"A study of THE PHYSICAL PROPERTIES of Barium Sulphate suspensions for use as Radiopaques"

By Roger John Simmonds.

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Chemistry department,
Bedford College
Regents Park
London, N.W.1.

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ABSTRACT.

The possible use of laboratory methods of (a) testing the efficacy of stabilised and unstabilised barium sulphate suspensions as radiopaques. (b) elucidating the interaction of barium meals with the gastro intestinal tract, and (c) predicting their efficiency as diagnostic tools, was investigated. Two methods involving model systems in which conditions in the gut after administration of a barium meal are closely approximated, were developed. These were established and used to investigate the effect of various physical and chemical parameters, such as concentration of barium sulphate, nature and concentration of stabiliser, pH, state of the mucous membrane of the gut surface upon the thickness and uniformity of the layer of barium sulphate adsorbed upon the gut surface.

The efficiency of various charged derivatives of methyl cellulose as stabilisers of barium sulphate suspensions was investigated, and the viscosity, stability, dispersibility of the suspension and the surface properties of particles characterised for suspensions containing various concentrations of different carboxy methyl cellulose grades.

The surface properties of particles derived from commercial stabilised suspensions were related to the additives used in their manufacture.

The site of adhesion of barium sulphate particles to the gut was shown to be the mucous membrane. The adhesion was not due either to charge/charge interaction of barium sulphate particles with the mucus surface, or to the stabiliser which coated the particle surface. Stabilisers increased the uniformity and reproducibility of the adsorbed layer of barium sulphate upon the gut surface, and particles in this layer become coated with mucus. There was a limiting concentration at which the gut surface was saturated with barium sulphate. The state of the gut surface mucus had a pronounced effect upon the amount of barium sulphate adsorbed on the surface.

The physical properties required of an efficient barium meal are discussed with reference to results obtained for the model systems.

CONTENTS.

SUMMARY

The thesis describes the results of studies of unstabilised and stabilised commercial and laboratory prepared barium sulphate suspensions in water, which are used in the X-ray examination of the gastro intestinal tract.

Very little systematic work of a physical chemical nature has been reported previously; papers, usually in medical journals, deal either with the physical properties of suspensions in a qualitative fashion, or with their performance under clinical conditions. Correlation between the physical properties of a suspension, such as concentration of barium sulphate, viscosity, stability, nature of stabiliser, particle size and its efficacy in vivo, when attempted is of a very unsatisfactory and qualitative nature. Complications arise not only because the physical properties are not well understood, but also because there are no accepted criteria whereby a radiograph may be judged; this is a subjective matter. A further difficulty is that the nature of the mucous membrane of the gastro intestinal surface, with which the suspension is assumed to react, is poorly understood.

The main physical properties of suspensions have been established (Goddard, 1970) including the role of the stabiliser, suspension stability, particle size and surface properties. It was demonstrated that the stabiliser fully coated barium sulphate particles; the stability of a suspension was related to the viscosity and degree of structure of the equeous phase. The dispersibility of a suspension in acid and in water was related to the degree of hydration, or hydrophilic character of the particles. No attempt was made to relate the investigated parameters to a suspensions effect in vivo, and little previous work upon this topic has been reported. Bircher et al (1971), however, investigated two widely differing suspensions under clinical conditions. Radiographs were expertly assessed under a number of criteria and this related statistically to the suspensions concentration and viscosity. These

authors concluded that the two suspensions gave differing results, but were not able to say which physical property or properties were responsible for this.

Theories of the interaction of barium sulphate suspension with the surface of the gastro intestinal tract are few; charge : charge interaction has been invoked, as has a process analagous to precipitation. Hitherto there has been no experimental evidence to support either view.

What is certain is that previous *in vitro* tests of radiopaques are quite inadequate, and give little indication of the performance of a barium meal *in vivo*. The conditions of interaction of a suspension in the gut with the gastro intestinal mucosa are very complicated, and no *in vitro* test has attempted to approximate these conditions to any great extent.

The present study follows on from work by Goddard and was an attempt to develop and apply a model system which would accurately reflect conditions in the *in vivo* situation, whilst allowing abjective assessment of mucous membrane/barium sulphate interaction. The study can be divided into;

(1) The development of an *in vitro* method of investigating the interaction of barium meals with the surface of the gastro intestinal tract.

(2) The development of an *in vivo* model system, and the use of the method to demonstrate the effect of changing the physiological conditions in the gut upon radiopaque efficiency.

(3) An investigation of surface properties of variously coated barium sulphate particles.

(4) An investigation of various carboxy methyl cellulose derivatives for use as stabilisers.

(1) This involved a study of the interaction of suspensions with small

sections of gut tissue derived from rat duodenum and small intestine. The reaction could take place under strictly controlled conditions, and many experiments were performed upon tissue obtained from a single animal; animal variation in a set of experiments was therefore minimised. Suspensions from various sources were investigated under various conditions of pH, stabiliser, concentration etc.; and the interaction of the suspension with the gut surface assessed by evaluation of derived microscopic preparations.

(2) This involved the perfusion of a barium sulphate suspension through the small intestine of an anaesthetised rat. The thickness of the resulting coat of barium sulphate was assessed by the weighing of the residue left adhering to a specific length of gut after all organic matter had been removed.

(3) The surface properties of particles coated with cmc. derivatives were investigated, and the effect of the degree of substitution upon surface charge demonstrated. The surface properties of particles from various commercial sources were investigated, enabling some general recognition of stabilisers used. Coated particles which had been recovered from the ileum of both normally fed animals and those subjected to various fasts, were examined and the interaction of suspensions with mucous solutions investigated.

(4) The variation of the stability of 60% w/v. suspensions with concentration of cmc. (0 - 1·0% w/v.) was investigated, and this was correlated with the viscosities of suspensions, and the degree of substitution of the cmc. derivative. The dispersibility of the prepared suspensions was measured qualitatively.

The model systems were not completely successful, because of experimental difficulties, notably the method of assessing results. Although there was considerable variation in some of the results obtained from the in vivo method (2), nevertheless, significant results were obtained. The systems were a great improvement upon previous laboratory tests, and the results obtained were capable of interpretation from the

point of view of physical and chemical conditions at the gut surface, and application to the clinical situation. Cmc. proved to be an effective stabiliser, especially when the degree of substitution was greater than 0·7 and the concentration of cmc. between 0·6 and 1·0% w/v. The stability of a suspension could be correlated with the viscosity of the suspension, and the dispersibility of a suspension, in both acid and water related not only to the surface properties of the particles but also to the degree of "structure" possessed by the aqueous phase.

The general conclusions obtained from this study of the model systems (1) and (2) may be summarised as follows:

(a) The site of adsorption is the mucous membrane of the gut surface. Penetration of particles could be deep.

(b) The barium sulphate particles reacted with the mucus at the surface. No adhering layer of barium sulphate was found upon surfaces devoid of mucus.

(c) The particles could react with free mucus present in the lumen of the gut resulting in ogglomeration (or flocculation) of the suspension and uneven coating of the gut.

(d) The mucous membrane becomes fully coated with barium sulphate when the comcentration of a stabilised suspension at the surface is 50% w/v. Increasing the concentration above this figure does not result in greater adsorption. Good firm coats of barium sulphate upon the gut surface were obtained with more dilute suspensions.

(e) The stabiliser was not responsible for adherance of particles to the gut surface. Unstabilised suspensions also resulted in an adsorbed layer, although this was irregular. For unstabilised suspensions no limiting concentration was found.

(f) The main action of the stabiliser, from the point of view of efficacy in use, was to increase the uniformity of the layer resulting in more consistent results. This was related to a reduction of flocculation and sedimentation of the suspension in the gut. Sodium citrate as a stabiliser

did not coat and protect the particles of barium sulphate, although it much reduced the viscosity of the suspensions, which behaved similarly to preparations containing no additives.

(g) The surface charge of the particles was not responsible for the adhesion of particles to the mucus. The adsorbed layer was unaffected by pH, and all stabilised suspensions, containing different stabilisers and thus having different surface properties, gave essentially similar adsorbed layers.

(h) Alteration of the state of the surface mucus, either by the topical action of a drug (N-acetyl-l-cysteine) or by previous fasting of the experimental animal,had a great effect upon the adsorbed layer, resulting in a 70% reduction in the weight of barium sulphate. This was correlated to the decrease in viscosity of the mucous membrane.

(i) In becoming attached to the surface of the gut at the mucous membrane the particles of barium sulphate, whether from unstabilised or stabilised suspensions, became recoated with mucus.

It was concluded that the model systems gave a good indication of the clinical performance of a barium meal and their application could be useful in the initial development of a barium meal. It is essential that studies of model systems be extended, in particular the results must be correlated and compared with actual X-ray plates.

CHAPTER ONE

INTRODUCTION

Chapter 1. Section 1. Barium meals.

Radiopaques are used for the medical examination of internal body organs. They are introduced into the organ under investigation and X-rays are directed through this region of the body, whereupon a photograph is taken. The organs appear on the radiograph as white images on a black background. The action of a radiopaque depends upon its ability to coat the surfaces, or fill the lumen, of the organ, and to absorb X-rays. There are two main categories; those based upon insoluble inorganic compounds of heavy metals, and those based upon soluble organic molecules containing iodine. In the present study only inorganic radiopaques based upon barium sulphate were investigated.

Suspensions of barium sulphate particles in water have long been the preferred radiopaque for investigation of the gastrointestinal tract, since barium sulphate is insoluble and non-toxic (when prepared under controlled conditions), cheap, easily administered, and gives good but by no means perfect visualisation of the organs under examination. Such suspensions, either available commercially, or prepared by radiographers according to their own specifications, usually have a number of additives to improve their efficiency as a diagnostic tool. These additives can be stabilisers, fluidisers, antibacterial agents (to improve storage ability) flavouring, and nutrients such as oil and sorbitol to increase the speed with which a suspension passes through the gastrointestinal tract.

Radiographers are agreed that suspensions should deposit a thin, even coat of barium sulphate particles upon the surface of the mucosae. The coat should be pliable, not flake unevenly, and resist peristalsis in the gut, and the manipulations of the gut by the examiner. Furthermore the layer should persist for several hours, and then be easily voided. It is generally agreed that unstabilised suspensions of barium sulphate are not efficient radiopaques, and that the addition of a stabiliser confers some of the above properties upon unstabilised

suspensions. There is, however, disagreement as to the best conditions for achieving a thin and even coat. Some radiographers recommend the use of suspensions of high barium sulphate concentration, and go to great troubles to achieve this, including the addition of coarse barium sulphate particles to a suspension of fine particles, and even the introduction of precipitating agents to the suspension in the gut. The patient is rolled over so the particles sediment over the whole surface of the gut. Nelson (1967) pleads for the use of small volumes and concentrations of suspension, adding that vast quantities will distort the organ under study; Root and Morgan (1969) come to similar conclusions.

Barium sulphate suspensions are unsatisfactory when the resulting radiograph gives an inadequate, and in some cases misleading representation on the surface of the gut. Failure in this respect can be due to a number of factors, the most important is flocculation in the lumen of the gut. This may be due to the instability of these suspensions to the conditions of pH and ionic strength in the gut, or reaction with free mucus. Astley and French (1951) showed the <u>in vitro</u> precipitation of unstabilised suspensions with salivary and gastric mucus. Commercial preparations which contained a stabiliser were not similarly affected by this treatment, but Pirk et al (1967) demonstrated that many available preparations had an increased rate of settling when gastric juice was added. Knoeffel et al (1956) suggested that flocculation is due to the uptake of a specific fraction of mucus by the barium sulphate particles.

The effect of flocculation is to give an appearance of patchiness due to uneven coating of the gut surface with aggregations of particles. The resulting thick coat masks important surface features.

Many stabilisers have been reported in the literature; their claimed effect is usually to increase the stability of the suspension

with respect to sedimentation on storage and *in vivo*, and to allow a more concentrated or fluid suspension to be administered, as well as decreasing flocculation *in vivo*. Alexander (1950) reported the use of purified mucin, and an improvement in radiographs was claimed. The resulting suspension was more stable, but too viscous to be used with ease. Other "suspending" agents include flour, acacia, gelatin, pectin, tragacanth, malted milk, egg yolk, and methyl cellulose, with various claims being made for their efficiency in use. Inorganic stabilising agents reported include aluminium hydroxide (Marks 1951) silicones (Keats and Whitrock, 1955), sodium or potassiam citrate or pyrophosphate (Gutcho, 1970) and dioctyl sodium sulpho succinate. Their invariable effect is to make the suspension more fluid *in vitro*, but in the gut the suspensions quickly become dehydrated, which reduces them to an insoluble immobile mass in the large bowel. Degraded carageenan and ghatti gum have been reported as giving good suspensions(Anderson, 1962), but synthetic stabilisers, rather than plant extracts have become more popular since they are of more uniform quality, cheap, and available with a wide range of properties. In particular cellulose derivations have had good reports (e.g. Kirsh and Spellberg 1953, Kotzmann, 1969, Gutcho, 1970) Knoeffel and Davis demonstrated that derivations containing charged groups were superior to neutral derivatives, Embring and Matsson (1968) reported, however, that carboxymethyl cellulose (cmc.) was unsuitable because the meals so produced were too viscous and tended to be immixible with gastric juices. Their findings are contrary to those of many other workers, and could be due to poor selection of a carboxymethyl cellulose derivative, as many, designed for specific purposes are commercially available. Carboxymethyl cellulose as an additive is cited by many patients for commercial preparations (e.g. US patent 3,236,735, 1966, US patent 3,201,317, 1965).

Goddard (1970) investigated the physical properties of various commercial stabilised suspensions, and laboratory prepared stabilised and unstabilised suspension of barium sulphate, from the point of view of particle size and surface properties; the effect of stabilisers from different sources and concentrations upon suspensions, and the dispersibilities of such suspensions in acid and water. It was shown that the majority of commercial preparations had particle sizes of between 0·07-0·5 μ m, as determined by an electron microscope, and that they were negatively charged at pH 7. The change of this charge with pH of such particles was demonstrated and contrasted with that of particles from unstabilised suspensions. It was concluded that the stabilisers coated the barium sulphate particles completely; barium sulphate itself did not contribute to the surface change. A Langmuir type adsorption isotherm was demonstrated for the uptake of a wide range of stabilisers by barium sulphate particles. The stabilities of stabilised suspensions was related to the viscosity of the aqueous phase, and the degree of structure of this phase (due to high concentrations of stabiliser). Stable suspensions were prepared with all stabilisers investigated, notably CD75, a plant gum extract and cmc. The capacity of suspensions to disperse readily in water and acid was related to the degree of hydration achieved by the surface of particles in suspension.

The most quoted desirable physical properties of barium meals are

(a) good stability on storage and in the gut,

(b) a concentration of barium sulphate 40 – 150% w/v., which can be diluted without precipitation for administration,

(c) uniform particle size,

(d) freedom from air bubbles and from foaming.

Although larger particles have been reported as giving better results (Perez and Friendenberg, 1967), Shufflelbarger (1953) reported

that suspensions where particle sizes ranged 0·3 - 1·5 μ m gave best results, whilst Brown (1963) quotes 1·0 - 2·0 μ m as the optimum. Confusion arises because no clear distinction is made between the sizes of individual particles, as determined by election microscopy, is generally under 0·5 μ m. Embring and Matsson (1968) stress that it is the size and stability of aggregates that determine the quality of the radiograph. Certainly aggregates must occur in suspension. Absolute particle stability is not required (Anderson, 1962) and whilst there is no general agreement concerning the viscosities of suspensions (confusion arises because it is not realised that many suspensions are non-Newtonian) it is agreed that suspensions should mix readily with the gastric juice and other physiological liquids in the gut. Gutcho (1970) adds that particles should remain in suspension for 30 minutes, and should not clog enema tips or associated tubing.

Radiologists disagree which commercial meals, or meals prepared by themselves are best, or why. This is no doubt due to the wide variety of administration techniques used, the great variability of physiological states encountered, either due to the illness of the patient or variation in patient preparation techniques, or the variability of commercial preparations stabilised with plant gums (Pirk et al, 1967). There are no agreed objective criteria in the assessment of radiographs (Bircher et al, 1971) and a fundamental lack of knowledge regarding the interaction of barium sulphate particles with the mucosae of the gut.

<u>In vitro</u> tests are limited to tests of dispersion in acid and water and the effect of addition of gastric juice to suspension, and assessing of adhesion of particles to glass (Miller 1965) and gelatin. The interpretation of such tests, and predictions made concerning <u>in vivo</u> behaviour of radiopaques are of limited value.

The effectiveness of clinical trials are beyond doubt, but these

are lengthy, costly and still depend upon the subjective assessment of radiographs by experts. Few systematic and controlled trials have been carried out, and only one had statistical design (Bircher et al 1971). Here the effectiveness of two widely differing commercial barium meals were compared, and the effectiveness of additives increasing the speed the suspension travels through the gut investigated. Both meals contained stabiliser, one was viscous and highly stable, the other suspension quickly sedimented. It was concluded that the two suspensions gave different results, the more stable suspension being slightly, but significantly better. The difference was not due to differences in viscosity, and the viscous suspensions were only advantageous in the oesophagus where peristaltic movements were greatest.

The physiological effect of the two suspensions on the gut were different, but the differences in performance could not be related to this. Results were difficult to interpret because of patient and observer variation, particularly because patients were diseased.

Because of confusion concerning the physical properties of barium meals, and their relation to the quality of radiographs, theories of adhesion to the surface of the gut are few. Adolph & Taplin (1950) compare it to precipitation, Embring and Mattson (1968) to a process of sedimentation. Miller (1965) assuming particles to be negatively charged, and the surface of the gut to be positively charged suggests an electronic interaction. It is obvious that a definite interaction occurs between barium sulphate particles and the mucus present at the surface of the gut, since the particles persist at the surface when all excess suspension is removed. The situation is further complicated because the structure and physical properties of mucus are not well defined.

Chapter 1. Section 2. The structure and function of mucus.

Mucus is a general term for any biologically derived viscous

liquid. It is virtually ubiquitous in the animal and plant kingdom. The organic constituents, which may or may not be soluble, of such liquids are known as mucins, and are present in quite low concentrations (0·2% w/v. for porcine gastric mucus). The mucins are macromolecular compounds containing both peptide and carbohydrate portions. The term glycoprotein is now in general use for such compounds. Glycoproteins can be further subdivided into mucoproteins, where protein is predominant, and mucopoly-saccharides, where the converse is the case. For both the key components are the amino sugars. The link between the carbohydrate and protein moeties of the molecule, ~~is~~ is covalent, anionic; the molecular weight of ~~which~~ the chain may be of the order of 2×10^6. ~~can be ionic or covalent.~~

Throughout the mammalian gastro-intestinal tract the epithelial surface is continuously protected by a flowing layer of mucus. This film is composed of a three dimensional gel which is synthesised and secreted by certain epethelial cells throughout the system. The mucus not only protects the villi of surface of the gastrointestinal tract from mechanical and chemical injury, but also provides a micro enviroment for the micro villi. The pH, ionic concentration and protein enviroment of the delicate micro villi are to sime degree independent of the biochemical events in the lumen of the gut. The term "glycocalyx" (Bennet 1963) has been used to describe such a system. Cells responsible for secretion of mucus are found in all regions of the stomach (in man and pig) as well as in the duodenum (notably in Brunner's glands) and the intestine (goblet cells).

The complete elucidation of the structure of any gastric or intestinal glycoprotein has not been accomplished. This is partly due to the complexity of the material and partly because of the difficulty of obtaining sufficient homogenous glycoprotein from a defined region of the gut, uncontaminated by secretions from other regions. Purification of such fractions that have been collected

also results in disruption of the tertiary structure of the macro molecule, and some loss of labile constituents (Kent, 1962). Variation of collected fractions can also be due to the state of the system from which they are collected (Menguy, 1969).

However, the sugar and amino acid units of glycoproteins derived from the gastrointestinal tract have been well established. Galactose, fucose, mannose, hexose and hexosamine have been found in practically all fractions as well as sialic acids. Sialic acids are acyl derivatives of the parent acid D-neuraminic acid, which itself does not occur naturally; three forms are commonly encountered: N-acetyl, N-glycollyl and 7 - O acetyl N-acetyl neuraminic acid, all common amino acids have been found, notably threonine, proline, alanine, serine glutumic acid, and cysteine. Chondroitin sulphate and Kerato sulphate have not been observed in mucus of the gastro intestinal tract to any great extent, and are generally thought to be absent.

The most recent work has resulted in the elucidation of the secondary and tertiary structure of a mucus fraction derived from mucosal scrapings from the Cardiac region of the porcine gastric mucosa (Allen & Snary, 1972). By using scrapings, problems associated with the enzymic attack of mucus samples were minimised, and in contrast to samples obtained by other methods the purified fractions of the scrapings formed gels.

