Our results show that cytochemical methods can be used to label the faces produced by freeze-fracturing of cells and tissues. They confirm our view that important surface groups of biological membranes (those with WGA and concanavalin A binding sites) may partition, during fracture, with the protoplasmic half of the membrane (*4*). We believe that these groups are part of integral membrane-transversing proteins that are dragged from the outer surface across the exoplasmic half of the membrane. In addition, splitting of the bilayer membrane continuum renders accessible for cytochemical labeling other groups associated at the outer surface with components that, upon fracture, partition with the exoplasmic half: lipid molecules of the outer half of the bilayer, peripheral membrane proteins at the outer surface, and putative integral proteins associated with the exoplasmic half. In consequence, fracture-labeling in its two forms (thin section and critical point drying) appears to provide a method for the identification of surface sites (including antigens and receptors) associated with transmembrane molecules or oligomeric complexes, as well as for the structural and cytochemical dissection of plasma and intracellular membranes.

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Serum Albumin Beads: An Injectable, Biodegradable System for the Sustained Release of Drugs

Abstract. *Biologically active compounds were entrapped in cross-linked serum albumin microbeads. Injection of these drug-impregnated beads into rabbits produced no adverse immunological reactions. Sustained release (20 days) of progesterone was demonstrated in vivo.*

The formulation of a device for controlled release of biologically active substances has been the goal of many researchers (*1*). For injectable preparations, it is advantageous to use for the matrix a material that can be assimilated. The matrix should not produce adverse immunological reactions, and the matrix material should be readily available and relatively inexpensive. Albumin is such a material; its concentration in the serum of higher mammals is high, 40 to 50 mg/ml, and it can be prepared from outdated blood by well-known fractionation methods (*2*). Injectable beads prepared from albumin under mild conditions should yield a nonimmunogenic, biodegradable device for drug delivery. Since native serum albumin binds many drugs strongly (*3*), this binding would in itself retard drug release from an injection site until the albumin is degraded by proteolytic enzymes. We used albumin beads prepared by chemical cross-linking of the protein as a device for the controlled release of progesterone in rabbits; there was no adverse immunological response (*4*, *5*).

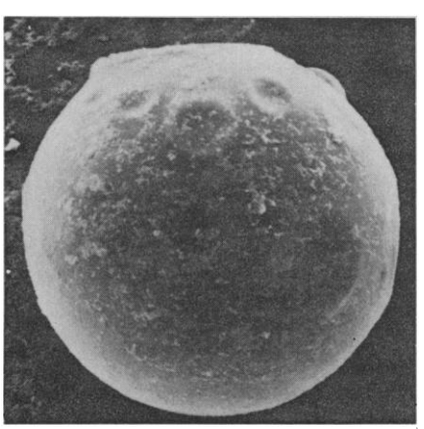

Fig. 1. A scanning electron micrograph of a norgestrel–serum albumin bead. The rough surface of the bead may be due to loosely adsorbed steroid or to abrasion of the polymer during stirring.

Progesterone (10 mg) was suspended in 0.8 ml of sodium phosphate buffer (1 m*M*, *p*H 7.5) containing sodium dodecyl sulfate (0.1 percent). Bovine serum albumin (200 mg) was then dissolved in the suspension and kept at 4°C. Polymerization was initiated by the addition of 0.2 ml of glutaraldehyde, making the final concentration 1 percent. The system was rapidly mixed, pipetted into 100 ml of an oil phase (corn oil and petroleum ether, 1:4 by volume), and stirred at room temperature. A water-in-oil emulsion formed. Although cross-linking of the protein in the emulsified droplets is complete in 10 minutes, the reaction mixture was stirred continuously for 1 hour before the oil phase was decanted. The resulting beads were washed three times with petroleum ether and dried in a vacuum desiccator. The size of the beads depends on the speed of stirring; the procedure outlined above consistently yielded beads with diameters of 100 to 200 μm (Fig. 1). Light microscopy at 100 times magnification revealed symmetrical beads with hormone crystals embedded in the matrix. The beads retained drug even after extensive washing with aqueous buffer solutions to get rid of excess reagents, as proved by release experiments in vitro. Drug content per milligram of beads can be controlled by varying the drug concentration in the reaction mixture. Beads with steroid concentrations of 5 to 30 percent were prepared in this manner.

