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SCIENTIFIC NOTES

An Autoradiographic Study of Prostaglandin E_1 and/or Its Metabolites in Mouse Uterus Tissue

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Prostaglandins (PG) have recently become the subject of extensive research. It has shown that prostaglandins are widely distributed in animal tissues. Recent advances have resulted in various hypotheses as to the action of these substances. The testing of these hypotheses is under way in a number of laboratories by either diffusing or injecting the prostaglandins into the animals. Observation that prostaglandins strongly stimulate smooth muscle has led to many studies of their effects on the uterus. These studies have been made both *in vitro* and *in vivo*. Most of the *in vitro* studies were concerned with the response of human myometrail strips to prostaglandins. The myometrial strip from a pregnant woman is often contracted by PGE_1, though higher doses can cause inhibition[2,3]. Most of the *in vivo* studies of human reproduction systems were done on volunteers. Bygdeman's group[12] reported that intravenous infusions of PGE_1, PGE_2 and $PGF_{2\alpha}$ induce abortion. Karim[8] reported that $PGF_{2\alpha}$ can be used to induce labor. Prostaglandin E_1 has also been used with success in termination of Pregnancy[4]. Further studies on the localization of the prostaglandins or their metabolites in uterus tissues after injection will yield valuable information which can be used to determine their pharmacological effects on human reproduction system.

Grain density autoradiography is beleived to be a useful tool and straight forward method for quantitative distribution studies. Liquid scintillation counting can be used for distribution studies, but in this case the classification of regions has to be made before the experiment is started. Using grain density autoradiography, however, one can classify the regions for counting according to the distribution of the grains on the autoradiogram so that a more valuable conclusion can be obtained. Moreover, the grain density autoradiogram provides a very precise location of radioactivity.

Relatively few papers have been published concerning the use of radioactivity labelled prostaglandins to study their tissue distribution in animals following injection. Liquid scintillation counting was used by Nakano[11] for subcellular localization in tissue and plasma distribution of intravenously injected ^3H-PGE_1 in rats and dogs. A similar counting technique was employed by Samuelsson[13] to study the organ distribution of radioactivity at different times after subcutaneous injection of ^3H-PGE_1 into female rats. Hansson and Samuelsson[7] reported an autoradiographic study using ^3H-PGE_1 and Green *et al.*[5] discussed an autoradiographic study using ^3H-$PGF_{2\alpha}$. Both of these studies involved the use of sagittal sections to indicate the distribution of radioactivity in various tissues. Grain density autoradiography for PGE_1 in mouse kidney was reported by Chen *et al.*[1]. Presently a similar quantitative technique for the distribution of radioactivity in various zones of mouse uterus following ^3H-PGE_1 injection was investigated.

MATERIALS AND METHODS

The solution of prostaglandin-E_1 [5,6-^{3}H(N)] (in a 7:3 ethanal-water mixture) was obtained with a stated specific activity of 68.5 Ci/mM (New England Nuclear). The radiochemical purity was determined by thin layer chromatography to be greater than 95%. The preparation of the injected solution was reported previously[1]. Fifty microcuries of ^{3}H-PGE_1 were injected into through tail vein of each of three 20-g Swiss-Cox female mice. A fourth mouse was served as control.

The three ^{3}H-PGE_1 injected mice, labelled as No. 1, No. 2 and No. 3, were sacrificed by ether at 20 min, 40 min and 45 hr correspondingly after injection, and the uteri were removed and frozen immediately by dipping into isopentane which was cooled by liquid nitrogen[6]. Tissue were sectioned in a thickness of 10 μ at -20°C.

The tissue sections were transfered from the microtome knife to slides which had been previously coated by dipping into kodak NTB liquid emulsion according to the method developed by Morris[9]. All slides were placed in light-tight plastic boxes containing dessicant and stored at -4°C for the duration of the exposure periods as listed in Table 1.

TABLE 1

Experimental Prameter.

Three mice labelled as No. 1, No. 2 and No. 3 are sacrificed at different time after injection. The autoradiograms made from different mouse have different exposure time.

Mouse No.	Time Between Injection and Sacrifice	Exposure Time of Autoradiogram (days)
1	20 min	8
2	40 min	24
3	45 hr	106

After the desired exposure the grain density emulsion slides were photographically processed and histologically stained using the procedure of Skierkowski[14] The distribution of radioactivity in the grain density autoradiograms was determined by microscopic counting of the number of exposed silver grains located over specific uterus regions using an oil-immersion objective (1000×). A grid was placed in the eyepiece of the microscope that superimposed an area of 2500 μ^2. Equally spaced fields in each region were chosen for grain counting. In each region the mean was calculated and the Students' statistic[10] was used to determine the 95% confidence interval of the mean. The mean grain density and its 95% confidence interval in each region were normalized by dividing by the number of days of exposure.

RESULTS AND DISCUSSION

The distribution of radioactivity was examined in various region of uterus tissue sections. When viewed with a microscope the violet stained nuclei could be very easily distinguished from the pink stained cytoplasm. Each region was recognized by its characteristic histological structure and staining. The normalized mean grain density versus region of uterus is plotted in Fig. 1. The 95% confidence intervals are shown as error bars on the histogram. It is shown in Fig. 1 that circular smooth muscle myometrium had the higest radioactivity. The radioactivity in the longitudinal smooth muscle myometrium is higher than that in endometrium. The distribution of grain density in each region was found to be more or less uniform. Background grain density was found to be considerably lower than the grain density on most tissue regions.