Analysis showed that the non-soluble (80%) and soluble mucus gel (20%) were very similar. Separation of the soluble fractions by gel filtration resulted in a high molecular weight mucoprotein A and a low molecular weight mucoprotein B. These were shown to be chemically identical, and it was suggested that A was composed of repeating units of B. It is significant that no sulphated esters were found in either fraction. A "bottle brush" structure for a mucoprotein subunit was suggested, similar to known structures of ovarian cyst blood group functions. The subunit has a molecular weight of 28,000 and consists

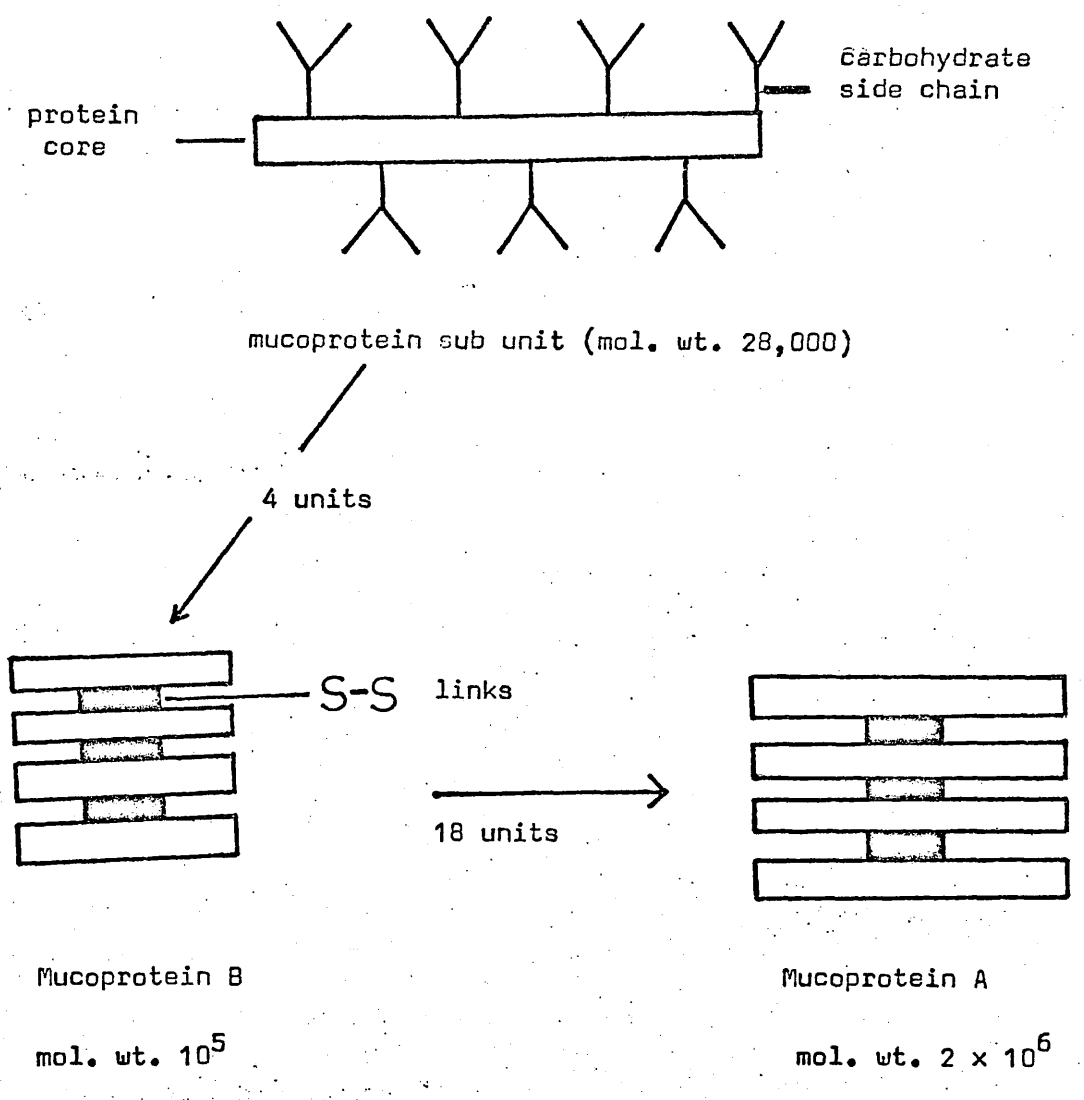
of a protein core with attached carbohydrate side chains. It has been proposed that each side chain consists of about fifteen sugar residues (Kochetkov et al 1970), repeating every three to four amino acid units forming a very tightly packed chain (Fig.1.1.). Four of the subunits are joined by disulphide bridges (across cysteine residues in the protein core), to form mucoprotein A (molecular weight 1×10^5). Mucoprotein B is in turn joined up to form the high molecular weight mucoprotein A (molecular weight 2×10^6) in such a way that it also is composed of four subunits joined by disulphide linkages. Since the molecular weight of mucoprotein B is not altered by treatment with CsCl solution it has been suggested that it is not polymerised by non-covalent interactions.

The proposed structure of mucoprotein A accounts for the high viscosity of the water soluble mucus, and, also for the reduction of viscosity of such solutions when treated with mucolytic agents, such as mercaptoethanal and N-acetyl-L-cysteine, which reduce the disulphide bonds. This would separate the subunits and cause a lowering of the molecular weight (Snary, Allen & Pain, 1972).

The high molecular weight mucoprotein and intact disulphide bridges are necessary for the formation of a gel *in vitro*; it is the irreversible formation of a gel *in vivo* that enables it to function. The *in vitro* gel is easily disrupted by salt solutions; gelling may simply involve the entangling of the mucromolecules (Snary, Allen & Pain, 1972), and its non-reversibility *in vivo* may simply be due to a greater concentration of mucoprotein and hence greater entanglement of the molecules. While it is evident that the integrity of mucoprotein A, which in its highly hydrated and expanded tertiary structure, is a prerequisite for the formation of a mucus gel, further differences in molecular structure between this water soluble mucoprotein and the water insoluble gel found *in vivo* cannot be ruled out.

Fig. 1.1.

STRUCTURE OF SOLUBLE GASTRIC MUCUS (AFTER ALLEN AND SNARY).



It is pertinent to ask how universal the proposed structure of gastric mucus may be. Many other mucous secretions have been shown to be broken down by disulphide bond splitting agents (Holden et al, 1971) into units of smaller molecular weight. This suggests other mucins may have a similar subunit structure. Those mucins unaffected by mercaptoethanal have a different proposed structure. Bovine sub maxillary mucins are an example and the mucoprotein molecules are thought to consist of a single chain "bottle brush". The analysis of human gastric mucus is closely similar to that of pig gastric mucus (Shrager, 1970) but demonstration of a similar three dimensional structure has yet to come.

The epithelial layer of mucus is constantly being removed and replaced. Turnover of mucins is very fast, as is shown by ^{14}C in corporation studies of tissue cultures in vitro. The secreted gel is coherent when first secreted, but soon imbibes more water and becomes swollen. Gastric acid causes flocculation and breakdown of the gel structure, and finally initiates the disintigration of the macromolecule with the liberation of fucose, sialic acid, and peptides. The rapidity with which gut epethelial cells divide and are shed from the surface of the gut must also contribute to the mucus level present in the gut (Stevens & Loblond, 1953).

Many drugs are known to have a great influence upon secretion of mucus in the gastro intestinal tract (Kent, 1962) when applied to the surface of the gut in vitro and in vivo, notably asprin, and the topical application of chemical irritants such as dilute mustard oil or HCl which cause rapid emptying of the goblet cells of the colon. Dieting and fasting (Wise & Ballinger, 1963) change the carbohydrate/ protein ratio of mucoproteins in the gut; stress is also known to affect the mucus layer. The effect of injected drugs is less well characterised, but those which cause an increase in gastric acid

secretion, such as pentagastrin, are also thought to increase the secretion of mucus. Oral administration of asprin is also known to affect the mucus barrier, but as yet little work has been done on its exact action.

Chapter 1. Section 3. Electrical double layer

It has been shown that coated particles of barium sulphate are ionogenic, and as such the surface of the particles must have an electrical double layer structure.

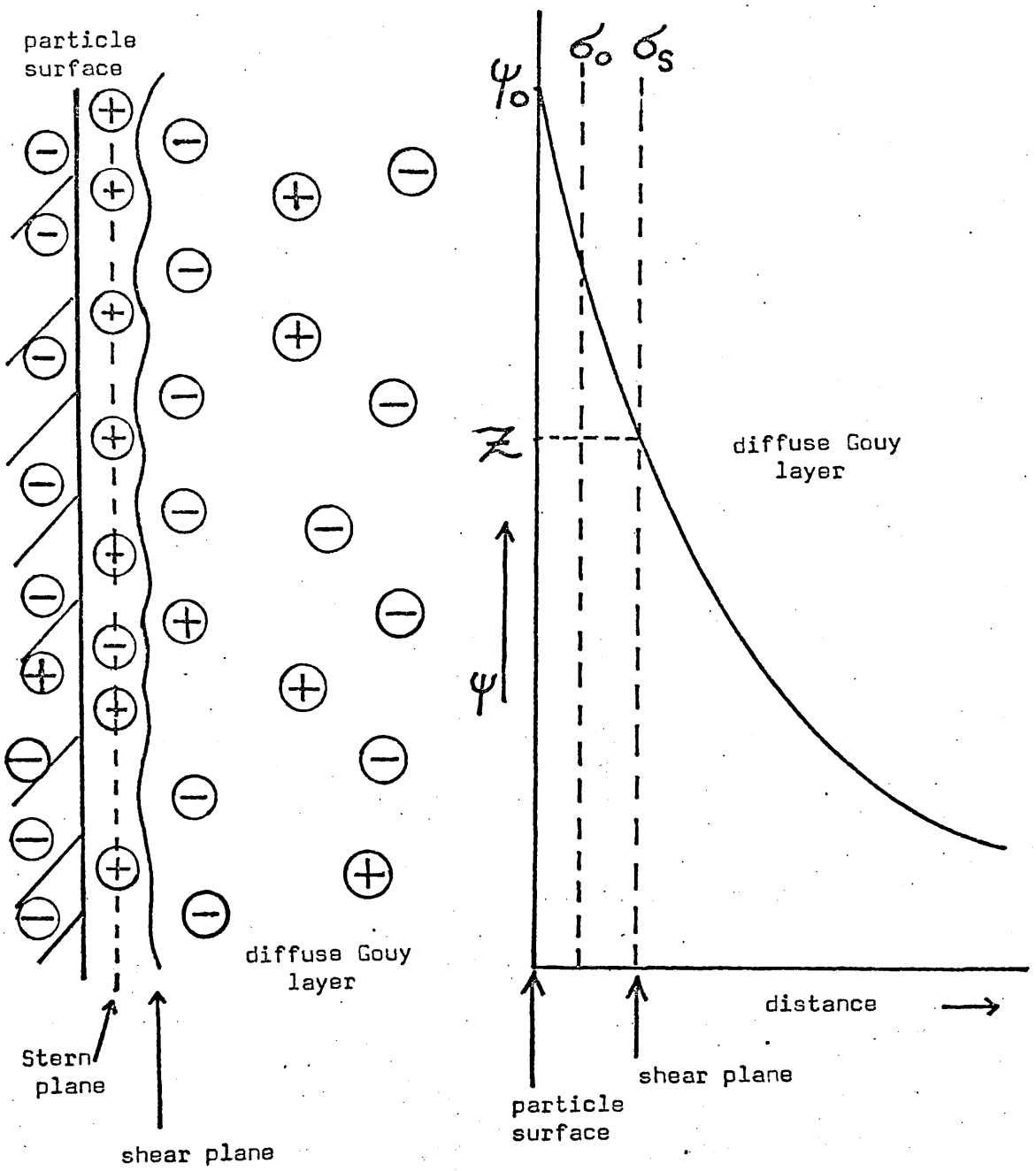
The theory of the electric double layer is concerned with the distribution of counter-ions and co-ions in the locality of the charged surface, and with the magnitude of the electric double layer can be regarded as consisting of two regions, an inner region of iongenic groups, or absorbed ions, and a diffuse region in which ions are distributed according to the influence of electrical forces and random thermal motion. The structure of the double layer, according to the stern theory and taking into account the finite size of ions, is shown in Fig.1.2. ψ_o is the electric potential at the surface, which is about 2 - 3 times ζ, the potential at the plane of shear. The potential exists when an applied electrical field causes one phase to move with respect to the other. σ_o and σ_s are the charge densities respectively at the surface and at the plane of shear. The mobility of a particle, the only directly measurable parameter (Chapter 2. Section 6.) is related to the zeta potential:

$$\bar{v} = C \frac{\epsilon r \zeta}{\pi \eta}$$

where r is the relative permitivity and η the Coefficient of viscosity in the double layer. C is a constant whose value depends upon K, the reciprocal thickness of the ionic atmosphere, and on a, the radius of the particle. When $Ka > 300$ Smoluchowski showed C to be equal to 0·25 so that ζ is independent of the size, shape, and orientation of the particle to the field.

Fig. 1.2.

STRUCTURE OF THE ELECTRICAL DOUBLE LAYER



The equation is not altogether satisfactory, and has been modified by Henry (1931) and Booth and Henry (1948) to take account of the surface conductance of the ionogenic particles. In view of the difficulties in calculating the zeta potential from measured mobility values it is customary to discuss electrophoretic results in terms of mobility. Provided the mobility values of different particles are determined under standard conditions of pH, I, etc: then a comparison of these values is analogous to a comparison of zeta potentials.

The charge density, σ_s, at the shear plane is a function of ζ, and can be calculated from the Gouy - Chapman equation, for symmetrical electrolyte:

$$\sigma = \left[\frac{N A E K T}{500}\right]^{\frac{1}{2}} C^{\frac{1}{2}} \sinh \left(\frac{z e}{Z K T}\right)$$

where NA is the Avagadro constant, K the Boltzmann constant, e the electronic charge and z the valence of the counter ion.

Chapter 1. Section 4. Applications of Electrophoretic mobility determination.

The variation of the electrophoretic mobility of a particle in suspension in an electrolytic solution, when the pH, ionic strength or composition of this solution is altered can be interpreted in terms of surface charged groups.

An increase in ionic strength initially causes an increase in the surface charge, but once this is complete the effect of decreasing thickness of the double layer reduces the zeta potential (Fig. 1.3.).

Information about the nature and amounts of surface groups may be obtained by varying the pH of the medium, maintaining a constant ionic strength. For acid groups on the surface the variation of mobility with pH produces curves (Fig. 1.4.) which can be explained by the dissociation of charged groups, the resulting curve is in effect a titiation curve. A particle coated with an acidic polysaccheride, such as cmc. will be titrated according to the equation.

Fig. 1.3.

VARIATION OF ζ WITH IONIC STRENGTH

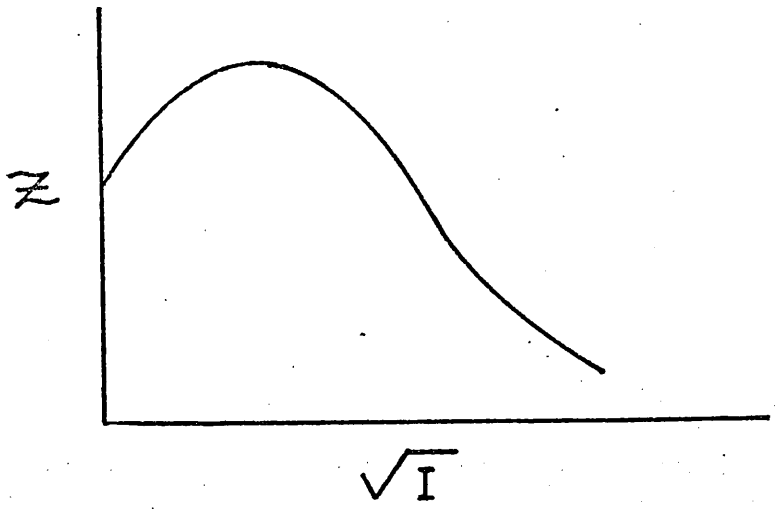
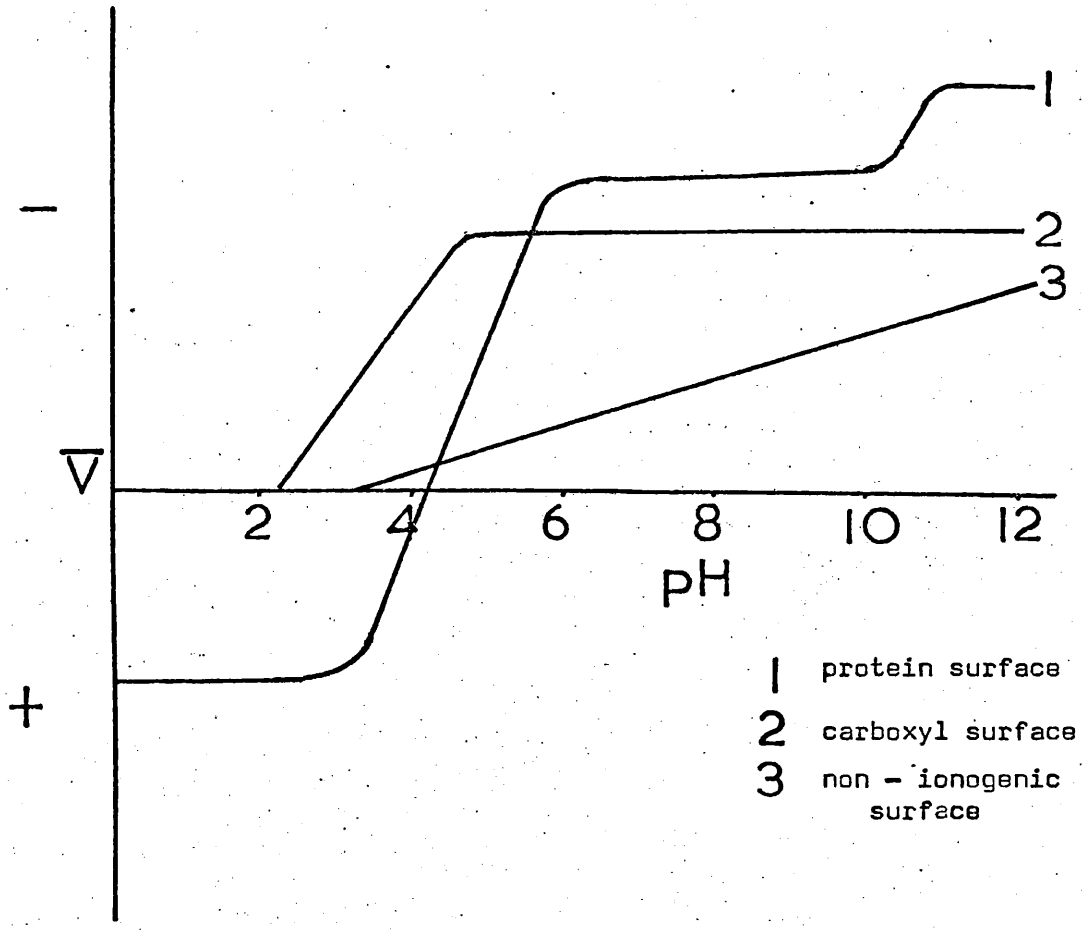


Fig. 1.4.

CHARACTERISTIC pH MOBILITY CURVES OF DIFFERENT SURFACES



$$-COO^- + H^+ \rightleftharpoons -COOH$$

At low pH the surface charge will be zero, but as the pH is increased ionisation of the carboxyl group will occur, with a resulting increase in negative mobility. At pH 4 - 5 a plateau is reached when dissocciation is complete, and no increase in surface charge occurs at higher pH values. The charge of a protein surface containing carboxyl and amino groups, will be the algebraic sum of the charge due to the dissociation of the two groups. At low pH the surface charge will be positive due to $-NH_3^+$, resulting in a positive mobility. On increasing the pH the net surface charge reduces to zero, as the amino groups loose a proton and the carboxyl groups become dissocciated. The mobility therefore changes from positive to negative and eventually reaches a plateau between pH 5 and 9. The increase above pH 9 is due to further ionisation of the amino groups.

Particles with non-ionogenic surfaces normally show a linear variation of mobility with pH, explained by disorption of hydrogen ions or adsorption of Hydroxyl ions with increasing pH.

The pK_a values of the acid groups may be found by taking the pH value at the point where the mobility has a value of half that at the plateau value. Ottewill and Shaw (1967) have shown that this method only approximates to the actual value since the concentration of hydrogen ions in the double layer is different from that in the bulk solution, but the values obtained are adequate for comparison purposes if the ionic strength of the buffer solution is always the same. Isoelectric points for amphoteric surfaces can also be found and correspond to the point when the coated particles have zero mobility.

Chapter 1. Section 5. Forcesin colloidal Systems.

Colloids may be defined as systems in which a significant proportion of the molecules lie in, or are associated with interfacial regions, and the contribution of the interfacial regions to the total

energy of the system is great. Simple considerations show that when matter is divided into particles of 1 μ m. (1,000 nm.) or less, a substantial percentage of the molecules are in, or close to the surface and make contribution to the energy different from those made by molecules in the interior. The lower size limit is determined by the size of the smallest aggregate for which it is meaningful to distinguish between "surface" and "interior" molecules. Conventially 1 nm. is taken as this limit.

Finely divided solids (or liquids) are thermodynamically unstable (or metastable) with respect to the bulk phases. When finely divided material is dispersed in such a phase, the energy associated with such interactions, the dispersed colloid may remain unstable (or metastable) with respect to the bulk phase (lyophobic dispersion), or may become thermodynamically stable (lyophilic dispersion). Fine particles of barium sulphate dispersed in water are unstable; the system is lyophobic (hydrophobic) and particle/water affinity is low.

When two particles in a lyophobic system collide they may adhere to one another. If the system is meta stable only a fraction of the collisions will result in adhesion. The metastability of lyophobic colloids arises from the existance of an energy barrier which has to be surmounted before two particles can adhere.

Particle – particle interactions have been treated quantitatively by Derjagian, Landau, Verwey and Durbeck. These workers showed that a delicate balance existed between the electrical forces of repulsion, the Vander Waals forces of attraction, thermal agitation and Brownian motion.

Attractive forces increase with decreasing particle distance. They are essentially short range forces, distances over which they operate being of the order of atomic dimensions.

Since a charged particle with its double layer is as a whole electrically neutral, no net coulombic forces exist between particles

at large distances from each other. As particles approach the diffuse regions of the double layers interpenetrate giving rise to repulsive forces. The magnitude of the forces depend upon ψ_o and $\frac{1}{Ka}$. The total energy of particle interaction is given by summating the attractive and repulsive forces. Characteristic forms of energy - particle distance curves are shown in Fig. 1.5.