For experiments in vivo, beads were prepared as described above, except that progesterone was entrapped in a matrix of serum albumin from rabbits. The steroid content was 20 percent of the bead weight. To facilitate the injection pro-

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cess, the beads were made smaller by increasing the stirring speed (1650 rev/min) during emulsification. The resulting beads were about 100 μm in diameter. Male rabbits (N = 3) were given intramuscular or subcutaneous injections of 70 mg of unwashed beads suspended in 3 ml of corn oil; an 18-gauge needle was used for the injection. The beads were not washed with aqueous solutions because we wanted to examine the applicability of this device in the simplest form. A control animal was injected with 10 mg of progesterone suspended in oil. Blood samples at various times were obtained by cardiac puncture, and the serum progesterone level was quantified by radioimmunoassay (*6*).

No significant fluctuations of body temperature or weight of the rabbits were noted during the experiment, and no inflammation or necrosis was observed at the injection site. Rabbits that had received injections for several months did not show any adverse immunological symptoms. One rabbit was killed 6 days after a subcutaneous injection, and dissection of the injection site revealed a pellet-like implant with intact beads; extraction of the recovered beads showed that progesterone was present. Examination of an intramuscular injection site after 2 months confirmed that the beads had biodegraded; the tissue looked normal and no beads were found.

Release experiments in vivo (Fig. 2) showed that the cross-linked protein matrix was able to sustain the serum progesterone level at about 1 ng/ml for about 20 days. The initial burst probably resulted from the release of surface progesterone in the preparation. In controls (data not shown), all of the unentrapped progesterone was released into the system in 1 day. The near zero rate of release from the matrix probably resulted from both the degradation of the protein matrix by intrinsic proteolytic enzymes and the diffusion of progesterone from the matrix. The drug was released at similar, predictable rates from the intramuscular and subcutaneous sites of implantation.

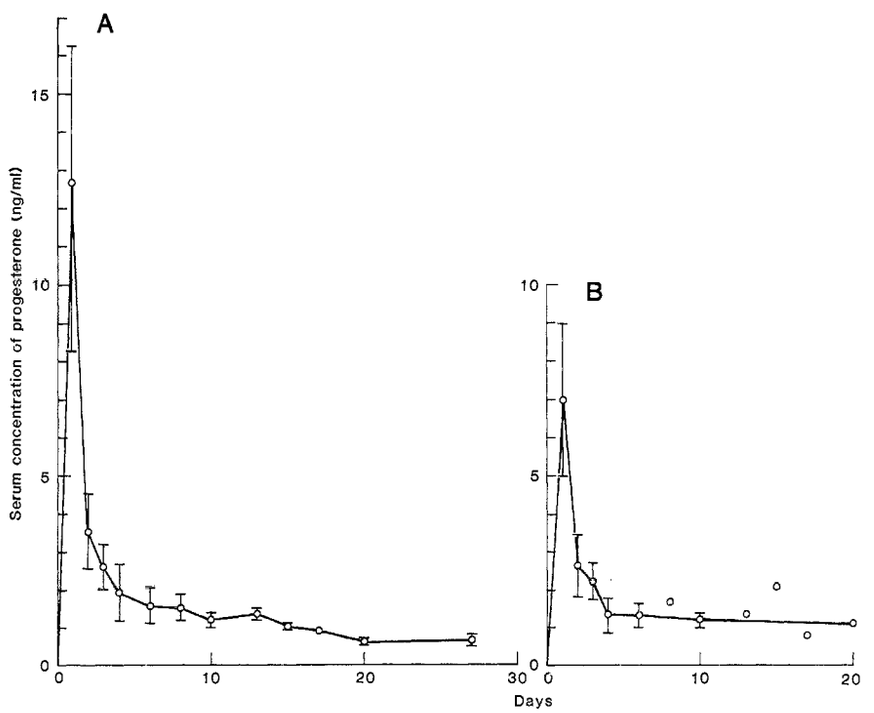
Analysis of the release of steroid hormones from serum albumin beads in vitro indicated that the density of the matrix significantly affected the rate of release. In one experiment, four batches of norgestrel–bovine serum albumin beads were prepared, with final concentrations of glutaraldehyde of 1, 2, 3, and 4 percent. Since the amount of drug per gram of beads was the same, the release rates could be compared. Ten milligrams of beads were suspended in 10 ml of 40 percent polyethylene glycol 300. The tubes were agitated by head-to-head rotation at room temperature. At different time intervals, the amount of norgestrel in polyethylene glycol solution was quantified spectrophotometrically at 245 nm. The ultraviolet absorption spectra of the released material and authentic norgestrel were identical.

