Synthesis and Evaluation of Some Bioadhesive Hydrogels

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In order to produce an oral drug delivery system that provides drug in a sustained and controlled manner for extended period of time, it is first necessary to overcome the usual prohibitive short gastrointestinal(GI) transit time. Several approaches have been suggested to delay GI transit of oral dosage forms. Recently the use of bioadhesive polymers for drug delivery has been suggested for the purpose of prolonging GI transit time of the drug delivery system, localization and intimate contact of drug with the absorbing membrane.

In a previous study, polycarbophil, a loosely crosslinked polyacrylic acid polymer, showed tenacious binding to cultured epithelial cell surfaces in vitro. In vivo, the use of polycarbophil showed improved bioavailability of the steroid fluorometholone in the rabbit eye when the drug was incorporated into the polymer. Also greatly increased bioavailability of chlorothiazide was observed in the rat when a capsule of polycarbophil, containing chlorothiazide in crosslinked albumin beads was surgically inserted into the rat stomach. Thus, an investigation of the bioadhesion of polycarbophil and several structurally related compounds, has been carried out for the purpose of gaining insight as to structural requirements for bioadhesion and secondarily to identify good bioadhesive synthetic polymer which might have

potential for oral bioadhesive drug delivery systems.

A series of lightly crosslinked, swellable, polymers were synthesized from monomers such as acrylic acid, methacrylic acid, and others, employing different crosslinking agents, to produce a range of polymers differing in charge density and hydrophobicity. Some of their physical properties were determined such as densities and apparent rate and extent of hydration. An increase in the number of hydrophobic groups in the polymer structure reduced hydration whereas density of the polymer was unaffected.

In order to characterize the polymers in terms of bioadhesiveness, a sensitive in vitro method, for measuring adhesion of polymer to tissue from the rabbit stomach, was developed. Polymers of acrylic acid loosely crosslinked (0.3 % w/w) with three different agents, divinyl glycol, 2,5-dimethyl-1,5-hexadiene, and divinyl benzene showed the same degree of bioadhesion while poly(methacrylic acid-divinyl benzene) showed reduced bioadhesion. The small percent of crosslinking agent, irrespective of physicochemical properties, did not seem to contribute substantially to bioadhesion, whereas the starting monomer had a large effect. The effect of pH on bioadhesion of poly(acrylic acid-divinyl glycol) was studied at constant temperature, ionic strength, and osmolality. The polymer showed maximum adhesion at pH 5 and 6 and a minimum at pH 7.

GI transit studies of crosslinked polymers in rats were conducted. Poly(acrylic acid-divinyl glycol) and poly(methacrylic acid-divinyl benzene) were shown to have substantially longer transit times than the control,

Amberlite 200 resin beads. The delay in transit time is due to bioadhesion of the polymer to the mucin/epithelial cell surface, which is clearly observable on animal autopsy. The acrylic acid polymer showed a longer GI transit time than the methacrylic acid polymer, and this in vivo GI transit result is consistent with in vitro bioadhesion test results.

Approved_____

Date_____

SYNTHESIS AND EVALUATION OF SOME BIOADHESIVE HYDROGELS

BY

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I. INTRODUCTION

A. Background

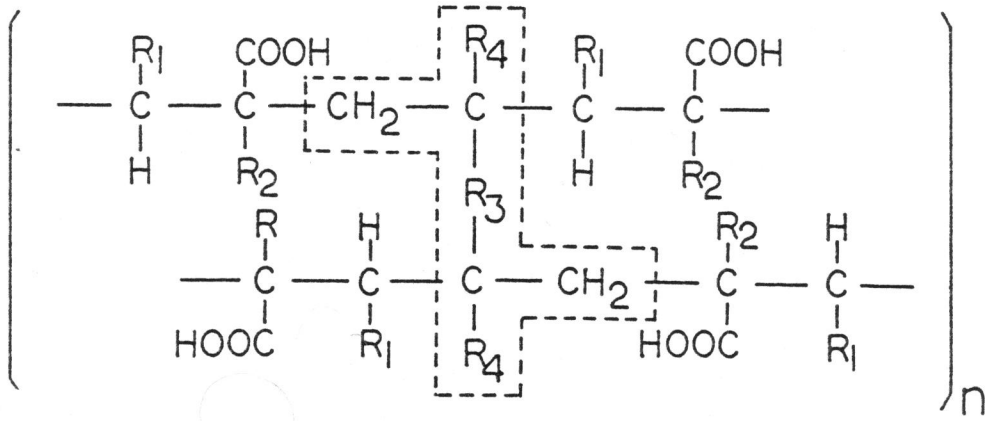
A significant limitation on oral controlled release drug delivery systems is gastrointestinal (GI) transit. The normal movement of solid and liquid dosage forms through various segments of the GI tract precludes specific localization of drug-delivery systems in selected regions of the GI tract. Moreover, the relatively brief GI transit time in humans, estimated to be about 8-10 hours from mouth to colon with considerable fluctuations in individual times, is a significant impediment in preparing a once daily dosage form for most drugs. It would be desirable to have a 24-hour transit time, to minimize patient compliance problems and maximize drug therapy, for the majority of controlled release systems and perhaps have a 3-4-day transit time for special conditions. Control of the placement of a drug delivery system in a specified region of the GI tract would give numerous potential advantages. Naturally, for drugs with a window for absorption, or perhaps for stability reasons, the ability to localize offers a means to improve drug absorption. Similarly, intimate contact of the drug delivery system with the absorbing membrane, with the potential to not only maximize total drug absorption but to also influence the rate of drug absorption, is a significant feature.

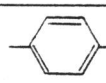
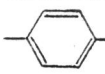
Several approaches have been reported as ways to prolong GI transit such as particle density (1, 2, 3), particle size (4), and the use of fibrous

materials. However, these approaches, some of which are controversial, generally lead to variable results and do not provide for intimate contact with the absorbing membrane. Polymers which can adhere to either hard or soft tissue have been used for many years in surgery and dentistry (5, 6). Among these "bioadhesive" polymers, monomeric α-cyanoacrylate esters has been most investigated (7, 8). This group of "instant" or "super" glues have been used for a number of conditions, such as repair of osteochondral fractures, and for hemostasis and soft tissue adhesives (9, 10). In dentistry they have been employed as adhesives for sealing extraction wounds and as a periodontal dressing (9). Other synthetic polymers such as polyurethanes, epoxy resins, polystyrene, acrylates, and natural products cement were also extensively investigated as bone glues (11, 12). These adhesives operate through formation of covalent bonds or noncovalent interactions. However, there has not been a systematic examination of polymers that adhere to the mucin/epithelial surface of the GI tract. We expect such polymers to be useful in oral drug delivery by prolonging GI transit time and improving oral drug absorption. A bioadhesive which can fulfill the above requirement should ideally have the following properties: be nontoxic, be nonabsorbable from the GI tract, preferably form a strong noncovalent bond with mucin/epithelial cell surfaces, adhere quickly to moist tissue, allow easy incorporation of drug and offer no hindrance as to its release, possess specific sites of attachment, and be economical. Earlier work (13) had examined a broad range of polymers as to their binding

affinity to mucin/epithelial surfaces. Interpretation of the results from that study suggested that a polyanionic polymer is preferred over a neutral or polycationic polymer and that a water insoluble polymer would offer advantages in design over a water-soluble polymer. Moreover, within the polyanionic polymer category, it was decided that carboxylic acid polymers would be a useful starting point in elucidating structural features as they relate to bioadhesion. Thus, a series of polymers were synthesized (Scheme 1) and tested. This report describes : (1) syntheses of a series of polymers, varying in hydrophobicity and charge density, (2) determination of some physical properties of these polymers, (3) an in vitro method to evaluate bioadhesion of these polymers to soft tissue, and (4) an in vivo method to evaluate GI transit of these polymers in experimental animals.

Scheme 1. The Structure of Cross-linked Swelling Polymers



Polymer	R_1	R_2	R_3	R_4
I[a]	H	H	$-CH(OH)-CH(OH)-$	H
II	H	H		H
III	H	CH_3	$-CH(OH)-CH(OH)-$	H
IV	H	CH_3		H
V	H	$-CH_2COOH$	$-CH(OH)-CH(OH)-$	H
VI	COOH	H	$-CH(OH)-CH(OH)-$	H
VII	H	H	$-CH_2-CH_2-$	CH_3
VIII	H	CH_3	$-CH_2-CH_2-$	CH_3

[a] Commercially available as polycarbophil (USP XX).

B. Bioadhesion

1. Definition

Bioadhesion can be defined as the process of uniting two biological surfaces, adhering of biological materials to artificial surfaces, or adherence of any material to biological surfaces. Included in this definition are intercellular adhesion, adhesion of cells to artificial surfaces, and adhesion of foreign materials to cell or tissue surfaces. The word 'bioadhesion' has been traditionally used to describe adhesion phenomena occurring between biological and nonbiological materials rather than interactions between biological materials. In contrast to numerous studies done for adhesion in artificial systems where both adhesive and adherend are nonbiological materials, there are not many research papers dealing with bioadhesion and consequently no established theory. Although there are many adhesion phenomena which are commonly referred to as bioadhesion, they refer to interaction between polymer and the mucin/epithelial cell surface in this report. Thus, a bioadhesive can be defined as any material which causes adherence between biological surfaces, causes adherence of biological materials onto itself, or adheres to biological surfaces. Thus, bioadhesives can be either natural or synthetic materials. The best bioadhesive candidates which can be used in vivo without any undesirable effect, would ostensibly be a mucomimetic polymer. In this sense, the search for bioadhesive polymers is essentially the same as that of finding mucomimetic polymers. Although the nature of the mucus gel is still not

not well understood, some observed properties and the structures of suggested mucin models can be used as a platform to study insoluble polymers. When bioadhesive properties of various, soluble or insoluble, polymers were tested, the observed bioadhesiveness is usually subjective and dependent on the characteristics of the substrate as well as the experimental technique. As a result, the absolute value varies from experiment to experiment and from laboratory to laboratory. Since experimental technique is one factor which significantly changes the result, it is necessary to briefly expound on experimental techniques, reported in the literature, to measure bioadhesiveness of various polymers.

2. Techniques for measuring bioadhesiveness

a. Methods for soluble polymers

a-1. Tensile test using mucus solutions

For soluble polymers, direct measurement of bioadhesiveness by a tensile test is not possible. Smart et al. (14) measured the interaction of soluble polymers with mucus molecules by using a tensiometer, after coating a glass plate with soluble polymers (Figure 1a.). The polymer coated glass plate was then immersed in the mucus solution and the interaction force measured. Obviously, there are a number of uncertainties in the method, but the results obtained agreed fairly well with those measured by other techniques.

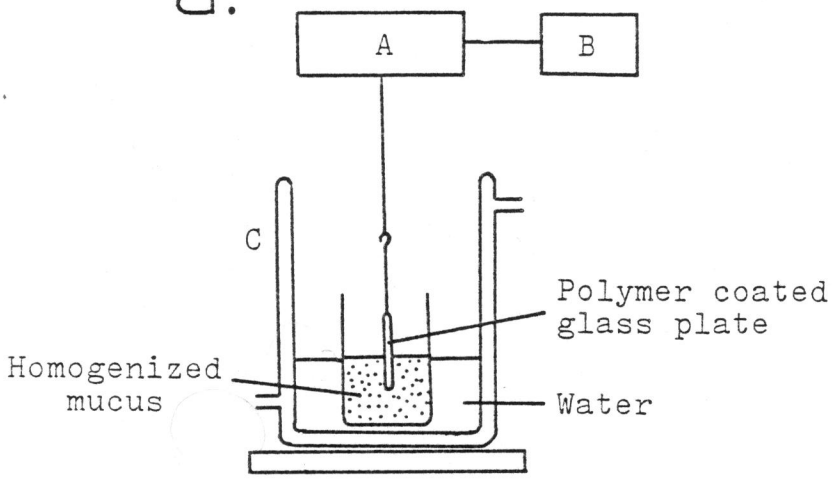
a-2. Fluorescent probe method using cell suspensions

Figure 1. Techniques for measuring bioadhesivness.

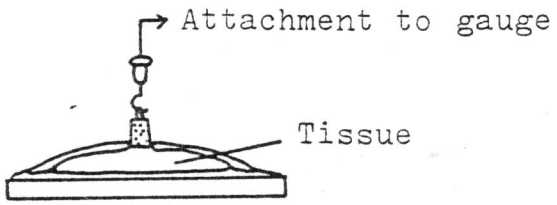
a. Tensile test using mucus solution; A, microbalance; B, chart recorder; C, water jacket

b. Tensile test using one tissue layer

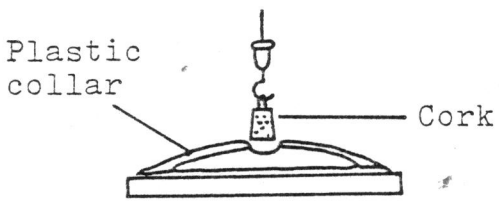
a.



b.



Before pulling



After pulling

Recently, a new technique employing a fluorescent probe was developed to measure binding abilities of various polymers to the cell membrane (13). The alteration in membrane viscosity after polymer binding was measured from the change in fluorescent spectra of pyrene which was incorporated into the lipid bilayer of the cell membrane. A number of charged and neutral polymers were tested and compared in a quantitative manner.

b. Methods for insoluble polymers

b-1. Tensile test using one tissue layer

This method has been used to measure the adhesiveness to skin or hard tissues. The adhesiveness of various synthetic adhesives to skin (15), parenchymal tissue (16) and dental enamel (17) were evaluated by a simple tension test (Figure 1b.). Although this method gives quantitative and reproducible results for some polymers such as acrylic and cyanoacrylate adhesives, it is not applicable for measuring bioadhesiveness of swellable water-insoluble polymer to the mucin/epithelial tissue in an aqueous medium. Thus it was necessary to develop a new test method to evaluate and compare the bioadhesiveness of polymers . This report describes a new technique using two tissue layers for measuring bioadhesiveness of swellable water-insoluble polymers. A bioadhesive polymer is placed between two tissue layers and the force to detach them is measured using a tensiometer. Surface irregularities and minute protuberances prevent perfect contact between adhesive and tissue and result in an imperfect interface. Thus, the effective area of contact is uncertain and difficult to determine, and the force

required to separate the surfaces gives no clue to the true strength of bonding at the points where contact does occur (18). However, with well controlled conditions, i. e., constant surface area, applied force, contact time, and peeling speed, reproducible results can be obtained.

