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ACCUMULATION AND APPARENT ACTIVE TRANSPORT OF PROSTAGLANDINS BY SOME RABBIT TISSUES *IN VITRO*

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SUMMARY

1. Slices or bits of rabbit tissues, not exceeding 100 mg, were incubated in tissue culture medium containing tritium-labelled prostaglandin ([^{3}H]PG). In some experiments, incubation medium also contained saturating concentrations of an unlabelled prostaglandin (PG), or [^{14}C]-sucrose for determination of extracellular space. At the end of the incubation period, usually 1 hr, the tissues were removed and weighed, and their ^{3}H (and ^{14}C) content were determined along with that of a unit volume of medium.

2. Tissues known to play a central role in PG metabolism (lung and liver) and in its excretion (kidney cortex) and tissues which have a known function in blood-brain and blood-ocular barriers (choroid plexuses and ciliary processes) show a large accumulation of ^{3}H when incubated in a medium containing [^{3}H]PGE_1. In addition, tissues of the female reproductive tract, and the aorta of the rabbit show similar ^{3}H accumulation. When uncorrected for tissue solid content or extracellular water volume, the extent of this accumulation is two- to sixfold. Calculated on the basis that all excess ^{3}H is present in the free form in the intracellular water, the accumulation ratio for ciliary processes, for example, indicates an over fortysix-fold gradient of PGE_1 across the cell membrane.

3. Tissues which accumulate [^{3}H]PGE_1 also accumulate [^{3}H]PGA_1, [^{3}H]$PGF_{1\alpha}$ and [^{3}H]$PGF_{2\alpha}$. In some tissues specificity is, however, apparent; in the lung accumulation of [^{3}H]PGA_1 was significantly greater than that of [^{3}H]$PGF_{1\alpha}$.

4. The extent of [^{3}H]PGE_1 accumulation was decreased, or in some tissues completely inhibited, by incubation at 2° C, or by addition of large concentrations of unlabelled PG.

5. Accumulation of [^{3}H]PGE_1 by the foetal liver is not apparent on the

20th day of gestation, but is fully developed by the 30th day of gestation. The foetal lung does not accumulate [^{3}H]PGE_1 at any stage of gestation.

6. In some tissues, most notably muscle, there appears to be full equilibrium of [^{3}H]PGE_1 between tissue water and medium within 1 hr of incubation.

7. PGs are partially excluded from the intracellular volume of some other tissues, most notably the spleen and subcutaneous connective tissues. This apparent exclusion cannot be blocked by incubation in the cold, or by the addition of saturating levels of unlabelled PG.

8. The simplest explanation for all observed results is that cell membranes are, in general, impermeable to PGs. However, there are specific, carrier-mediated mechanisms across some membranes which facilitate the entry of PGs. In some cells these transport mechanisms are linked to a source of metabolic energy, and/or to the counter-transport of some other substance, thus allowing net accumulation of PGs against a concentration gradient. Alternatively, ^{3}H accumulation may represent adsorption of [^{3}H]PGs or one of their labelled metabolites on to specific adsorption sites.

INTRODUCTION

Prostaglandins (PGs) are present in, and released by, a variety of mammalian tissues (cf. Ramwell, 1970; Ramwell & Shaw, 1971). After entering the general circulation PGs are inactivated, degraded and ultimately eliminated from the body by the lungs, liver and kidneys (cf. Ramwell & Shaw, 1971). PGs released by the brain (Ramwell & Shaw, 1966; Holmes, 1970), intraocular tissue (Ambache, Kavanagh & Whiting, 1965) or the foetus must cross the blood–brain, blood–intraocular fluid or the blood–placental barriers before entering the general circulation.

Since PGs have a profound effect on both the brain (Horton, 1964; Horton & Main, 1967) and the eye (cf. Eakins, 1972), we must consider that there are specific mechanisms to prevent the accumulation of these autocoids in the extracellular fluids of these organs. This could imply that tissues that are associated with blood–brain and blood–intraocular fluid barriers such as the choroid plexuses and the ciliary processes may have specific absorptive PG transport function. The presence of PGs in the amniotic fluid (Karim, 1966) also suggests release of these autocoids by the foetus. To prevent accumulation of locally released PGs in the amniotic fluid or the foetal circulation, a transport of PGs across the blood–placental barrier or, in some species, by the yolk sac and/or endometrium may be required.

It should also be considered that the first step in the metabolism of PGs by the liver should require its uptake from the circulation into cells, since

PG metabolism is presumably intracellular (cf. Hamberg, 1968). Rat liver perfused with [^{14}C]PGE_1 removes 89–95 % of the radioactivity from the perfusate in one circulation (Dawson, Jessup, McDonald-Gibson, Ramwell & Shaw, 1970). Such a high rate of extraction again indicates facilitated transfer and possibly active transport of PG from circulation into the intracellular compartments of the liver. Efficient excretion of PGs by the kidneys may also require tubular transport, thus the possibility of accumulative transport of PGs in the kidney cortex is also worth considering.