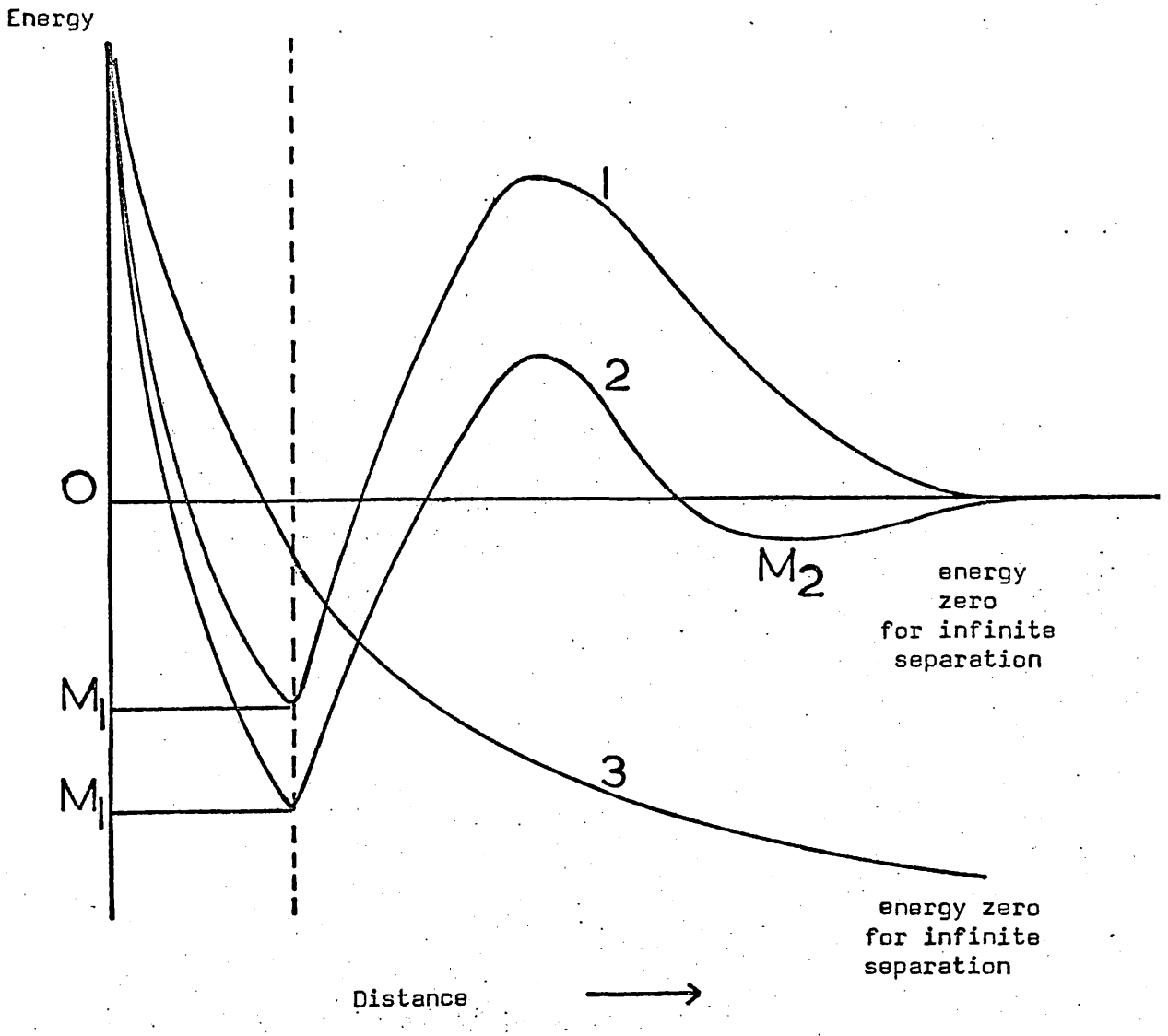
In (i) a collision in which the associated Kinetic energy is sufficient to carry the system over the potential energy barrier (primary maximum P) to the minimum M, (primary minimum) association of particles (coagulation) will result. If the potential energy barrier is high in comparison to Kinetic energies, the particles will repulse one another and the dispersion will remain stable indefinitely. In (ii) a third possibility arises; upon collision the two particles may become associated in the state represented by M_2 (flocculation) and may later either dissociate or pass over into the primary minimum. In (iii) the dispersed state is stable, and dispersion of the particles occurs spontaneously as in lyophilic sols.

Putting energy into a lyophobic system, by heating, or stirring rapidly increases the number of collisions likely to lead to adhesion, and thus flocculation will occur more rapidly changing the chemical conditions of the bulk phase, i.e. by addition of ions, or a change in ionic strength can also bring about the rapid flocculation of such a system. The added electrolyte causes a compression of the diffuse parts of double layers around the particles, and may, in addition exert a specific effect through ion-absorption into the Stern layer. The primary maximum P is reduced and allows particles to approach close enough for vander Waals forces to predominate.

The addition of a lyophilic colloid to a lyophobic colloid promotes the stability of the latter. In the case when polymeric material is adsorbed into the surface of the particle, particle/solvent affinity is increased, and this promotes stability by

Fig. 1.5.

MUTUAL POTENTIAL ENERGY OF TWO COLLOID PARTICLES AS A FUNCTION OF THE SEPARATION BETWEEN THEIR SURFACES.



mechanical means. The solvent in the neighbourhood of a particle has a higher viscosity than that in the bulk medium, acting as a protective impenetrable layer. This protective action is shown best by lyophilic colloids that normally carry charged groups at the surface.

Chapter 1. Section 6. Aims of present Study.

The limited range of *in vitro* tests, and the doubtful value of results of such tests extrapolated to the *in vivo* situation have already been discussed (chapter 1. section 1.). It was hoped (a) to develope a model system which, whilst approximating more closely to *in vivo* would allow strict supervision of chemical and physiological conditions, and demonstrate the effect of variation of such conditions upon the interaction of a radiopaque and the surface of the gut; (b) that results would be capable of interpretation not only from the point of view of the physical and chemical parameters of the system, but also in terms of the *in vivo* situation; so that (c) the conditions of barium meal administration necessary for good visualisation of the gastrointestinal tract could be elucidated.

The surface properties of particles derived from barium meals tested were characterised, and the efficiency of carboxymethyl cellulose as a stabiliser investigated.

CHAPTER TWO

PHYSICAL CHEMICAL TECHNIQUES.

Chapter 2. Section 1. Microelectrophoresis Technique.

The cell and electrode compartments are shown in Fig. 2.1. The cell was constructed by the fusion of two optically flat Hysil glass plates (40 x 25 x 0·5 mm.) such that the cell depth was 0·5 mm. The arms were constructed of 10 mm. bore pyrex tubing, this being sealed directly into the cell and bent in the plane of the cell so that the cell could be immersed completely in a water bath. Glass rod around the cell gave structural rigidity. Spherical quick fit joints were used to attech the cell arms to the electrode compartments, and clips were used to ensure a leak proof seal.

The electrode compartments were constructed with high quality vacuum taps. The No.2. sintered glass discs were sealed as near the side arms as possible, thus eliminating dead space and easing the removal of air bubbles.

The electrodes were made by coiling 25 cm. 2 mm. diameter silver wire and sealing these into rubber bungs. These were briefly cleaned in 50% nitric acid, using a platinum cathode; a grey-purple coating was deposited. Electrical connections to the power supply were made by barrel connectors.

A Watson "Service" microscope was employed. This gave a magnification of x 600, achieved by a x 40 phase contrast objective, x 1·5 angled eye piece and x 10 eye piece containing a cross hatch graticule. A high intensity microscope lamp, focused directly on the phase plate of the condenser provided the illumination. Both the condenser and objective were waterproofed with nail laquer. The microscope was mounted horizontally, the normal stage being replaced by a 10 mm. thick rectangular (240 x 140 mm.) metal plate. This had a hole cut to allow the condenser to pass through it. The cell was mounted in a vertical position between the condenser and eye piece by means of bolts attached to perspex clamps, these being screwed into the metal plate. Sponge rubber was wrapped around the side arms before

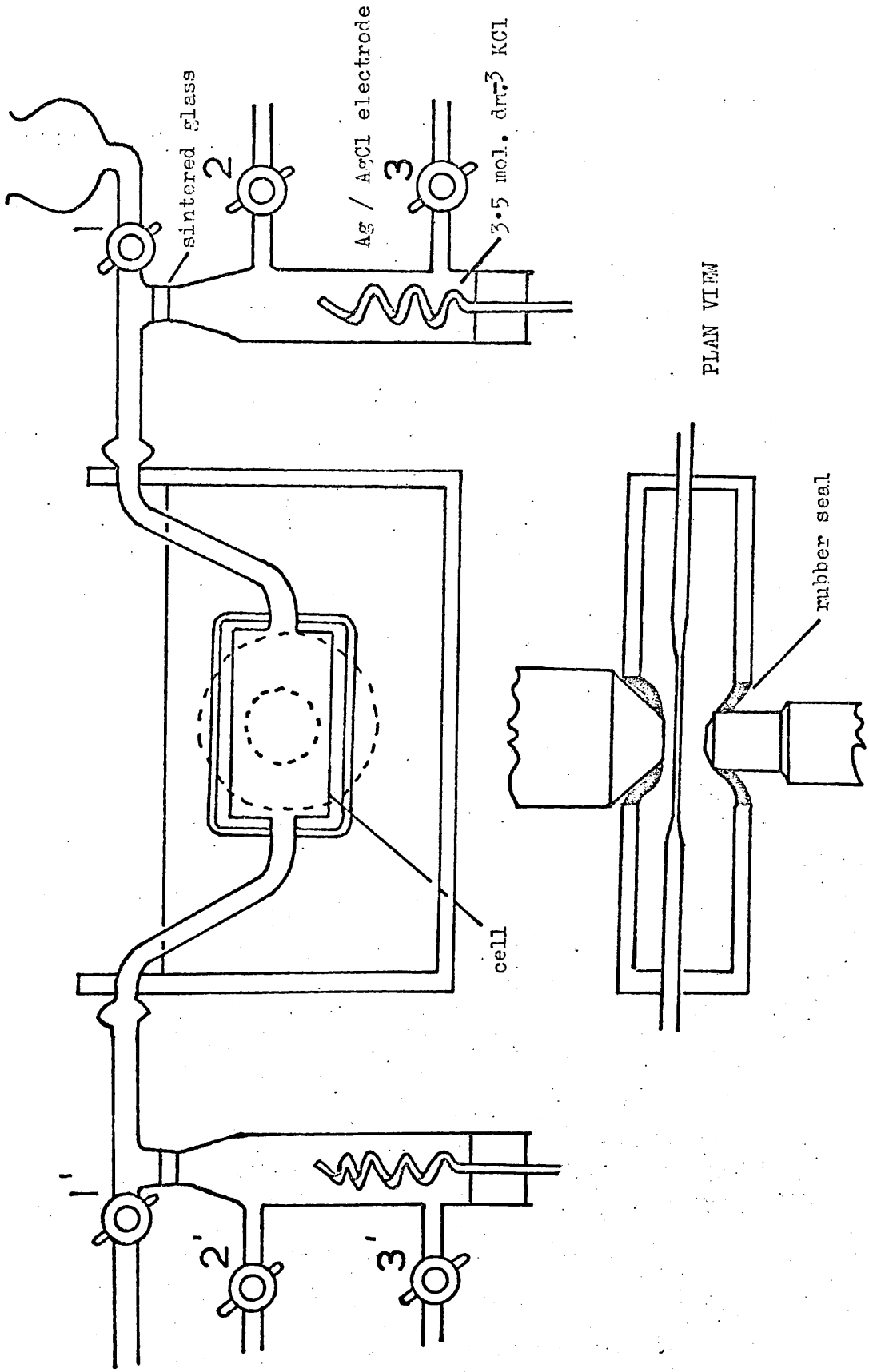


Fig. 2.1. MICROELECTROPHORESIS CELL AND ELECTRODE COMPARTMENTS

tightening the bolts.

The water bath was constructed of perspex and had two circular holes back and front. Rubber flanges, glued, and clamped in position with aluminium rings, fitted over the condenser and the objective. The rubber/metal joint was glued with cow gum; the whole provided a flexible seal and allowed movement of both the condenser and the objective in the water bath.

Water was pumped from a thermostatically controlled reservoir under the bench into the water bath to maintain a constant temperature of 25°. The water was retained by a "constant head" device, adjusted so that the whole cell and part of the side arms were immersed.

The electrical circuit (Fig. 2.2.) was powered by 3 x 60 volt H.T. batteries. r_1 and r_2 were wire wound potentiometers. The actual current passing through the cell was adjusted by r_2 and this was measured using a works calibrated Sangamo - Weston multi range milliameter.

The suspension under investigation was introduced through the funnel attached to the right hand side of the apparatus and flushed out (through rubber tubing) into a reservoir below the bench. The cell was fed by gravity, but a partial vacuum was applied on just setting up the apparatus to remove air bubbles. The elctrode compartments were fed by gravity from reservoirs containing 3·5 mol. dm^{-3} KCl solution.

The microscope was focused by movement of the objective, the fine adjustment screw, which was calibrated over the whole of its range was used to determine the depth of the cell. In practice, appreciable slack was found in the screw drive and thus only the mid range of the calibrated scale was used and the screw turned in one direction only.

Chapter 2. Section 2. Assembly and use of cell.

In order to locate the positions of the inner front and back

Fig.2.2. THE ELECTRICAL CIRCUIT FOR THE MICROELECTROPHORESIS APPARATUS.

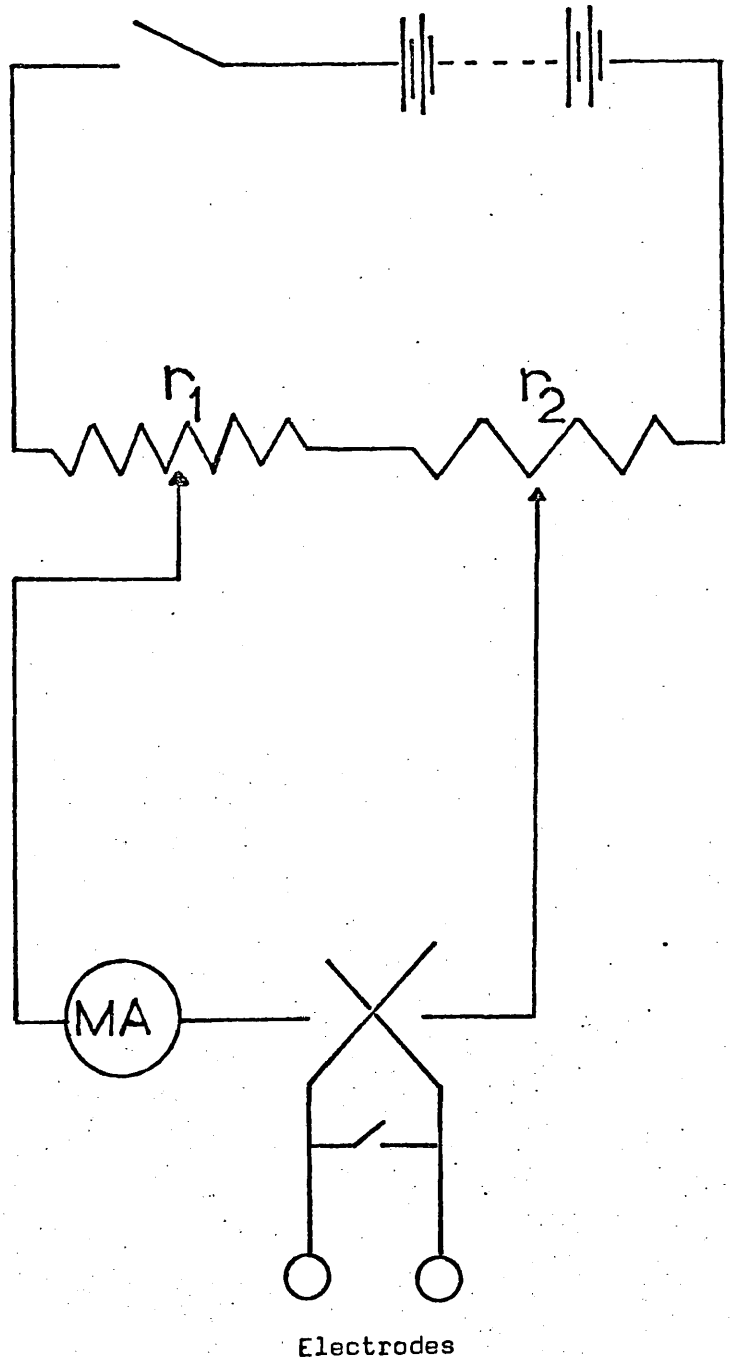
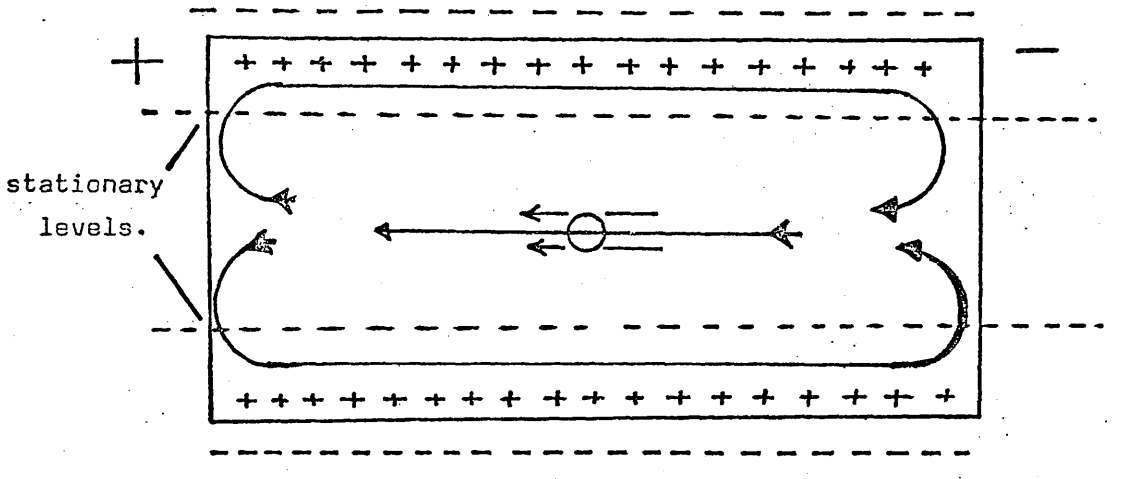
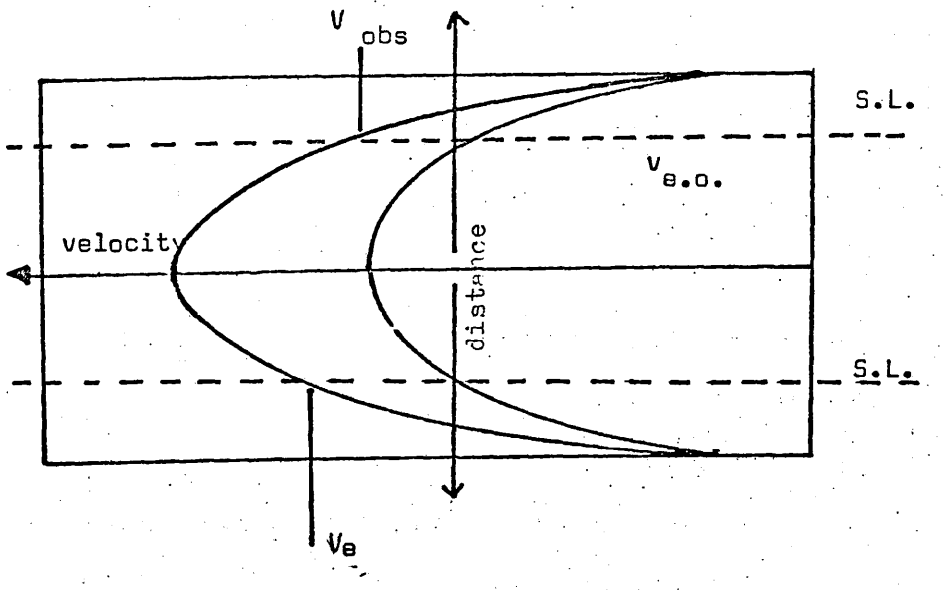


Fig.2.3.

(a) ELECTROOSMOTIC FLOW IN MICROELECTROPHORESIS CELL.





(b) VELOCITY GRADIENT IN MICROELECTROPHORESIS CELL.

surfaces of the cell, these were coated with bacteria before assembly of the apparatus. Cells of Klebsiella aerogenies were harvested from a plate and taken up in ethanol and the resulting suspension pipetted into the cell, the ethanol was evaporated at 37° in an oven, with suitable manipulations of the cell to ensure that both surfaces were coated. Subsequent washing the cell with distilled water removed the vast majority of the adhering bacteria but left the cell with sufficient cells adhering for easy location of the inner surfaces.

The cell was mounted firmly in the perspex clamps, the spherical ground joints lightly greased and the electrode compartments attached. Care was taken to ensure that the cell was truly horizontal when viewed from the front, adjustments being made with reference to a spirit level, and vertical when viewed from the side. The cell was also square to the microscope eye piece and the condenser.

Water was flushed through the cell, air bubbles removed, and the apparatus left overnight with the electrodes shorted out.

The mode of operation of the apparatus has been well established by previous workers and only slight modifications, usually due to the nature of the particles studied, were made.

(a) The thermostat was switched on and adjusted to give a temperature 25° in the perspex bath (in hot weather a cooling unit was employed in the lower reservoir).

(b) The electrode compartments were filled with fresh 3·5 mol. dm^{-3} KCl from the reservoirs, and a little of this solution was forced through the sintered glass discs by opening taps 3 or 3^1 (which were connected to the reservoir), with taps 2 or 2^1 closed and 1 open. All taps were then closed and the cell and side arms washed with distilled water to remove all traces of electrolyte.

(c) The suspension was introduced into the cell, and taps 1 and 11 were closed.

(d) The microscope was focused on the front inner surface using the

fine adjustment, and then racked down the required amount to the front stationary level (Fig. 2.3.). The microscope was used only in this way so that any error due to backlash of the screw drive occured in all readings and was therefore automatically cancelled.

