15 animals. ^bSE based on 7 animals.

Results and discussion. Quantitation of proliferative cell activity (table) yielded 2.67 cpm per crypt (wet wt). This figure can be used for further quantitation since dry vs wet weights have no bearing on this number. The cpm per mg data was 1.01×10^4 dry wt and $1.60 = 10^3$ wet wt. Proliferative cells per mg were calculated to be $1.71 = 10^5$ dry wt and $2.71 = 10^4$ wet wt. The data between these groups are consistent since the number of proliferative cells per crypt is $4.5 = 10^1$ for both dry and wet parameters.

Our calculations on the quantitation of proliferative activity of the intestine give figures which are lower by at least a power of ten than those of Hagemann et al.¹⁰ for the mouse. This difference was true for both dry and wet weights. There was a substantial difference in sampling since our samples consisted of mucosal scrapings only and did not include the muscle layer. Elimination of the weight of the muscle layers which take up little ³HTdr would result in a higher uptake per mg in animals with the same

proliferative rates. Our figures are nevertheless lower and thus indicate a substantial decrease in proliferative activity in the gerbil gut.

Two factors which may affect the various parameters were analyzed: age and diurnal variation. Both variables were carefully monitored. Crypt size might also affect the cpm/mg determination, since the gerbil's crypt size is 181 cells as compared to 450 in rats¹³ and 500 in mice¹⁴.

The gerbil, *Meriones unguiculatus*, is an important addition to cell kinetic modeling. It has been useful in grafting experiments, and may provide a base for the establishment of a number of new transplantable tumor lines. The gerbil has been shown to have high radioresistance^{11,12} and an extremely slow intestinal transit time⁷⁻⁹.

The slower proliferative activity in the small intestine of the gerbil may prove useful in models for studies on the small intestine and on radioresistance.

- 1 This work was supported by National Science Foundation grant No. GB35522. Reprint requests should be addressed to: H.S., Health Physics, Merck Sharp & Dohme Research Laboratories, West Point (Pennsylvania 19486, USA).
- 2 S.I. Magalini, A.H. Handler and G.P. Mascioli, Fedn Proc. 24, 437 (1965).
- 3 A.H. Handler, S.I. Magalini and D.Pav, Cancer Res. 26, 844 (1966).
- 4 H. Haas, J. Hilfrich, N. Knoch and U. Mohr, J. natl Cancer Inst. 55, 637 (1975a).
- 5 H. Haas, N. Knoch, U. Mohr and A. Cardesa, Z. Krebsforsch. klin. Onkol. 83, 233 (1975b).
- 6 R.E. Billingham and W.K. Silvers, Scient. Am. 208, 118 (1963).
- 7 J.M. Nelson, Ph.D. thesis, University of Michigan, Michigan 1969.
- 8 J.M. Nelson, Radiat. Res. 43, 367 (1972).
- 9 C.P. Sigdestadt, A.M. Connor and O.R. Czerwonka, Experientia 30, 344 (1974).
- 10 R.F. Hagemann, R.F. Sigdestadt and S.W. Leshner, Cell Tissue Kinetics 3, 21 (1970).
- 11 R.J. Flynn, ANL Biological and Med. Res. Semiannl. Rept. (ANI-5841), 31 (1957).
- 12 M.C. Chang, D.M. Hunt and C. Turbyfill, Nature 203, 536 (1964).
- 13 D.G. Bader and A.F. Hopper, Radiat. Res. 34, 555 (1968).
- 14 F. Devik, Acta radiol. ther. 10, 129 (1971).

Brain prostaglandin content in rats sacrificed by decapitation vs focused microwave irradiation

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Summary. Our experiments have shown that using microwave irradiation to sacrifice the animals prevents further post-mortem synthesis of prostaglandins in the rat brain.

In this preliminary study the possible correlation between total prostaglandin E and E₂ contents have been evaluated in rat brain hypothalamus after decapitation in comparison to the basal values obtained by using the microwave technique for sacrificing animals.

Male Sprague-Dawley rats (250-350 g) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) to avoid possible effects on prostaglandin content caused by the brief immobilization stress that occurs in the microwave technique (Poddubiuk, unpublished results) and killed between 02.00 h and 14.00 h by decapitation with a guillotine at 24°C or by microwave radiation by focusing the microwave radiation on the head for 5 sec (5 kW; 2450 MHz, General Medical Engineering, Peabody, Mass.). Brains were removed and placed on an ice-cooled plate for dissection. Hypothalami were used for further procedures. Prostaglandins were extracted by homogenizing 200 mg of tissue in a mixture of 1 ml 0.9% saline and 0.4 ml 0.1 N HCl at 40°C. The homogenate was then extracted with 2.6 ml of ethyl acetate:isopropanol (1:1; vol:vol) for 15 min with constant shaking. The ethyl acetate phase was separated by the addition of 3 ml 0.9% saline and 2 ml ethyl acetate.

Following centrifugation at 1000-2000×g the upper ethyl acetate phase was removed, evaporated under a stream of nitrogen, dissolved in 0.2 ml benzene:ethyl acetate:methanol (60:40:10; vol:vol:Vol) and stored at -20°C. The radioimmunoassay procedure was performed as previously described².

The analysis of our experimental data (table) indicates that prostaglandin content in brain is differentially affected by

Total prostaglandin and E₂ content in hypothalamus^a of rats sacrificed by microwave irradiation and by decapitation

	Method killed	
	Guillotine	Microwave irradiation
Brain total PGE content (ng/200 mg tissue)	33 ± 6.8	20 ± 5.4
Brain PGE ₂ content (ng/200 mg tissue)	24 ± 3.8	12 ± 3.0*

^a Each sample is a pool of the hypothalami from 3 rats. Results are mean values (± SE) for 8 rats. Significance expressed as *p < 0.05.