It has been shown that tissues such as the kidney cortex (Cross & Taggart, 1950), ciliary processes (Becker, 1962) and the choroid plexus (Becker, 1961; Csáky & Rigor, 1968), when incubated *in vitro*, accumulate a variety of substances which they actively transport *in vivo*. Thus, a tissue-to-medium distribution ratio (T/M) of greater than unity for such substances can be used as a preliminary indication of the existence of *in vivo* transport mechanisms. The present experiments were undertaken to determine whether tissues which are involved with blood–brain, blood-ocular and blood–placental barrier systems or with PG metabolism and excretion show such accumulation of radioactivity when incubated with tritium labelled prostaglandins ([^{3}H]PGs). Most other tissues were also surveyed to determine whether this activity represents a specialized function or a more general cellular phenomenon.

METHODS

New Zealand white rabbits of either sex (2·5–5·2 kg) were killed with intravenous sodium pentobarbitone injection. Tissue bits or slices not exceeding 100 mg were incubated in Eagle's basal medium (with Earle's balanced salt solution; Microbiological Associates, Inc., Bethesda, Maryland) containing a [^{3}H]PG. The medium was gassed throughout the incubation period with 5 % CO_2 in O_2. In all but two experiments (Table 3 and Fig. 1) the duration of incubation was 1 hr, and with the exception of one experiment (Table 2) the incubation medium was kept at 37° C.

The ^{3}H-labelled prostaglandins (New England Nuclear Corporation, Boston, Mass. 02118) had the following specific activity: PGE_1, 87·3; $PGF_{1\alpha}$, 104; $PGF_{2\alpha}$, 14·3; and PGA_1, 81 c/m-mole. The radiochemical purity of all the labelled PGs was greater than 97 %. The PGs A_1, E_1 and $F_{1\alpha}$ were labelled with tritium on C_5 and C_6 and $F_{2\alpha}$ on C_9. Sufficient [^{3}H]PG was used in each experiment to obtain 10,000–30,000 counts/min per ml. of medium with the solvent scintillation cocktail used for the particular experiment. This amount of radioactivity corresponded to less than 5×10^{-9} g PG/ml. medium. In one set of experiments non-radioactive PGA_1 in the concentration of 1 or 50 μg/ml. was added to the incubation medium to study the saturability of the accumulation of PGE_1.

At the end of the incubation period the tissue bits were removed from the medium gently blotted on filter paper, and weighed within 3 sec on a Cahn Model 7500 digital-readout electrobalance (Ventrum Instrument Co., Paramount, California 90723). The tissues were then either transferred to a scintillation counting vial containing 10 or 20 ml. of a scintillation cocktail-solvent mixture or were homogenized

in scintillation solvent. Early experiments indicated that the labelled PG was completely eluted by the solvent system within a few hours. Paired homogenized and eluted tissues gave similar corrected counts; in fact, the eluted pieces gave higher absolute readings since they showed less quenching than the homogenized samples. The solvent-scintillation system used was 35 g hyamine chloride/l. toluene containing 4 g POPOP and 50 mg PPO. Scintillation solutes were obtained from the Packard Instrument Co. (Downers Grove, Illinois 60515). The samples were counted for a minimum of 5000 (typically 20,000–50,000) counts in a Packard Tricarb liquid scintillation counter. Quenching was monitored, and corrected for, when necessary, by the channel ratio technique. Incubation medium (100 μl. per scintillation vial) was taken in duplicate at the end of the incubation period and counted with each group of corresponding tissue samples.

Rate of uptake and elution. The rate of ^{3}H uptake was studied by incubating twenty-eight pieces of vaginal tissue (4 × 4 mm pieces of vaginal wall, about 1 mm thick) in a large volume of medium containing [^{3}H]PGE_1. Two pieces of tissue were removed at fixed intervals up to 2 hr. The pieces that remained after 2 hr were repeatedly transferred through several changes of tissue culture medium containing no [^{3}H]PGE_1. Further samples were taken during this period of elution. The tissue pieces and samples of medium were counted for ^{3}H activity as usual.

Double label experiments. Tissue bits to be doubly labelled were incubated for 1 hr at 37° C in a medium containing [^{3}H]PGE_1 and [^{14}C]sucrose, then dried overnight at 75° C in vacuum to determine dry weight, remoistened in the counting vial and dissolved in Soluene (Packard). The ^{3}H and ^{14}C activities were counted simultaneously on the three-channel scintillation counter using appropriate settings for double labelling. Isotope spill-over was monitored and corrected for. In these experiments total water content and extracellular and intracellular water volume were calculated from the wet and dry weights and from the sucrose space of each piece of tissue.

Presentation of results. Results of most of the experiments are reported as the tissue-to-medium ratio (T/M). ^{3}H counts per unit time were corrected for tissue weight (unit weight = 100 mg) and were divided by the counts per unit time per unit volume (100 μl.) of medium. The samples were not corrected for solid content. All values except those presented in Table 6 are the mean T/M ratios obtained on (n) number of tissue pieces. The S.E. of the mean is given.

The Tw/M ratio and the Iw/M ratios presented in Table 6 were calculated as follows: Tw/M; ^{3}H or ^{14}C counts per min of tissue divided by total water content of tissue (in mg) divided by ^{3}H or ^{14}C counts per min per μl. medium; Iw/M for ^{3}H, (total ^{3}H counts per min of tissue) − [(sucrose space of tissue in mg of water) × (^{3}H counts per min per μl. of medium)]/[(total tissue water) − (sucrose space of tissue in mg of water)].