(e) Particles were allowed to come to thermal equilibrium, and after establishing that they exhibited only Brownian motion (i.e. no systematic drift of particles, due to various causes) the current was switched on and adjusted to give a suitable transit time (2 - 4 seconds) across one square of the graticule.

The time for a particle in focus to traverse a fixed distance of the graticule was then determined, using a cumulative stop watch reading to 0.015s.. The polarity of the electrodes was reversed and the process repeated. A minimum of 50 readings on different particles were taken for each suspension. The value of the current was kept constant throughout, and this was recorded. Deciding which particles were in focus at the stationary levels and at the cell surfaces was a subjective matter, but a constant particle appearance was used at all these levels, to minimise errors.

Some of the particles studied sedimented rapidly, but if the cell was truly vertical these remained in focus at the stationary level. If the suspensions were very dilute the mobility determinations were not affected, as the particles sedemented into the part of the cell where their presence is hydronamically unimportant.

The cell depth was measured before each experiment, but was found to be effectively constant.

(f) The electrodes were shorted out and the cell flushed and left filled with distilled water. Tap 1 was left open when the cell was not in use.

Because of the abrasive nature of the particles studied, taps 1 and 1^1 quickly showed signs of wear and became susceptible to leaks. Since the particles tended to sediment the cell, side arms and sintered

disc soon became coated with particulate matter. From time to time therefore the apparatus was dismantled and the cell and side arms washed with 50% sulphuric acid and copious amounts of distilled water. The sintered glass discs were cleaned with tissue soaked in distilled water.

Taps 1 and 1^1 were cleaned and regreased after every day. Any adhering particles used for determination of the cell depth washed off in the cleaning processes were quickly replaced in normal use, and in contrast to previous work these were not removed by the suspensions under investigation.

Chapter 2. Section 3. Preparation of suspension for Electrophoresis.

Radiopaques supplied as powders (Chapter 3. Sections 6 and 7) were made into suspensions using the appropiate buffer solution and those supplied as suspensions were diluted with buffer solution so that the final concentration was of the order 0·01 – 0·1% w/v. At these concentrations the particles when reviewed under the microscope appeared as discreet entities and suffered no interference from neighbouring particles.

Acetate-veronal buffer solutions (Michaelis, 1931)(herinafter called barbiturate buffer solutions) of ionic strength 0·02 mol. $dm.^{-3}$ were used for determination of mobility values. The stock solution was prepared from:-

Sodium barbitone	154·64 g.
Sodium acetate trihydate	102·07 g.
Sodium chloride	58·4 g.

dissolved in 5 $dm.^3$ distilled water to give I = 0·5 mol. $dm.^{-3}$. This stock solution was kept at 5° to prevent bacterial growth. It was diluted to 0·02 mol. $dm.^{-3}$ HCl or NaOH to the diluted buffer solution. The ionic strength of the buffer remains constant for the pH range 2·5 to 11, but outside this range I increases. The conductivity of the buffer solutions was determined using a bottle type conductivity cell with shiny platinum electrodes. The cell was mounted in a water thermostat maintained at 25·0 $\mp$ 0·1° and the conductance measured with

a Wayne-Kerr B221 conductance bridge at 1992 H_z.

Chapter 2. Section 4. Theory of the closed cell.

The method of determination of the electrophoretic mobility of a particle is based upon that of Ellis (1911), where the migration of particles under the influence of an electric field is observed using a microscope. The measurements are complicated by the simultaneous occurence of electro-osmosis. The internal surfaces of the cell are charged, and the applied electric field causes not only electrophoretic migration of the particles but also an electro-osmotic flow of liquid near to the inner surfaces. Since the cell is closed there is a compensating return flow of liquid, with maximum velocity at the centre of the cell (Fig. 2.3.). This results in a parabolic distribution of liquid speeds with depth (Fig. 2.4.) and the true electrophoretic velocity of the particles relative to the stationary liquid is only observed at the levels in the cell where the electro-osmotic and return flow of liquid cancel each other. These are known as the stationary levels.

Although it is possible to calculate the position of the stationary levels theoretically (Komogata 1933: Vangils et al 1936) an experimental determination is more accurate and also confirms the symmetry of the cell. Ellis (1912) showed that since there was no net liquid flow in a closed cell, the average particle velocity over all depths will be equal to the actual electrophoretic velocity, v_e.

$$\text{Thus} \quad v_e = \frac{1}{x_1} \int_0^{x_1} v_{obs} \, dx \qquad \text{.....2.1.}$$

where x_1 is the cell depth.

Thus if v_{obs} is determined at different values of x_1 and the equation of the velocity depth - parabola determined, v_e can be determined. Substituting v_e into 2.1. for the parabola yields values for x at which the liquid velocity is zero.

Chapter 2.Section 5. Determination of the position of the stationary levels and estimation of cell symmetry.

The symmetry of the cell was examined by determining the velocity -depth curve for the cell, using a suspension of Micropaque particles (i.e. coated Barium sulphate particles) in barbiturate buffer solution (pH 7·0). The time for particles in focus at a known cell depth at a constant electric field strength was determined and the reciprocal time plotted against the cell depth, this being expressed as fractional depth measured from the centre of the cell (Fig. 2.4.). The equation of the parabola was determined as follows.

Let x denote the fractional depth from the centre of the cell and y the velocity (i.e. reciprocal time measured at this depth). The equation for the velocity depth parabola is of the form:

$$y = a + bx + cx^2 \quad \text{.....2.2.}$$

Substituting the values of the above equations and solving gives the experimental equation for the cell under study. This was

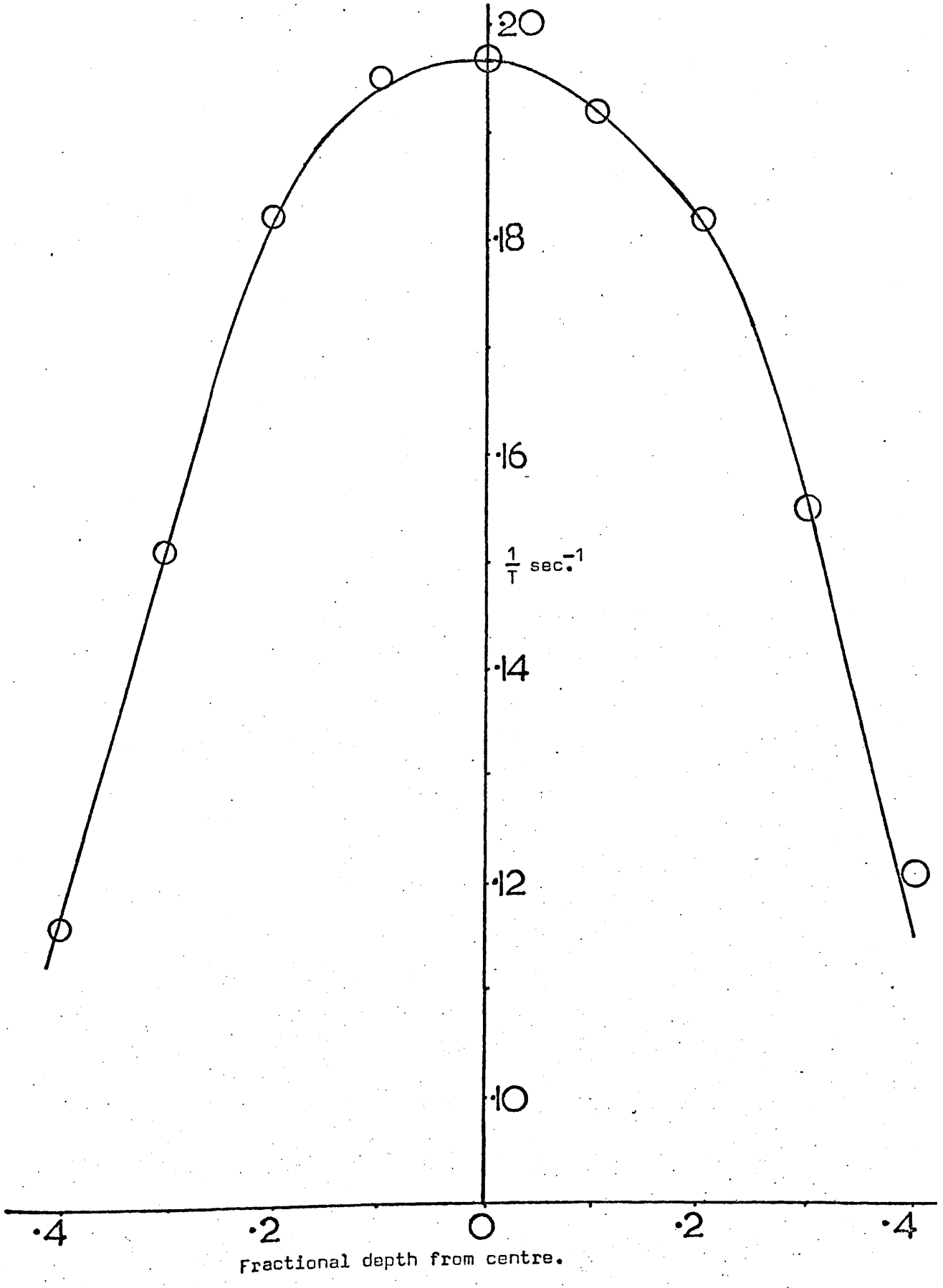
$$y = 0{\cdot}1942 - 0{\cdot}0067x - 0{\cdot}42bx^2 \quad \text{.....2.3.}$$

The small value of b indicates that the calculated centre of the parabola is slightly displaced from the geometrical centre. Integration of equation 2.3. between $x = \pm 0{\cdot}5$ gives the experimental value of the electrophoretic velocity, i.e. $0{\cdot}157 s^{-1}$. Substitution of this value back into the equation 2.3. gives a value for x corresponding to the stationary levels of + 0·287 and - 0·304 from the centre of the cell. This indicates that the stationary levels are at 0·213 and 0·696 of the cell depth from the front inside surface of the cell, and conform to the predictions of the Smoluchowski equation.

Chapter 2. Section 6. Determination of cell constant and Electrophoretic mobility.

The electrophoretic mobility $\bar{v} / m^2\ s^{-1}\ v^{-1}$, i.e. the particle velocity v / ms^{-1} per unit potential gradient in the stationary level

Fig.2.4. VELOCITY DEPTH CURVE OF MICROELECTROPHORESIS CELL.



is given by

$$\bar{v} = \frac{v}{x} = \frac{nL}{r}\,\frac{qK}{I}\,s = \frac{nL}{r}\,\frac{q}{I}\,JG \quad \ldots\ldots\ldots 2.4.$$

where nL/m. is the distance travelled (n being the number of squares of side L in time t/s, q/m^2 the area of cross section of the cell, and I/A the current flowing). $K_s/ohm^{-1}\ m^{-1}$ is the conductivity of the suspension obtained from the measured conductance G/ohm^{-1} and the cell constant J/m^{-1} of the conductance cell. The values of G, I, t are obtained experimentally. Since it was not possible to measure the cross sectional area of the cell, a standard particle of known mobility $\bar{v}_s$ was timed in the apparatus and an apparatus constant K, which included the cell constant J of the conductance cell determined. K is given by

$$K = LqJ = \frac{\bar{v}_s\ t^1\ I^1}{nG^1} \quad \ldots\ldots\ldots\ldots\ldots 2.5.$$

where t^1 I^1 G^1 refer to values for the standard particle. combining equations 2.4. and L, J the mobility of the particle under study can be calculated.

$$\bar{v} = \frac{KnG}{rI} \quad \ldots\ldots\ldots\ldots\ldots\ldots 2.6.$$

In this study two standard particles were used for the determination of the all symmetry and the position of the stationary level.

1) Cells of Aerobactor aerogenes suspended in Barbiturate buffer solution, pH = 7·0; I = 0·02 mol. dm^{-3}

$\bar{v} = 1{\cdot}67 \pm 0{\cdot}02 \times 10^{-8}\ m^2\ s^{-1}\ V^{-1}$ (Gittens & James).

2) Micropaque suspended in barbiturate buffer solution

pH = 7·0 I = 0·02 mol. dm^{-3}

$\bar{v} = 1{\cdot}46 \pm 0{\cdot}02 \times 10^{-8}\ m^2\ s^{-1}\ V^{-1}$ (Goddard & James).

A suspension of Micropaque was used for daily calibration of the apparatus.

Hence-forth values of electrophoretic mobility will be quoted as a single number. A value of 1·46 indicates a negative mobility of $1{\cdot}46 \times 10^{-8}\ m^2\ s^{-1}\ V^{-1}$ the particle being negatively charged. In some instances particles having positive mobility were encountered,

mobility values for such paricles will be prefixed by +.

In practice particles of Micropaque were very convenient since suspensions could be quickly and easily prepared and were not subject to biological variation. They did not sedimentate to any great extent during experiments, but did tend to cause abrasion of all greased seals, making their reconstruction practically a daily occurance.

Chapter 2. Section 7. Laboratory preparation of Barium sulphate suspensions.

Barium sulphate paste was supplied by Aspro Nicholas Ltd. This contained 65 - 67% solid material as determined gravimetrically; this was 95% barium sulphate. Impurities included salts of heavy metals such as lead, and salts of calcium and magnesium. The barium sulphate particles, precipitated under acid conditions, had a characteristic diameter of 0·07 - 0·6 μm. average 0·13 μm. (Goddard & James, 1971).

Initially, the paste was washed by dispersion in a large amount of distilled water, allowed to settle, and the supernatant removed. The sediment was dried in an oven at 200°, broken up, and mill ground.

It was found that suspensions prepared from the washed and dried paste tended to exhibit grittiness, due to the presence of aggregates of barium sulphate formed in the drying process. These larger particles quickly sedimented out of suspension leaving a preparation of uncertain concentration, and had a marked effect upon viscosity and stability determinations.

Suspensions were therefore prepared from the untreated paste. An amount of the paste was thoroughly mixed in a plastic bag. By drying weighed samples of the mixed paste in an oven at 200° to a constant weight the concentration of solid barium sulphate could be determined.

Unstabilised suspensions were prepared by introducing an amount of the mixed paste, containing the equivalent of 360 g. of solid barium

sulphate into a 600 cm^3 polythene or thick glass, screw top bottle. 200 cm^3 of distilled water was then added and the mixture blended for 30 minutes with a "Premier" colloid mixer.

The nature of the mixer is such that agglomerations of particles are broken up by causing fast moving particles to change direction suddenly. The mixer has no grinding action and thus the size of barium sulphate particles was strictly comparable to those in Micropaque, which is prepared industrially with the same paste, and to other laboratory meals which needed extended mixing to ensure a homogenous preparation.

After blending the suspension was treated for 30 minutes in an ultra-sonic bath, and could be diluted, if required with distilled water.

Two methods of preparation of stabilised meals were employed, one based upon the method of Goddard (1970) which employs processes similar to the industrial preparation of Micropaque and the other based upon Gutcho (1971). The latter method was more convenient and quicker in practice, and no differences in meals containing the same amounts of a given stabiliser, but prepared differently, could be detected.

In the first method a 2% solution of the given cmc. powder was prepared by introducing the powder into warm water vigorously stirred with the colloid mixer. The apparent viscosity of the cmc. solution was determined, and adjusted, if necessary, by dilution with distilled water so that the viscosity of the solution was comparable to that of the natural gum extract solution used in Micropaque. The equivilent of 360 g. of barium sulphate was introduced as the paste into a 600 cm^3 bottle and 200 cm^3 of the cmc. solution added. The mixture was blended for 30 minutes, and then made up to 600 cm^3 with distilled water. It was treated in an ultra-sonic bath for 30 minutes. This procedure resulted in a homogenous suspension containing 60% w/v. of stabilised barium sulphate. Suspensions were allowed to stand 24 hours before they were further investigated.

In the second method paste was introduced into a 600 cm^3 bottle, and the appropriate amount of dry cmc. powder added. The mixture was blended for 15 minutes with the colloid mixer, the cmc. fluidised the barium sulphate paste and efficient mixing was achieved. 200 cm^3 of warm (40°) distilled water was added and the suspension blended for a further 15 minutes. It was then made up to 600 cm^3 and treated in an ultra-sonic bath, and left 24 hours to equilibrate.

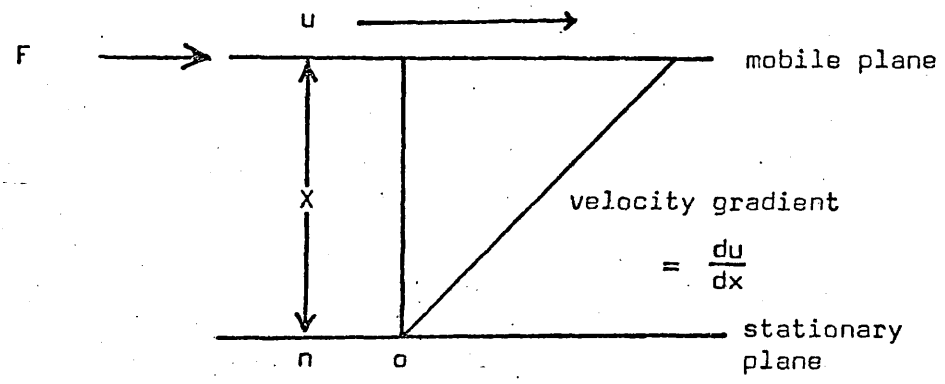
All prepared meals required some degree of stirring before any tests were performed on them. Usually 5 minutes of blending in the "Premier" mixer followed by 5 minutes in an ultra sonic bath was sufficient to redisperse all sedimented particles and achieve a homogenous suspension. With the less stable meals, especially those containing high (above 0·6% w/v.) amounts of stabiliser needed longer to disperse the "cement" which formed on the bottom of the bottle.

Chapter 2. Section 8. Determination of the viscosity of Barium meal.

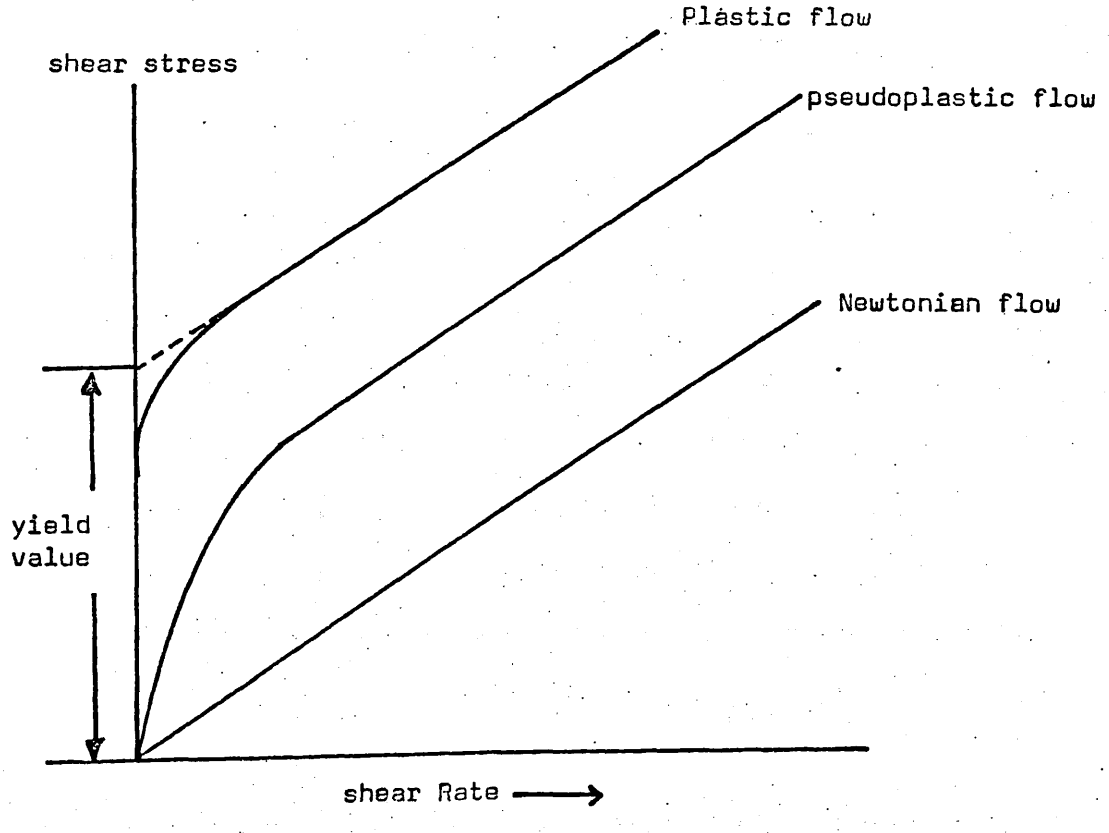
A liquid flows when a force is applied to it, and this flow will be characteristic of Newtonian or non-Newtonian behaviour. Fig. 2.5(a). shows a simple model consisting of a liquid between two parallel planes. When a force F is applied to the upper plane it moves with a velocity u. All the liquid enclosed by the plane does not move with the same velocity, however, as the rate of flow varies with the distance from the upper plane, approaching zero at the lower plane. The layers of liquid in contact with the planes are stationary relative to the planes. At any point in the liquid, the liquid velocity is given by $x \frac{du}{dx}$. The force $\frac{F}{A}$ applied to unit area of the upper plane is the shearing force, or stress, and $\frac{du}{dx}$ is the shear rate. For Newtonian liquids the ratio of shear stress to shear rate is constant and defines the viscosity η . A plot of shear rate against shear stress gives a straight line, passing through the origin of slope η_{App} (Fig.2.5(b).).

Fig. 2.5.

