depressor response to dopamine with propranolol alone. In contrast, Day & Blower (1975) reported no antagonism with propranolol but dose-related reductions in the depressor response to dopamine with metoclopramide. Wardell et al (1979) showed that although the dopamine receptor antagonists bulbocapnine and metoclopramide exerted a slight inhibition of the dopamine-induced depressor response, this blockade could be explained by the weak β-receptor antagonist activity of these agents. They concluded, in agreement with Blackwell & Marley (1967), that stimulation of β-receptors is primarily responsible for dopamine's depressor response in phenoxybenzamine-pretreated normotensive rats. Chapman et al (1980) by using sulpiride, a more selective dopaminergic antagonist, concluded that the depressor response to dopamine in normotensive rats is partly due to an action on β-receptors and partly due to an action on specific dopamine receptors.

Our results in the SHR although with small numbers of animals were in the same direction and to the same degree among animals and show that as in the normotensive rat, dopamine's pressor response is due to an α-receptor stimulation, and that the depressor response after α-receptor blockade is caused by an action on both β-adrenergic and dopamine receptors. The more effective blockade by propranolol vis-a-vis sulpiride suggests that the dopamine induced depressor response is primarily a result of β-receptor stimulation. Thus, the SHR may not be the ideal model in which to explore selective dopamine agonists.

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Reconstituted collagen nanoparticles, a novel drug carrier delivery system

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Recent colloidal drug delivery systems (Marty et al 1978; Kreuter 1978; El-Samaligy & Rohdewald 1982) have shown promise for optimal drug activity. Cross-linked coacervated gelatin and human serum albumin systems have been used as carriers for carcinogenic agents (Oppenheim & Stewarts 1979) and flukicides (Marty 1977). Gelatin, even in extremely dilute solutions, is capable of aggregate formation (Boedtker & Doty 1954; Engel 1962). This aggregation is temperature-dependent at all temperatures below the equilibrium melting point. The aggregate size, however, is dependent not only on temperature, but also on the process thermal path. It was concluded by Boedtker & Doty (1954) and Beyer (1954) that the crystallites in the gel aggregates appear as multiple chain segments in the collagen-fold configuration.

Such a property has been used to prepare aggregates in the nanometer range to act as colloidal drug-delivery carriers. The nanoparticles have also been investigated for their particle size distribution, biodegradability and their drug-holding capacity. The in-vitro release of the sorbed drugs from such nanoparticle products was also studied.

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Methods

Materials. Gelatin, type II from swine skin (IEP 5.6): adriamycin and dactinomycin (Sigma Chemical Co., W. Germany), triamcinolone diacetate (Cyanamide GmbH, W. Germany), Tween 20 (Atlas Chemical Industries Inc., W. Germany). Sephadex G-25m, and Sephadex G-10f (Pharmacia Fine Chemicals, Sweden).

Methods. To determine the pH most suitable for the preparation of the colloidal nanoparticle system, 0·25% aqueous gelatin solution containing 0·5% Tween 20 was prepared and adjusted to different pH values in the range 3–9 using ammonia or HCl (to avoid the effect of ionic strength of buffer solution). The solutions were left at 25 °C for 48 h. The colloidal solutions obtained were examined for colloidal stability and particle size distribution at 5 day intervals for one month.

Accordingly the nanoparticles were prepared by dissolving 0·25 g gelatin and 0·5 g Tween 20 in 100 ml of bidistilled water, adjusting the solution to the optimum pH determined using dilute HCl solution, then centrifuging the solution at 14 000 rev min^{-1} for 15 min. The clear solution was heated at 40 °C for 1 h, quenched to 4 °C for one day, and then left at 25 °C for 48 h. The

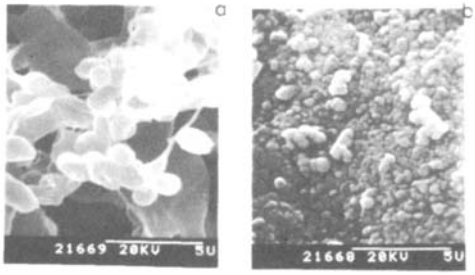

FIG. 1. Scanning electron micrographs of reconstituted collagen nanoparticles, (a) before purification, (b) after purification through gel filtration technique.

colloidal system was filtered through a sintered glass filter G3, and 2 ml glutaraldehyde was added with continuous stirring at 1200 rev min^{-1} at 35 °C. Cross-linking was stopped after 10 min by the addition of 2 ml of 12% sodium metabisulphite solution. The crude colloidal system was purified by passing it through 200 ml of Sephadex G 25m column and then through 100 ml of Sephadex G 10f column to separate the electrolytes and the excess macromolecules present. The colloidal solution was then freeze-dried overnight.