RESULTS

Following 1 hr of incubation in medium containing [^{3}H]PGE_1, a net accumulation of radioactivity was evident in the choroid plexuses, iris-ciliary processes complex, tissue pieces from liver, renal cortex, aorta, uterus, vagina, and most notably, the cervix of rabbits. After 1 hr of incubation in [^{3}H]PGE_1 the ^{3}H activity of the spleen, adipose tissue, pancreas and the brain was appreciably less than that of the medium (Table 1). In other tissues, the T/M ratio is very close to unity, suggesting a free passive equilibrium of PGE_1 between tissue compartments and the incubation medium.

TABLE 1. Relative tritium content (T/M)* of rabbit tissues incubated at 37° C for 1 hr in medium containing [^{3}H]PGE$_1$

System; tissue	$T/M \pm$ S.E. (n)	System; tissue	$T/M \pm$ S.E. (n)
C.N.S. and eye		Male reproductive	
Brain	0·67 ± 0·05 (6)	Testes	0·80 ± 0·04 (9)
Choroid plexus	4·92 ± 0·95 (8)	Epididymis	0·58 ± 0·03 (10)
Iris-ciliary processes	3·48 ± 0·50 (8)	Vas deferens	0·85 ± 0·12 (6)
Cornea	1·06 ± 0·05 (8)	Prostate	0·70 ± 0·09 (7)
Respiratory		Seminal vesicle	0·81 ± 0·03 (8)
Trachea	0·76 ± 0·04 (6)	Female reproductive non-gravid	
Lung	1·25 ± 0·13 (8)	Ovary	0·83 ± 0·04 (6)
Gastro-intestinal		Uterus	2·59 ± 0·38 (8)
Oesophagus			
inner lining	0·60 ± 0·08 (8)	Cervix	7·98 ± 1·14 (16)
muscle coat	0·73 ± 0·02 (8)	Vagina	2·93 ± 0·58 (7)
Stomach		Other	
inner lining	0·68 ± 0·05 (6)	Adipose	0·43 ± 0·08 (8)
muscle coat	0·89 ± 0·06 (6)	Adrenal	0·60 ± 0·08 (7)
Intestine	0·47 ± 0·02 (8)	Aorta	1·40 ± 0·14 (6)
Liver	1·41 ± 0·18 (11)	Diaphragm	0·97 ± 0·05 (6)
Pancreas	0·60 ± 0·07 (6)	Heart	1·05 ± 0·07 (7)
Gall-bladder	0·87 ± 0·05 (6)	Spleen	0·35 ± 0·01 (8)
Urinary		Skin	0·80 ± 0·04 (7)
Kidney cortex	1·82 ± 0·09 (8)	Subcutaneous	0·88 ± 0·03 (6)
medulla	0·84 ± 0·03 (6)	connective	
Bladder	0·52 ± 0·04 (8)		

* Tissue to medium ratio (T/M) = (Counts per minute per 100 mg tissue)/(Counts per minute per 100 μl. medium). The numbers given are the mean ratio followed by the S.E. of the mean and the number of tissue pieces used (n). For each value presented tissues were obtained from two or more animals.

TABLE 2. The effect of incubation temperature on the extent of [^{3}H]PGE$_1$ uptake into rabbit tissues. Values given are tissue ^{3}H activity/medium ^{3}H activity (T/M) after 1 hr of incubation

	Incubation temperature		
	2° C	25° C	37° C
Brain	0·40 ± 0·05 (3)	—	0·67 ± 0·05 (6)*
Choroid plexus	2·91 ± 0·31 (7)	—	4·92 ± 0·95 (8)*
Iris-ciliary processes	1·34 ± 0·19 (6)	—	3·48 ± 0·50 (8)*
Liver	1·16 ± 0·04 (13)	1·29 ± 0·10 (13)	1·38 ± 0·11 (13)
Kidney cortex	1·67 ± 0·20 (11)	2·78 ± 0·48 (11)	3·27 ± 0·60 (11)
Uterus	0·72 ± 0·15 (9)	1·63 ± 0·18 (14)	2·40 ± 0·24 (12)
Spleen	0·29 ± 0·03 (6)	0·42 ± 0·05 (6)	0·49 ± 0·24 (6)

* Values taken from Table 1; not a direct comparison with 2° C values, since tissues were not taken from the same rabbits.

The extent of ^3H accumulation depends upon incubation temperature (Table 2). Accumulation of PGE_1 in the uterus was completely inhibited by incubation at 2° C, although kidney cortex, choroid plexus and the iris-ciliary processes still accumulated significant amounts of ^3H when