3. Bioadhesive polymers

a. Water-soluble polymers

For most soluble polymers, quantitative measurement of bioadhesive strength is not available yet and each material is tested only to determine relative performance in a qualitative or semiquantitative manner. Table I compares the bioadhesive abilities of various polymers measured from different studies (14, 19, 20). Although absolute numbers are absent, it appears that the rank order of adhesiveness of various polymers are in agreement.

Recently bioadhesive properties of various polymers to the cell membrane were reexamined using a fluorescent probe (13). Their result (Table II) also falls in the same trend as shown in Table I, although significant differences were observed. The difference may be due to the different adherends used, i.e., cell membrane vs. mucus layer.

b. Water-insoluble polymers

For practical application, it is preferable to use an insoluble bioadhesive polymer for drug delivery systems. However no systematic study has been made to test the bioadhesiveness of insoluble polymers. In

Table I. Rank Order of Adhesive Properties of Various Polymers.

	Mucoadhesive Force[a]	Adhesive Performance[b]	Gastric Half Emptying Time[c]
Carboxymethylcellulose	193	excellent	
Carbopol 934	185	excellent	
Polycarbophil		excellent	5.0 hrs
Polymethacrylic acid			2.5 hrs
Trangacanth	154	excellent	
Alginate, sodium	126	excellent	
Hydroxyethyl cellulose		excellent	
Karaya gum	125	good	
Gelatin	116	fair	
Gua gum		fair	0.5 hrs
Pectin	100	poor	
Polyvinylpyrrolidone	98	poor	
Acacia	98	poor	
Polyethylene glycol	96	poor	
Psyllium		poor	0.2 hrs

[a] From reference 14
[b] From reference 20
[c] From reference 19

Table II. Results of Polymer Binding to Cultured Cell Monolayer. The higher △(E/M) value implies higher binding affinity to the cell membrane[a].

Polymer		△(E/M)
Cationic polymer	Polylysine	0.08-0.24
	Polyvinyl methyl	0.03-0.13
	Polybrene	0.03-0.18
Anionic polymer	C. M. C.	0.21
	Polyacrylic acid	0.76
	Polyglutamic acid	0.18
	Déxtran sulfate	0.01
	Lambda-Carragenan	0.14
Neutral polymer	Dextran	0.08-0.12
	Polyethylene glycol	0
Mixed charge polymer	Gelatin	0.29

[a] From reference 13

earlier work(13), using a fluorescent probe, three insoluble polymers were tested polycarbophil, guar gum and psyllium. Polycarbophil showed tenacious binding ability to the surface of cultured monolayer cells, although it was not possible to measure quantitatively the binding potential of insoluble polymers using this technique.

Polycarbophil is a synthethic polymer of acrylic acid crosslinked with divinyl glycol (3,4-dihydroxy-1,5-hexadiene). The amount of divinyl glycol is usually 0.01-2.0 % by weight. Polycarbophil is prepared by a free radical polymerization mechanism using benzoyl peroxide or azobisisobutyronitrile as a free radical initiator. The structure of polycarbophil is shown in Scheme 1. In acid form, polycarbophil swells extensively in water and the swelling characteristics are dependent upon the pH of the media in which it is placed, with the amount of swelling increasing as pH rises. Thus, in low pH regions, one gram of the polymer absorbs 15-35 ml of the solution while the polymer absorbs about 100 ml of neutral solution per gram of polymer. Polycarbophil is transparent in the swollen state. In clinical applications, polycarbophil has been used as an antidiarrheal agent due to its capacity to absorb considerable amounts of water at the pH of the small and large bowel.

4. Proposed mechanisms of bioadhesion

Wet adhesion can be defined as bioadhesion occurring in the presence of water. In contrast to numerous studies done for adhesion in artificial systems in which adhesives and adherends are both non-biological materials,

there are not many research papers dealing with bioadhesion, and consequently no established theory. In 1970, Chen and Cyr (20) proposed a mechanism of wet adhesion based on the relationship between properties of soluble polymers and adhesion performance. Hydrated polymer chains are free to move and stretch and thus entangle or twist with substrates when brought into close contact. Once entangled, they are able to match their active sites with those on the substrate to form adhesive bonds or to match each other to form cohesive bonds. In this process, the amount of water at the interface controls adhesive performances. Excessive hydration may overextend polymer chains to make them stiffer and more difficult to interact or entangle. This suggested mechanism can explain many of the observed bioadhesive phenomena. The role of physical entanglement between polymer chains seems to be most prominent in polycarbophil adhesion to gastric mucus gel. When polycarbophil is separated from the mucus gel layer, some mucin molecules are detached from the tissue surface and stay inside of the polymer. Dried hydrogels display extremely aggressive adhesion to moist soft tissues. They may function by dehydrating moist tissue surfaces, swelling and penetrating surface depressions (78) as in wet adhesion. The adhesion of dental adhesives, such as polyacrylic acid, to enamel was explained by the ability of free carboxyl groups to ensure excellent wetting (79). It was suggested that polyanion chains of adhesive actually penetrate into the enamel apatite, and carboxyl groups displace phosphate ions from the apatite matrix which ensures very intimate contact between adhesive and

adherend (80). The adhesion of the glass-ionomer cement to tooth surfaces was attributed to ionic forces operating across the interface (79, 81).

C. The Mucus Layer

Mucus is a unique secretion that occurs naturally as a viscous, sticky water-insoluble glycoprotein gel : it adheres to the epithelial surfaces and is found in all internal tracts of the body. In the GI tract, where mucus is the principal secretion, it acts as the major barrier separation the 'outside' world from the 'inside' world of GI cells. Its major component is large glycoproteins (mucins) secreted from mucus cells located in the epithelium. Mucus glycoproteins are high molecular weight compounds with large numbers of carbohydrate side chains attached to a protein core through an O-glycosidic bond. The physicochemical properties of mucus are known to depend on the composition and structure of its glycoprotein constituents.

1. Chemical composition of gastrointestinal mucins

Mucins are macromolecules in which a peptide core comprising 10-30 % by weight, is linked to oligosaccharide chains via O-glycosidic bonds between serine and/or threonine and N-acetylgalactosamine residues (21, 22, 23). the sugar composition of some representative purified mucins are shown in Table III (23). Lengths of the carbohydrate chains vary from 2 to 15 sugars (24). Sulphate is usually present (25), however its linkage point has not been identified and its concentration is variable. The peptide core of the mucin macromolecule shows much more uniformity in profile than oligosaccharide chains. Generally, for each mucin molecule, almost two thirds of the protein consists of threonine, serine, proline, alanine and

Table III. Carbohydrate Composition of Representative Gastrointestinal Mucins (mg/mg protein)[a].

	Porcine gastric mucin	Human salivary glyco-protein	Rabbit small intestinal mucin	Rat small intestinal goblet cell mucin	Rat small intestinal mucin
Galactose	1.91	2.34	1.13	1.92	1.15
Hexosamine	2.04	1.32	1.33	1.87	1.71
GlcNac	1.43	-	-	-	0.94
GalNac	0.61	-	-	-	0.77
Fucose	0.83	0.69	0.22	0.55	0.68
N-acetylneuraminic acid	0.01	0.24	0.24	0.83	0.92
Sulphate	0.23	0.07	0.08	0.03	0.2

[a] From reference 23

glycine (26, 27, 28, 29). Aspartic and glutamic acids are also relatively prominent. Hydrophobic, basic, aromatic and sulfur-containing residues are observed to be low in concentration.

It has been proposed by many investigators (30, 31, 32) that mucins are crosslinked *in vivo* by non-glycosylated peptides which differ from the common peptide core of mucins by being rich in cysteine residues. A disulfide bond between cysteine residues is formed and in some mucins these bonds appear to link mucin into large aggregates. It is reported that treatment with sulfhydryl reagent causes partial degradation of the mucin aggregates (33, 34).

Various models of the mucin tertiary structure have been proposed (Figure 2). Allen et al. (35) proposed a 'windmill' model for porcine gastric mucin. Four typical, but not necessarily identical glycoprotein arms are connected centrally through disulfide bonds between non-glycosylated peptide areas. The windmill structure polymerizes to form aggregate of molecular weight 2×10^6. By treating with sulfhydryl agents or proteolytic enzyme digestion, the windmill structure is partially degraded into four units of approximately 5×10^5 molecular weight (37).

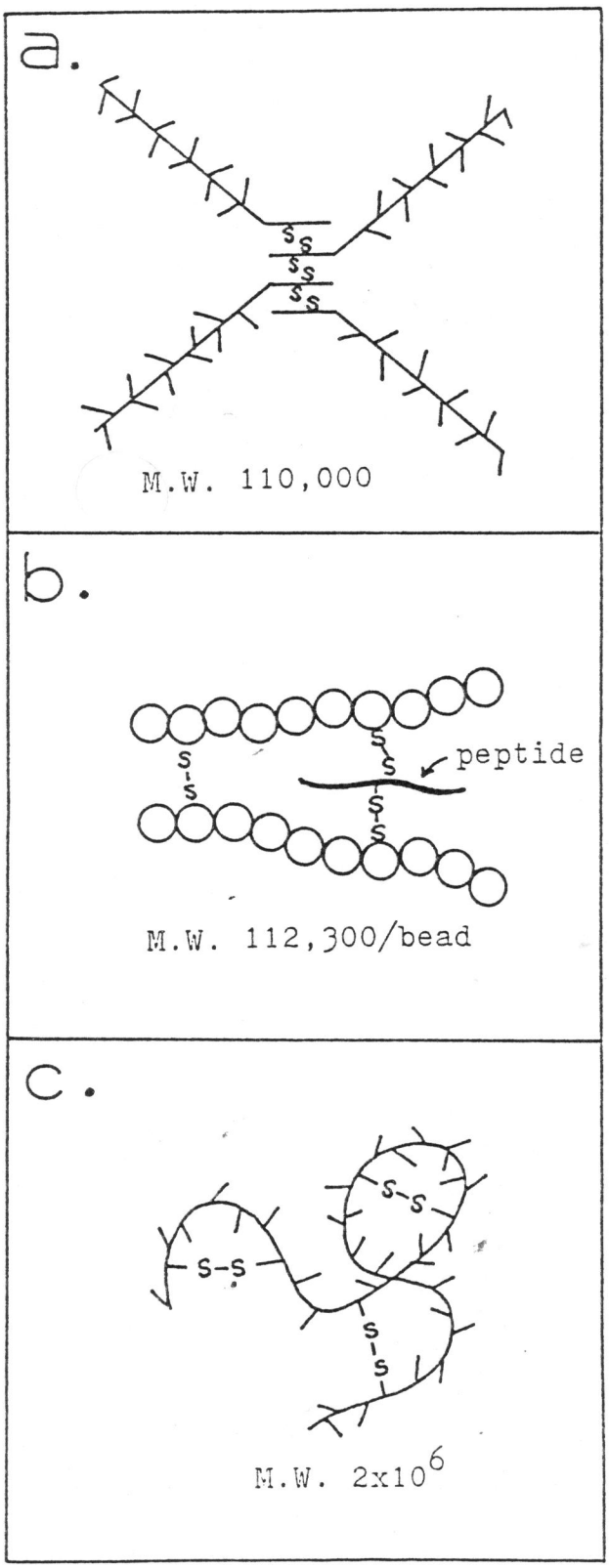
Robinson and Monsey (36) proposed a 'beaded mucin' model for egg-white β-ovomucin. As illustrated in Figure 2 the linear mucin is pictured as a string of ten spherical glycoprotein beads each with a molecular weight of 112,300. In this model, the beads are associated by strong hydrophobic bonds, and individual strands of beads are connected to each other by a

Figure 2. Proposed models of mucin structure.

a. 'Windmill' model for porcine gastric mucin by Starkey et. al. (28)

b. 'Beaded chain' model for β-ovomucin by Robinson and Monsey (36)

c. 'Flexible thread' model for rat intestinal mucin by Forstner et. al. (26)


a.
S-S
S-S
S-S
M.W. 110,000
b.
S-S
S-S
peptide
S-S
S-S
M.W. 112,300/bead
c.
S-S
S-S
S-S
M.W. 2x10^6

disulfide bridge. A 'flexible thread' configuration stabilized by non-covalent bonds and intramolecular s-s bonds was proposed to describe rat soluble goblet cell mucin by Forstner et al. (26). They observed that the molecule is not reduced into subunits by sulfhydryl reagents in spite of 34 s-s bonds per molecule (26). It was not identified however, and they speculated the presence of small peptides in vivo to crosslink glycoprotein threads through non-covalent interactions.

2. The proposed roles of the mucus gel layer

Over the years, a number of suggestions of mucus function have been proposed and these are listed in Table IV however, direct evidence of an in vivo function for mucus does not exist.

a. Protection

One of the major functions of the mucus layer is protection of the underlying mucosa from machanical damage by shear forces during blinking in the eye or the passage of food in the intestine. In the stomach and duodenum epithelial cells are protected from acid and pepsin in the gastric juice by virtue of this mucus layer (38, 39). Mucin also protects the oral cavity from desiccation and exogenous insult (40). The mucus layer has been proposed as the 'first line of defense' (41).

b. Selective permeability barrier

Mucin secreted by goblet cells appears to be the decisive luminal barrier to the passage of a compound through the gut wall (42). The mucus

Table IV. Proposed Functions of GI Mucus Layer

Protection
Selective permeability barrier
Antibacterial(viral) action
Lubricant
Hydration

layer can influence the concentration of substances in the immediate vicinity of the cell membrane through filtration of solute or foreign particles by its gel network (23). The mucus gel, however, is composed mostly of water and diffution of small ions should not be affected more than by an unstirred water layer (43). The upper limit on the size of the molecule that can penetrate the mucus gel has not yet been determined.

c. Antibacterial (viral) action

Mucin can protect tissues by favoring attachment and subsequent proliferation of certain microorganisms and/or by promoting the clearance of others (40). The proliferation of microorganisms in the large, but not the small bowel is evidence of this activity.

d. Lubrication

Extremely viscous gestagenic mucins impede sperm transport whereas oestrogenic mucin facilitates the locomotion of sperm cells (23). It was suggested that lubrication is dependent on the presence of the terminal sialic acids (44). The optimum mucin concentration for the lubrication effect on ciliary propulsion in tubes was measured to range between 1.7 and 4.1 % mucin.