An equation that applies to a release process in which diffusion through a growing path length is the rate-limiting step (*7*) is

$$Q = \sqrt{D_m(2A - C_s)C_p t}$$

where Q is the cumulative amount of drug per unit area of device that is released; A is the total amount of drug contained in a unit volume of device; C_s and C_p are the solubilities of the drug in the elution medium and the polymer phase, respectively; t is the time; and D_m is the effective diffusivity of the drug species, which most likely is a function of the density of the matrix. In our experiments, A, C_s, C_p, and the effective area of the device were held constant. If the model holds, the cumulative amount of drug released should be proportional to the square root of time, with the slope dependent on D_m or the density of the matrix. A plot of Q versus $t^{1/2}$ is linear (Fig. 3). The initial lag time for the wetting of the beads and for breakthrough of the diffusional barrier was expected. A decrease in release rate with an increase in the concentration of cross-linking reagent is observed.

As further proof that the compactness of the matrix is a function of cross-



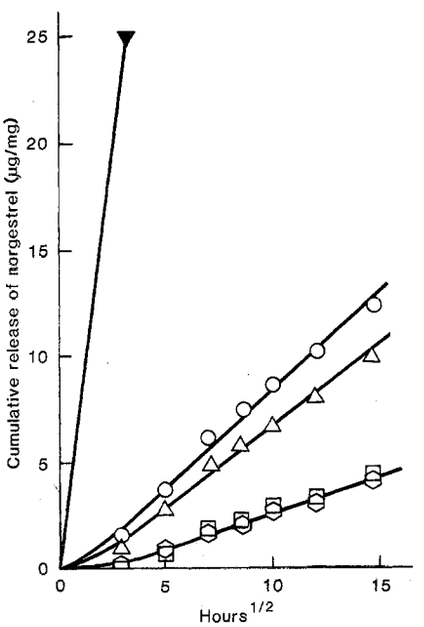


Fig. 2 (left). The daily serum concentration of progesterone in rabbits after (A) intramuscular or (B) subcutaneous injection of rabbit serum albumin-progesterone beads. Values are means ± standard deviations. Fig. 3 (right). The effect of concentration of the cross-linking agent, glutaraldehyde, on the rate of release of norgestrel from bovine serum albumin beads, expressed in micrograms of norgestrel per milligram of beads. Symbols: ▼, unentrapped norgestrel; ○, 1 percent glutaraldehyde–cross-linked beads; △, 2 percent glutaraldehyde–cross-linked beads; ⬡, 3 percent and □, 4 percent glutaraldehyde–cross-linked beads. The latter two have a similar rate, as expected, since the number of lysine residues modified do not differ significantly (refer to text).

linking, we hydrolyzed the beads (6*N* HCl, for 24 hours at 110°C) and examined the resulting amino acid composition. A progressive increase in the number of modified lysine residues (21, 33, 44, and 47) was found as the concentration of glutaraldehyde was increased (1, 2, 3, and 4 percent, respectively). Although the rate of release could be controlled by varying the density of the matrix, only the 1 percent glutaraldehyde–cross-linked beads were susceptible to chymotrypsin digestion, an indication of their biodegradability. The other three systems were resistant to proteolytic destabilization even after prolonged incubation (days) with fresh enzyme. However, even with the lowest concentration of glutaraldehyde, the rate of release is at least ten times slower than that of unentrapped drug.