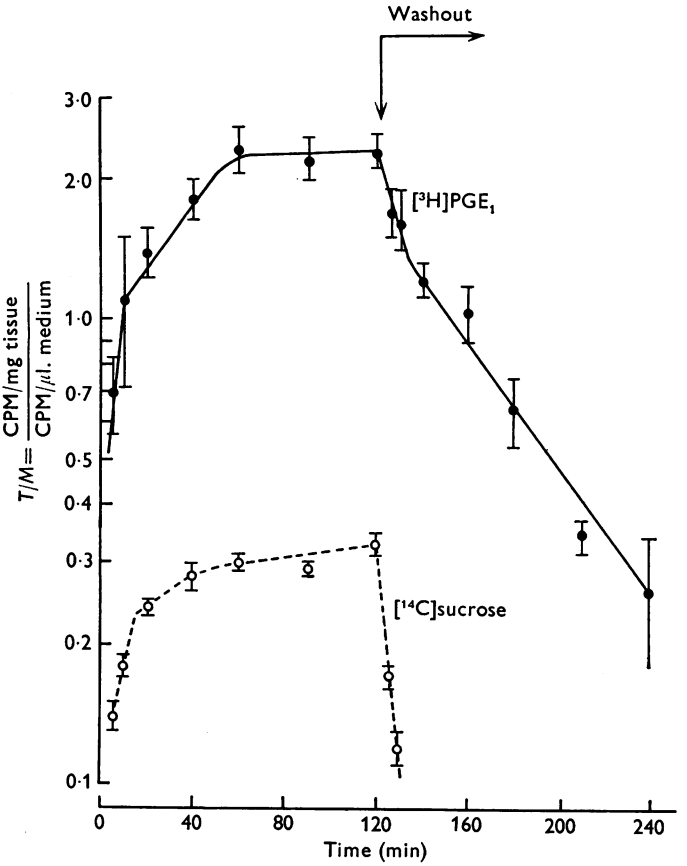

Fig. 1. The time course of PG accumulation by, and elution from, pieces of rabbit vaginal tissue. Tissue pieces were placed in medium containing [^3H]PGE_1 and [^{14}C]sucrose at zero time. Two pieces of tissue were removed at each point indicated on the graph. After 2 hr of incubation the remaining tissue pieces were transferred to medium containing no radioactivity; periodic removal of the tissue pieces continued. Each point represents the mean T/M ratio of six tissue pieces from three separate experiments. The limits are S.E. of the mean.

incubated at 2° C. At 25° C T/M ratios were between those obtained in the cold and at physiological temperature.

The time course of ^3H accumulation by vaginal tissues indicates that a maximum T/M ratio is achieved between 1 and 2 hr (Fig. 1). The rate of

uptake appears to be biphasic, a T/M ratio of 1·0 being achieved in less than 10 min, while doubling of the ratio requires about 50 min. The elution is also biphasic, about one third of the ^3H activity being lost from the tissue during the first 10 min of washout. This rapid phase presumably represents the elution of primarily extracellular ^3H. The remaining ^3H activity is lost at a much lower rate (half-time about 30 min).

With the notable exception of the uterus, the tissue ^3H accumulation increased very little between 1 and 2 hr of incubation. In some tissues, such as the liver and choroid plexuses, a maximum ^3H accumulation was already evident after 0·5 hr of incubation (Table 3). Since ^3H accumulation

TABLE 3. The effect of duration of incubation on the extent of ^3H accumulation by various rabbit tissues. The medium contained [^3H]PGE_1. Incubation temperature 37° C

	Tissue ^3H activity/Medium ^3H activity = (T/M) Duration of incubation		
	30 min	60 min	120 min
Brain	0·56 ± 0·03 (3)	0·67 ± 0·05 (6)	—
Choroid plexus	6·02 ± 0·86 (6)	4·92 ± 0·95 (8)	5·86 ± 1·46 (5)
Iris-ciliary processes	3·14 ± 0·53 (8)	3·48 ± 0·50 (8)	—
Liver	1·57 ± 0·20 (6)	1·48 ± 0·24 (6)	1·29 ± 0·13 (6)
Uterus	1·40 ± 0·18 (6)	2·14 ± 0·30 (5)	3·12 ± 0·32 (5)
Cervix	9·73 ± 1·37 (6)	12·53 ± 3·11 (4)	12·37 ± 0·81 (2)
Adipose	0·30 ± 0·02 (6)	0·43 ± 0·04 (6)	0·40 ± 0·02 (6)
Spleen	0·16 ± 0·01 (6)	0·24 ± 0·02 (6)	0·29 ± 0·02 (6)
Subcutaneous connective	0·80 ± 0·07 (6)	0·88 ± 0·03 (6)	0·92 ± 0·02 (6)

was, as a rule, affected very little by extending the duration of the incubation from 1 to 2 hr, there was no indication that the T/M ratio in tissues which seem to exclude PGE_1, such as the spleen and adipose tissues, would approach unity on further incubation. Incubation was, therefore, kept at 1 hr in all further experiments.

The effect of pregnancy on the apparent accumulation of PGE_1 by tissues of the female reproductive system, and the time course of development of PG accumulating activity in the foetus is shown in Table 4. It is clear that pregnancy and the associated hormonal changes do not alter the accumulation of PGE_1 by the rabbit reproductive system. Vagina, cervix and uterus of gravid rabbits showed ^3H accumulation comparable to that of non-pregnant adults (see Table 1). However, implanted regions of the rabbit uterus which were incubated with pieces of adherent placental tissues did not show PGE_1 accumulation. In the foetal rabbit there was some PGE_1 accumulation by the liver, but not by the lung, after 24 days of gestation. The ability of the liver to accumulate PGE_1 seems to be fully

developed before term (30th day of gestation), while this capacity does not develop in the lung until after birth.

