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Diffusion in Polymer Gel Implants

(^{125}I-solutes/polyacrylamide and polyvinylpyrrolidone gels/hamsters/retarded release)

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ABSTRACT **Crosslinked polyacrylamide and polyvinylpyrrolidone gels have been used to subcutaneously implant ^{125}I-labeled immunoglobulin, ^{125}I-labeled luteinizing hormone, ^{125}I-labeled bovine serum albumin, ^{125}I-labeled insulin, [^{3}H]prostaglandin $F_{2\alpha}$, and Na^{125}I into hamsters. From the rates of absorption of the solutes, their diffusion coefficients were determined. The diffusion coefficients showed a logarithmic dependence on implant polymer concentration and solute molecular weight. Release of the solutes from gel preparations incubated 10 mM phosphate buffer (pH 7.2) at 37° revealed a similar relationship between solute diffusion coefficient, molecular weight, and the concentration of polymer. A general equation was derived that gives the expected diffusion coefficient of a substance in a polymer gel from its molecular weight, diffusion coefficient in solvent, and polymer concentration of the gel.**

It is possible to significantly lengthen the life of diabetic rats with subcutaneously implanted polyacrylamide (PA) gels containing insulin (1). In addition, PA implants bearing prostaglandin $F_{2\alpha}$ (2) and ethinyl estradiol (3) have been used to suppress fertility in female hamsters. As steroid and protein hormones were both effectively released from these polymer gel implants, it is apparent that they have application to a wide variety of substances. Implantation of protein hormones is noteworthy since previous methods involving compaction (4–6) or encapsulation by silicone rubber (7–9) have failed with polar substances. They either did not significantly prolong absorption (10, 11) or virtually blocked it (2). Another significant feature of polymer gel implants is that solute release can be changed by modifying gel porosity, which is subject to monomer concentration in the polymerization reaction mixture. The implants were not resorbed and they produced no observed bad effects among implanted animals.

The possibility of using polymer gels as drug delivery systems prompted this endeavor aimed at making their performance reasonably quantitative. A primary objective was to relate the release of a solute to its molecular weight and the concentration of polymer in an implant. Diffusion in polymer gels under *in vitro* conditions appears to be determined in a relatively simple way by these factors (12). In the present investigation, absorption of various radioactively labeled substances was observed from cylindrical PA and polyvinylpyrrolidone (PVP) implants placed under the skin of hamsters. Solute release from these polymer gel preparations was also established during incubation *in vitro*, for the purpose of comparison with absorption *in vivo*.

Abbreviations: PA, polyacrylamide; PVP, polyvinylpyrrolidone; BIS, *N,N'*-methylenebisacrylamide; LH, luteinizing hormone; IgG, immunoglobulin G; PG, prostaglandin.

MATERIALS AND METHODS

Solutes. Six solutes were used in this work; bovine serum albumin (Sigma), rabbit immunoglobulin (Nutritional Biochemicals), bovine pancreatic insulin (Sigma), and rat luteinizing hormone (L.E.R. 1056, prepared by Dr. L. Reichert), which were all iodinated, Na^{125}I (New England Nuclear Corp.), and [^{3}H]prostaglandin $F_{2\alpha}$ (7.5 Ci/mmole, New England Nuclear Corp.).

Iodination of Protein Solutes. Protein iodination was performed in the presence of chloroamine-T (13, 14) with Na^{125}I. The risk of protein denaturation by chloramine-T was reduced by stepwise addition of the oxidant (15). At each addition, 10–30 μl of chloramine-T (2 mg/ml) (Eastman) were introduced with a microcapillary pipette (Bolab) into 1 ml of buffer solution containing 0.2 g of protein per 100 ml and I^- ions (1.5 mole of I/mole of protein). ^{125}I uptake by protein was monitored during the reaction from trichloroacetic acid-precipitable radioactivity in 10-μl samples withdrawn about 2 min after each addition of choramine-T. When uptake ceased to follow oxidant addition, the reaction was stopped by lowering the redox potential with $Na_2S_2O_5$. Labeled proteins were recovered after gel filtration of the reaction mixture on a Sephadex-G75 (Pharmacia) column (1 × 18 cm), and they had the following specific activities: 34 μCi/mg, ^{125}I-labeled insulin; 12.4 μCi/mg, ^{125}I-labeled immunoglobulin G (IgG); and 3.2 μCi/mg, ^{125}I-labeled bovine serum albumin. Insulin, IgG, and bovine serum albumin were iodinated with Na^{125}I at a specific activity 0.375 mCi/μg. Phosphate-buffered saline, 10 mM, pH 7.8, was used in the preparation of ^{125}I-labeled bovine serum albumin, and 19 mM ethylenediaminetetraacetate buffer, pH 8.4, was used for preparation of ^{125}I-labeled insulin and ^{125}I-labeled IgG. ^{125}I-labeled luteinizing hormone (LH) (41.2 μCi/μg) was prepared with carrier free ^{125}I by the method of Greenwood *et al.* (14). (^{125}I-labeled LH used in these experiments was obtained from C. Robinson.)