(a) NEWTONIAN FLOW DIAGRAM



(b) NON-NEWTONIAN FLOW DIAGRAM



Most of the suspensions under study, however, exhibited non-Newtonian behaviour, i.e. the viscosity did not vary in a linear way with the shear rate. Most suspensions were psuedo plastic, which is characterised by a stress that increases more rapidly at low shear rates than at high shear rates. In plastic behaviour, which resembles the former, and indeed is difficult to differentiate from it experimentally, the shear rate does not aquire a finite value until the stress exceeds a given value, known as the yield value (yv.). Theories of psuedo plastic behaviour all involve the formation of links between particles in the system; these are distorted in shear and eventually break but later may reform. The breaking of such interparticulate forces, whatever their nature, requires work and thus this contributes to the viscosity of the system. The rate of formation of "bonds" will not increase as quickly as the rate of shear, hence the work in breaking them and the shearing stress required will not increase so rapidly as the shear rate. When a system is at rest the formation of "bonds" is able to proceed to a point where the system can be thought of as having a definite structure. To disrupt this a definite stress is necessary, if a force below this minimum stress is applied no flow will result. The minimum stress required is the yield value, when it is applied interparticulate bonds are broken and flow will commence. The value of yv. can be thought of as a measure of the strength of the bonds or the degree of structure associated with the colloid.

Viscosity measurements were made using a Ferranti Portable viscometer, model VM., supplied with VM. cylinders and fitted with a 20 g./cm. spring. The apparatus consists of an outer rotating cylinder driven by a synchronous motor, with an inner cylinder located coaxially inside it. This inner cylinder is attached to the spring, and rotates around it. Between the top of the inner cylinder and the outer cylinder is a guard ring which prevents end effects. The inner cylinder activates a pointer, which moves over a linear scale. The

instrument is supplied with three calibrated cylinders of different sizes and the instrument has a gear box capable of three different speeds, thus nine different shear rates can be produced ranging from $8 - 160 s^{-1}$ To obtain the value of η of a suspension at any given shear rate the scale reading is multiplied by the appropriate calibration factor, (determined by the size of the inner cylinder and the speed of rotation of the outer cylinders). Values of the shear stress were calculated, and a graph of shear rate against shear stress was drawn for each suspension. The slope of the graph gives the apparent viscosity $\eta_{app.}$ of a suspension, and the values of yv. obtained from extrapolution of the line.

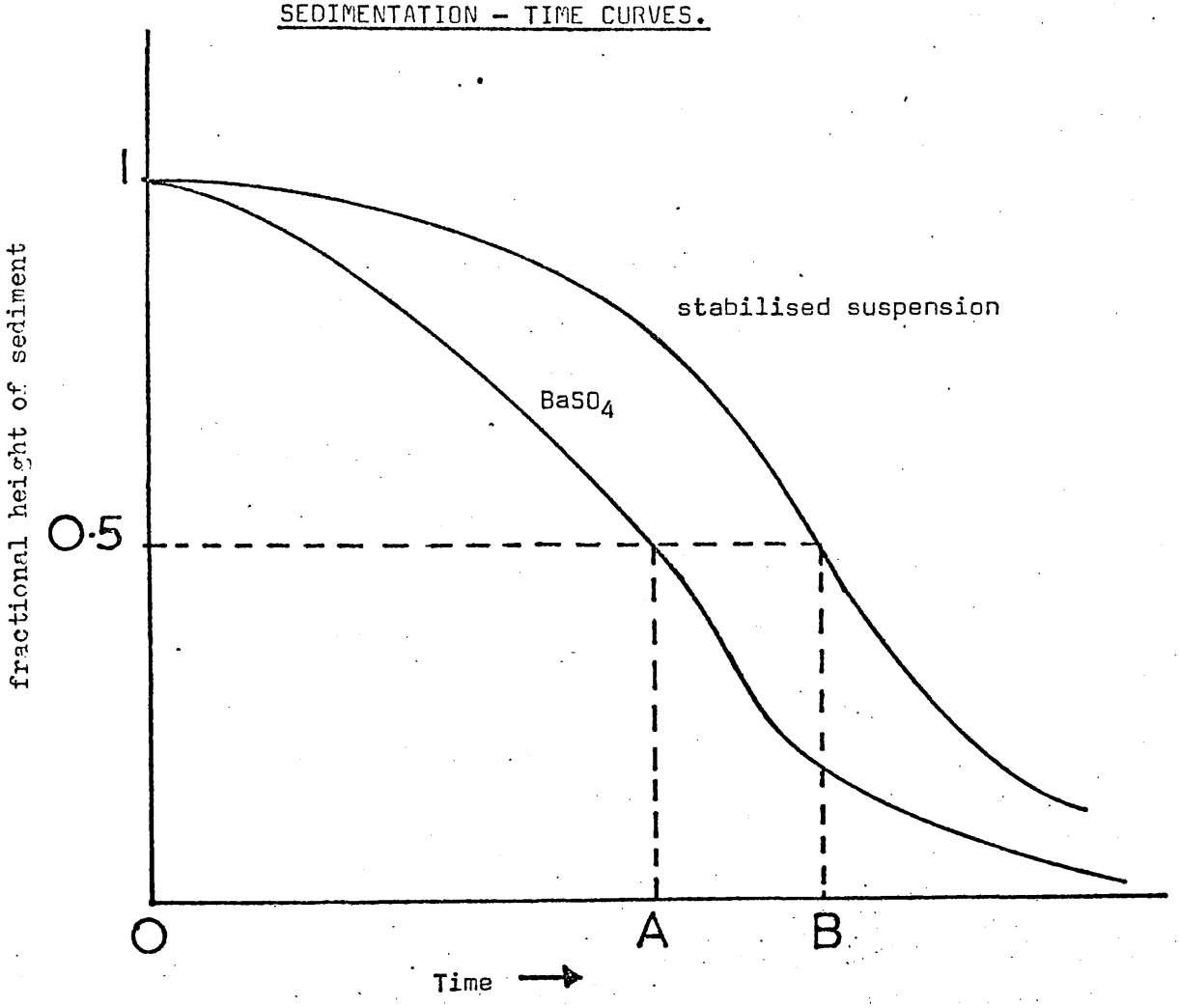
Since $\eta_{app.}$ is independent of shear rate, this parameter of the various meals can be directly compared.

Chapter 2. Section 9. Determination of the stability of Barium meals.

Generally, suspensions prepared in the laboratory were quite stable in that settling was appreciable only over long periods of standing, and thus the relative stabilities were measured under centrifugation.

Suspensions containing 0.5% w/v. or less carboxy methyl cellulose, and plain suspensions of barium sulphate separate into two distinct phases when centrifuged. This is theoretically predictable since a suspension containing particles with an approximately monotonous size distribution should settle out when subjected to a gravitational field in such a way that at a given time all particles whose equivilent spherical diameter is greater than a given value are completely sedimented. The samples, contained in "Universal" bottles were centrifuged at a constant speed for set periods of time. The height of the precipitate was then measured after each time interval. A plot of fractional height of precipitate against time was then drawn. The

Fig.2.6. DETERMINATION OF R.S. VALUE FROM SEDIMENTATION – TIME CURVES.



$$R\,S = \frac{OB}{OA}$$

time taken for half precipitation to occur was directly proportional to the stability of the suspension. In each centrifugation run two suspensions of 60% w/v. barium sulphate were included. Times taken for half sedimentation of the samples were compared to the time taken by the control barium sulphate suspension to reach the same stage. The ratio time for half sedimentation of sample/time for half sedimentation of control barium sulphate suspension is called the relative stability (RS.) of the suspension. Variations in centrifugation procedure were thus automatically allowed for since 60% w/v. barium sulphate suspensions provided an arbitary standard, and this means RS. values of different suspensions can be meaningfully compared.

Suspensions containing concentrations of stabiliser in excess of 0·6% w/v. could not be sedimentated to give two distinct phases in under two hours, in a centrifugal field of 900 g., and even after this time separation of the two phases was not clear. They did however give a definite and measurable sediment in the bottom of the "Universal" bottle, and a plot of fraction sedimentated (i.e. height of precipitate /height of precipitate after complete sedimentation) gave an analogous graph to Fig. 2.7. When such suspensions were centrifuged, two samples of a stabilised suspension of known RS. (by the first method) were included. By comparing times of half precipitation of the suspensions of known RS. to those of the others, the RS. of the more stable suspensions could be determined. The RS. values of some suspensions, usually those containing 0·5 - 0·6% w/v. stabiliser were open to determination by both methods. It was found that the values of RS. obtained agreed closely for such suspensions.

Chapter 2. Section 10. Dispersive properties of suspensions.

It is generally held that to be an efficient radiopaque, i.e. one that adheres evenly and uniformally to the gastro intestinal tract so that diagnosis is possible, a barium meal must disperse readily in a

larger volume of liquid, either distilled water, or as the gastric fluid is acid, solutions of hydrochloric acid. The dispersive properties of preparaed meals was therefore investigated.

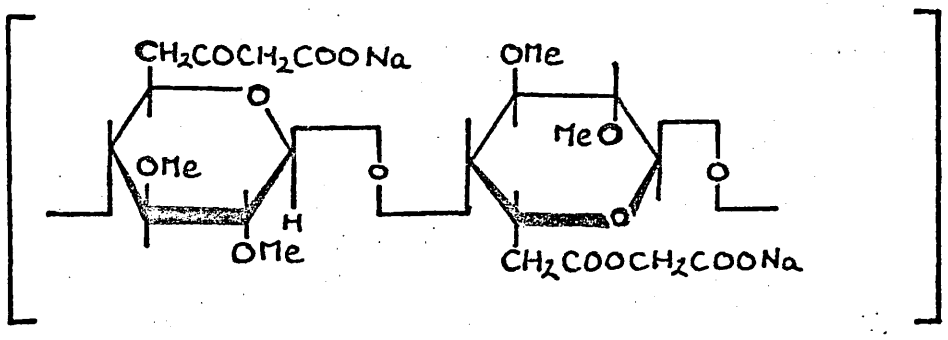
A 2000 cm^3 measuring cylinder was filled with either distilled water or 0·25 mol. dm^{-3} HCl. 1 – 2 cm^3 of a suspension was then slowly discharged from a Pasteur pipette into the top of the cylinder. The ease with which the suspension dispersed was then observed. Flocculation, the presence of a "snow" of tiny leaflets of suspension, and even the rapid sinking of droplets of undispersed material were evidence of poor performance.

CHAPTER THREE

PHYSICAL PROPERTIES OF COMMERCIAL AND LABORATORY PREPARED BARIUM MEALS

Chapter 3. Section 1. Introduction.

Carboxymethyl cellulose (cmc.) is reported to be a good stabiliser of barium sulphate suspensions and this material has the advantage that it is readily available, cheap, non-toxic, and easily soluble. Furthermore it is not variable in quality, as is the case for some vegetable gum extracts (Chapter 3. Section 7.) and its properties can be easily altered and controlled to make it suitable for a specific purpose. cmc. is a macro molecule consisting of repeating carboxymethyl o-methyl glucose units joined by β , 1.4. linkages:



The degree of substitution (DS) of a cmc. molecule is defined as the average number of $-CH_2COO^-$ groups in each anhydro-glucose unit. The DS value is important in determining its dispersibility in water and a minimum DS. value of 0·35 is necessary for its solubility. cmc is usually supplied as the sodium salt.

Chapter 3. Section 2. cmc types investigated in the laboratory

All samples were supplied by Hercules Ltd., and had the following codes: (i) 4M6F : DS = 0·4. Food grade (i.e.can be used as supplied in foods) viscosity of a 2% w/v. solution 300 - 600 cp. (at unspecified shear rate) at 25°.

(ii) 7HF : DS = 0·7. Food grade, 2% solutions have a viscosity over 1,000 cp.

(iii) 9M8F : DS = 0·9 viscosity of a 2% solution 400 - 800 cp.

(iv) 12M8P : DS = 1·2. pharmaceutical grade. 2% solutions have a

"medium" viscosity (hence the M) of 400 - 800 cp.

The aim of the investigation was to determine the effect of the degree of substitution upon stabilities and dispersibilities of suspensions; to characterise the electrophoretic mobilities of particles coated with these stabilisers, enabling the recognition of such stabilisers in other commercial products; essential if a comprehensive study of barium meals is to be made (Miller, 1965). The Langmuir adsorption isotherm of cmc in barium sulphate has been well characterised (Goddard, 1970) and was not further investigated.

Chapter 3. Section 3. Results.

Table 3.1.

Physical properties of 60% w/v. barium sulphate suspensions.

NO.	stabiliser	conc. % w/v.	η App. /cp.	yield value (yv) /cp.	Relative stability (RS.)
1	7HF	0•19	7•5	0	0•9
2		0•66	57•7	7,000	6•3
3	4M6F	0•49	8•3	0	1•5
4		0•77	8•5	2,000	1•1
5	9M8F	0•64	29•6	33	10
6		0•95	62•0	300	12
7	12M8P	0•68	28	0	11
8		0•87	53	0	15

Fig. 3.1.

VARIATION OF VISCOSITY WITH SHEAR RATE FOR VARIOUS CMC. SOLUTIONS

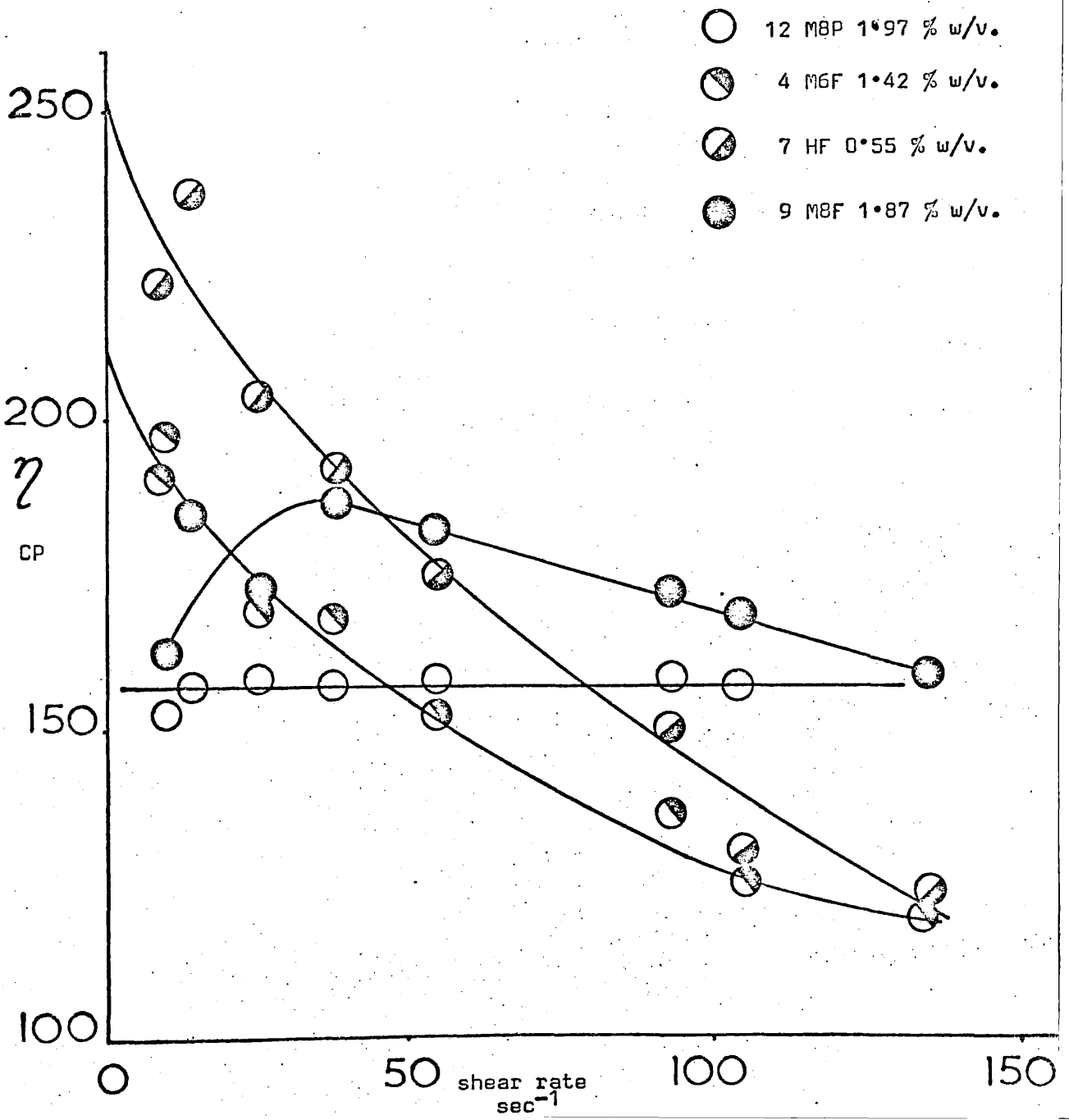
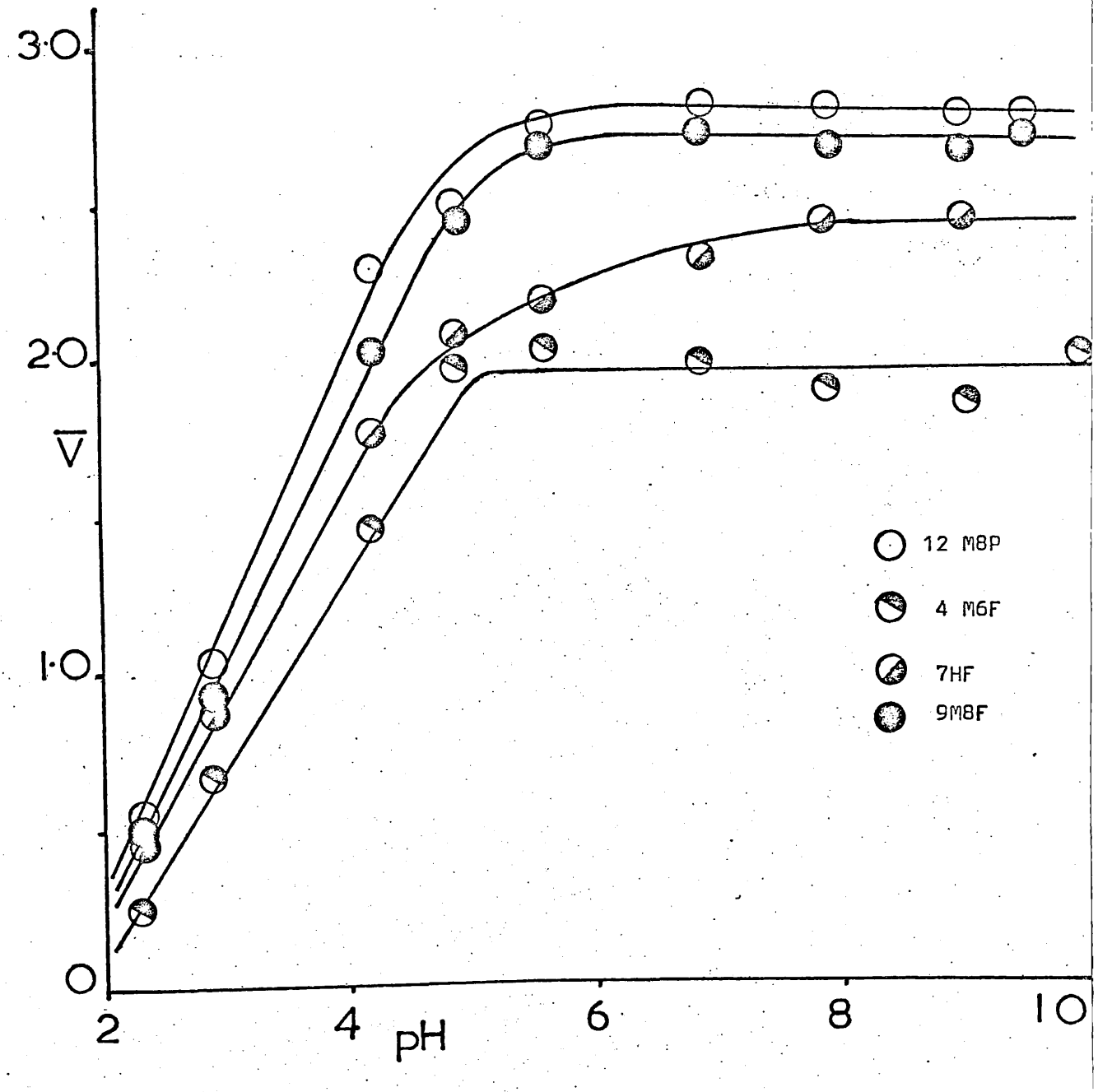


Fig. 3.2.

pH MOBILITY CURVES OF LABORATORY PREPARED CMC. STABILISED MEALS, MEASURED IN ACETATE VERONAL BUFFER SOLUTION I = 0·02 MOL. DM^{-3}



The dispersibilities of the suspensions in water and 0·25 mol. dm^{-3} HCl. (measured as described in Chapter 2. Section 10.) are shown in Table 3.3.

Table 3.3.

Dispersibility of cmc. stabilised barium sulphate suspensions.

No.	stabiliser & conc.	dispersibility	
		water	0·25 mol. dm^{-3} HCl.
1	7HF 0·19% w/v.	Fine dispersion	Good dispersion, slight "snow" of flocculated particles.
2	7HF 0·66% w/v.	Poor dispersion, heavy "snow"	Very poor dispersion, heavy "snow"
3	4M6F 0·49% w/v.	Fine even dispersion, slight snow	Good dispersion, slight flocculation
4	4M6F 0·77% w/v.	dispersion quite good, but marked flocculation	flocculation more marked
5	9M8F 0·64% w/v.	Fine dispersion, very slight "snow"	Fine dispersion, slight "snow"
6	9M8F 0·95% w/v.	Fine dispersion	Poor dispersion, heavy "snow"
7	12M8P 0·68% w/v.	Fine even dispersion, no flocculation	Fine even dispersion
8	12M8P 0·87% w/v.	Fine dispersion	Fine dispersion, no "snow"

Samples were prepared by the method of Goddard. For samples 1, 3, 5, 7, the concentration of cmc. in the original stabiliser solution was adjusted to give a viscosity at a shear rate of 25 sec^{-1} similar to that of CD75 solutions used in the industrial preparation of Micropaque. The variation of η with shear rate for the various stabiliser solutions is shown in Fig. 3.1. Use of these solutions did not result in suspensions of similar viscosity and stability to CD75 stabilised suspensions. Solutions were therefore prepared containing the maximum concentration of cmc. concomitant with accurate measurement of their viscosities, and these solutions were used to prepare samples 2, 4, 6, 8.