The site of PGE_1 accumulating activity in the uterus was studied by separating the endometrium from the bulk of the myometrium (Table 5). Pieces of uterine wall about 1 cm^2 were placed in the Stadie–Riggs tissue slicer with the endometrium facing up. Two cuts were made in the plane

TABLE 4. Relative tritium content (T/M) of tissues of the gravid reproductive system and of second and third trimester rabbit foetuses incubated in medium containing [^{3}H]PGE_1

	Duration of gestation in days		
	20	24	30
Cervix	7·49 ± 0·49 (4)	5·20 ± 0·28 (3)	7·84 ± 1·22 (6)
Vagina	4·20 ± 0·38 (4)	2·88 ± 0·34 (4)	3·02 ± 0·30 (3)
Uterus (non-implanted)	2·37 ± 0·31 (5)	—	2·37 ± 0·16 (3)
Placenta	0·61 ± 0·23 (3)	—	0·59 ± 0·11 (4)
Placenta-uterus complex	0·86 ± 0·04 (2)	—	0·67 ± 0·04 (4)
Foetal lung	—	0·76 ± 0·05 (3)	0·73 ± 0·07 (4)
Foetal liver	0·61 ± 0·07 (4)	1·22 ± 0·14 (3)	2·16 ± 0·36 (4)

TABLE 5. The site of ^{3}H accumulation in the uterus. The uterine wall was sectioned in the plane parallel to its surface before or after 1 hr incubation at 37° C in medium containing [^{3}H]PGE_1, or after being kept in moist chamber for an additional 30 min

	(n)	Section 1 (endometrium)	Section 2 (myometrium and endometrium)	Section 3 (myometrium)	Whole uterus
Sectioned before incubation	(6)	2·69 ± 0·39	1·33 ± 0·15	0·96 ± 0·10	—
Sectioned after incubation	(6)	3·12 ± 0·21	1·42 ± 0·10	1·17 ± 0·05	—
Sectioned after incubation and 30 min in moist chamber	(6)	2·01 ± 0·13	1·65 ± 0·15	1·43 ± 0·11	—
Not sectioned	(6)	—	—	—	2·51 ± 0·14

of the uterine wall so that the first slice contained endometrium with some underlying myometrium, the second contained some islands of endometrium, and the remaining tissue slice consisted of myometrium. The uterine wall was thus sectioned either before or after incubation in the [^{3}H]PGE_1-containing medium. In either case the greatest T/M ratio was evident in the endometrium, and the least was obtained on the second slice of myometrium.

In all the previous tables the results were expressed as T/M ratios. If PG accumulation is limited to intracellular compartments this expression must underestimate the actual accumulation gradient of PGs. When the sucrose space and the water content were estimated together with the T/M of ^3H activity, corrections for solid content and for the ^3H content of the extracellular water volume could be made (see Table 6). These corrections are especially important in tissues which have large solid content,

TABLE 6. The apparent [^3H]PGE_1 accumulation expressed on the basis of total tissue water (*Tw*)* and intracellular water (*Iw*)*. Tissue pieces were incubated for 1 hr in medium containing [^3H]PGE_1 and [^{14}C]sucrose; solid content was determined by drying overnight at 75° C in partial vacuum

Tissues	(*n*)	*T/M* ^3H	*Tw/M* ^3H	*T/M* ^{14}C	% H_2O	*Iw/M* ^3H
Brain	(6)	0·53 ± 0·04	0·63 ± 0·04	0·36 ± 0·02	83·8 ± 0·72	0·43 ± 0·05
Ciliary processes‡	(6)	7·02 ± 1·01	8·12 ± 1·14	0·83 ± 0·02	86·3 ± 1·19	46·62 ± 7·22
Liver	(9)	1·58 ± 0·07	2·23 ± 0·09	0·44 ± 0·04	70·9 ± 1·18	3·34 ± 0·34
Kidney cortex	(6)	2·43 ± 0·14	3·04 ± 0·17	0·38 ± 0·03	79·7 ± 0·60	4·38 ± 0·37
Cervix	(10)	6·31 ± 1·29	7·55 ± 1·56	0·51 ± 0·02	83·7 ± 0·72	15·35 ± 3·50
Vagina	(8)	2·70 ± 0·36	3·28 ± 0·44	0·54 ± 0·01	82·3 ± 0·94	6·15 ± 1·06
Spleen	(4)	0·33 ± 0·01	0·43 ± 0·02	0·08 ± 0·01	77·2 ± 1·37	0·37 ± 0·01
Subcutaneous connective	(6)	0·70 ± 0·03	0·84 ± 0·02	0·79 ± 0·02	83·2 ± 0·76	0·25 ± 0·08

* T = ^3H activity per unit weight of tissue; Tw = ^3H or ^{14}C activity per unit volume of total tissue water; Iw = ^3H activity in intracellular water; M = ^3H or ^{14}C activity per unit volume of incubation medium. For the actual calculation of the distribution ratio see presentation of results in Methods.

‡ The bulk of the iris was removed, only a ring of tissue about 2 mm wide, containing most of the ciliary processes, was used in this experiment.

such as the liver, or tissues with very large extracellular space, such as the ciliary processes (Iw/M for ^3H = 46·6 as compared to T/M = 7·2), or the subcutaneous connective tissues (Iw/M for ^3H = 0·25 as compared to T/M = 0·70).