Preparation of Implants. Polymer gels composed of PA and PVP crosslinked with 20% (w/w) methylenebisacrylamide (BIS) were used. Implants containing proteins were photopolymerized in 3-cm glass capillaries placed near a fluorescent lamp. The freshly prepared polymerization reaction mixture (pH 7.6) contained 3.8 mM *N,N,N',N'*-tetramethylethylenediamine (Eastman), 62 mM tris(hydroxymethyl) aminomethane (Canalco), 14 μM riboflavin (Nutritional Biochemicals) as catalyst, about 5 × 10^4 cpm/ml of ^{125}I-labeled solute suspended in 10 mM phosphate-buffered saline, recrystallized acrylamide (Eastman) or *N*-vinyl-2-pyrrolidone (GAF), at selected concentrations (g/100 ml), and BIS repre-

sented 20% (w/w) total monomer. After polymerization, the gels were removed from the capillaries and weighed. The polymer concentration was established from the gel dry weight after use. The cylindrical implants were 2.0-cm long and 0.15 cm in width, with wet weights around 50 mg. To avoid dehydration before use, we placed them in sealed containers. $Na^{125}I$ and [^{3}H]prostaglandin ([^{3}H]PG) containing gels were polymerized inside a silicone rubber tube (Silastic, no. 601-261, Dow) that was implanted together with the gel preparation to prevent radial diffusion.

In Vitro Incubation. Gels containing the radioactively labeled solutes were immersed in 1000 volume of 10 mM phosphate-buffered saline, pH 7.2, and maintained at 37° in a heated water bath subjected to moderate shaking with a Dubnoff incubator. At intervals during incubation the solvent was removed, and radioactivity in ^{125}I-containing gels was determined with a γ-ray counter. [^{3}H]PG-containing gels were liquified in an oxidizer (Packard) and assayed for radioactivity in a liquid scintillation counter (Packard), with a phosphor miscible with water.

Implantation into Hamsters. Mature female golden hamsters (*Mesocricetus auratus*) with a mean body weight of 182 g were obtained from a local breeder. The animals were kept at constant room temperature (21 ± 1°) and light (700–1900) and freely provided with Purina Chow and water. One implant was placed subcutaneously on the right side of each animal. Radioactivity in implants containing ^{125}I label was measured with the bearer placed inside a cylindrical, wire retaining cage (4.5 cm in diameter, 18-cm long) and positioned in front of the Geiger–Müller tube of a γ-ray counter (Nuclear Chicago). Screening of radioactivity by skin and implant was determined to be not significant. Correction for radioactivity in the general circulation was made after measurements at a body site remote from the implant. This was observed to be a relatively minor factor. The fraction of solute retained by an implant at a given time was estimated from the radioactivity that remained after correction for isotope decay. [^{3}H]PG-containing gels were removed from implanted animals at intervals, liquified in a tritium oxidizer, and assayed for radioactivity in a liquid scintillation counter.

Determination of Diffusion Coefficient. Radial diffusion in a long circular cylinder (radius = a) by a solute with a diffusion coefficient, D, can be expected to result in a fraction of the solute, q, being left in the cylinder that is given by the following equation obtained from Barrer (16):

$$q = \bar{C}/C_0 = \sum_{n=1}^{\infty} (4/j_n^2) \exp[-j_n^2\tau^2] \qquad [1]$$

where $\tau = \sqrt{Dt}/a$, $\bar{C}$ is the average concentration of solute in the cylinder at time, t, and C_0 is the initial concentration, and j_n represents the nth term in a Bessel series of the first kind and zero order. Boundary conditions for Eq. 1 require that solute concentration is initially uniform within the cylinder and that it remains negligible at the surface throughout release. Diffusion coefficients were obtained from a table (17) giving accurate values of τ for each q. Average diffusion coefficients with their standard errors were then calculated. Nonradial diffusion from the ends contributed about 5% to solute release from cylindrical implants. Diffusion coefficients in implants free to release solute only from the ends were determined by a similar procedure with the following equation for planar diffusion (16)

$$q = \sum_{n=0}^{\infty} (8/(2n+1)^2\pi^2) \exp[-(2n+1)^2\pi^2\tau^2] \qquad [2]$$

with $\tau = \sqrt{Dt}/2l$ and where l is half the implant length.