D. Gastric Emptying

The stomach can be divided into two functional compartments. The proximal stomach, corresponding to the fundus and upper body of the stomach, acts as a reservoir for food, while the distal stomach, which includes the lower body and antrum, is primarily responsible for mixing and breakdown of solids (45). The food that enters the stomach is usually a mixture of liquids and solids, but the chyme that leaves, by way of the pylorus, is essentially a viscous liquid. Gastric emptying of a soluble and insoluble component of food has been extensively studied by numerous investigators and this work has accelerated during the last 15 years together with the development of a scintiscanning technique using an external gamma camera.

1. Gastric emptying of liquid

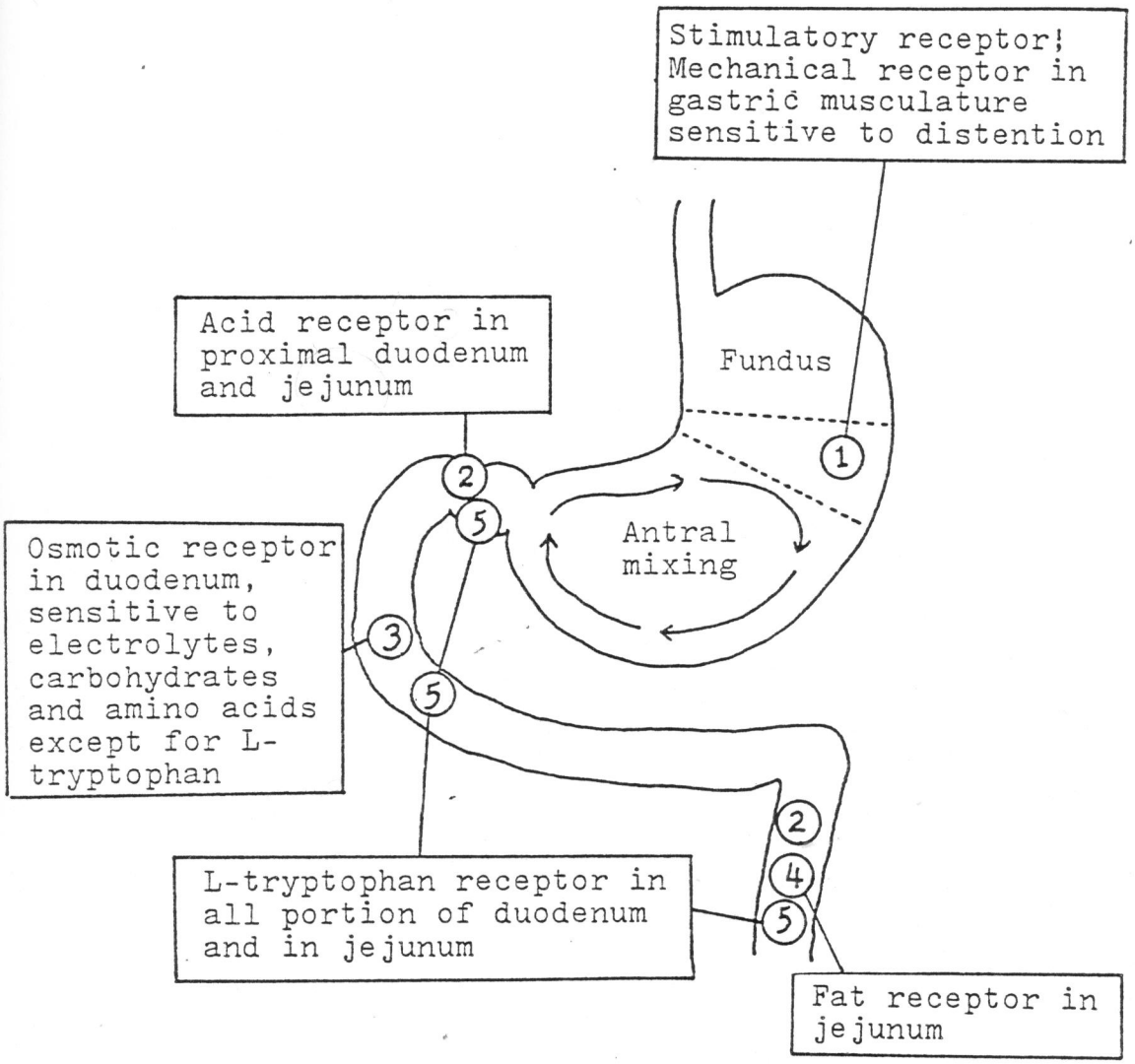
Gastric emptying of neutral, isoosmolar and calorically inert solutions is rapid and the primary determinant of the emptying of liquids from the stomach is volume (56, 57). For saline, the volume remaining in the stomach determines the rate of emptying, usually following a typical exponential pattern (45, 46, 47). In humans, the gastric half emptying time of a 500 ml saline solution is reported to be 12 minutes (54). The emptying of liquid is thought to be primarily a function of the pressure gradient between the stomach and duodenum (45, 46, 47). Owing to their low viscosity and low resistance to flow, liquids are propelled from the fundus

and body into the pyloric antrum. Due to their low resistance to flow, the increase of internal pressure is small and the slowly adapting tension receptors are activated to only a small extent (48). Therefore the gastric reflex mechanism for relaxation of the gastric reservoir is not activated (49). It is believed that gastric emptying is also altered by the particular mechanisms arising from the duodenum, in response to such elements in the effluent from the stomach as osmolality (61), calorie-bearing nutrients and hydrogen ion (51, 62). Many investigators demonstrated that the rate of gastric emptying of a meal could be predicted by its nutritive density. McHugh et al. (52) reported that in rhesus monkeys, glucose solutions with a high calorie content followed a more linear than exponential course of emptying from the stomach with the steady state delivery rate of 0.4 Kcal/min, regardless of the concentration, osmolality or volume of the intragastric meal, within limits. Later Brener et al. (51) also demonstrated that different concentrations of glucose solutions emptied at an isocaloric gastric emptying rate, i,e., 2.1 Kcal/min in human. Thus they suggested that a regulatory control on gastric emptying for nutrient solutions does arise from postpyloric signals which might come from interactions between nutritive component and the receptors located in the duodenum which are as yet unidentified and uncharacterised.

2. Gastric emptying of solids

Digestible solids are emptied from the stomach only when they have been changed to a liquified or suspended form. It is reported that particles larger than 2.0 mm in size are retained in the stomach (52). Emptying of solid contents is thought to be due to contractions of the pyloric part, while emptying of liquid is mainly influenced by volume. The frequency and velocity of distal gastric contractions are determined by the the pacesetter potentials, normally 3 to 4 cycles per minute in humans. However, not every pacemaker electrical event results in a muscle contraction. The gastric pacemaker is known to be located in the stomach muscle cells in the upper body of the stomach on the greater curvature (70). Contractions of the distal stomach are the peristaltic waves. These peristaltic contractions increase in amplitude and velocity as they move toward the pyloric part. These peristaltic waves constitute the digestive pattern of the distal stomach (54). As these waves approach the distal antrum, the terminal antrum and pylorus close. Large solid particles retained in the stomach are retropelled and triturated in the antral mill where pressures up to 60 cm H_2O have been measured (55). Through this grinding action, along with enzymatic digestion, most solid particles are reduced to chymouslike consistency allowing outflow into the duodenum. It is believed and demonstrated by many investigators that the activities of the stomach, including its emptying, are under the influence of the receiving duodenum. Figure 3 illustrates the position of postulated various receptors in the small intestine through which nutritive components probably exert their influence on gastric emptying of

Figure 3. Location of various receptors that influence gastric emptying. (From reference 54.) 1 and 3 was determined by human studies and other receptors localized by studies in dogs. Numbers 2 to 5 are small bowel inhibitory receptors, which are mediated by neural and/or hormonal pathway. Hormonal candidates include gastrin, cholecystokinin, secretin, gastric inhibitory polypeptide, vasoactive intestinal polypeptide, glucagon, and somatostatin.


Stimulatory receptor; Mechanical receptor in gastric musculature sensitive to distention
Acid receptor in proximal duodenum and jejunum
Fundus
Antral mixing
Osmotic receptor in duodenum, sensitive to electrolytes, carbohydrates and amino acids except for L-tryptophan
L-tryptophan receptor in all portion of duodenum and in jejunum
Fat receptor in jejunum
1
2
3
4
5

contents. For example, carbohydrates and aminoacids retard gastric emptying via osmoreceptors. However, these receptors are yet unidentified. In summary, gastric emptying is influenced by several factors (54, 56): (1) the volume of the gastric content, (2) the viscosity of the contents which determines resistance to flow, (3) chemical composition of the chyme (in the order of inhibitory potency): fats, fatty acids, proteoses, peptones, aminoacids, sugars and other products of starch and hydrogen ion , (4) the reservoir function of the fundus and body, which is regulated by the volume, consistency of the contents, and the state of disease (5) frequency and force of contraction of the pyloric part and (6) the type of contracton of the pyloric canal.

Because so many factors are involved, it is not surprising that there are not always strong correlations between one of above factors and gastric emptying of solids.

3. Techniques for measuring gastric emptying

A great deal of effort was expended in the past to develop appropriate method to study GI transit of food. Three currently used general methods are summarised below.

a. Intubative technique

This technique has the longest history clinically and has been widely used for measuring gastric emptying of liquid (48, 57, 58, 59). Clinically the well known 'saline load test' is a typical intubation test in which 750 ml of

normal saline is instilled in the stomach followed by aspiration and measurement of the volume of solution remaining in the stomach at a certain period of time. Usually a single measurement is performed in one day and the test continues several days until the gastric emptying-time profile is completed. This technique has the disadvantage that it does not take into account gastric secretion. Another method is the use of nonabsorbable markers such as phenol red or polyethylene glycol in a specified volume of test solution. After withdrawing the remaining test solution from the stomach, the volume of the contents and the concentration of the marker are determined to disclose the contribution of gastric secretion (60, 61, 62).

b. Radiological technique

Use of liquid barium sulfate or radioopaque plastic spheres, has been used extensively due to its ease of performance as well as safety (64, 65). However , this technique is so qualitative, because the volume of residual barium in the stomach cannot be accurately determined from a radiograph, that only the complete gastric emptying time can be assessed.

C. Radioisotope method

This technique, using external <u>in vivo</u> scanning, provides the most reliable, quantitative measure of GI transit of γ-emitting isotope labeled material (66, 67, 68). It has the problem of variation in count efficiency of radioactivity, due to the changing of depth of radioactivity under the gamma camera. The exact method of performing the test may differ from laboratory to laboratory. However, generally, the subject eats the radiolabeled test

meal. External scanning is done with the patient in a supine position using the gamma camera at various time intervals. With the advent of more sophisticated gamma cameras, which are capable of simultaneously tracking two nucleotide markers of different energies, solid and liquid emptying or emptying of different components can be assessed simultaneously. Radioisotope methods, at present, are regarded as the most tolerable and accurate means of evaluating gastric emptying of liquids and solid materials in humans. Most frequently used isotopes are chromium 51, technetium 99m and indium 113m. These isotopes are found to be neither absorbable nor adsorbable in the GI tract (66, 67, 68, 69). ^{51}Cr has been regarded as a suitable isotope to incorporate into a meal. However, due to its long half-life (28 days) it is not appropriate for successive tests on the same subjects. The most widely used isotope for studies of gastric emptying of food is technetium 99m, which has advantages over chromium 51. It has a shorter half-life (6 hours) than ^{51}Cr, making it a safer isotope and allowing repeated nuclide examinations without interference from previous studies. Indium isotope ($t_{1/2}$ = 1.7 hours) is also preferred to ^{51}Cr for the same reason to measure gastric emptying of a meal.

In this report, an <u>in vivo</u> method is described for measuring GI transit of ^{51}Cr labeled insoluble polymers in rats using gamma scintillation counting.

II. EXPERIMENTAL

A. Reagents

Acrylic acid, methacrylic acid, itaconic acid, benzene, maleic anhydride, benzoyl peroxide and magnesium sulfate heptahydrate were obtained from Aldrich Chemical Company (Milwaukee, WI). Divinyl benzene, 2,5-dimethyl-1,5-hexadiene and azobisisobutyronitrile were obtained from Polyscience Inc. Anhydrous sodium phosphate dibasic, sodium phosphate monobasic, acetic acid and ether were purchased from Mallinkrodt Inc. (St. Louis, MO). Sodium acetate and mannitol were obtained from Sigma Chemical Company (St. Louis, MO). Concentrated hydrochloric acid was from Hi-Pure Chemical Inc. (Nazareth, PA) and sodium chloride was from Columbus Chemical Industries Inc. (Columbus, WI). ^{51}Chromium chloride was from New England Nuclear (Boston, MA) and Amberlite 200 resin beads were from Rohm and Haas (Philadelphia, PA). All of the above chemicals were either reagent or analytical grade and were used as received.

B. Materials

Aluminum vial caps were obtained from Wheaton Scientific (Milleville, NJ) and hard gelatin capsules were from Parke-Davis (Morris Plains, NJ). Surgical silk thread was from David and Geck (Manati, PR). Suturing needle (3/8 circle cut edge. 1 inch length) was from Torrington Medical Supplies (Fairfield, CT). Scintillation vials (polypropylene, 11 i.d. x 160 mm) were

from Packard Instrument Company (Downers Grove, IL).

C. Methods

1. Preparation of polymers

Salient features of the synthesis are shown in Table V. The procedures are modified from conditions reported by Markus (71). A mixture of 1 g initiator dissolved in 100 g of monomer and 0.05 g to 1.5 g of a crosslinking agent was added, with stirring to a solution containing 800 g of magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$) in 100 ml of distilled water and refluxed at the required temperature. Polymerization occurred within a short period of time. After polymerization, the mixture was maintained at the same temperature with stirring for the required curing time. At the end of the reaction, the mixture was diluted with 150 ml of hot water, strained through a 40 mesh stainless steel screen then repeatedly washed with one liter portions of water. The washed crosslinked polymer was dried in a hot air oven at 90°C for a specified time before being ground to the required size.