The specificity of PG accumulation is shown in Table 7. Tissue slices or bits taken from the same rabbit were randomly divided, three to four pieces of each tissue being incubated in two, three, or all four different prostaglandins which are presently available with ^3H label. For the most part, tissues which were shown to accumulate [^3H]PGE_1 also accumulate $PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGA_1. The extent of ^3H accumulation does, however, depend on the [^3H]PG used. For example, the rabbit lung accumulates significantly more ^3H when incubated in medium containing [^3H]PGA_1 (T/M = 2·14) as compared to [^3H]PGE_1 and the PGFs, most notably

$PGF_{1\alpha}$ (T/M = 1·13). This difference in the extent of ^3H accumulation is significant ($P < 0·01$).

The extent of ^3H accumulation could be greatly decreased when, in addition to the usual amount of [^3H]PGE_1, small quantities of unlabelled PG were added to the incubation medium (Table 8). Addition of PGA_1

TABLE 7. Comparison* of the extent of ^3H accumulation by rabbit tissues incubated for 1 hr at 37° C in media containing ^3H labelled prostaglandins E_1, $F_{1\alpha}$, $F_{2\alpha}$ or A_1

	Tissue ^3H activity/Medium ^3H activity = (T/M)			
	[^3H]PGE_1	[^3H]$PGF_{1\alpha}$	[^3H]$PGF_{2\alpha}$	[^3H]PGA_1
Brain	0·70 ± 0·03 (4)	0·60 ± 0·09 (4)	0·60 ± 0·03 (4)	0·89 ± 0·25 (4)
Iris-ciliary	5·62 ± 1·50 (4)	—	6·93 ± 1·77 (4)	—
processes	—	6·20 ± 1·55 (2)	—	6·72 ± 1·73 (4)
Lung	1·45 ± 0·11 (8)	1·13 ± 0·08 (8)	1·50 ± 0·14 (8)	2·14 ± 0·27 (8)
Liver	—	1·09 ± 0·04 (4)	1·65 ± 0·12 (4)	1·16 ± 0·14 (4)
Kidney cortex	2·21 ± 0·49 (4)	—	4·42 ± 0·59 (4)	—
	—	4·15 ± 1·26 (4)	—	3·16 ± 0·33 (4)
Testes	0·70 ± 0·03 (8)	0·63 ± 0·04 (8)	0·72 ± 0·08 (4)	0·98 ± 0·05 (8)
Vagina	3·78 ± 0·40 (4)	—	3·59 ± 0·27 (4)	—
	—	3·64 ± 1·56 (6)	—	3·78 ± 0·19 (6)
Adipose	—	0·28 ± 0·01 (4)	0·20 ± 0·01 (4)	0·39 ± 0·02 (4)
Spleen	0·34 ± 0·04 (4)	0·48 ± 0·01 (4)	0·30 ± 0·03 (4)	—
	—	—	—	0·55 ± 0·03 (6)

* Values given on the same line are direct comparisons: tissue pieces taken from each animal were divided equally using the incubation vials containing the different tritiated prostaglandins.

TABLE 8. The effect of PGA_1 on the accumulation of [^3H]PGE_1 by various tissues of the rabbit. Tissues were incubated for 1 hr at 37° C in medium containing [^3H]PGE_1 and unlabelled PGA_1, 0, 1 μg or 50 μg/ml.

	(n)	[^3H]PGE_1	[^3H]PGE_1 + PGA_1 1 μg/ml.	[^3H]PGE_1 + PGA_1 50 μg/ml.
Kidney cortex	(4)	2·75 ± 0·32	2·54 ± 0·22	1·50 ± 0·05
Uterus	(7)	1·65 ± 0·05	0·93 ± 0·02	0·82 ± 0·02
Vagina	(4)	6·36 ± 1·33	2·28 ± 0·15	1·34 ± 0·05
Spleen	(10)	0·53 ± 0·03	0·54 ± 0·03	0·48 ± 0·02

1 μg/ml. decreased the T/M for [^3H]PGE_1 in three out of four tissues, while PGA_1 50 μg/ml. completely blocked PGE_1 accumulation in the uterus and further decreased the T/M for the kidney cortex and vagina. The T/M for the spleen, normally less than unity, was not significantly affected by the addition of PGA_1 to the incubation medium.

DISCUSSION

The present results demonstrate that tissues associated with blood-brain, blood-aqueous and possibly blood-placental barriers, or with the metabolism and excretion of PGs, exhibit a concentrative accumulation of these autocoids or their labelled metabolites. As indicated by the T/M ratio, some of these tissues can contain 3–8 times the ^3H activity of a corresponding volume of incubation medium. Most other tissues of the body, which were also surveyed, either show no evidence of PG accumulation, or, like connective tissue, spleen and red blood cells, effectively exclude PG from some, presumably intracellular, tissue compartments.

A diversity of PG distribution was also evident from studies by Samuelsson and co-workers (Hansson & Samuelsson, 1965; Gréen, Hansson & Samuelsson, 1967). These authors autoradiographed sagittal sections of whole mice which had been injected with [^3H]PGE_1 or $PGF_{2\alpha}$ before they were killed. They also reported high radioactivity in the liver and kidneys. However, contrary to our findings, they also found high activity in connective tissues and myometrium, but not in the endometrium. However, the lack of constant concentration of the labelled compound in blood, and consequently in extracellular fluids, makes even qualitative interpretation of these earlier results equivocal. A continual decrease in blood radioactivity resulting from uptake into cells and from excretion can lead to a transitory state in which there is a higher concentration of labelled substances in some extracellular fluids than in blood. Thus, the high radioactivity observed by Samuelsson and co-workers in loose connective tissues could represent retention of labelled PGs in a relatively stagnant extracellular fluid compartment rather than a 'specific uptake' of these compounds by formed elements.