Particle size measurements and surface inspections of the nanoparticles were carried out from electron micrographs. Specimens for scanning were prepared by spraying the nanoparticle colloidal suspensions onto glass slides, drying under vacuum, then coating with carbon-gold layer (50 nm thick) under vacuum.

Biodegradability testing of the nanoparticle products was done on a colloidal solution (10 mg ml^{-1}) in phosphate buffer of pH 7·2 containing 1 mg percent of either trypsin or collaginase or an equal mixture of both. The biodegradation was measured turbidimetrically as the percentage light transmission.

Drug uptake by the nanoparticles could be measured through its adsorptive capacity for different drugs. Adsorption isotherms were determined for adriamycin and dactinomycin in buffer solutions of pH 7·2, and for triamcinolone diacetate in the same buffer containing 1% Tween 20, to solubilize the steroid.

Drug release was carried out using 250 mg of the ultrafiltered nanoparticles after equilibrium with the drug (drug adsorbed amounted to 7·5 mg/250 mg solid nanoparticle matter). The nanoparticle product was redispersed in 900 ml of phosphate buffer of pH 7·2 in a glass beaker, continuously stirred at 50 rev min^{-1} and incubated at 37 °C in a thermostatted water bath. Five ml samples were withdrawn at definite time intervals to be analysed after ultracentrifugation. The samples withdrawn were replaced by 5 ml of fresh phosphate buffer solution.

Table 1. Biodegradability of reconstituted collagen nanoparticles through turbidimetric measurements of their colloidal solutions containing trypsin or collaginase enzymes.

	Percentage light transmission of colloidal nanoparticle dispersions containing:			
Time (h)	—	1 mg % trypsin	1 mg % collaginase	0·5 mg % trypsin + 0·5 mg % collaginase
0·0	21·0	20·8	5·0	11·7
1·0	21·6	24·8	7·2	13·9
2·0	22·1	28·6	8·8	15·8
3·0	22·4	31·5	10·4	17·1
5·0	23·1	37·7	13·5	19·2
10·0	25·0	49·3	20·5	28·3
First-Order Clarity Rate Constant (K)	0·0048	0·0443	0·0175	0·0202

Results and discussion

The optimal pH range for aggregate formation in the nanometer range was found to be 5·5–6·5. The gelatin solutions adjusted at lower pH range produced no aggregates or very dilute opalescent solutions, while those of higher pH led to thready products which precipitated on storage. The colloidal nanoparticle solution prepared accordingly, remained stable for two months without sign of aggregation or agglomeration. The electron micrographs of the nanoparticle suspension, before purification through gel filtration techniques, showed the spherical particles distributed through the non-aggregated gelatin net-work (Fig. 1a). After purification, the nanoparticle populations appeared as almost perfect spheres with nearly smooth surfaces. It was found that the measured particle size diameters of the purified products was in the range of 180–300 nm, with a mean value of 232 ± 58 nm (Fig. 1b).

Biodegradability investigations showed that the nanoparticle colloidal solutions clarified more rapidly in the

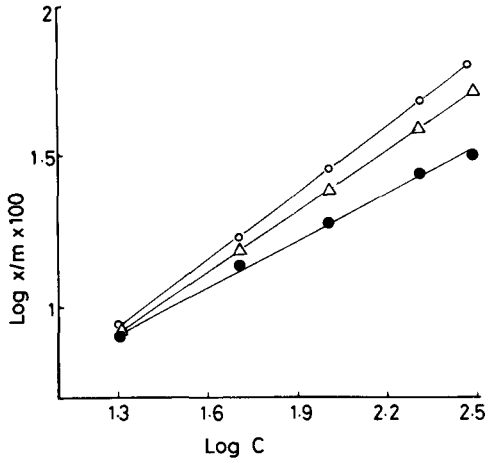

FIG. 2. Plots of the adsorption isotherms of adriamycin, dactinomycin, and triamcinolone diacetate onto reconstituted collagen nanoparticles at 25 °C; (○) adriamycin, (△) dactionomycin, (□) triamcinolone diacetate. C in μg ml^{-1} and x/m in μg drug mg^{-1} nanoparticles.