2. Determination of some physical properties of the polymers

a. Determination of apparent polymer hydration

0.5 g of each polymer was allowed to hydrate in 25 ml of distilled water at 25°C in a 25 ml graduated cylinder. The volume of the polymer was measured at 5-minute intervals until there was no further hydration.

b. Determination of polymer density

Table V. The Synthesis of Crosslinked Swelling Polymers

Polymer	Monomer and Quantity (g)	Crosslinking agent and Quantity (g)	Initiator and Quantity (g)	Polymerization Condition		Curing Time	Yield (%)
				Time (min.)	Temp. (°C)		
I	(A) 100	(a) 0.3[a]	(1) 1.0	15	95	2 hr.	83
II	(A) 100	(b) 0.3	(1) 1.0	25	95	18 hr.	67
III	(B) 100	(a) 1.0	(1) 1.0	30	95	48 hr.	53
IV	(B) 100	(b) 0.3	(1) 1.0	30	95	48 hr.	87
V	(C) 100	(a) 1.0	(1) 1.0	30	65	24 hr.	11
VI	(D) 100	(a) 0.2	(2) 1.0	20	95	72 hr.	10
VII	(A) 100	(c) 0.3	(1) 1.0	10	95	4 hr.	98
VIII	(B) 100	(c) 0.2	(1) 1.0	10	95	20 hr.	93

(A) Acrylic acid
(B) Methacrylic acid
(C) Itaconic acid
(D) Maleic anhydride

(a) Divinyl glycol
(b) Divinyl benzene
(c) 2,5-dimethyl-1,5-hexadiene

(1) Benzoyl peroxide
(2) Azobisisobutyronitrile

The density of each polymer was determined in a 2-ml specific gravity bottle at 25°C. Benzene of known density (0.874 g/cc) was used as the medium. No swelling of the polymer in benzene was evident.

c. Effect of pH on equilibrium swelling of polycarbophil

Water sorption of polycarbophil as a function of pH was determined as the apparent volume expansion of polycarbophil. 50 mg of 30-40 mesh polycarbophil was placed in 100 ml of the solution being tested and allowed to hydrate at 37°C for 24 hours. The polymer was periodically stirred to remove trapped air bubbles and the pH of the solution was checked and adjusted with saturated sodium hydroxide solution, if necessary, to maintain constant pH. After 24 hours, fully hydrated polymer was transferred to a 10-ml graduated cylinder and allowed to settle. After several hours, the meniscus of the interface between the fully hydrated polymer and the test solutions was measured. The test solution used were hydrochloric acid diluted to pH 0.5 and 1.2; 0.1 M phosphate buffer at pH 2, 3, 4, 6, and 7; and 0.1 M sodium acetate buffer at pH 5. Ionic strength was kept constant at 0.12 M using a calculated amount of sodium chloride, and osmolality was kept constant at 290 mOsmol using mannitol. Both pH[a] and osmolality[b] were measured.

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[a] Digital Ionizer, Model 701, Orion Research (Cambridge, MA)

[b] Osmette S Automatic Osmometer, Model 4002, Precision Systems (Walthan, MA)

3. In vitro evaluation of polymer bioadhesion

a. In vitro bioadhesion test of polymers

The force required to separate a polymer specimen from freshly excised rabbit stomach tissue was measured using a precision balance[a] adapted for bioadhesion measurements. A schematic of the apparatus is shown in Figure 4. After sacrificing a rabbit by rapidly injecting 5-ml of 5 % sodium pentobarbital solution into the ear vein, the stomach was excised and placed in a beaker of normal saline solution (4°C). The isolated stomach was opened with scissors and carefully washed several times with chilled saline solution (4°C) to remove its contents. The washed stomach was then placed in a beaker containing aerated saline (4°C), and kept until used. A section of tissue was cut from the fundus of the rabbit stomach and secured, mucosal side out, onto a weighted glass vial using a rubber band. The vial was placed in a 500-ml beaker containing USP simulated gastric fluid (pH 1.2), without pepsin, and positioned under the scale. The remaining tissue was placed, after carefully removing the external longitudinal layer of muscle, mucosal side out, over a #2 rubber stopper and secured with an aluminum vial cap which had a hole 10 mm in diameter in its center. Four mg of polymer, previously fully hydrated with test solution, was spread in a uniform monolayer over the exposed tissue on the rubber stopper. The stopper was

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[a] Roller Smith, Biolar Inc. (North Grafton, MA)

then suspended from the scale into the beaker containing the test solution and weighted vial (Figure 4). The scale was zeroed with the tissue on the vial at a depth equal to the layer of polymer. The scale was locked and the beaker repositioned so that the vial was directly below the rubber stopper. The beaker was then slowly raised until the tissues came into contact. The contact is initiated by the weight of the rubber stopper (1.8 g in this system). After 1 minute the lock was released and a force was applied to remove contact between polymer and tissue. The force was increased at a constant rate of 10 mg/sec until the polymer became detached from the mucus.

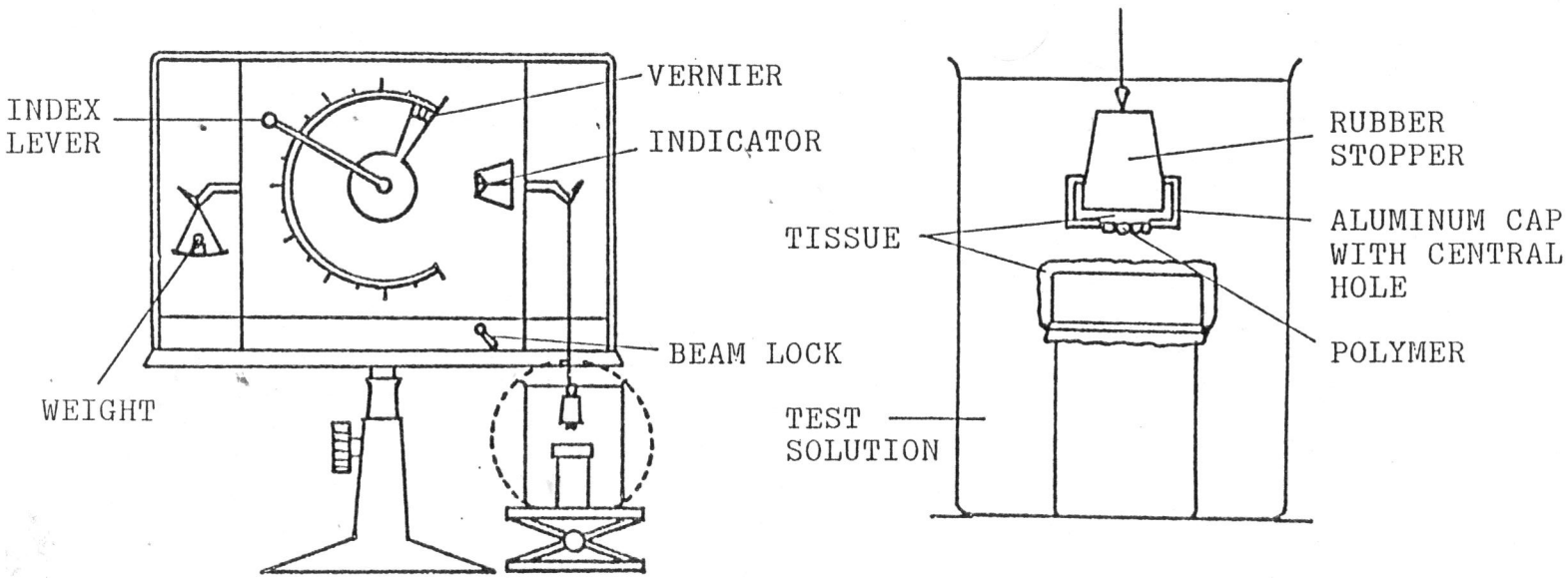
b. Effect of pH on polycarbophil bioadhesion

The effect of pH on the bioadhesion of polycarbophil was studied using the buffers described earlier. Solutions were prepared as described in the equilibrium swelling test, and the actual pH tested were 0.46, 1.2, 2.0. 3.0, 4.0, 5.0, 6.0, and 7.0.

c. Effect of applied force for contact on bioadhesion

Effect of applied force on <u>in vitro</u> bioadhesion of polycarbophil, poly(methacrylic acid-divinyl benzene) and poly(HEMA), to the rabbit stomach tissue was studied using different weights of rubber stopper. The weight of stopper used were, 0.57, 1.13, 1.62, 2.00, 3.70, 5.05, 7.06 and 8.80 gram. The force to separate the polymer was measured in the same manner previously described and USP simulated gastric fluid (pH 1.2), without pepsin, was used.

Figure 4. A modified surface tensiometer for *in vitro* evaluation of bioadhesion.


INDEX LEVER
VERNIER
INDICATOR
BEAM LOCK
WEIGHT
RUBBER STOPPER
ALUMINUM CAP WITH CENTRAL HOLE
TISSUE
POLYMER
TEST SOLUTION

4. In vivo Gastrointestinal studies

a. Labeling of polymers with ^{51}Chromium

Ten g of polymer were allowed to swell in 50 ml of distilled water containing 1 mCi of ^{51}Chromium chloride for 24 hours. The suspension was filtered and the residue resuspended in 600 ml of distilled water. After overnight stirring, the suspension was filtered. The same procedure was repeated several times with USP gastric fluid without pepsin until no radioactivity was detected in the filtrate. The labeled polymer was dried in the oven for 24 hours then sieved and the 30/40 mesh fraction retained was used for all experiments. 150 mg of ^{51}Cr-polymer were packed in a number 3 gelatin capsule and its radioactivity measured[a].

b. Labeling normal saline solution with ^{51}Chromium

0.1 ml of ^{51}chromium chloride solution containing 1 mCi radioactivity was diluted to 50 ml with normal saline. The radioactivity per milliliter of the solution was measured.

c. Preparation of Amberlite 200 resin bead system

A known number of 30/40 mesh size Amberlite beads, weighing 150 mg, were packed in a number 3 gelatin capsule.

d. Gastrointestinal transit studies

Male Sprague-Dawley rats, each weighing 150 to 200 g, were fasted with free access to water for 48 hours prior to the experiment. Each animal was

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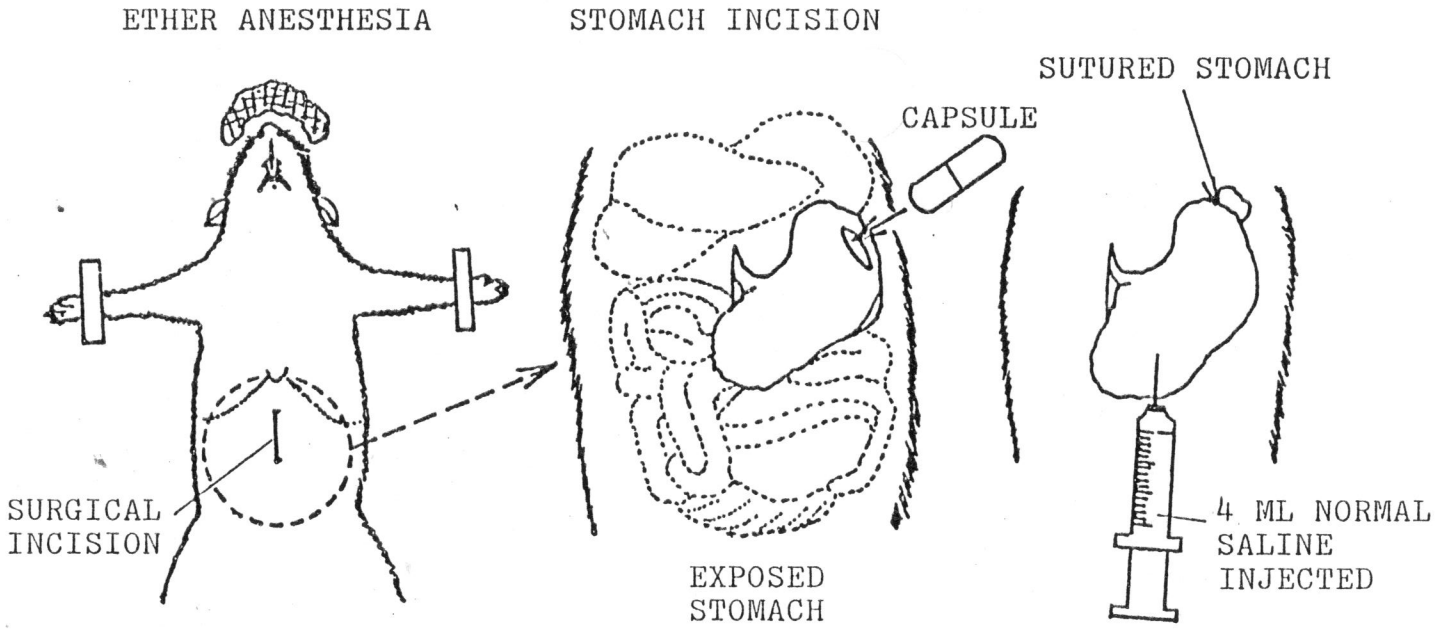
[a] Packard Auto-gamma Scintillation Spectrometer, Model 5236 (Chicago, IL)

anesthetized in an ether saturated chamber then secured on a surgical board in the supine position with adhesive tape after trimming its abdominal hair with animal clippers. A longitudinal incision, about 2 centimeters, was made a few millimeters distal from the base of the sternum (Figure 5). The stomach was located and carefully elevated from the abdominal cavity, and an opening 2-3 mm in diameter was cut into the fundus with surgical scissors. A capsule containing either test material or solid control was inserted into the stomach through the opening. The opening was then tied with a loop of surgical silk thread. The stomach was replaced in the abdominal cavity and 4 ml of normal saline injected into the stomach to assist in dissolution of the gelatin capsule. The external incision was closed with at least four stitches in both the peritoneum and epidermis. Anesthesia was maintaind throughout the operation by using a 50 ml beaker containing an ether-moistened gauze pad placed over the nose of the rat. The time interval for surgery was approximately 2 minutes. The rat was allowed to recover from the anesthesia in a well ventilated cage. At selected time intervals, the rat was sacrificed with an overdose of ether. The stomach and small intestine were removed from the rat. The small intestine was further cut into 20 equal segments and the radioactivity of each segment and the stomach determined. In the case of the Amberlite resin beads, the number of beads in each segment and the stomach were counted under a magnifying glass. For the ^{51}Cr-labeled normal saline solution, the surgical procedures were identical to the above except that no capsule was inserted,

Figure 5. Surgical procedure for gastrointestinal transit studies.