All cmc. solutions except those prepared with 12M8P showed non-Newtonian behaviour, and confusion arises if viscosities of suspensions are not quoted at a specific shear rate, as occurs too frequently in the literature.

The variation of the mobility values of particles derived from suspensions containing the higher concentration of stabiliser are shown in Fig. 3.2. pK_a values for the ionogenic groups at the surfaces of the particles are given in Table 3.2.

Table 3.2.

Mobility values of cmc. coated particles at pH 7·0, I = 0·02 mol. dm^{-3}

stabiliser	DS	PKa	$\bar{v}$
12M8P	1·2	3·25	2·85
9M8F	0·9	3·4	2·72
7HF	0·7	3·25	2·42
4M6F	0·4	3·45	2·00

Chapter 3. Section 4. Discussion.

The pH mobility curves of the coated particles show the expected trend, 12M8P giving the most negative particle and 4M6F the least. Values of PKa are typical of the carboxyl ion, the final plateau values achieved by particles in all suspensions are indicative of the various degrees of substitution of the cmc. preparations. 12M8P and 9M8F gave the most stable suspensions, a result of the high viscosities exhibited. Although the suspension stabilised by 7HF at a concentration of 0•66% also had a high apparent viscosity (58 cp.) the RS value was lower than suspensions stabilised with either 9M8F or 12M8P. 12M8P gave a suspension that exhibited Newtonian behaviour at both concentrations (since yv = o), 9M8F showed a slight deviation from Newtonian behaviour. 4M6F gave unsatisfactory suspensions, both in terms of stability and dispersibility in water and acid. 7HF, though giving unstable suspensions was acceptable because sedimented particles could be easily redispersed by shaking and in this characteristic it is similar to some commercial products (Chapter 3. Section 7.). However its dispersive properties in water and acid were unsatisfactory.

Although suspensions containing 12M8P and 9M8F as stabilisers settled only slowly, the resulting precipitate was not easily resuspended, especially at the higher concentrations, as it set as a "clay". Their dispersive properties were acceptable, and these two stabilisers were selected for further experiments. Suspensions were prepared by the second method (Chapter 2. Section 7.). 60% w/v. suspensions were prepared containing a range of concentrations of the two stabilisers and the viscosity, yield value, relative stabilities and dispersibilities of these investigated (Tables 3.4. - 3.7.). The viscosities and yield value are plotted as a function of stabiliser concentration (Fig. 3.3. and 3.4.) and the relative stability is plotted as a function of stabiliser concentration (Fig. 3.5.) and apparent viscosity (Fig. 3.6.).

Fig. 3.3.

VARIATION OF VISCOSITY OF STABILISED SUSPENSIONS WITH CONCENTRATION OF CMC.

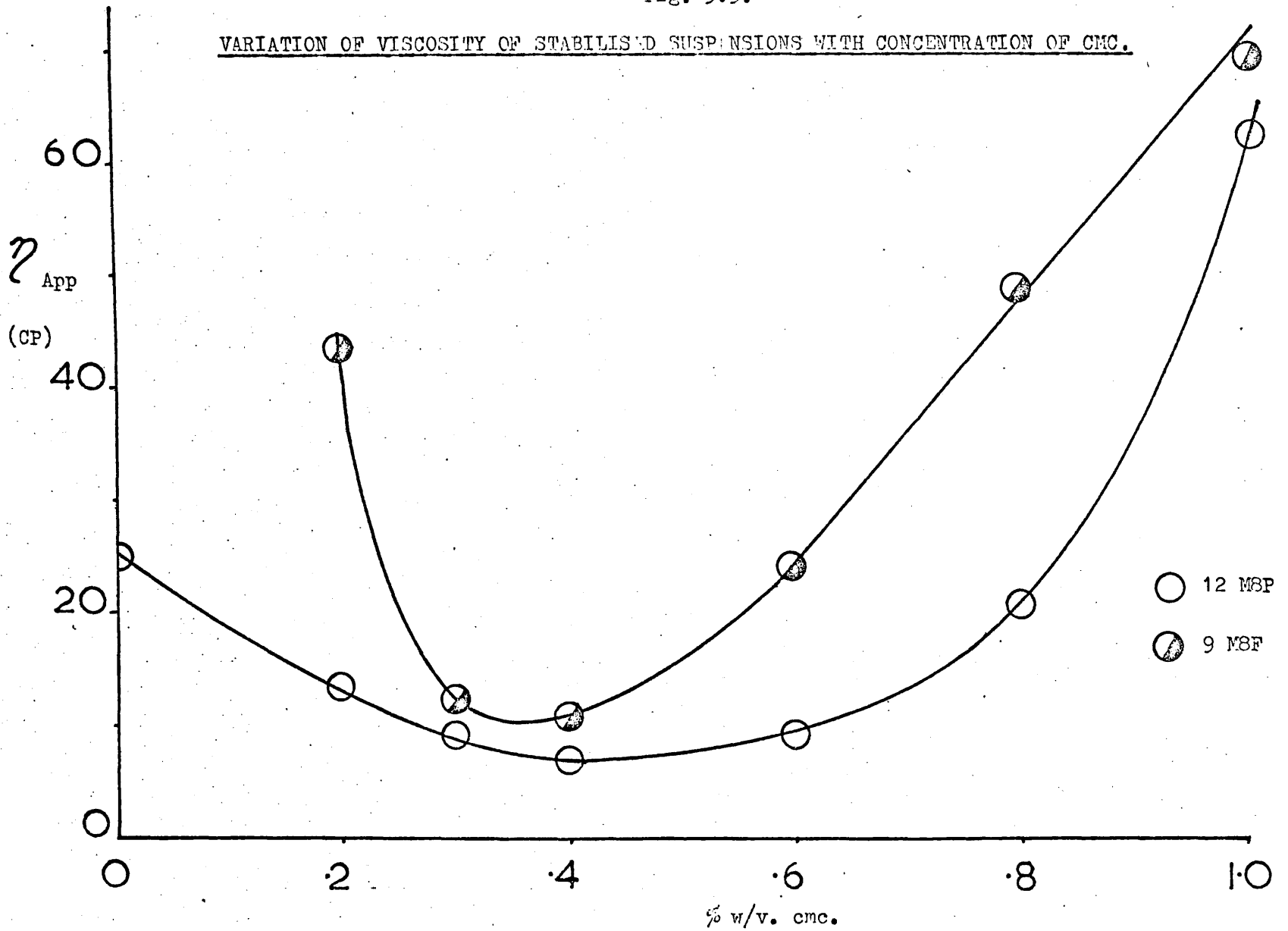


Fig. 3.4.

VARIATION OF YIELD VALUES OF STABILISED SUSPENSIONS WITH CONCENTRATION OF CMC.

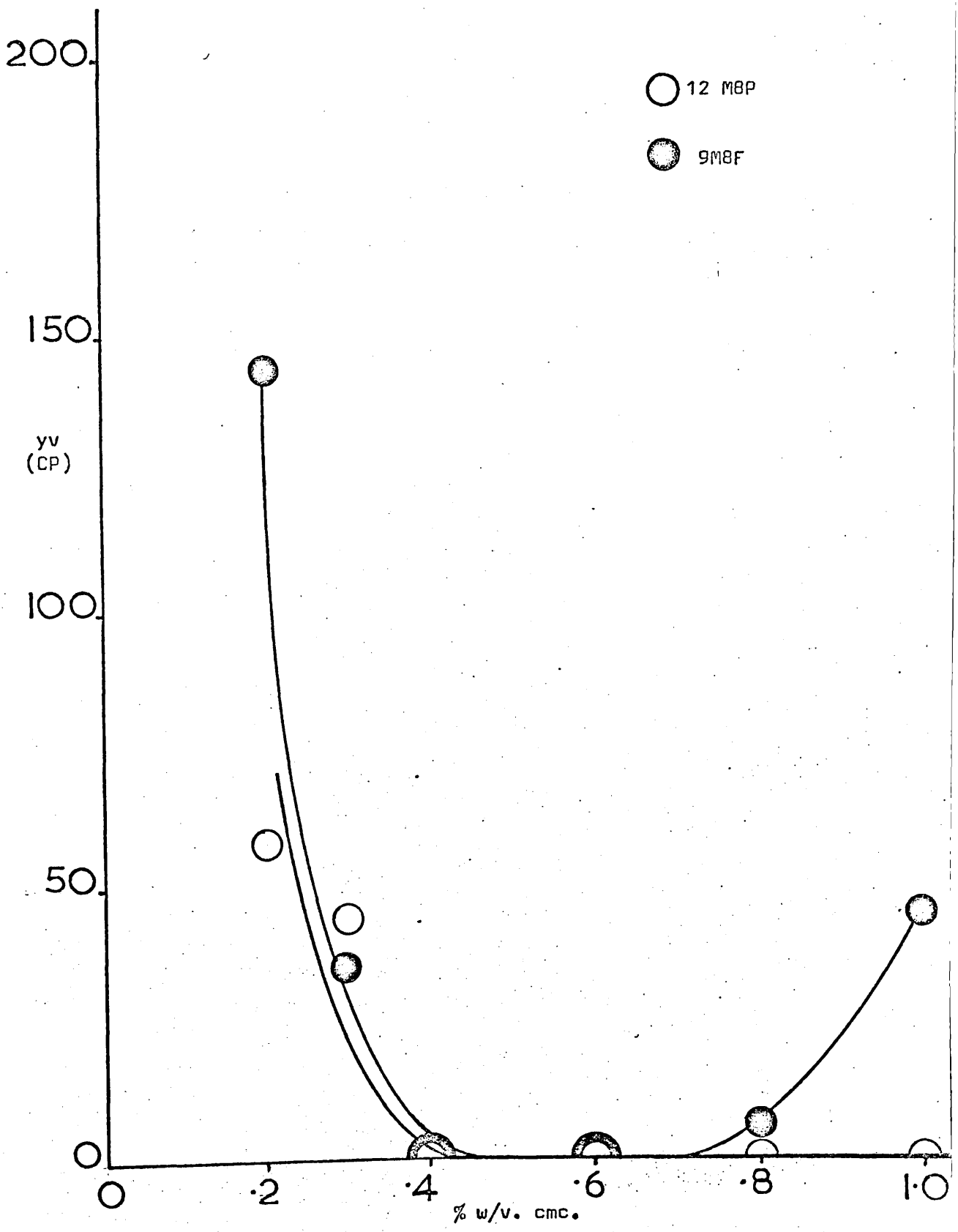


Fig. 3.5.

VARIATION OF STABILITIES OF SUSPENSIONS WITH CONCENTRATION OF CMC.

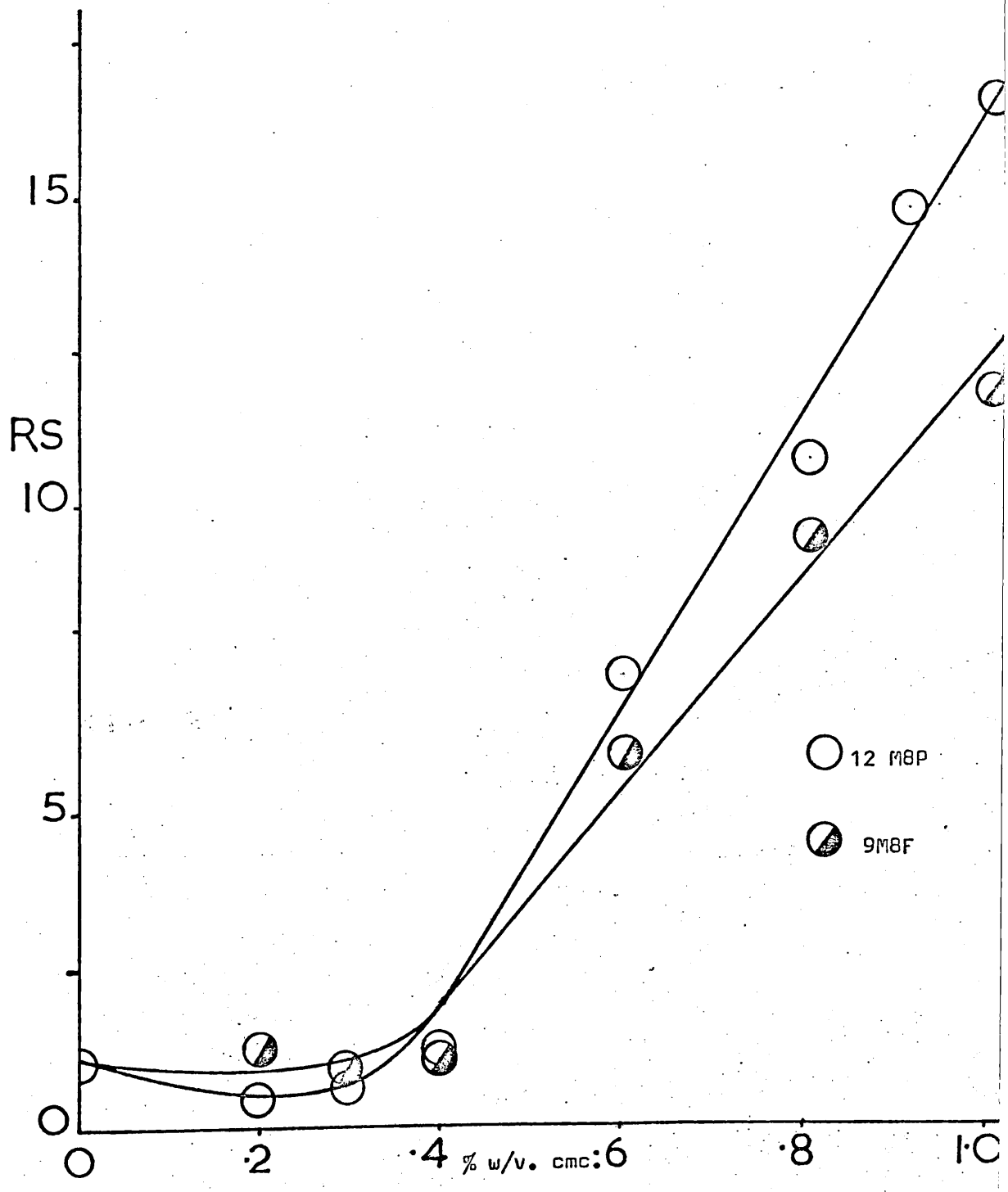


Fig. 3.6.

VARIATION OF STABILITIES OF CMC. STABILISED SUSPENSIONS WITH APPARENT VISCOSITY

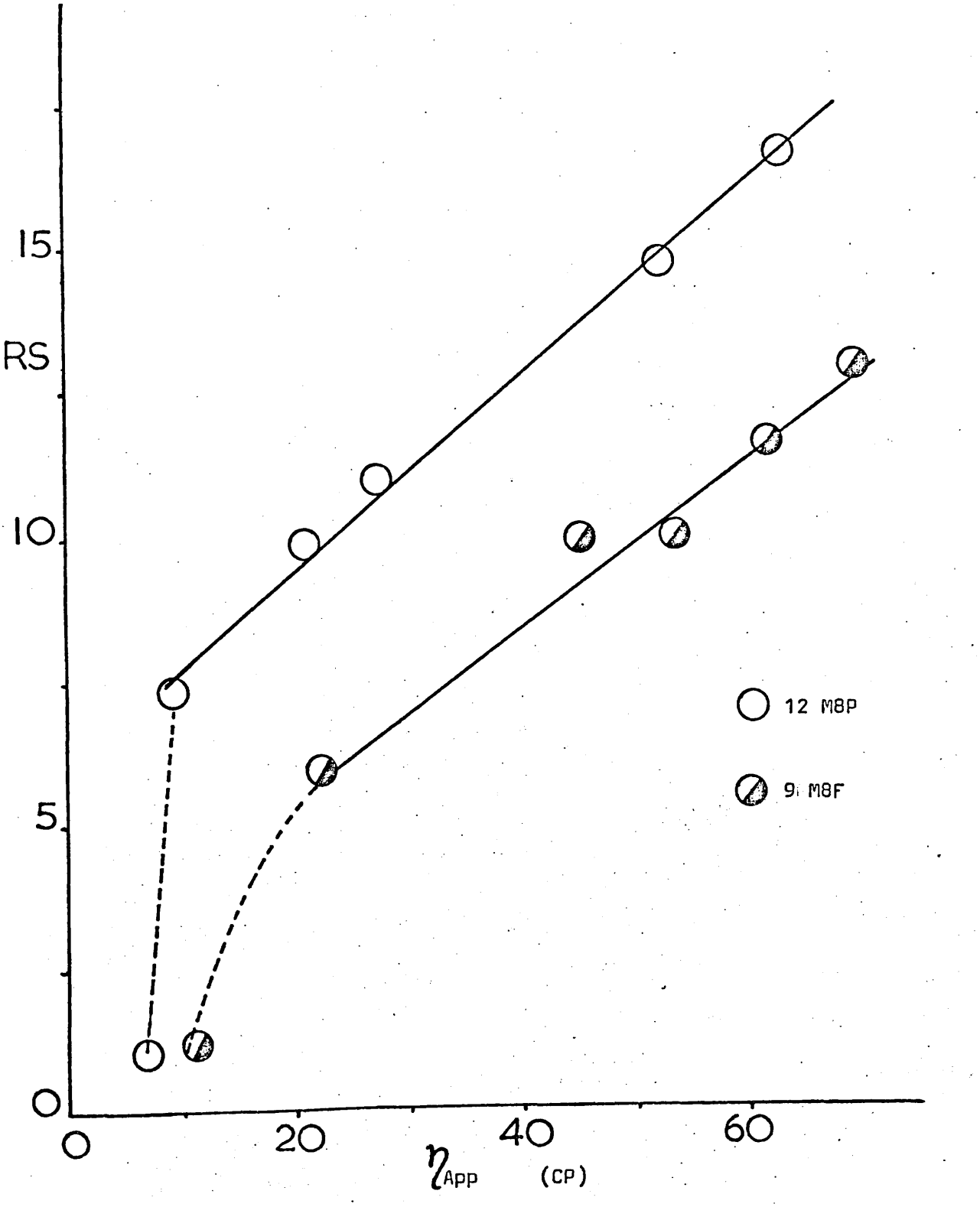


Table 3.4.

Physical parameters of 60% barium sulphate suspensions stabilised with cmc. (9M8F).

conc./% w/v.	η App./cp.	yv./cp.	RS.
0•2	43•0	144	1•2
0•3	12•0	35	0•98
0•4	11•1	0	1•0
0•6	24•0	0	6•0
0•8	47•0	6	10
1•0	68•0	45	12•0

Table 3.5.

Physical parameters of 60% barium sulphate suspensions stabilised with cmc. (12M8P).

Conc. 12M8P/% w/v.	η App./cp.	yv./cp.	RS
0•2	14	59	0•5
0•3	9•0	44	0•5
0•4	7•0	0	1•0
0•6	8•0	0	7•0
0•8	21	0	10•0
1•0	62	0	17•0

Table 3.6.

Dispersibilities in water and acid of suspensions stabilised with 9M8F.

Conc. % w/v.	Dispersibility. water	0·25 mol. dm^{-3} HCl.
0·2	heavy flocculation	very heavy flocculation
0·3	heavy snow, no dispersion,	heavy snow, some dispersion
0·4	fine dispersion, slight flocculation	fine dispersion, slight snow
0·6	fine dispersion, slight flocculation	fine dispersion, some snow
0·8	dispersion less good, slight flocculation	dispersion quite good, some flocculation
1·0	slight flocculation	good dispersion, slight flocculation

Table 3.7.

Dispersibilities in water and acid of suspensions stabilised with 12M8P

Conc. % w/v.	Dispersibility	
	water	0·25 mol. dm^{-3} HCl.
0·2	heavy snow, no dispersion	severe flocculation
0·3	heavy snow, no dispersion	heavy snow, flocculation
0·4	Fine even dispersion, no snow	Fine dispersion slight snow
0·6	Fine dispersion	Fine dispersion, slight snow
0·8	Fine dispersion, very slight snow	good even dispersion
1·0	Fine dispersion, slight snow	dispersion good, very slight snow

Chapter 3. Section 5. Discussion.

(a) Shapes graphs.

(i) Variation of yv. and viscosity of suspensions with concentration of stabiliser (Figs. 3.3. and 3.4.).

The viscosity of a suspension can best be considered as resulting from particle -particle interaction, and the viscosity of the liquid

phase. Initially the viscosity of the liquid phase (i.e. of the unstabilised barium sulphate suspension) is very low, but particle - particle interaction is high. The uncoated particles are rough and thus great force is required to produce shearing. The added stabiliser coats the particle, reducing particle - particle interaction, but little stabiliser passes into the liquid phase, thus there is a decrease in η App and yv. When the particles become fully coated particle - particle interaction remains constant and increasing amounts of stabiliser now enter the liquid phase. The viscosity of the suspension therefore rises. If the suspension is Newtonian, as is the case for those containing 12M8P, the yield value remains at zero. For those suspensions stabilised with 9M8F the yv increases after 0·6% w/v. stabiliser added, and this is due to the increasing "structure" assumed by the liquid phase, and is the minimum energy required to break this structure. Suspensions containing above 0·6% w/v. 9M8F were non-Newtonian. The minimum in the yield value is thought to coincide with the concentration of gum necessary to fully coat the barium sulphate particles. Thus particles became fully coated at 0·4% w/v. stabiliser concentration.

(ii) Variation of Relative stability value and concentration of stabiliser (Fig. 3.5.).

The graphs are of a similar shape to those of η App and yv. as a function of concentration, and this gives the clue to their interpretation. Stability is least when particles first become fully coated, and therefore offer little resistance to their motion through the liquid phase which contains little stabiliser in solution. As the stabiliser concentration increases particles move through an increasingly viscous medium, and the force resisting their motion increases as concentration of stabiliser in the liquid phase increases. 12M8P gave more stable suspensions, and this must be due to the greater degree of substitution, since 12M8P suspensions were less viscous than those containing 9M8F at the same concentration.