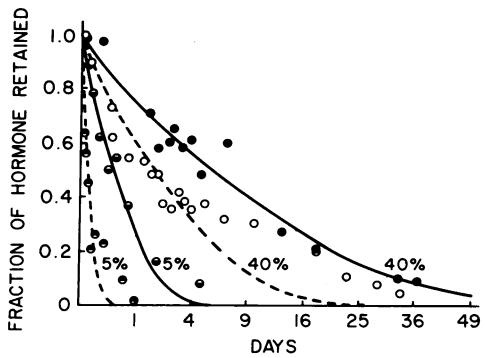

FIG. 1. Depletion of ^{125}I-labeled LH from 5% and 40% PA gels (20% crosslinked with BIS) during incubation in 10 mM phosphate-buffered saline, pH 7.2, at 37°, and implantation in mature female hamsters. The curves were drawn with a calculator (Hewlett Packard, model 9810 A) attached to an X-Y plotter, that was programmed to calculate solute depletion by radial diffusion from a cylinder (radius = 0.075 cm), using the average apparent diffusion coefficients obtained from the amount of solute retained by the gels at indicated times. *Continuous lines* denote implants, and *broken lines* are for depletion during incubation *in vitro*.

RESULTS

Release of ^{125}I-labeled LH from 5 and 40% PA gels that were subcutaneously implanted in hamsters or incubated in 10 mM phosphate-buffered saline pH 7.2, at 37°, is depicted in Fig. 1. The duration of hormone release is significantly longer in the dense gel. Comparable results were obtained, as anticipated, with the other substances studied in these experiments. Solute release followed an exponential time course until depletion was virtually complete. In view of this, crosslinking of the solute to polymer chains during polymerization does not appear to have been a significant factor. Parenthetically, preparation of the sample gel in disc electrophoresis (18) can involve polymerization of a PA gel in the presence of sample protein. Agreement between release data and theoretical curves (Fig. 1) indicates that the conditions assumed for diffusion in the polymer gels were reasonable. These conditions required that solute concentration in the implant be uniform initially and that during release, "back-diffusion" was negligible. It may be noticed in Fig. 1 that LH release was slower *in vivo* than during incubation in buffer. Comparatively fast release *in vitro* was also observed with the other solutes under study. Depletion of LH *in vitro* became retarded after 4 days in 40% PA (Fig. 1). Conceivably, the protein was subject to some denaturation after long intervals under the conditions used for incubation *in vitro*. A dependence of LH depletion on concentration seems ruled out by the performance of the other gel preparations.

The logarithm of the diffusion coefficients in Fig. 2 appears to be a linear function of polymer concentration. The results also indicate that diffusion by these solutes was slower *in vivo* than it was during incubation with 10 mM phosphate-buffered

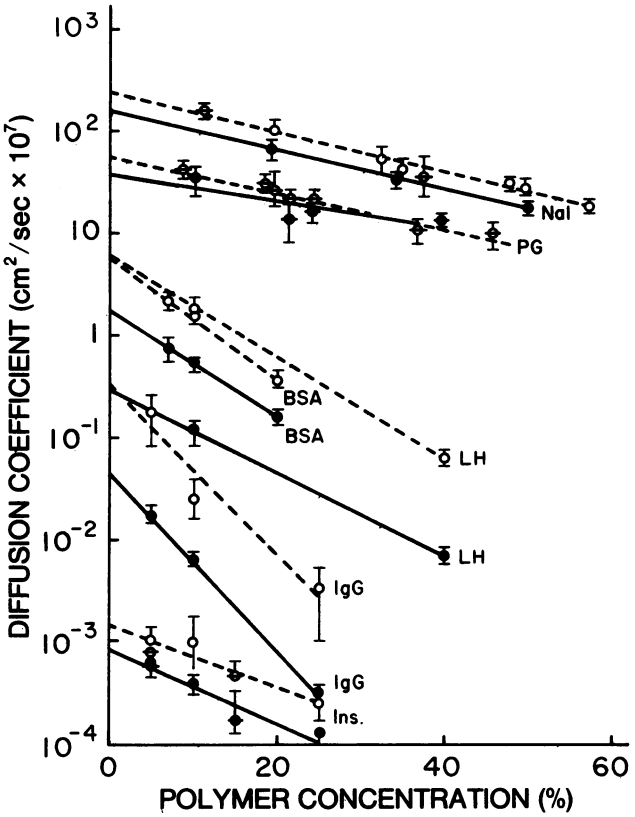

FIG. 2. Relationship between apparent diffusion coefficients for various substances and gel polymer concentration. Average diffusion coefficients, with standard errors, were determined after implantation in adult hamsters or incubation *in vitro* at 37°. *Continuous lines* with *solid circles* refer to implants; *broken lines* with *open circles* are for incubated gels. O denotes PA; ⊖ indicates PVP. *BSA*, bovine serum albumin; *Ins.*, insulin.