Sample from mouse No. 3, which was sacrificed at 45 hr after injection, the radioactivity was so low in uterus tissue that very long exposure time was needed. Unfortunately, a long exposure not only accumulates a high background but also introduces errors as a result of latent image fading. Therefore, the results of mouse No. 3 were only considered as reference information and not for quantitative comparison. However, it is very clear from results No. 3 that the radioactivity in uterus was very

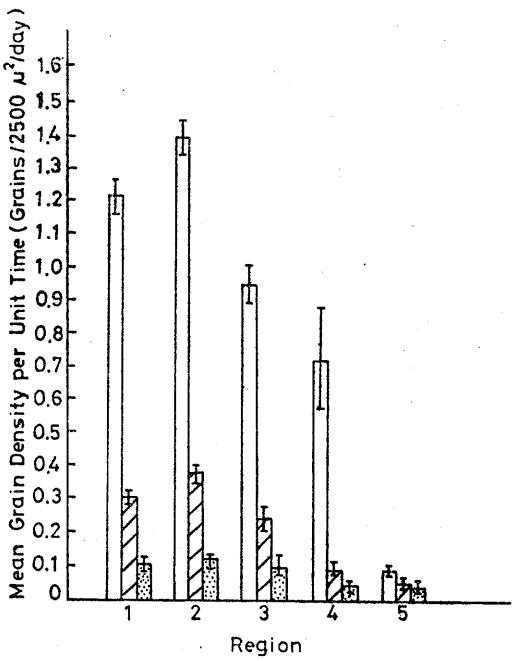

Fig. 1. Histogram of Uterus Grain Density Measurements. The normalized mean grain density of each region is plotted. Results from three mice are plotted together in this figure, ☐ representing results from mouse No. 1, ▨ from mouse No. 2 and ⁞⁞ from mouse No. 3. The 95% confidence intervals are shown as error bars in each mean.

Regions

1. Endometrium.
2. Circular Smooth Muscle of the Myometrium.
3. Longitudinal Smooth Muscle of the Myometrium.
4. Emulsion Region Immediately Adjacent to the Tissue Section.
5. Background of the Emulsion at a Considerable Distance from the Tissue Section.

low at 45 hr after injection.

In order to examine the rat of removal of radioactivity, the net mean grain density and the percentage decrease of the net mean of mouse No. 2 from that of mouse No. 1 in each region were calculated and are given in Table 2. From this table one can see that the percentage decreases in endometrium, circular smooth muscle of myometrium and longitudinal smooth muscle of myometrium are similar. Therefore the distribution of radioactivity in various regions of the uterus remains constant between 20 and 40 minutes after injection. In other words, the binding ability of these three regions are close to each other.

The degree of precision for location of radioactivity of a grain density autoradiogram depends upon how detailed the histological structure can be deduced from the histological staining. It also depends upon the fixation of the compound in the tissue, because translocation of radioactivity during preparation of the autoradiogram may give considerable error in the results. This was prevented in this research by quickly freezing the tissue immediately after it was removed from the animal. The results of this research are acceptable because the region which was considered was quite large in comparison to the possible translocation during sectioning. If a very small region is being considered, such as a nucleus, than a further test of the degree of prevention of translocation would have to be made.

It was found that if the grain density was too high then counting errors occured as a result of counting grains more than once. However, as long as the grain density was below about 150 grains/2500 μ^2, the grain counting is very reproducible. For those regions in which the grain density was greater than 150 grains/2500μ^2 a large number of fields were counted so that satisfying statistical results could be obtained. This can be seen from Fig. 1. The 95% confidence interval was small compared to the mean.

Since prostaglandins have such a wide spectrum of pharmacological effects more autoradiographic studies of their distribution in organs after injection seem warranted. For example, a distribution study in lungs might be helpful in understanding the metabolism of prostaglandins in the lungs. Also., a comparison

TABLB 2.

Percentage Change of the Net Mean Grain Density in Various Uterus Regions.

The percentage decrease of the net mean of mouse No. 2 from that of mouse No. 1 in the same region is given as percentage change of each region.

Region	Net Mean Grain Density Mouse No. 1	Net Mean Grain Density Mouse No. 2	% Change
Endometrium	1.127	0.246	78%
Circular Smooth Muscle of Myometrium	1.315	0.322	76%
Longitudinal Smooth Muscle of Myometrium	0.858	0.181	79%

of the distribution of PGE_2 in the uterus with that of PGE_1 would be interesting since PGE_2 has been found to be very active at inducing labor.

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以放射自動印相法研究前列腺素 E_1 及其代謝物在小鼠子宮之分佈

王 淑 霞

此研究工作爲用黑點密度自動印相 (grain density autoradiography) 之方法測得前列腺素 (prostaglandin) E_1，以及/或它的代謝物在小鼠子宮組織的分佈。實驗結果顯示在子宮肌膜的環狀平滑肌裏黑點的密度最大。而在子宮肌膜的環狀平滑肌，縱走平滑肌以及子宮內膜三區域內放射性在注射後 20 分鐘到 40 分鐘隨時間降低的比例相似。