The quantitative extent of ^3H accumulation or exclusion observed in this study depends on the expression used for comparing the amount of ^3H activity in the tissue to that of the medium. Usually, simple T/M ratios have been presented. This expression makes no assumption concerning the state or distribution of the ^3H activity within the tissue and does not require the determination of water content or sucrose space. It simply states the amount of ^3H contained per unit weight of tissue in relation to a unit volume of incubation medium. In general, T/M ratios between 0·8 and 1·2 can be assumed to indicate free penetration of the label into all tissue compartments; T/M values less than 0·8 tend to indicate exclusion of the label from some tissue compartments, while values greater than 1·2 indicate accumulation in some compartments or adsorption on to some tissue constituents. In the present study the T/M ratio ranged from 0·35 for spleen to 7·98 for cervix. It is clear, therefore, that there is a tremendous

variation in the extent of PG entry into the various tissues of the body. The simple T/M ratios, however, underestimate the ^3H accumulation in most tissues and the tissue-to-tissue variation in PG uptake.

Accumulation of a substance in a tissue is usually assumed to result from its transport into the intracellular fluid compartment. Consequently T/M ratios are frequently presented as the calculated concentration of a substance in the intracellular water divided by the concentration in the incubation medium (these values are more appropriately referred to as Iw/M ratios). When water content and sucrose space were determined in our experiments, a greater than fortyfold accumulation of ^3H activity was apparent in one tissue, the ciliary processes, while the Iw/M for some others, e.g. connective tissue, was as low as 0·25. This implies that following exposure of different tissues to the same concentration of PGs the intracellular PG concentration of different cell types may differ by a factor of 100 or more.

Such expressions of the extent of apparent tissue-to-medium distribution of a substance must be used cautiously, however, because they require three questionable assumptions: (1) that the concentration of the substance in question is the same in the extracellular space as in the medium, i.e. that it is not bound to membranes or other formed elements of the intercellular matrix; (2) that the substance present in the intracellular compartment is evenly distributed in all cell types of the tissue; (3) that the tissue ^3H activity is still associated with the original compound rather than a metabolite. In some cases, these assumptions may be relatively valid. In the case of PGs, however, we cannot exclude the possibility of binding to either extracellular or intracellular structures, and it is quite unlikely that PGs penetrate or accumulate in all the cell types of a given tissue, or intracellular compartments, at the same rate. Furthermore, PG is rapidly metabolized and identification of the labelled compound extracted from very small tissue pieces proved difficult and equivocal with our present techniques. The ^3H on the PGs used in this study were on stable positions and should be present in all known primary metabolites. Thus, the distribution of ^3H activity observed must represent distribution of PG or closely similar derivatives.

In the present paper the Iw/M ratios are given for a few selected tissues to indicate the magnitude of the ^3H accumulation after appropriate correction for extracellular water volume (sucrose space) and solid content were made for these tissues. But before the state of accumulated ^3H activity within these tissues is known, the Iw/M values should not be taken to imply the actual concentration gradient of PGs across the cell membranes of any given tissue.

The state of ^3H within the tissues is of obvious concern with respect to

interpretation of the present results. It could be argued that differences in tissue distribution simply represent the passive partition of PGs or their metabolites between the medium and some tissue components. Such passive partitioning into non-specific cellular constituents cannot play an important role in PG accumulation, since accumulation of radioactivity cannot be correlated with lipid content or with cellularity of the tissue. On the contrary, tissues of high cellularity and/or lipid content, such as adipose tissue or brain, showed no net PG accumulation. The fact that the ^3H activity can be readily eluted from accumulating tissues clearly demonstrates that the ^3H accumulation is not due to the incorporation of [^3H]PG or one of its labelled metabolites into macromolecular or structural elements. Thus, the observed ^3H accumulation must result either from mediated transport or reversible adsorption on to tissue components.

The kinetics of mediated transport and adsorption are essentially the same; in fact, adsorption and desorption are clearly involved in carrier-mediated transport. Thus the inhibitory effect of the addition of PGA_1 to the incubation medium on the accumulation of [^3H]PGE_1 into kidney cortex, uterus or vagina can indicate either that the same carrier, or the same stable adsorption sites can interact with both PGE_1 and PGA_1. The temperature dependence of ^3H accumulation in the uterus indicates an active energy-dependent process, since this accumulation is completely inhibited by cold (2° C). However, in several other tissues, most notably the choroid plexuses, large accumulation of ^3H was still evident following incubation in [^3H]PGE_1 at 2° C. Since accumulation at 2° C is unlikely to represent a primary, energy-dependent, active transport, it may, instead, represent adsorption. It should be kept in mind, however, that secondary or tertiary transport systems (cf. Stein, 1967) could operate in the cold, utilizing the pre-existing concentration gradients of other substances. On the basis of the present experiments it can only be concluded that the accumulation of ^3H by some tissues must represent specific adsorption on to a limited number of specific adsorption sites or, alternately, a carrier-mediated concentrative transport of PGs or their labelled metabolites into intracellular fluid compartments.