saline. Extrapolation of the regression lines given for PG in Fig. 2 to zero polymer concentration yielded diffusion coefficients (D_0) of 56 and 39 × 10^{-7} cm²/sec *in vitro* and *in vivo*, respectively. This difference is consistent with the relative viscosity of 1.40 found between serum and phosphate-buffered saline at 37° with a modified Ostwald viscometer. NaI had D_0 values of 236 and 149 × 10^{-7} cm²/sec, respectively, during *in vitro* and *in vivo* diffusion, and this variation could result from anticipated differences in solvent viscosity. The divergence between D_0 values for bovine serum albumin and LH seems too large, however, to attribute to solvent viscosity. Encapsulation of the gels during implantation and a relative lack of "solvent" supply may have been implicated. As noted already, LH release from 40% PA gels *in vitro*, unlike that during implantation, became significantly retarded after the half-time of the hormone deposit. Diffusion coefficients given for this preparation in Fig. 2 are from the early phase of release. The slow diffusion of ^{125}I-labeled insulin, apparent in Fig. 2, may have resulted from some absorption by the gel or from low solubility. Diffusion coefficients obtained for insulin and NaI implanted in PVP and PA gels revealed no significant difference between the polymers. Consequently, data from both sources have been pooled to calculate the linear regressions given in Fig. 2. Some of the substances used in this work are potentially antigenic to the hamster; however, this did not appear to influence their release.

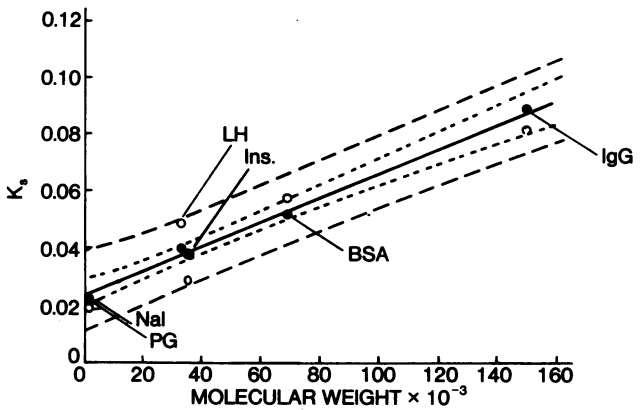

FIG. 3. A plot of K_S and solute molecular weight. O, K_S values determined *in vitro*; ●, values determined *in vivo*. The *solid line* is a least square regression involving both sets of K_S data and is expressed by the equation $\hat{Y} = 0.022 + 4.4 \times 10^{-7} X$. Molecular weights for these substances were: IgG, 150,000 (20); bovine serum albumin (BSA), 67,000 (20); LH, 33,000 (24); insulin (Ins.), 36,000 (20); PG, 354; and I (NaI), 125. Confidence limits ($P = 0.95$) for the standard curve (- - -) and individual observations (·— —) are given.

Results presented by Fig. 2 suggest ^{125}I-ions have a diffusion coefficient (D_0) in aqueous solution of 236 × 10^{-7} cm²/sec at 37°; a D_0 of 254 × 10^{-7} cm²/sec for I^- ions has been published (19). In this study, D_0 was 5.4 × 10^{-7} cm²/sec for bovine serum albumin; it has been reported (20) as 5.9 to 6.0 × 10^{-7} cm²/sec in aqueous solution at 20°. Rat LH had a value of 5.9 × 10^{-7} cm²/sec in Fig. 2; using gel filtration chromatography, Reichert *et al.* (21) reported it is 7.62 and 7.75 × 10^{-7} cm²/sec at 20° with human and bovine LH, respectively. $PGF_{2\alpha}$ had D_0 equal to 56 × 10^{-7} cm²/sec in phosphate-buffered saline; it may be anticipated to have a value about 47 × 10^{-7} cm²/sec from the Einstein–Stokes equation (22), given that the molecule has a Stokes radius of 0.48 nm.