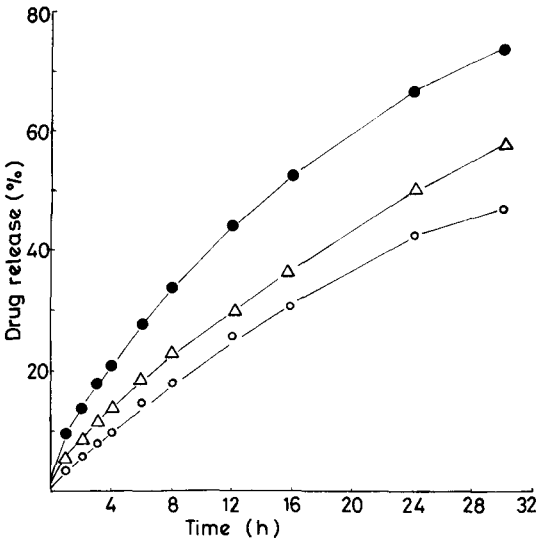

FIG. 3. Release patterns of adriamycin, dactinomycin, and triamcinolone diacetate from their reconstituted collagen nanoparticle sorbates in phosphate buffer (pH 7·2) at 37 °C; (○) adriamycin, (△) dactinomycin, (●) triamcinolone diacetate.

presence of trypsin than those containing collaginase or a mixture of the two enzymes, in relation to the control buffer solution of the nanoparticles (Table 1). Kinetic calculations, using the least square method, showed that the mean light transmission values as a function of time acquired the percentage coefficients of variation of 1·7734 and 0·0675 according to zero- and first-order rate pattern respectively. This finding illustrated that the colloidal solutions acquired a first-order rate pathway, with the clarity rate constants (K) shown in the Table. The clarity rate constant values verified the sequence of decreased clarity in the presence of trypsin, trypsin/collaginase mixture, and lastly collaginase. The data illustrated that the product could be a partially reconstituted collagen/cross linked gelatin mixture. Such enzyme-dependent degradation illustrates the suitability of the products as targeting drug carriers releasing their drug contents in the body tissues through the digesting lysosomal systems of tissue cells.

Fig. 2 shows the adsorption isotherms of the drugs tested on the colloidal nanoparticles. It is obvious that the colloidal nanoparticles have a high adsorptive capacity towards these drugs. Adriamycin showed the highest adsorptive affinity, followed by dactinomycin, and then triamcinolone diacetate from its surfactant solutions. Taking the water solubility of the drugs as a measure for their polarity, the more polar the drug the higher is its adsorptive affinity to the nanoparticle colloids. Such findings might give an indication that the nanoparticle surfaces acquired semipolar characteristics with a slight tendency towards hydrophilicity. In comparison with the drug uptake capacities of either liposomes or polyalkylcyanoacrylate nanoparticles (Kante et al 1980), partially reconstituted collagen nanoparticles show a high adsorptive capacity especially from dilute drug solutions.

The release of the drugs from the nanoparticle adsorbates showed that they leached in sustained patterns (Fig. 3), the release rates going parallel with what has been found in the adsorption investigation. Triamcinolone diacetate was liberated at the highest rate, followed by dactinomycin and then adriamycin which had the least release pattern. Kinetically it was found that the release of the tested medicaments followed an apparent first-order pathway. The first-order rate constants were found to be 0·02145, 0·02733, and 0·04226 h^{-1} for adriamycin, dactinomycin, and triamcinolone diacetate respectively. The differences in the release rate constants reflect the decreased affinities of the medicaments to the nanoparticle polymer colloid in the order stated. The final ultracentrifuge deposits left after the sample treatment decreased gradually with time, finally in the 30 h sample reaching about 56% (calculated as a function of the deposit left after ultracentrifugation of the corresponding freshly prepared nanoparticle colloid). This last finding may indicate that some biodegradation of the nanoparticle polymer has occurred even in simple phosphate buffer. No correlation was observed between amounts of drug released and polymer biodegradability, since the deposit loss was equally observed during the release of the three medicaments.

In conclusion, it is evident that reconstituted collagen nanoparticles can be prepared with controlled particle size. Through their high surface area and high adsorptive capacity of the drugs tested, the nanoparticles showed that they have a promising potential as a colloidal drug delivery system. Furthermore, the safe parenteral utility of such proteins and the highly retarded drug release suggested the usefulness of this system as a depot injectable dosage form.

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