Other experiments now in progress (Bito, 1971) indicate, however, that in at least some tissues a mechanism for transmembrane transport of [^3H]PGE_1 does exist. Sacks of ligated rabbit vaginas filled with medium containing [^3H]PGE_1 and suspended in an incubation medium containing the same [^3H]PGE_1 concentration establish, within 2 hr, a ^3H gradient of as much as eightfold between the inside and outside media. The net flux is from inside to outside. In the ciliary processes evidence of PG transport function was also obtained *in vivo*. When [^3H]PGE_1 is injected into the vitreous together with [^{14}C]sucrose, ^3H activity disappears from the eye

at a rate higher than the rate of sucrose loss. No appreciable ^3H enters the aqueous humour, while the major site of sucrose exit is through the anterior chamber. This demonstrates that [^3H]PGE_1, which diffuses from the vitreous body into the posterior chamber, is quantitatively removed from the posterior aqueous humour before it enters the anterior chamber. Thus, it seems that the *in vitro* accumulation of PGE_1, at least by the ciliary processes, and most likely also by the choroid plexuses which have a similar function, may indeed reflect an active absorptive transport mechanism.

Accumulation of ^3H activity by the uterus could clearly be associated with a 'blood-placental' barrier function since our experiments indicate that this accumulation is mainly due to the endometrium and at least *in vitro*, the accumulation in the myometrium is the result of apparent transport across the endometrium into the myometrium. In the living animal any substance transported from the lumen of the uterus across the endometrium will presumably be carried away by the circulating blood. Thus, the apparent endometrial transport could prevent accumulation of PGs in the foetal circulation. It could also minimize entry of PG from the maternal into the foetal circulation and, provided that the yolk sack and amniotic sacks are permeable to PGs, it could also contribute to PG removal from the amniotic fluid.

The accumulation of PGs by the rabbit cervix and vaginal wall may also represent part of a foetal barrier system. Under *in vivo* conditions such uptake is likely to represent transport of PGs from the vaginal tissues into the local circulation; such removal of PGs from the vagina should minimize the entry of PGs from the ejaculate into the uterine cavity. It has been shown in this regard, that PGs deposited into the rabbit vagina have a systemic hypotensive effect; i.e. the PGs or some of their physiologically active metabolites can enter the general circulation from the vaginal lumen (Apslund, 1947). The present experiments suggest that, in the rabbit, transfer of PG from the vaginal lumen to blood may occur against a concentration gradient; i.e. it may represent an active absorptive transport mechanism. Absorption of [^3H]PGE_1 from the human vagina is also evident (Sandberg, Ingelman-Sundberg, Rydén & Joelsson, 1968).

PG transport processes in the female reproductive tract may also play a role in the delivery of PGs to their apparent target organs, the ovaries. Evidence has recently been presented (Barrett, Blockey, Brown, Cumming, Goding, Mole & Obst, 1971; cf. McCracken, 1971) which indicates that PGs from uterine venous blood are preferentially delivered to the ipsilateral ovaries. If this apparent countercurrent exchange between venous blood and the arterial system of the ovaries were extended into the vaginal venous effluent, an active PG transport by the vaginal mucosa into the vaginal venous circulation would greatly facilitate the delivery of intra-

vaginally deposited PGs to the ovaries. Such a transport mechanism may play a reproductive role in the rabbit, in which the seminal plasma has a very low PG content. In species with high seminal fluid PG concentration accumulative PG transport may not be necessary, or functional.

It must be emphasized that the present results pertain to the rabbit only. Results of experiments on species variation (Bito, 1972) indicate that most tissues that accumulate PGs in the rabbit also show similar activity in cats, rats and several fishes (cf. Bito, Turansky & Van Voris, 1971). A notable exception is the female reproductive tract of the cat which shows no *in vitro* accumulation of PGE_1 in the normal, pregnant or oestrous states.

Clearly, most tissues of the body show no net PG accumulation; in many tissues the intracellular [^{3}H]PG does not approach the extracellular ^{3}H level, even in apparent steady-state. This apparent exclusion from the intracellular fluid is not due to an active PG transport in the outward direction, since the exclusion cannot be prevented by incubation in the cold or by saturating levels of an unlabelled PG. It should be noted that the Iw/M ratio for subcutaneous connective tissue (0·25), which was the lowest found in this study, was still significantly greater than zero. Thus, the Tw/M ratio for [^{3}H]PGE_1 was in all cases greater than that for [^{14}C]-sucrose. This tends to indicate that either some cell types or some cellular subcompartments, in subcutaneous connective tissue comprising some 25% of the total intracellular space, are accessible to [^{3}H]PG.

The simplest explanation for all the present results is that, while all cell membranes are basically impermeable to PGs, some have a mechanism to facilitate the passage of PGs. In some tissues concentrative accumulation of PGs can take place. In these tissues facilitation of cellular PG transport is either active (linked to the expenditure of metabolic energy) or linked to the efflux of another substance by exchange diffusion. Such basic impermeability of cell membrane to PG and the selective facilitation or transport of PGs across some cell membranes can account completely for the diversity of PG entry and accumulation observed in the present survey of some thirty-four different tissues of the rabbit. Such variation in PG distribution must be considered in the interpretation of the effects of PGs on various mammalian systems and in the choice of the route of administration of these pharmacologically active compounds. The interactions of active and physiologically inactive PGs at the transport sites may offer a tool to modify the normal distribution pattern and excretion rate of PGs.

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