From Fig. 2 it appears the diffusion coefficient D_P in a gel containing P% polymer is related to its diffusion coefficient (D_0) in solvent by the expression,

$$\log D_P = \log D_0 - K_S P \quad [3]$$

K_S is comparable to a retardation coefficient (12, 23) and it may be defined analogously; $K_S = -d \log D_P/dP$. Results given in Fig. 3 show K_S increased linearly with solute molecular weight (M) according to the following equation,

$$K_S = 0.022 + 4.4 \times 10^{-7} M \quad [4]$$

Differences between K_S values determined *in vitro* and *in vivo* were within experimental error, and both were used to derive Eq. 4. Combining Eqs. 3 and 4 gives the following empirical equation,

$$D_P = D_0 \exp [-(0.05 + 10^{-6} M) P] \quad [5]$$

According to this result, the absorption of a solute is determined by its molecular weight, diffusion coefficient in solvent, and the polymer concentration of the gel.

DISCUSSION

Absorption from subcutaneous PA and PVP implants was shown to depend on polymer concentration and solute molecular weight. This is consistent with previous findings (12, 23, 25–28) concerning solute behavior in polymer gels during

chromatography and electrophoresis. Both the rate of uptake by implanted hamsters and release during incubation in phosphate-buffered saline decreased logarithmically with increases in polymer concentration for the six substances used in these experiments. A similar relationship was established *in vitro* by White and Dorion (29), who determined diffusion coefficients for four small solutes (molecular weight < 400) in PA gels with various polymer densities. Present observations extend this finding to solutes ranging in molecular weight from 148 to 150,000 for diffusion under conditions both *in vitro* and *in vivo*.

The logarithmic relationship observed between absorption and polymer concentration would be anticipated from Ogston's expression for porosity in polymer networks (30). In this regard, Eq. **5** can be redefined with relatively little change in complexity and mathematical accuracy in order that its exponential term becomes equivalent to an expression for porosity (porosity being defined by the space available for collision-free diffusion). For these highly crosslinked gels (O-D type, 12), K_S may be appropriately expressed from the data in Fig. 3 as follows:

$$K_S^{1/3} = 0.05\ (\bar{R} + 5.0) \qquad [6]$$

where $\bar{R}$ (geometric mean radius of solute) = $(0.75\,M\bar{v}/\pi N)^{1/3}$, M is molecular weight, $\bar{v}$ (partial specific volume) = 0.74 cm^3/g, and N is Avogadro's number. Eq. **6** indicates that polymer chains in the gels used were clustered with a mean fiber cross-sectional radius of about 5.0 nm. The value expected for PA fiber radius (r) with Fawcett and Morris' empirical relationship (26) ($r = 0.5 + 0.1C$) is 2.5 nm, as crosslinking (C) was 20%. In 15% crosslinked PA gels they reported the fiber radius was 3 nm, which exceeds by 50% the anticipated value, and they point out, moreover, the relationship applies well only when the extent of crosslinking is below 10%. Analogy with studies of solute behavior during chromatography and electrophoresis (12) suggests, furthermore, the rate of change in the number of PA "points"/nm^3 (n) relative to PA concentration (P) is related to the coefficient 0.05 in Eq. **6**. $dn/dP = (0.05)^3/(4\pi/3)$. Combining Eqs. **3** and **6** yields

$$D_P = D_0 \exp[-0.0003(\bar{R} + 5.0)^3 P] \qquad [7]$$

Eq. **7** states that the diffusion coefficient of a substance in a polymer gel is given by the product of its diffusion coefficient in solvent and a Poisson term giving the probability of unhindered diffusion. The relationship is apparently unconstrained by solute shape (12). Apart from modifications in the exponent that depend on interchain crosslinking (26, 28, 31, 32), Eq. **7** evidently applies generally to solute diffusion in polymers gels.

The duration of drug delivery from implants complying with Eq. **7** can be expected to rise sharply at high polymer concentrations. To prepare implants with long delivery schedules, it is consequently necessary to control their polymer content carefully, and the burden of this requirement increases with the length of the desired term of administration. Absorption followed an exponential time course with these implants, and solute delivery rate characteristically was relatively rapid and ever-decreasing during the first half-time of uptake for an implanted substance. After this time, the release rate was relatively more uniform. Two possible approaches to securing more uniform delivery are preincubation until the initial rapid release period has elapsed and preparation of implants possessing a concentration gradient that furnishes the desired pattern of delivery.

The application of these polymer gel implants can be extended from insulin supplementation during diabetes (1) and fertility control (2, 3), and they may merit consideration, for example, in cancer therapy, treatment with narcotics antagonists, radiation protection, and disorders involving certain metabolic deficiencies.

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