A wide range of compounds with different chemical and physical properties can be entrapped in the serum albumin matrix. These include water-soluble dyes (for example, methyl orange); morphine and its antagonist, naloxone; peptides; and proteins. This versatility stems from the mild conditions required for formation of beads under which most biologically active molecules are stable.

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Brain Tumors in Children and Occupational Exposure of Parents

Abstract. *Ninety-two cases of brain tumor in children less than 10 years old were compared with 92 matched controls for parental occupational history. Cases were more likely than controls to show maternal occupations involving chemical exposure, paternal occupations involving solvents, and employment of father in the aircraft industry. These three factors were not affected by adjustment for the potential confounding variables examined in this study.*

The issue of whether occupational exposures of parents cause malignant disease in offspring has been gaining attention. The results of five studies (*1–5*) that we reviewed, in which different populations, different techniques, and different age groups were used, are not consistent.

We investigated the possibility that parents of children with brain tumors are more likely to have had occupational exposure to chemicals than are parents of controls. Cases of brain tumors in children less than 10 years of age at diagnosis, identified from 1972 to 1977 by the Los Angeles County Cancer Surveillance Program (CSP) (*6*), were selected for study. A control was identified by an algorithm that matched patients by sex, race, and year of birth (within 3 years). Social class was matched by first attempting to locate a control among friends of the case (57 such controls were found) and, if no friend was available, by selecting a matching neighbor (35 controls). Mothers of 98 cases (84 percent of available mothers) and 92 controls (86 percent of first-match friends and 80 percent of first-match neighbors) were interviewed by telephone. The 92 matching pairs were analyzed.

Information sought about the mother included the work history during the year before pregnancy, during the three trimesters of pregnancy, during nursing, and at the time of diagnosis of the case. This included information on job title, actual job, name of employer, and what the company made or did. Other questions concerned whether protective equipment or clothing was used; whether chemical solvents, dust, or other fumes were inhaled; whether chemicals, solvents, oils, dust, and so forth, got on clothes or skin; and whether radiation or radioactive material was used. Each mother was asked similar questions about the job experiences of the child's father before conception, during the pregnancy, and at the time of diagnosis of the case. Information was also collected about the mother's smoking habits and use of drugs, alcohol, hair dyes, and certain foods during the pregnancy. Statistical analysis was done by standard matched-pair methods (*7–8*); pairs in which either the mother of a case or the control mother did not answer the relevant question were eliminated. All statistical significance levels are based on one-sided tests.

Questions about chemical exposure were more often answered in the affirmative by mothers of cases than of controls (Table 1). Mothers of cases reported skin exposure to chemicals more than three times as frequently as mothers of controls—relative risk (*RR*) 3.3—and that they inhaled chemicals, fumes, and dusts more often (*RR* = 3.0). To pursue the question of fathers' exposure to chemicals, all jobs and exposures mentioned for fathers of cases and of controls were listed and enumerated. Exposure to solvents (*RR* = 2.8), paints in particular (*RR* = 7.0), was more frequent for fa-

Table 1. Matched-pair comparison of parental occupational exposure of cases and controls. The mother was considered to have been exposed if exposed at any time from 1 year before conception through lactation. The father was considered to have been exposed if exposed during that period or at the time of diagnosis of a case.

Factor	Concordant pairs: both exposed	Discordant pairs		Relative risk	One-sided test (*P*)
		Cases exposed	Controls exposed		
	Mother				
Got chemicals on skin	0	10	3	3.3	.05
Inhaled chemicals or fumes	1	12	4	3.0	.04
One or both of the above	1	14	5	2.8	.03
	Father				
Exposed to chemical solvents	3	17	6	2.8	.02
Exposed to paints	0	7	1	7.0	.04
Worked in aircraft industry	2	10	0	∞	.001

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