(iii) Variation of RS with apparent viscosity of suspension (Fig. 3.6.).

For concentration of stabiliser 0•4 – 1•0% w/v. the RS is clearly a linear function of the apparent viscosity of the suspension, and hence of concentration stabiliser present. 12M8P for the same viscosity gives more stable suspensions than 9M8F, yet only the latter displays non-Newtonian behaviour.

(b) Dispersibilities in acid and water. (Tables 3.6. and 3.7.).

Generally for both stabilising agents the dispersive properties of suspensions containing 0•4 – 1•0% w/v. of stabiliser i.e. from when they just become fully coated, were very good and comparable to best commercial preparations in this respect. Best dispersions were given by those containing between 0•6 and 0•8% w/v. stabiliser, above and below this concentration good dispersions were less readily obtained and slight snow was apparent, especially under acid conditions.

(c) General observations.

The bench life of the suspension was fairly long, especially for those containing higher concentrations of stabiliser, the precipitates which eventually over a period of days set as a "cement", this claying being more apparent for 12M8P than for 9M8F; vigorous stirring was needed to break the sediment up and redisperse it. This is not a good feature, but such a situation obtains in many commercial preparations, e.g. Micropaque, Raybar and Baritop which contain high concentration of stabiliser. Claying can obviously be prevented if the barium meal is supplied as a spray dried powder, where the characteristics desired for a barium meal can be incorporated into a product without the bench life of a suspension being considered.

No differences in physical characteristics could be assigned to suspensions prepared by different methods, and agreement between similar samples prepared by the two methods was good. The second method was by far the most convenient and might well have commercial applications.

Chapter 3. Section 6. Barium meals used in Animal work.

(i) Micropaque.

Micropaque is perhaps the most popular Barium meal manufactured in the British Isles. It is supplied either as a suspension or as a spray dried powder. Only the former was investigated. This contains 100% w/v. of barium sulphate (av. diameter 0·2 μ m.) stabilised by 2 - 3% of CD75, an extract of a plant gum. Various other additives are present including flavourings and antibacterial agents, but these do not affect the stability of the suspension. CD75 is of variable composition (according to source) and this results in the variation of Micropaque from different production runs. Because of the known variability of Micropaque two dm^3 samples from a single batch were reserved for use in all in vitro and in vivo investigations. The physical parameters of Micropaque have been well defined by Goddard and James (1972). Micropaque has a long bench life, being highly stable, and good dispersive properties in acid and water. The pH mobility curve of Micropaque particles is shown in Fig. 3.9.

(ii) Barosperse.

Barosperse was investigated because its physical properties are very different from Micropaque, and yet is very popular with radiographers. It is supplied as a powder of dry coated barium sulphate particles. Suspensions are easily prepared in the laboratory by the simple addition of water followed by slight agitation to ensure mixing. The suspension is pink, and contains flavouring and various other additives, but there is little excess stabiliser in the aqeous phase. Consequently prepared suspensions quickly settle, although these are easily resuspended by agitation. Suspensions show less good dispersive characteristics in acid and in water. The coated barium sulphate particles are characterised by a high negative charge, the mobility at pH 7 is 2·7. (Fig. 3.8.).

(iii) Other commercial preparations.

Other commercial preparations are similar either to Micropaque, being supplied as ready made stabilised suspensions containing from

60 - 90% w/v. barium sulphate, including

(a) Baritop (Fig. 3.9.).

(b) Raybar, basically Micropaque, 75% w/v. barium sulphate with added hydroxy ethyl cellulose.

or to Barosperse, supplied as a dry powder e.g.

(c) Eugnost (Fig. 3.9.).

(d) Roussell UCLAF (Fig. 3.8.).

Because some commercial preparations as e.g. Eugnost and Roussell UCLAF were only available in very small quantities it was not possible to determine their relative stabilities. All commercial preparations had good dispersive properties in water and in acid.

(iv) Citrate stabilised suspensions.

Citric acid has long been known as an additive to barium sulphate suspensions. It greatly reduces the viscosities of such suspensions, and allows more concentrated suspensions having a viscosity low enough for oral administration to be prepared. The preparation was supplied as a dry powder containing 2% w/v. citric acid and sodium bicarbonate.

pH mobility curves of barium sulphate particles from the various suspensions are given in Fig. 3.7. and 3.8. All provide evidence that the particles are coated with stabiliser, since the curves are unlike those for unstabilised barium sulphate particles prepared under various conditions (Goddard 1970). Particles of Micropaque and Baritop give curves typical of carboxyl surfaces (surface $pK_a = 3{\cdot}4 - 3{\cdot}5$). The pK of Barosperse could not be measured accurately as the mobility never approached zero. This might indicate the presence of a stronger acid residue at the surface, e.g. phosphate or sulphate. Since, however, the main suspending agent in Barosperse is cmc., the high mobility of particles in suspensions of high pH might indicate interaction of some other component in the preparation affecting the surface.

The pH mobility curve for particles of Eugnost are peculiar and give little clue as to the nature of the stabiliser, while the particle of UCLAF appears to be coated with a stabiliser carrying both anionic

and cationic groups (possibly carboxyl and imino groups). The pH mobility curve for citrate stabilised particles gives evidence of the physical adsorption of citrate by the particles, producing highly charged negative particles.

Chapter 3. Section 7. Surface properties of other commercial preparations.

The pH mobility curves are shown in Fig. 3.10. Ryubari sol.B, x sol.B, and Baryten sol. are of Japenese manufacture. The"cmc. Micropaque" preparation was supplied as a 100% w/v. suspension similar in all respects to Micropaque, but stabilised with 2% cmc. instead of CD75.(Fig.3.7.) Liquibarine was supplied as a powder.

The pH mobility curves are all similar, and characteristic of surfaces containing carboxyl groups (pKa 3·5) for each preparation. All particles are thus coated with stabiliser. For "cmc. Micropaque" the limiting mobility value (2·1) suggests a degree of substitution of 0·5 (Chapter 3. Section 3., Table 3.2.). The stabiliser for the other preparations cannot be defined so confidently, but their great similarity to the pH mobility curves previously obtained for laboratory prepared meals (Fig. 3.2.) strongly suggest that cmc. is the stabiliser, although, of course, there may be other additives present.

Fig. 3.7 .

pH MOBILITY CURVES OF COMMERCIAL BARIUM MEALS MEASURED IN ACETATE VERONAL BUFFER SOLUTION (I = 0·02 MOL. DM^{-3})

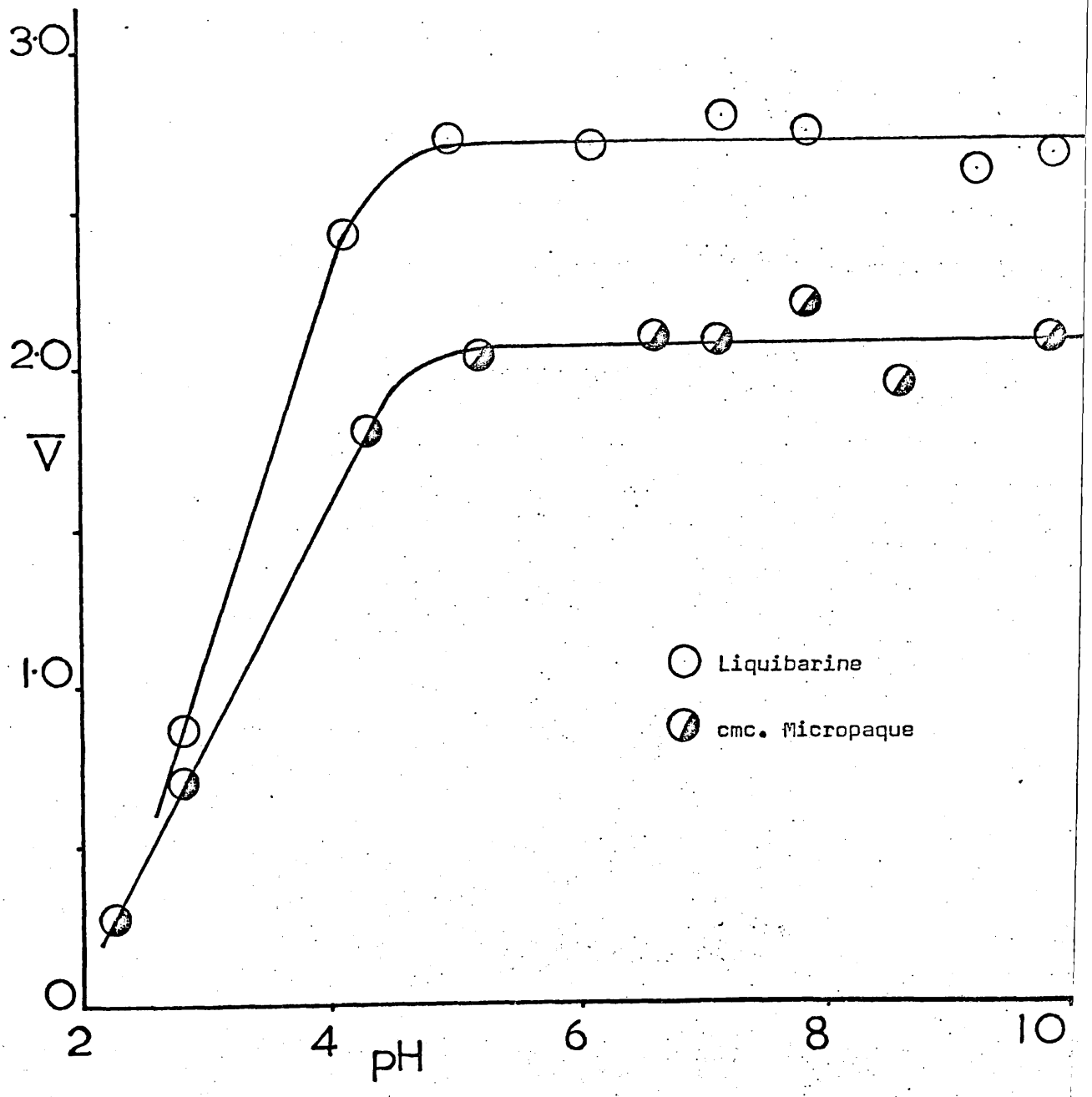


Fig. 3.8.

pH MOBILITY CURVES OF VARIOUS COMMERCIAL PREPARATIONS MEASURED IN ACETATE VERONAL BUFFER SOLUTION ($I = 0{\cdot}02$ MOL. DM^{-3})

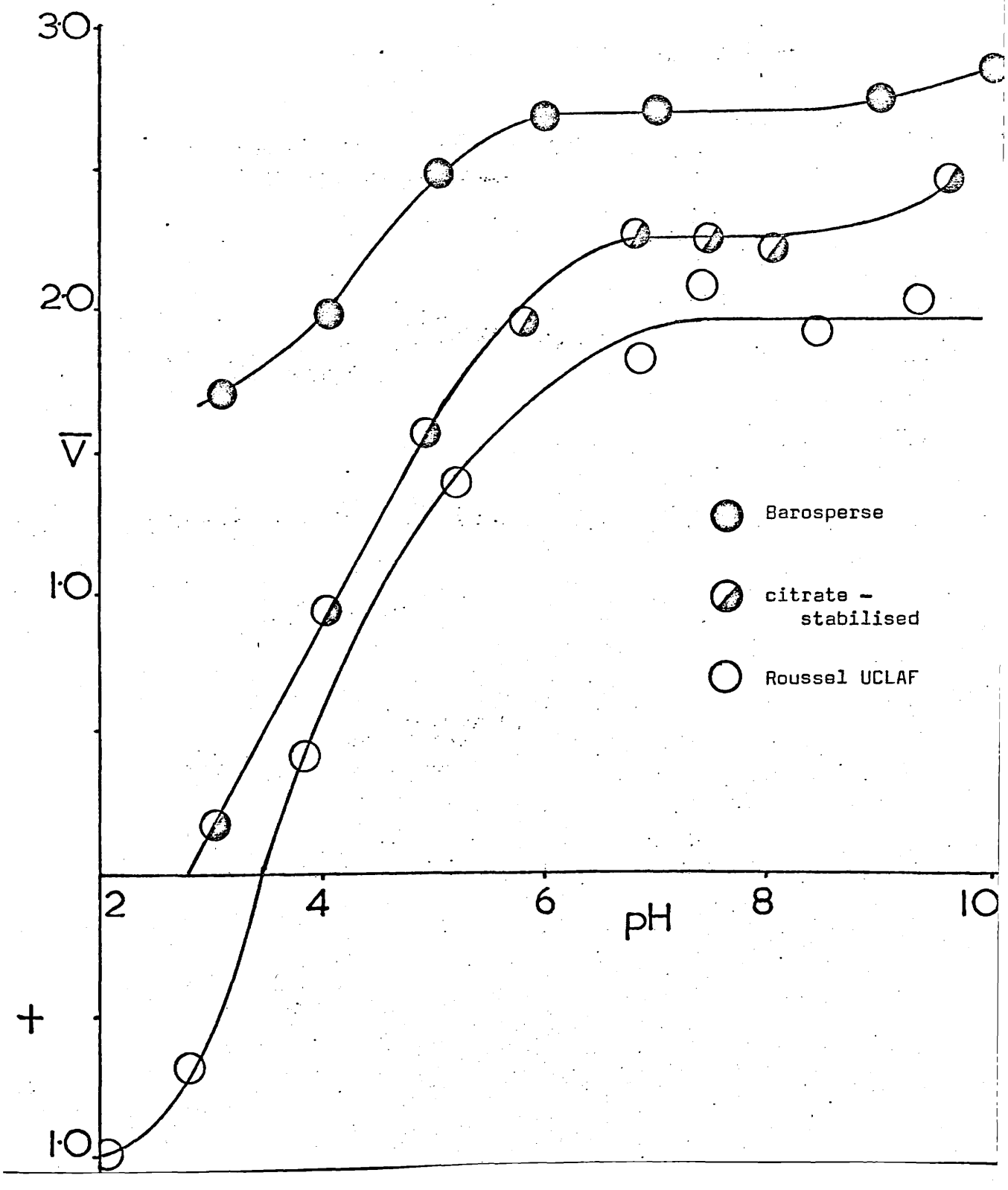


Fig. 3.9.

pH MOBILITY CURVES OF COMMERCIAL PREPARATIONS MEASURED IN ACETATE VERONAL BUFFER SOLUTION I = 0·02 MOL. DM^{-3}

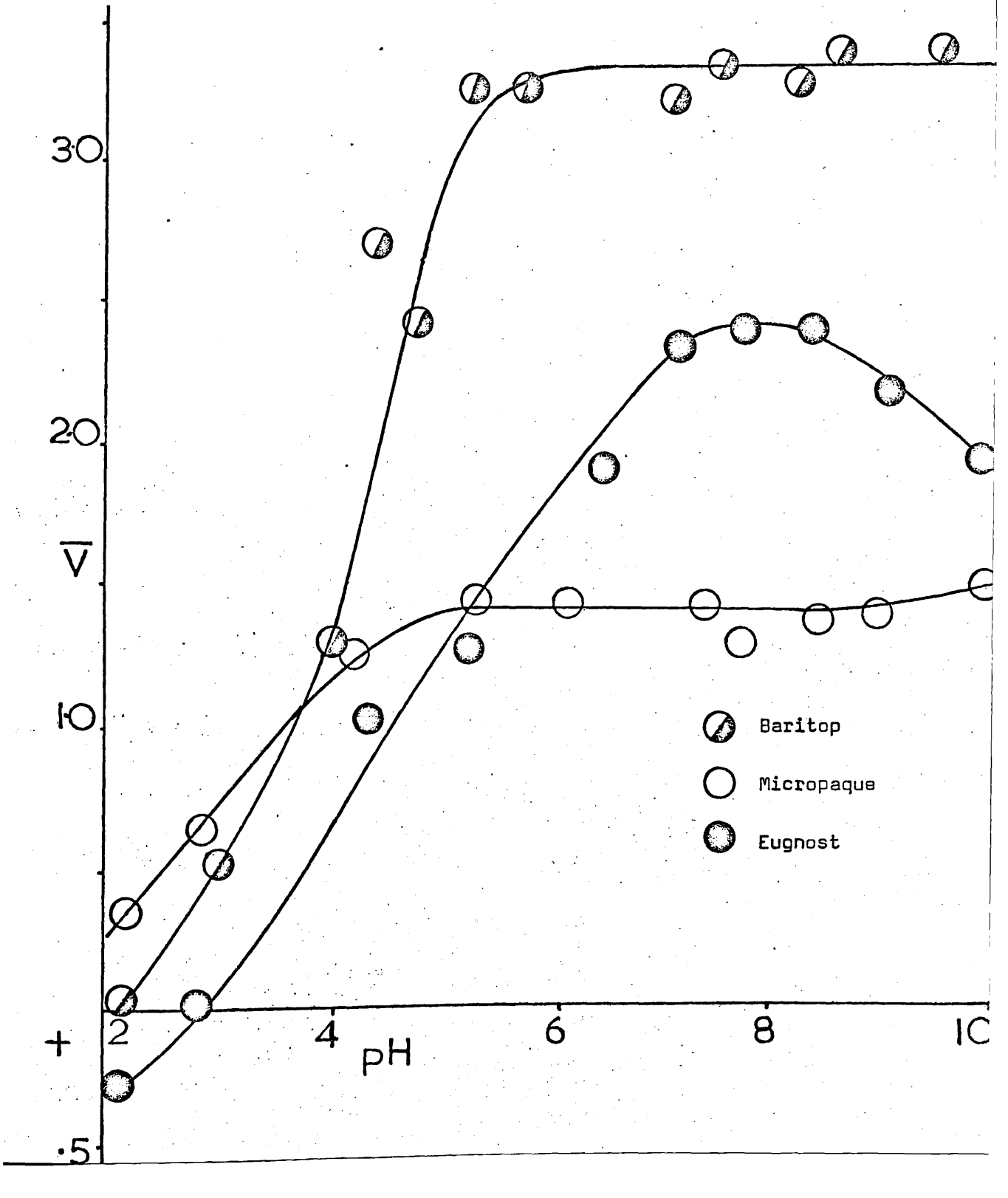
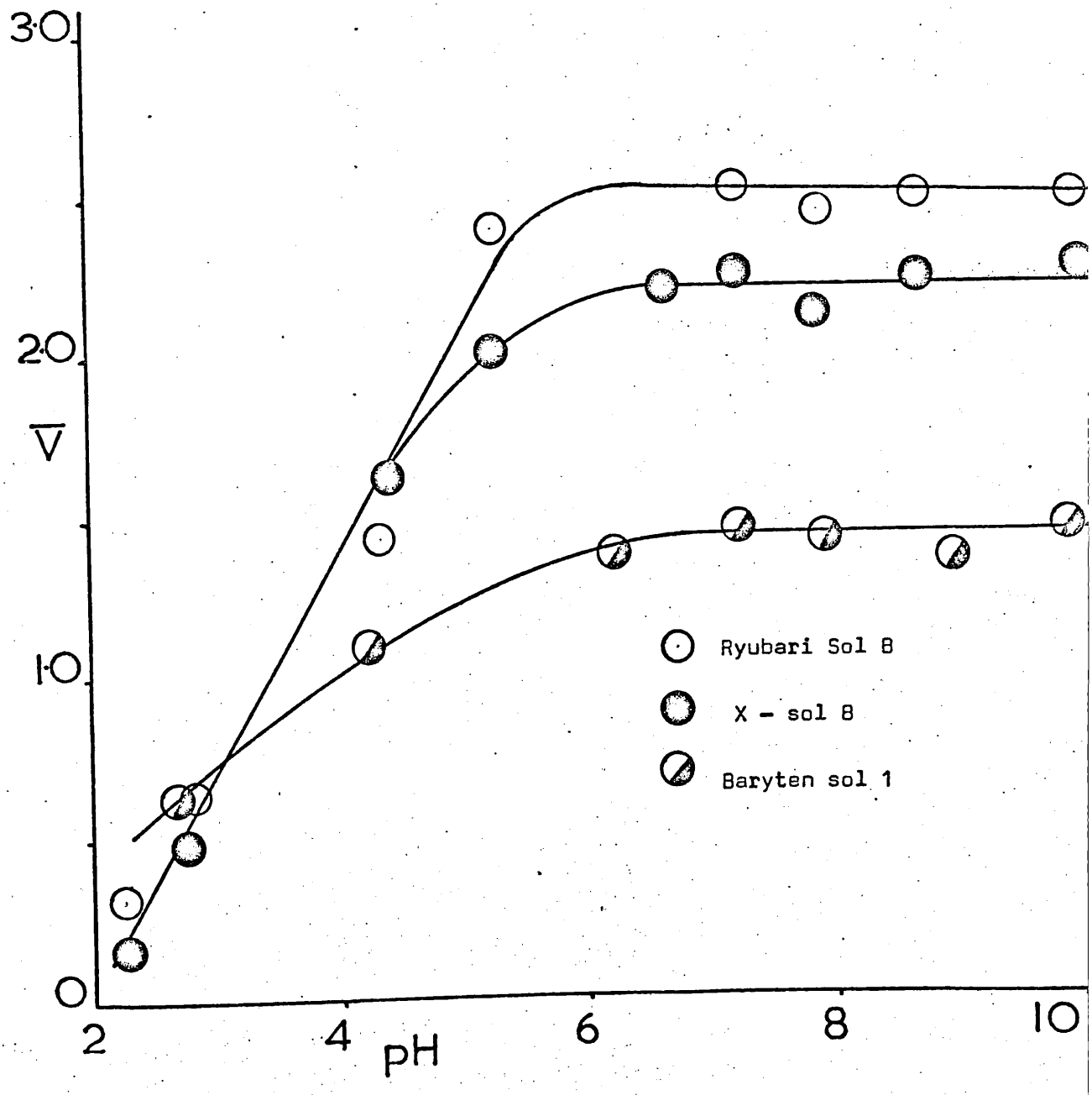


Fig. 3.10.

pH MOBILITY CURVES OF VARIOUS COMMERCIAL PREPARATIONS MEASURED IN ACETATE VERONAL BUFFER SOLUTION I = 0·02 MOL. DM^{-3}



CHAPTER FOUR

DEVELOPMENT AND APPLICATION OF IN VITRO INVESTIGATION OF BARIUM MEALS

Chapter 4. Section 1. Introduction.