ETHER ANESTHESIA
STOMACH INCISION
SUTURED STOMACH
CAPSULE
SURGICAL INCISION
EXPOSED STOMACH
4 ML NORMAL SALINE INJECTED

and instead of normal saline, 4 ml of ^{51}Cr-labeled normal saline was injected into the stomach. A minimum of 5 rats were used for each time point.

III. RESULTS AND DISCUSSION

A. Preparation of Polymers

Polycarbophil and related polymers were synthesized according to the method of Markus (71), with suitable modification. Reasonably high yields were obtained for most of the polymers as shown in Table V, except for itaconic acid-divinyl glycol and maleic anhydride-divinyl glycol, which yielded only 11% and 10%, respectively. In most cases, polymerization occurred within 10 to 30 minutes and a granular mass was obtained. The polymers were prevented from extensive swelling by the saturated magnesium sulfate solution which also served as a suspending medium. Because of their insolubility and inertness, conventional methods for structural identification cannot be used. Thus, with the exception of polycarbophil which meets the USP criteria for identification and purity, identification of the remaining polymers is assumed. Further work is needed to confirm identity.

B. Physical Properties of the Polymers

The synthesized polymers were examined as to densities and extent of hydration. As anticipated, the percent crosslinking has a small effect on the resulting density as shown in Table VI. However, a somewhat greater effect on density is observed for different starting monomers as shown in Table VII. The relationship between starting monomer structure and density is at

Table VI. Densities of Polycarbophil with Different Percent of Crosslinking Agent.

% Crosslinking Agent[a]	Density (g/cc)
0.05	1.49
0.30	1.56
0.60	1.57
1.20	1.62
2.00	1.65

[a] Starting percent of crosslinking agent in the reaction mixture.

Table VII. Some Physical Properties of Adhesive Polymers.

Polymer	Maximum Hydration[a] of 1 g Polymer (ml)	Density[b] (g/cc)
Polycarbophil	74	1.56
Poly(acrylic acid-2,5-dimethyl-1,5-hexadiene)	42	1.62
Poly(methacrylic acid-2,5-dimethyl-1,5-hexadiene)	15	1.47
Poly(methacrylic acid-divinyl benzene)	27	1.36

[a] In distilled water
[b] All polymers synthesized with 0.3 % crosslinking agent.

present unknown. The effect of structural modification on extent of hydration is clear. Polycarbophil being least substituted has the greatest extent of hydration, with 1 gram of polymer being able to swell to 74 ml of volume. The rate of hydration is an important parameter and has some practical implications in that swelling increases the surface area and hence bioadhesion and any oral drug delivery system would have a finite residence time in the stomach and intestine. Figure 6 shows that all of the polymers tested hydrate quickly in water, reaching equilibrium in 30–60 minutes. Smaller sized particles hydrate more quickly than larger sized particles. Equilibrium swelling of polycarbophil was measured at different pH's, and corresponding profiles are shown in Figure 7. In acid conditions up to pH 4, swelling of the polymer increased very slightly. Swelling was greatly increased between pH 4 and pH 5 and continued to increase at pH 6 and pH 7 with a reduction at more alkaline pH's. In the process of polymer hydration, the dependence of water movement into the polymer network in the presence of electrolytes is known to be a characteristic typical of Donnan membrane equilibrium (72). Since the pKa of polyacrylic acid is 4.75 (73), a dependence of equilibrium swelling of polycarbophil on pH was expected.

C. In vitro Bioadhesion Studies

Various methods have been used to measure the adhesive strength of soluble and sticky polymers (15, 16, 20). However, none of these methods are suitable for assessing swelling insoluble bioadhesive polymers. The

Figure 6. Apparent rate of hydration of crosslinked polymers in water. Particle size of all polymers was 30/40 mesh. ○, polycarbophil; △, poly[methacrylic acid-divinylbenzene (0.3%)]; ●, poly[methacrylic acid-(2.5-dimethyl-1.5-hexadiene) (0.3%)]; (w/w %), initial feed amount of crosslinking agent in the reaction mixture by weight.

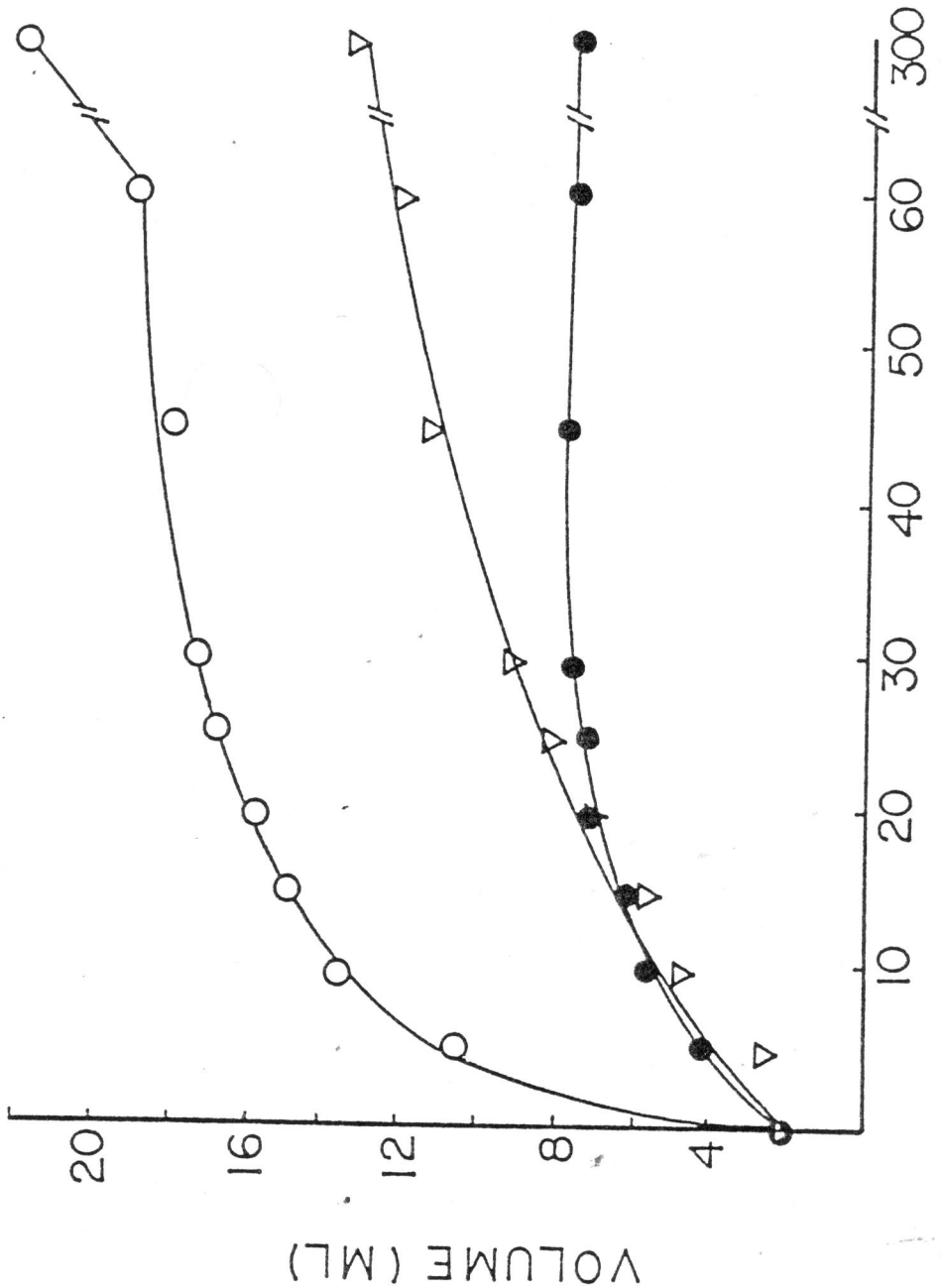
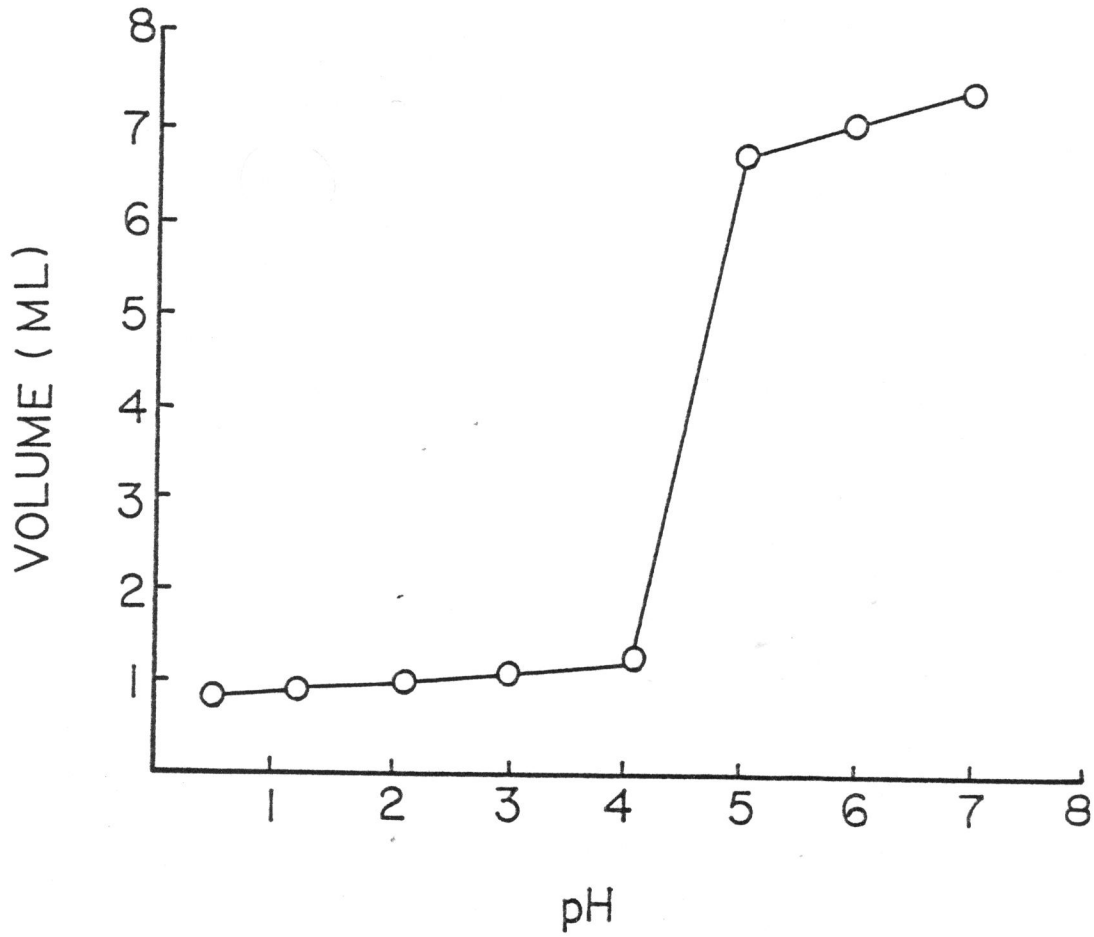

VOLUME (ML)
20
16
12
8
4
10
20
30
40
50
60
300
TIME (MIN)

Figure 7. Apparent volume of equilibrium swelling of 50 mg polycarbophil at various pH's. The buffer systems were diluted hydrochloric acid (pH 0.46, 1.2 and 2.0) 0.1M phosphate buffer (pH 3.0, 4.0, 6.0 and 7.0) and 0.1M acetate buffer (pH 5.0). Ionic strength was adjusted to 0.12 M with sodium chloride and osmolality was maintained to 290 mOsmol using mannitol. Temperature was maintained at 37°C. Each point is the mean of two measurements.


VOLUME (ML)
8
7
6
5
4
3
2
1
1
2
3
4
5
6
7
8
pH

modified surface tensiometer method gives a reasonably sensitive and reproducible method to measure adhesion of polymer to animal tissue, provided precautions are taken to ensure that the conditions of each test are the same. Four mg of fully swollen polymer was sufficient to provide a good range of forces required for detachment. As shown in Table VIII, bioadhesion of three polyacrylic acid polymers did not show any significant difference for different crosslinking agents. However, methyl substitution in the polyacrylic acid backbone reduced bioadhesion significantly (Student's t-test, $p<0.01$). The structural features, in terms of hydrophobicity and charge density, responsible for bioadhesion, are currently unknown, but are being explored. Poly(2-hydroxyethyl methacrylate), a neutral polymer, requires a very much lower force for detachment compared with charged polymers. Amberlite resin and crosslinked gelatin microcapsules possess no adhesion to rabbit stomach tissue, within the limits of the detecting systems.

D. Effect of pH on In Vitro Bioadhesion of Polycarbophil

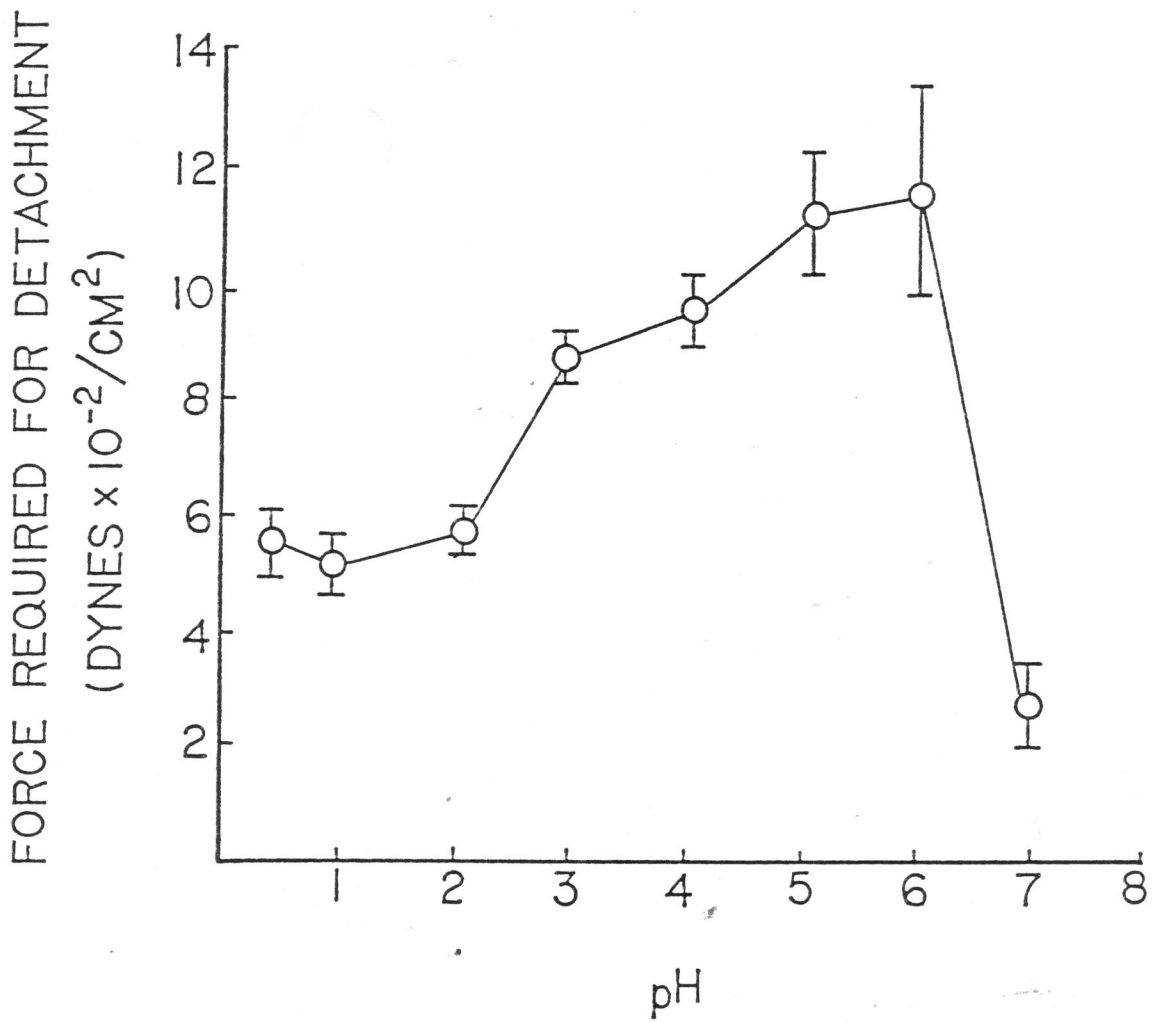
In vitro bioadhesion of polycarbophil was also measured at different pH's. The force measured to separate the polymer from the tissue at various pH between pH 0.5 to 7 are shown in Figure 8. As seen in this figure, maximum adhesion was observed at pH 5 and 6 which is more than twice the adhesive force required for pH 0.46, 1.2, or 2.0. At pH 7, bioadhesion of polycarbophil to rabbit stomach tissue was significantly reduced (Student's

Table VIII. In Vitro Evaluation of Polymer Bioadhesion to Rabbit Tissue using a Modified Surface Tensiometer. All studies used 4 mg of 30/40 mesh material.

Test Material	Weight Required for Detachment (mg)	Force/Area (dyne/cm^2)
Polycarbophil	855±55[a] (13)[b]	1,061±68
II	876±57 (13)	1,086±71
IV	306±45 (8)	380±56
VII	864±56 (12)	1,072±68
Poly(HEMA)[c]	30±8 (8)	37±10
Amberlite 200 Resin Beads	0 (4)	0
Gelatin Microcapsules[d]	0 (4)	0

[a] Mean±S.E.M.
[b] Number of tests
[c] Poly(2-hydroxyethyl methacrylate)
[d] Crosslinked with formaldehyde
II Polymer of acrylic acid-divinyl benzene
IV Polymer of methacrylic acid-divinyl benzene
VII Polymer of acrylic acid-(2,5-dimethyl-1,5-hexadiene)

Figure 8. Effect of pH on *in vitro* bioadhesion of polycarbophil to rabbit stomach tissue, each point represents the average of eight to ten measurements (± S.E.M.)


FORCE REQUIRED FOR DETACHMENT
(DYNES x 10⁻²/CM²)
14
12
10
8
6
4
2
1
2
3
4
5
6
7
8
pH

t-test, $p<0.01$). This reduction in bioadhesion at neutral pH is unanticipated. In order to explain this finding, it is necessary to involve pathological changes in the tissue or to examine changes in gastric mucin. Changes in mucin structure as a function of pH have been studied by several investigators, using rheological measurements. However the mechanism(s) of change in viscosity due to pH are at present unknown. James et al. (74) investigated the effect of pH on rheology of purified and unpurified hog gastric mucin and found that the intrinsic viscosity was not affected by changes in pH in unpurified mucin while purified mucin showed a decrease in viscosity at low pH. Forstner at al. (75) observed a decrease in specific viscosity of semipurified, hog gastric mucin as pH increased from pH 4 to 9 although the solubility of mucin was not strongly affected by changes in shear rate at pH 7.0, which they attributed to an instability of mucin structure or a greater sensitivity to shear at physiological pH values. Although the in vivo mucus layer includes other agents such as water, electrolytes, sloughed cells, albumin, bacteria and bacterial products, digested food, plasma proteins, bile salts, pancreatic enzymes, and other constituents normally found in the gastrointestinal juice, its major component is mucins (23). Changes in pH will affect ionization of the sialic acid group and amino acids in the peptide backbone of the glycoprotein. For example, the pKa of sialic acid is about 2.6 (76); consequently, at pH values of 4 and above, this acid group will be almost fully ionized (33). Polycarbophil with a pKa of around 4.75 will be almost fully ionized at pH 7. Thus above pH 5, negative charge repulsion will

be considerably increased in the mucus-polycarbophil interaction. In addition, above pH 4, due to swelling, the polymer gel network expands greatly as pH increases, especially between pH 4 and 5. Although the precise adhesion mechanism of polycarbophil to the mucin/epithelial suface is not known, the degree of swelling seems to be one of the factors affecting adhesion of polycarbophil up to pH 6.

During the in vitro adhesion test, it was observed that, when polycarbophil was detached from the tissue, mucus remained bound to the polymer and the break occurred within the mucus network. Thus, it can be deduced that the interaction force between polycarbophil and mucus is greater than the mucus-mucus cohesive force in rabbit stomach. Numerous analytical studies have been carried out on mucus glycoproteins from many organs and many species. The common feature of these macromolecules synthesized and secreted by epithelial cells of the underlying mucosa is that they consist of subunits held together by interchain disulfide bonds, ionic interactions, hydrogen and hydrophobic bonding and further stabilized by noncovalent interaction. These macromolecules consist of a peptide core, comprising 10 to 30% by weight of the molecule, linked to multiple oligosaccharide branches of varying length via O-glycosidic bonds. The intermolecular interactions create an extended and random gel network, which imparts to mucus secretions their characteristic property of viscoelasticity. Considering the observation that interaction failure occurs between mucus molecules rather than mucus-polycarbophil, it is assumed that the polycarbophil-mucus

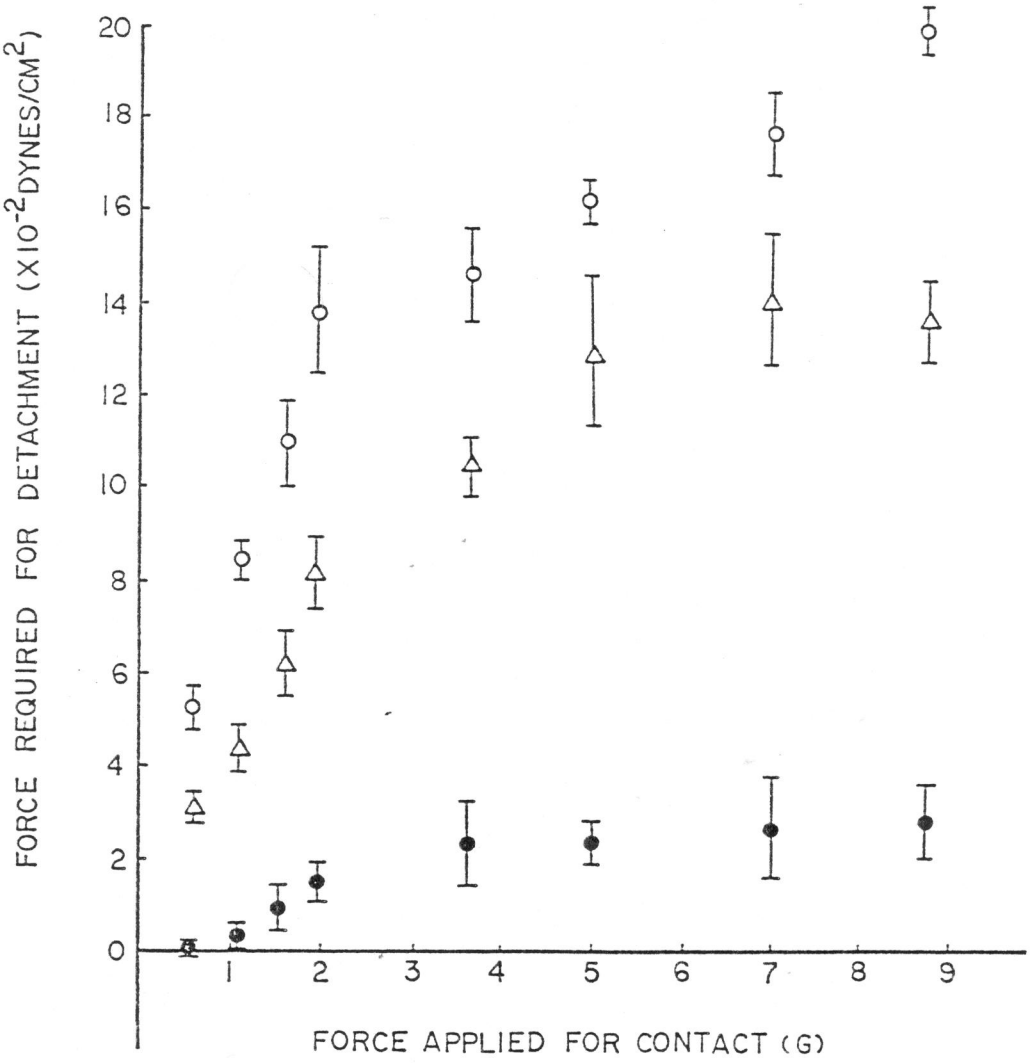
interaction is greater than the sum of various interactions between mucin molecules including mucin-mucin entanglement.

Several mechanisms of bioadhesion have been proposed by many investigators. Chen and Cyr (20) proposed the mechanism of wet adhesion based on the relationship between properties of polymers used and adhesion performances. Hydrated polymer chains are free to move and stretch and thus become entangled or twisted when brought in close contact with the substrate. Once entangled, they are able to match their active adhesive sites with those on the substrate to form adhesive bonds or to match each other to form cohesive bonds. In this process, they claimed that the amount of water at the interface controls adhesive performance. Excessive hydration may overextend polymer chains to make them stiffer and more difficult to entangle. This suggested mechanism, for wet bioadhesion of linear hydrogels, may be applicable to bioadhesion of crosslinked hydrogels and can explain some of the observed bioadhesive properties of polycarbophil, i.e., sudden drop in bioadhesiveness of polycarbophil at pH 7 due to a small increase in apparent swelling at this pH. Although changes in bioadhesion cannot be explained entirely by changes in the physical state of the polymer, the amount of water in the polymer network might be one of the determinant factors in bioadhesion of crosslinked hydrogels. However, direct proof of this mechanism is lacking.

E. Effect of Applied Force on Bioadhesion

One of the critical factors affecting overall bioadhesive performance was the pressure applied to contact tissue layer and the polymer. In order to eliminate the artifact produced by contact pressure, bioadhesiveness was measured at various pressures. The result is shown in Figure 9. Below 2 gram of applied force, the measured force for detachment of polymer from the tissue was increased. The sensitivity of adhesion to applied pressure was reduced greatly above 2 gram of force. For any applied force, polycarbophil showed the best bioadhesion, followed by methacrylic acid polymer and poly(HEMA). The initial pressure sensitive phase was extrapolated back to the y-axis to obtain the theoretical force required to separate the polymer from the tissue when no force was applied for contact which is beyond the detection limit in this system. Linear regression analysis showed a positive extrapolated y-intercept value for polycarbophil and methacrylic acid polymer, of 0.164 and 0.072 gram respectively. For poly(HEMA), the extrapolated value is negative which can be interpreted that poly(HEMA) will not adhere to the tissue without external force. The critical force required for contact for poly(HEMA) to adhere to the tissue can be also obtained as the x-intercept value from linear regression, which has been calculated as o.688 gram for poly(HEMA).

Figure 9. Effect of applied force on *in vitro* bioadhesion of polymers. ○, polycarbophil; △, poly[methacrylic acid-divinyl benzene (0.3%)]; ●, poly(HEMA), each point represents the average of six to ten measurements (± S.E.M.)


FORCE REQUIRED FOR DETACHMENT (X10^{-2}DYNES/CM^2)
20
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
FORCE APPLIED FOR CONTACT (G)

F. In Vivo GI Transit Studies

The ^{51}Cr ions were strongly sorbed onto the polycarbophil polymer. After repeated washing, no further removal of the ^{51}Cr occurred. Migration of ^{51}Cr ions to the surface of the polymer during drying did not appear to occur since no radioactivity was detected from the filtrate after rehydration of dried ^{51}Cr impregnated polycarbophil. The clear gastrointestinal fluid from the test animal did not show any radioactivity, confirming that no leaching of ^{51}Cr ion occurred during the experiment however, the mechanism of ^{51}Cr labeling on the polymer is not known.

Rats appear to be an acceptable animal for these studies, as judged by ease of use, cost, and the fact that comparative differences in GI transit were observed for different polymers and the normal saline solution. Table IX indicates that emptying of ^{51}Cr-labeled normal saline occurs at a very rapid rate; only 5.0 ± 0.9% remains in the stomach at 60 minutes postdosing. On the other hand, for the solid control, Amberlite 200 resin beads, 3.8 ± 1.4 %, remained in the stomach after 8 hours. Of the two polymers tested, ^{51}Cr-polycarbophil has a significantly slower GI transit compared with ^{51}Cr-poly(methacrylic acid-divinyl benzene). The former had 9.0 ± 3.2% left after 24 hours compared with 8.3 ± 2.7% after 16 hours for the latter. The plot of percent of material remaining in the stomach versus time is shown in Figure 10ab. A semilogarithmic plot of the data, shown in Figure 11, produces a straight line for all materials tested with good correlation coefficients. The control and the test materials appear to follow first order

Table IX. Percent of Test Material Remaining in Rat Gastrointestinal Tract.

Time (Hour)	^{51}Cr-Polycarbophil		
	Stomach	Small Intestine	Total
1	96.9[a]±1.2[b]	3.1±1.2	100
2	91.3±2.5	8.7±2.5	100
4	84.8±2.4	15.2±2.4	100
6	78.3±1.5	21.7±1.5	100
8	-	-	-
10	54.6±1.0	17.2±6.0	71.8
16	-	-	-
17	37.0±3.4	17.4±2.2	54.4
24	9.0±3.2	12.4±3.6	21.4

[a] Mean of five rats
[b] S.E.M.

Table IX. (Continued)

Time (Hour)	^{51}Cr-poly(methacrylic acid-divinyl benzene)		
	Stomach	Small Intestine	Total
1	-	-	-
2	82.3±3.8	17.7±3.8	100
4	69.0±4.7	31.0±4.7	100
6	64.1±5.4	35.9±5.4	100
8	33.9±5.6	44.5±6.6	78
16	8.3±2.7	14.8±1.4	23

Table IX. (Continued)

Time (Hour)	Amberlite 200 Bead		
	Stomach	Small Intestine	Total
1	95.8±1.9	4.2±1.9	100
2	62.4±3.3	37.6±3.3	100
3	26.8±5.4	73.2±5.4	100
4	11.8±3.5	88.2±3.5	100
6	3.6±1.2	79.1±9.7	82.7
8	3.8±1.4	47.0±10.2	50.8

Table IX. (Continued)

Time (MIN.)	^{51}Cr-Normal Saline		
	Stomach	Small Intestine	Total
5	37.7[a]±4.4[b]	62.3±4.4	100
10	26.5±5.1	64.3±5.5	91
20	20.3±2.6	71.8±6.3	92
30	14.7±1.5	79.5±1.4	94
60	5.0±0.9	94.0±1.1	98

[a] Mean of five rats

[b] S.E.M.

Figure 10a. Rat gastrointestinal transit of polymers. ○, polycarbophil; ▽, poly(methacrylic acid-divinyl benzene); □, Amberlite 200 resin bead.

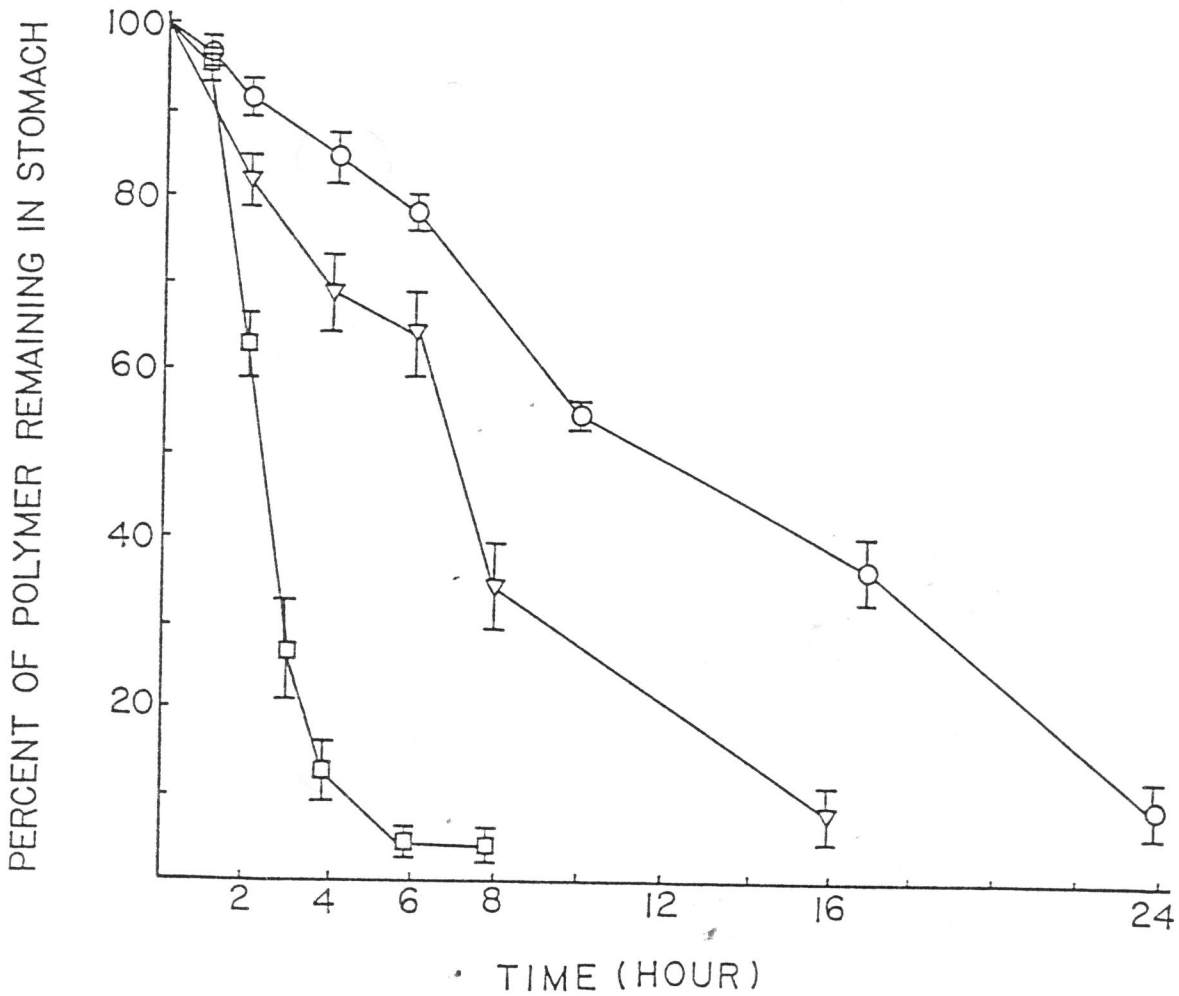

PERCENT OF POLYMER REMAINING IN STOMACH
100
80
60
40
20
2
4
6
8
12
16
24
TIME (HOUR)

Figure 10b. Rat gastrointestinal transit of ^{51}Cr labeled normal saline.

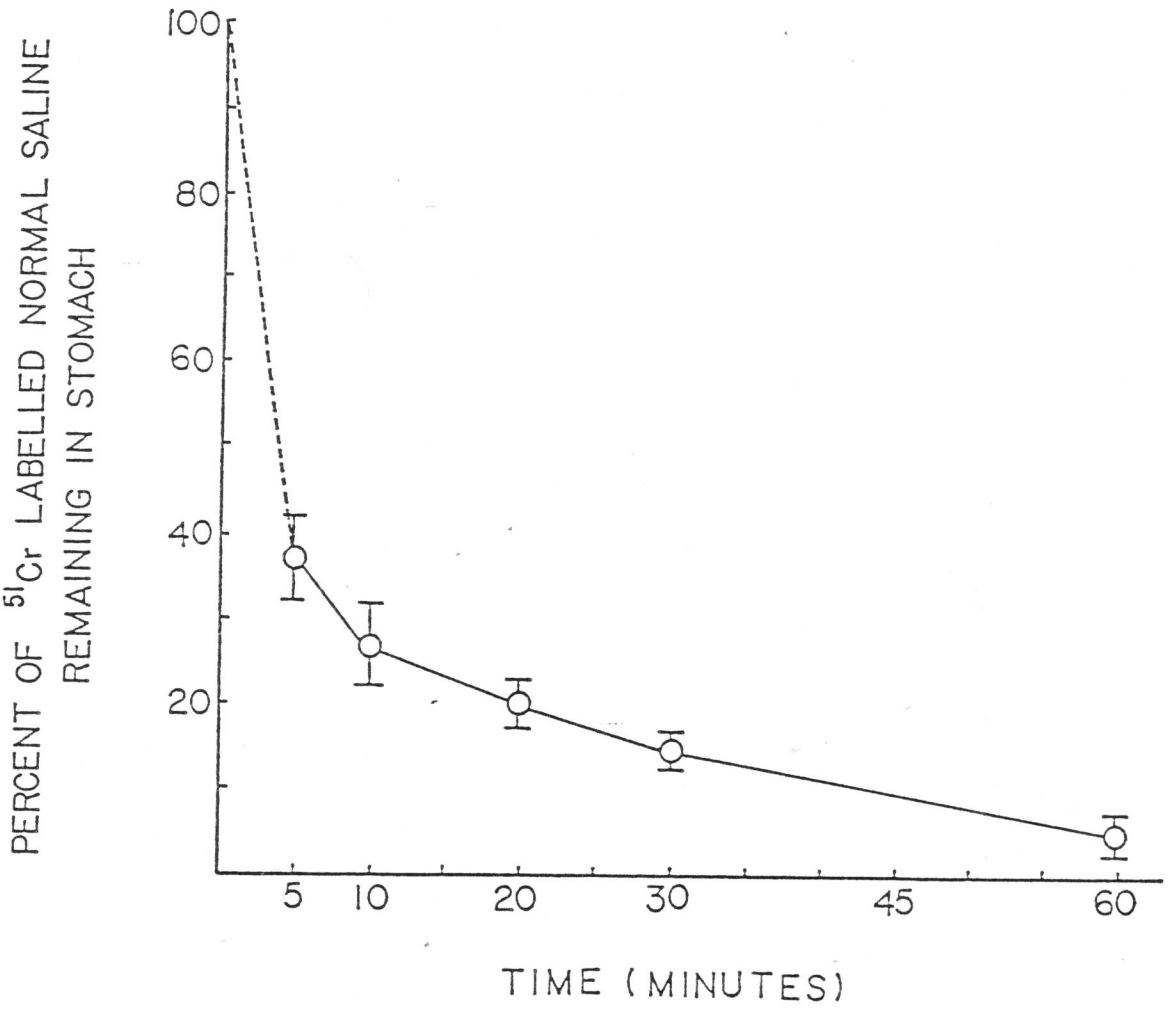
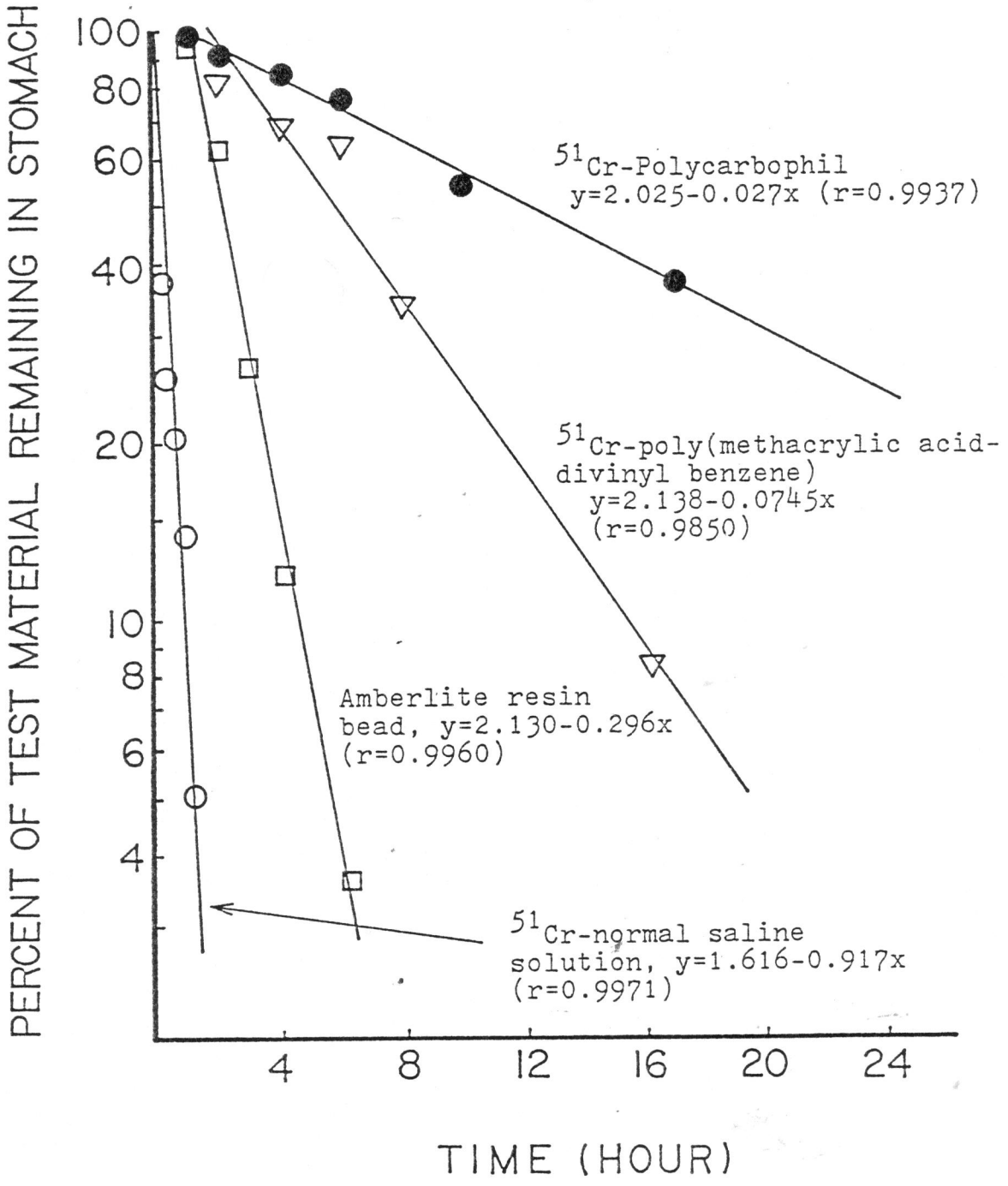

PERCENT OF ^{51}Cr LABELLED NORMAL SALINE REMAINING IN STOMACH
100
80
60
40
20
5
10
20
30
45
60
TIME (MINUTES)

Figure 11. Semilogarithmic plot of percent of test materials remaining in rat stomach versus time.


PERCENT OF TEST MATERIAL REMAINING IN STOMACH
100
80
60
40
20
10
8
6
4
4
8
12
16
20
24
TIME (HOUR)
51Cr-Polycarbophil
y=2.025-0.027x (r=0.9937)
51Cr-poly(methacrylic acid-
divinyl benzene)
y=2.138-0.0745x
(r=0.9850)
Amberlite resin
bead, y=2.130-0.296x
(r=0.9960)
51Cr-normal saline
solution, y=1.616-0.917x
(r=0.9971)

kinetics with the associated gastric half transit times shown in Table X.

The above findings suggest that polycarbophil, whose structure is shown in Scheme 1 and which has less hydrophobic groups as compared with poly(methacrylic acid-divinyl benzene), adheres better to the mucin/epithelial cell surface. Therefore it can be deduced that certain physical properties such as the extent of hydration and charge density did not contribute extensively to the difference in bioadhesion of the polymers in this experiment because these parameters were kept approximately the same for the test materials and control. Figures 12-14 shows movement of test bioadhesive polymers and control through the rat small intestine. The rates of emptying of materials from the small intestine occurred in the same rank order as in the stomach with Amberlite 200 resin beads emptying at 12.3% per hour; poly(methacrylic acid-divinyl benzene), 7.7% per hour: and polycarbophil, 4.4% per hour. The results indicate that bioadhesion also occurred in the small intestine. However, in order for the polymer to adhere to the mucin/epithelial cell surface of the small intestine, there must be some free binding sites on the surface of the polymer available for adhesion. This can only occur when the surface of the polymer is not fully covered with mucin when it is discharged from the stomach.

It was noted throughout these studies that contamination of the tissue surface with blood significantly reduced bioadhesion. Rapid surgical technique and care during surgery minimized or eliminated contaminating blood. Nevertheless, some blood might have been present during these

Table X. Gastric Half Transit Times of Test Materials in Rat.

Testing Material	Gastric Half Transit Time
^{51}Cr-Polycarbophil	12 hours
^{51}Cr-poly(methacrylic acid-divinyl benzene)	5 hours 48 min.
Amberlite 200 resin bead	2 hours
^{51}Cr-normal saline	12 min.

Figure 12. Movement of Amberlite resin beads in rat small intestine.

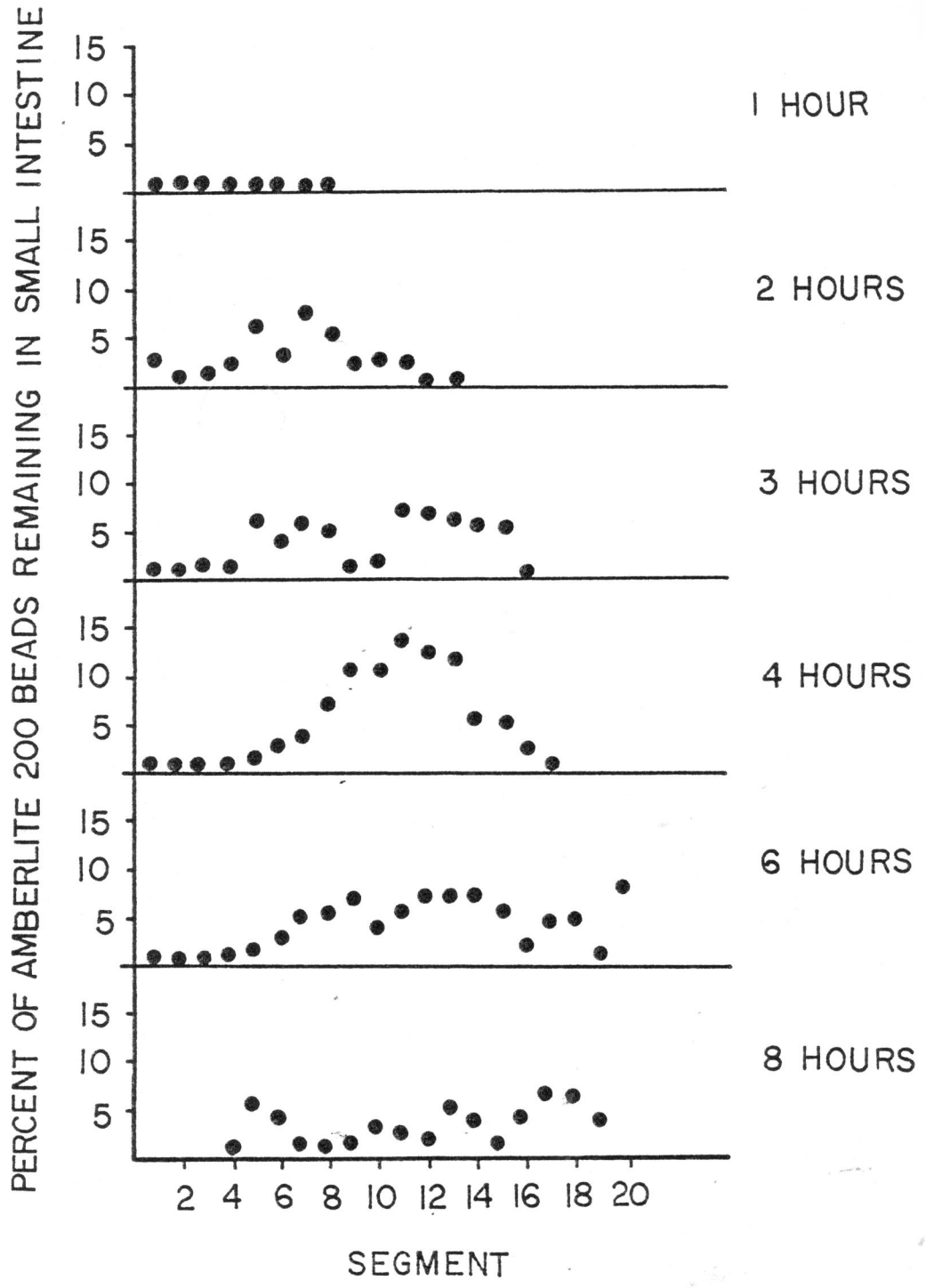

PERCENT OF AMBERLITE 200 BEADS REMAINING IN SMALL INTESTINE
15
10
5
1 HOUR
2 HOURS
3 HOURS
4 HOURS
6 HOURS
8 HOURS
2 4 6 8 10 12 14 16 18 20
SEGMENT

Figure 13. Movement of ^{51}Cr-poly(methacrylic acid-divinyl benzene) in rat small intestine.

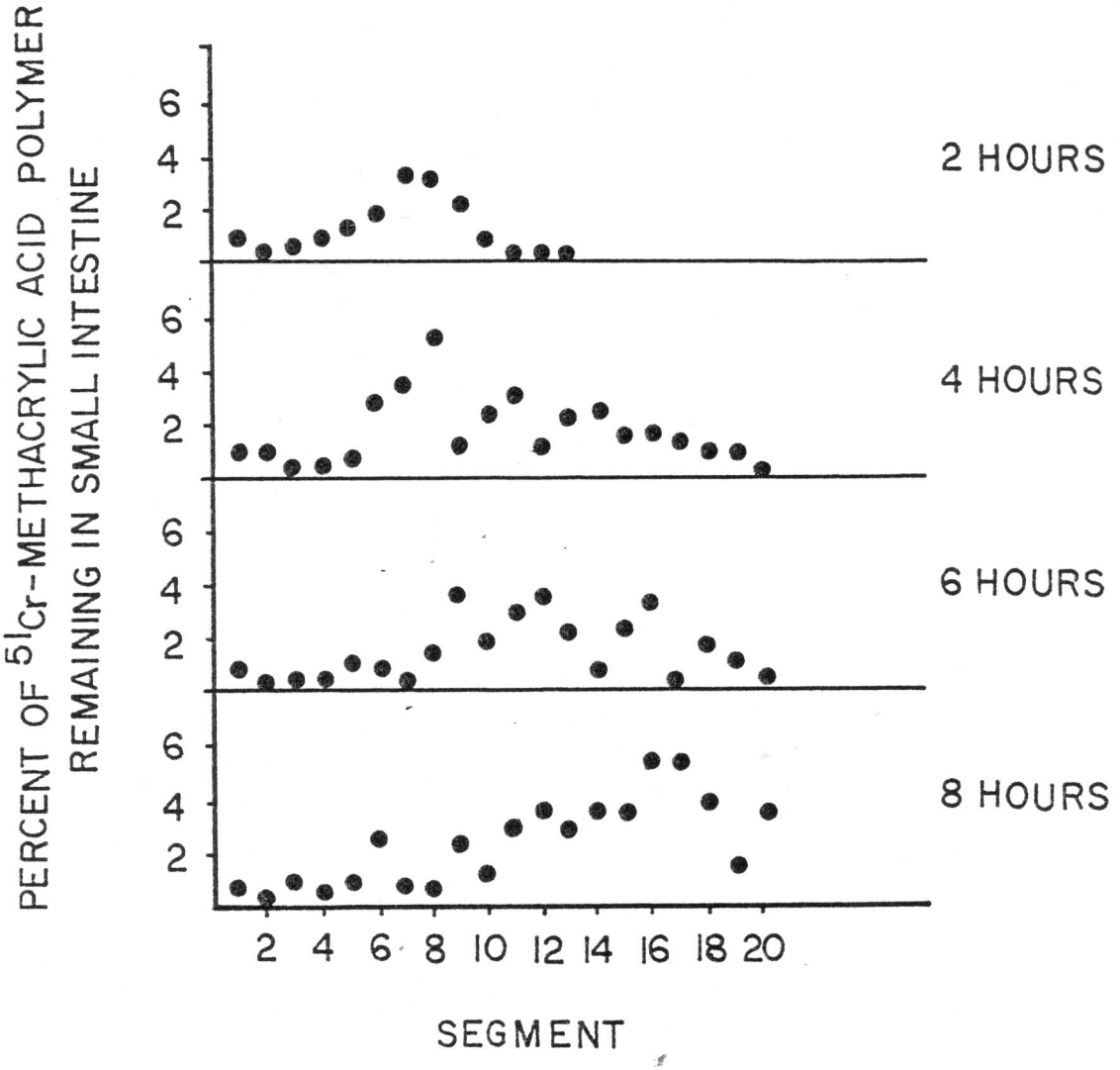
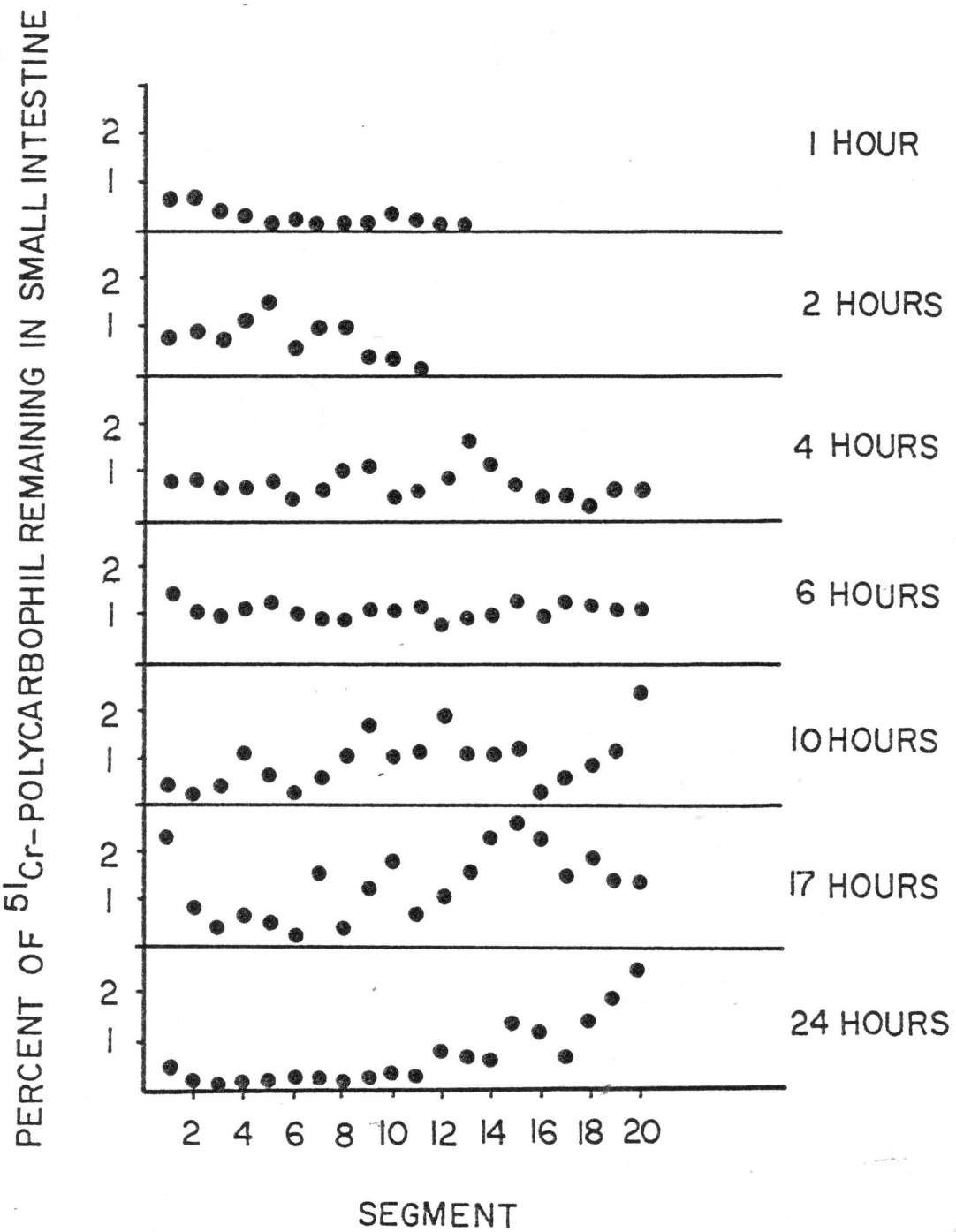

PERCENT OF ^{51}Cr-METHACRYLIC ACID POLYMER REMAINING IN SMALL INTESTINE
2 HOURS
4 HOURS
6 HOURS
8 HOURS
2 4 6 8 10 12 14 16 18 20
SEGMENT

Figure 14. Movement of ^{51}Cr-polycarbophil in rat small intestine.


PERCENT OF ^{51}Cr-POLYCARBOPHIL REMAINING IN SMALL INTESTINE
2
1
1 HOUR
2 HOURS
4 HOURS
6 HOURS
10 HOURS
17 HOURS
24 HOURS
2 4 6 8 10 12 14 16 18 20
SEGMENT

studies; and if so, even greater bioadhesion would be expected when the polymers are administered orally instead of being surgically implanted.

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