The need for objective testing of radiopaques has already been discussed. Ideally the test should be so constructed that the experimental conditions can be easily altered and those parameters thought to be important for efficient radiopaques investigated, so that the conditions most favourable for an even coating of the gastro intestinal mucosa *in vivo* can be elucidated.

It was not possible to conduct controlled clinical trials of radiopaques, and even here the results of such trials as have been carried out, rely upon expert appraisal of X-ray photographs obtained. A model system had therefore to be developed, in which a standard mucosal surface could be brought into contact with a radiopaque of known concentration, and the interaction of the surface with the radiopaque under controlled conditions of pH, ionic strength and duration, assessed. The essence of the developed technique involves use of small samples of fresh ileal tissue and their interaction with radiopaques is assessed by microscopic examination of sections removed from the tissue sample, frozen at completion of the experiment.

Criteria considered in the development of the technique were:

(a) Mucosal surface, i.e. thickness of mucus and the state of villi, to be reproducible, so that results from different experiments could be directly compared;

(b) Concentration of barium sulphate suspensions, pH and ionic strengths to be easily varied and known accurately.

(c) Assessment of adhesion to be non-subjective and open to interpretation from the point of view of both thickness and uniformity of any adhering layer.

(d) Results to be applicable to clinical *in vivo* technique.

Chapter 4. Section 2. Development of technique.

A fasted rat (male, 250 g.) was sacrificed and the body cavity opened to reveal the duodenum and the large and small intestines.

5 cm^3 of a Micropaque suspension, diluted to 50% w/v. with phosphate buffer (pH 7·0. I = 0·1 mol. dm^{-3}) was injected into the duodenum. This was allowed to find its way along the small and large intestines and 2 cm. lengths, starting from the stomach were tied off and removed. Each was further divided into 0·5 cm. lengths, and washed in isotonic medium (Difco Trowell medium T.S. adjusted to pH 7). Care was taken not to disturb the mucosal surface in any other way. Two 0.5 cm. samples from each 2 cm. sample were briefly immersed in a solution of 10% PVA (poly vinyl alcohol) in water. These and the other two 0.5 cm. portions were then flash frozen by dropping into 20 - 25 cm^3 of n-hexane maintained at -65°C by a solid carbon dioxide/ethanol cooling bath. The frozen pieces were removed, briefly dried on kleenex tissue, and stored in labelled bottles at -70°.

The samples of ileal and duodenal tissue were subsequently sectioned in a cryostat to give 12 ρ m tangenital cross sections. The cryostat chamber was maintained at -25° and the knife at -70°. Sections were cut from equivalent distances along the blocks of tissue that is 0·25, 0·5 and 0·75 of the length; in this way a standard procedure for each tissue sample was maintained.

The sections were mounted on standard microscope slides at room temperature, dried briefly on a warm (50°) hotplate and stained in a bath of Toluidine blue (0·2% w/v. in 0·1 mol. dm^{-3} citrate buffer pH 6·0), washed in distilled water, dried and labelled. For each original 2 cm. of the gut 16 stained preparations were made. The preparations were examined microscopically. Results of the examination were:

a) The Intestinal tissue withstood the various manipulations well. There was no evidence of any degradation of the mucosal or muscle layers, or the villi, which were intact.

b) Barium sulphate particles remained adhering to the mucosa throughout the freezing, cutting and mounting procedures, in samples treated with PVA., and also in the untreated samples. Although it was possible that

these operations might remove barium sulphate, this was not the case, and all samples from all regions of the gut showed a thick layer of barium sulphate particles. In some instances, especially in the lower gut, this layer extended into the lumen of the gut.

c) Adhesion was very variable even within a single region of the gut, and appeared to depend upon the state of the mucus on the mucosal surface, the presence of free mucus in the lumen of the gut, and the actual position in the gut from which the sample had been taken. It was not possible to compare directly samples taken from the small intestine and the large intestine, due to morphological differences in the samples. In the duodenum villi are short and compact, whilst in the small intestine they are long and filamentous. The surface areas of the mucosa are thus very different in these two regions, as is the state of the mucous lining.

Because of the non uniformity of the adhering layer it was not possible to estimate the barium sulphate with a microphoto-densitometer. Estimation of the layer gravimetrically, involving the enzymic digestion of the section and weighing of the barium sulphate was open to the objection that sections varied in size, i.e. the surface areas of the mounted sections was variable and therefore the weight of barium sulphate would depend to a great extent upon this. Attempts to assess the area of gut surface, and the area with an adhering layer were also abandoned due to the presence of barium sulphate in the crypts and the great variability and diversity of the mucosal surface, which meant that barium sulphate particles did not always penetrate to the surface of the gut. Such a calculation of the area would thus have little meaning. (Plate 4.1.).

However, the experiment was valuable in showing that a standard technique using a standard region of the intestine was required, and that the state of the mucus lining would have to be very carefully controlled if any claim for the standardisation of the method was to be made.

This was underlined when a similar experiment was carried out with

pig ileum. Fresh pig ileum obtained from a slaughter house was washed in Difco medium (pH 7·0) and 30 cm. tied off into 2 cm. lengths. Each length was injected with 50% w/v. Micropaque suspensions (pH 7·0) and left for five minutes, after which it was opened and portions (0·5 cm. x 1 cm.) removed. Two from each of these were treated with PVA., and a total of four small sections from each tied off section were flash frozen as before, sectioned, mounted and stained.

Microscopic observation of the sections revealed that very little barium sulphate had adhered to the mucosal surface, and this was variable in a random fashion.

This was due to handling of the intestine before its aquisition which caused damage to the mucous lining, and to the cutting up of the gut into small portions for freezing after the introduction of the barium sulphate suspension; when further disruption of the mucosal surface could not be avoided.

No differences could be found between those samples treated with PVA and those not treated. It was concluded that pig ileum obtained in this way was unsuitable since the state of the mucosal surface was not in any way controllable or reproducible.

Further experiments were therefore carried out only upon rat ileum, since the diet of the animal could be easily controlled, the material always fresh, could be taken from a known region of the gut, and handling of the tissue could be kept to an absolute minimum. The ileum and not the stomach was chosen for a number of reasons: (i) The rat stomach is unlike the human or pig stomach since approximately half of lumen is a continuation of the oesophagus, and as such has no villi, gastric or mucus-secreting cells. (ii) The morphology of different regions of the glandular stomach are also different, and preparation of the material for experiments would require more handling than for the ileum. (iii) The ileum, or small intestine of the rat is a well defined organ and homogenous region of the gut. The villi are coated with a thick layer

of mucus which is able to withstand the various manipulations required. (iv) Since the ileum is characteristically 30 - 35 cm. long. One animal will provide enough tissue for many experiments. The results of such experiments will be strictly comparable since there is no animal variation.

Chapter 4. Section 3. Experimental.

(a) Apparatus.

Glass rings, such as are used for mounting large biological specimens for microscopic examination were glued to standard microscope slides, two per slide, with "Araldite". It was in these cells, approximately 1•5 cm. in diameter by 0•5 cm. deep that the experiments were performed. The bottom of each cell was lined with two squares (1 cm. x 1 cm.) of lens tissue to provide a seat for the ileal tissue.

(b) Experimental Technique.

A fasted rat (male, 24 hours on water only) was sacrificed and the small intestine removed. This was placed in a shallow dish containing Difco medium. Further operations were carried out only in this dish so that the tissue was subjected to a minimum of shock, and drying out of the tissue was prevented. The mesentry was removed and the anterior end of the small intestine cut into rings of 0•2 cm. lengths, starting 5 cm. in from the duodenum. Each ring was further divided down one side, so that gross deformation of the tissue, due to curling of the peritoneal surface was prevented. Twenty of these sections were then transferred to the prepared cells, which had been filled with 0•5 $cm.^3$ of the same Difco medium solution. The cells were then temporarily fixed to pieces of card and labelled. The contents of the cells were mixed by gentle shaking of these cards, this ensured that all cells received the same treatment. The medium solution of each cell, now containing tiny particles of removed food was drawn off with a 2 $cm.^3$ syringe. This procedure ensured that mucosal surfaces were clean and free of food. Care was taken to fill and drain the cells only

from the sides so that the ileal tissue was not disturbed. The cells were again drained and 0·5 cm^3 of preparaed barium sulphate suspensions added to all cells except four controls for each experiment. The controls were carried through all manipulations; 0·5 cm^3 of phosphate buffer solution (I = 0·1 mol. dm^3, pH according to the experiment) was added instead of the suspension. For each different suspension preparation there was a minimum of two cells. During exposure of the tissue slices to the suspensions the cells were gently agitated, to minimise the effect of settling of the suspensions. After 10 minutes the suspensions (and buffer solution of controls) were removed with a syringe and 0·5 cm^3 of fresh medium introduced. The cells were again agitated, fresh medium introduced, and the washingprocedure repeated. The washing was necessary to remove any particles of barium sulphate loosely held on the gut, as would be the case for particles not in actual contact with the mucus lining of the gut.

The ileal pieces were removed with fine forceps and frozen in hexane as before. The frozen blocks were cut in a standard manner, 12 μ m. sections being taken and these were mounted and stained as before. Flash freezing of samples ensured that ice crystals did not form in the tissue, with consequent loss of structure. If frozen in such a manner the inter cellular water sets as a glass and the integrity of the tissue is retained.

The technique used only 0·5 cm^3 of a given suspension. This was useful when only a small amount of suspension was available, and also meant that the effect of pH and concentration of the suspension upon adhesion could be investigated using the same batch of a suspension. Since Commercial suspensions are known to be variable unwanted factors could easily be introduced if the same suspension was not used throughout the experiments.

The method was quick and easy in practice, and parameters such as the pH and ionic strengths of suspensions could easily be controlled.

The absolute concentration of barium sulphate could also be controlled, though because of sedimentation this was less easily accomplished with unstable preparations.

The mounted samples were examined under the microscope, and evaluated for the thickness and uniformity of the adhering layer. because this was a subjective matter a further control was introduced into each experiment. This was Micropaque, diluted to 12•5% w/v. with buffer (pH 7•0). In each assessment of the various samples Micropaque at this concentration and pH was given an arbitary value of 10, and the other samples judged using this standard. Experimental results from different animals could then be directly compared, and variation from animal to animal could be assessed and allowed for.

All animals used in experiments were fasted overnight before the experiment. This ensured that the small intestine was reasonably clear of food and required a minimum of washing, and differences due to variation of feed minimised.

Chapter 4. Section 4. Experimental parameters.

All sections cut from ileal tissue treated with barium sulphate suspensions exhibited, to a greater or lesser extent a layer of barium sulphate adhering to the mucous membrane. In no case was a similar layer found on the periteneal surface of a section. This showed that a definite interaction between the layer of mucus and barium sulphate suspension had occured, and the coating upon a section was not due only to settling of barium sulphate particles.

The ileal tissue, even at pH 3 and 8 showed no degradation, in either those [redacted] treated with various suspensions and those not treated (controls).

A comparison of sections taken from tissue treated with 12•5% w/v. Micropaque at pH 7, included in all experiments, revealed that animal variation was less than anticipated, since these standards were all

comparable in terms of eveness and thickness of adhering layer.

(a) Effect of concentration of Micropaque.

Micropaque was diluted with phosphate buffer solution (I = 0•1 mol. dm^{-3}, pH = 7) to give concentrations of 50, 25, and 12•5% w/v. 2 cm^3 of each suspension was taken up in a disposable syringe, and were well shaken before introduction into the glass cells to ensure a hormogenous suspension. Difco medium was adjusted to pH 7 for all washings. A 50% w/v. unstabilised suspension of barium sulphate was also included in the experiment.

An expected trend in the thickness of the adhering layer was found, the thickness decreasing in the order 50 $>$ 25 $>$ 12•5% w/v. Micropaque. The uniformity of the layer showed the reverse trend, 12•5 $\gg$ 25 $>$ 50% w/v. (Plates 4.2. - 4.4.) 50% w/v. unstabilised barium sulphate gave a thicker coat than any of the Micropaque dilutions.

The thickness of the adhering layer was definitely related to the concentration of the suspension. The uniformity of the layer increased with dilution, and therefore with decrease in viscosity of the suspension. Uncoated particles of barium sulphate adhered strongly to the mucosal surface, but the layer was by no means uniform over the whole surface, and aggregations of barium sulphate as well as bare areas of mucosa were common. The unstabilised suspension was very viscous, and the washing procedure tended to remove aggregations of barium sulphate in a random manner.

It was concluded that greater dilutions gave the best results, since the adhering layer was more uniform from sample to sample and within a sample. This led to the selection of 12•5% w/v. suspension of Micropaque for use as a standard in all subsequent experiments (Plates 4.2. - 4.5.).

(b) Effect of pH of Micropaque suspensions.

Micropaque, 12•5% w/v. was prepared from the original suspension by dilution with buffer solutions of pH 3, 4, 5, 6, 7, 8, and 9. Samples of Difco medium solution were also adjusted with 0•1 mol. dm^{-3}

HCl or NaOH solutions to these pH values for washing of the tissue pieces before and after introduction of the suspensions.

Microscope sections from all pH values were very similar (Table 4.1., Plates 4.6. and 4.7.).

(c) Comparison of suspensions.

Commercial and laboratory meals were compared with Micropaque, at 12·5% w/v. These were Barospere, Baritop, Eugnost, Rousel UCLAF, Raybar 75, unstabilised barium sulphate (chapter 3. section 7.) and cmc. stabilised suspensions. These were prepared by the method of Goddard (1970) and contained 0·8% w/v. carboxy methyl cellulose of medium viscosity, D.S. = 0·4. The suspensions were made up to 12·5% w/v. in phosphate buffer solution (pH 7) and used in a similar manner to Micropaque. Because several of the suspensions were much less stable than Micropaque, notably Barospere and Eugnost and Roussel UCLAF, the cells containing the tissue and these suspensions were agitated every 30 seconds. The experiments were repeated for all suspensions at pH 3 and 8.

Generally, the less stable suspension scored more highly than Micropaque (Table 4.2.) but gave a greater variation of results. This was the case for Barospere and cmc. stabilised suspensions, especially at extremes of pH. The adhering barium sulphate was not consistent from sample to sample, and even within a sample variation of sections, taken from different parts of the tissue block, was evident.

Raybar gave consistantly poor covering, especially at low and high pH, which was non uniform, aggregates and areas of very sparse covering being common. At pH 3 the covering exhibited by different meals taking account of thickhess and uniformity was in order:

cmc. > Baritop > Micropaque > Eugnost = Roussel > Barospere > Barium sulphate > Raybar 75

and at pH 7:

cmc. >> Barosperse > Baritop > Roussel = Eugnost > Micropaque > Barium sulphate > Raybar 75

Table 4.1.

Scores acheived by 12•5% w/v. Micropaque suspensions at different pH values.

pH	score		average
	(a)	(b)	
3	10	12	11
4	10	10	10
5	7	10	8•5
6	10	11	10•5
7 (control)	10	10	10
8	11	12	11•5
9	10	10	10

Table 4.2.

Scores achieved by 12·5% w/v. suspensions at pH 3, 7 and 9.

Suspensions.	Average score.		
	pH 3	pH 7	pH 9
Barospere	8 – 10	11 – 13	11 – 13
Baritop	12	12	12
Eugnost	8 – 11	8 – 11	8 – 11
Roussel UCLAF	8 – 11	8 – 11	8 – 11
Raybar 75	5	6 – 10	6 – 10
Barium sulphate	8 – 10	10	10
cmc.	9 – 15	12 – 14	9 – 14
Micropaque (control)	9 – 11	10	9 – 11

at pH 8 - 9:

Barospere ⟩/ cmc. ⟩ Baritop ⟩ Eugnost = Roussel ⟩ Barium sulphate ⟩ Micropaque ⟩⟩ Raybar.

(d) The effect of viscosity of suspensions upon adhesion.

Micropaque was diluted to 12•5% w/v. and centrifuged. The stabiliser, i.e. the gum solution was removed and the barium sulphate particles resuspended in distilled water. This suspension was centrifuged, the supernatant removed, and particles resuspended in distilled water to give a concentration of 12•5% w/v.

The suspension was homogenised in an ultrasonic bath. The separated gum solution was used to dilute a fresh sample of 100% w/v. Micropaque to 12•5% w/v. The two suspensions were then compared at pH 3 and 8 with 12•5% w/v. Micropaque prepared in the usual way (i.e. by dilution with water) and a 12•5% w/v. Barospere suspension in a further experiment.

Results.

Micropaque diluted with gum showed no detectable difference from the standard, but the suspension not containing gum was significantly better than the standard at both pH values, and was comparable to Barospere. Score at pH 3 was 11 - 13; at pH 8 it was 13.

(e) Discussion.

It would seem that the charge carried by a particle plays little part in the adhesive process, as is shown by the similarity of the adhering layer exhibited by Micropaque and the other suspensions at extremes of pH. Differences in this layer shown by different suspensions can not be attributed to differences in surface charge carried by the particles, but they are most probably due to the great variation in stabilities of suspensions. Since Micropaque particles in unstable suspension gave a better average than the standard indicates the effect which the stability has upon the adhering layer of barium sulphate under the conditions of the experiment. This may be due to the greater ease with which a less viscous suspension can wet the surface of the

mucosa, or to sedimentation during the experiment, thereby achieving a greater concentration of barium sulphate at the mucosal surface. When differences in performance were detected at different pH values, as in the case of Raybar and unstabilised suspensions, these could be assigned to precipitation or flocculation of the suspensions at low pH resulting in non-uniform coating of the gut surface.

The indications are that interaction of electrostatic charges play little part in the adhesive process, and that a mechanical process is more probably occuring. The site of adhesion of these particles was demonstrated as the mucosal surface, and showed that quite deep penetration into the crypts between the villi is possible. It is not surprising therefore that barium meals are known to persist in the body for a long time after administration. It could well be that barium sulphate is only removed from the gastro intestinal tract when fresh mucus is secreted and the old is sloughed off.

The main critism of this experimental technique is that it is not fully quantitative in that the performance of a meal is assessed subjectively. Adhesion to the ileal mucosa was shown on the microscopic level and it was not possible to extend the method to obtain a macroscopic picture of the adhesion, as would be obtained from an *in vivo* X-ray examination. The ileal tissue was possibly in a shocked condition at the time of the experiment, due to the killing of the animal, and disruption of the mucus lining was possible due to the various manipulations employed.

The predictions of the method were less stable suspensions, or more concentrated suspensions would be helpful in the production of a firm even coat of barium sulphate on the gastro intestinal tract, in support of the conclusions of many radiographers.

PLATE 4.1.

SECTION OF SMALL INTESTINE OF RAT TREATED WITH 50% w/v. MICROPAQUE (DARK AREA).

(× 75.)

PLATE 4.2.

MICROPAQUE 50% w/v., pH 7 ADHERING TO RAT INTESTINAL MUCOSA

(x 133)

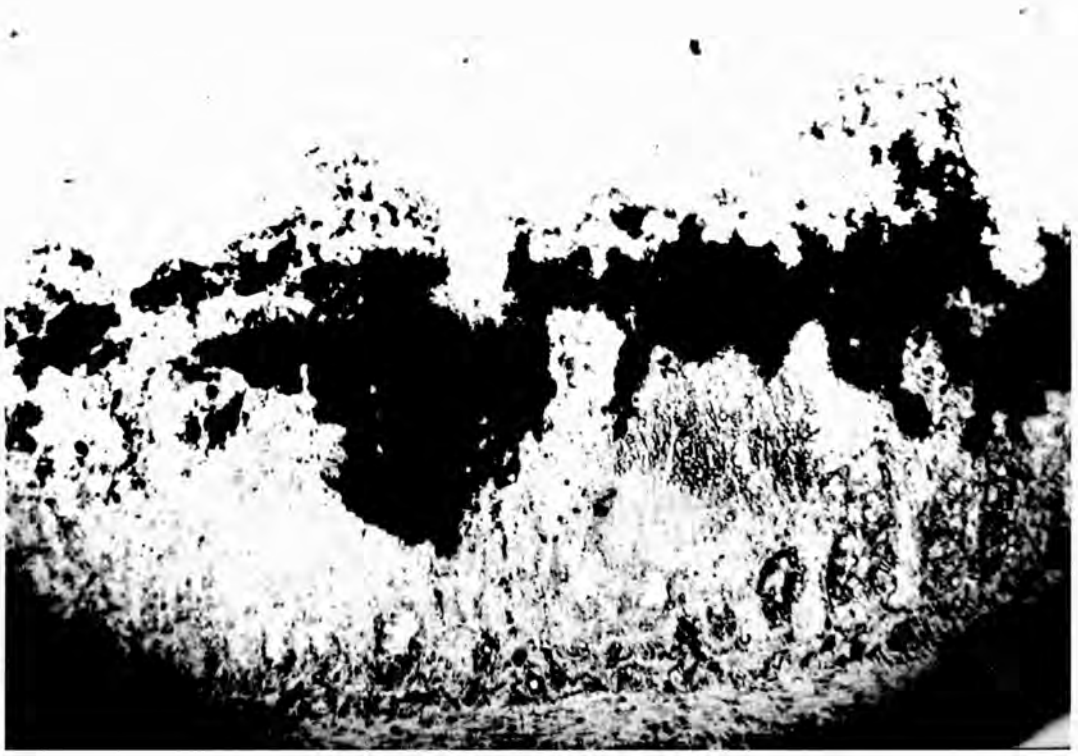


PLATE 4.3.

MICROPAQUE 25% w/v.

(x 133)

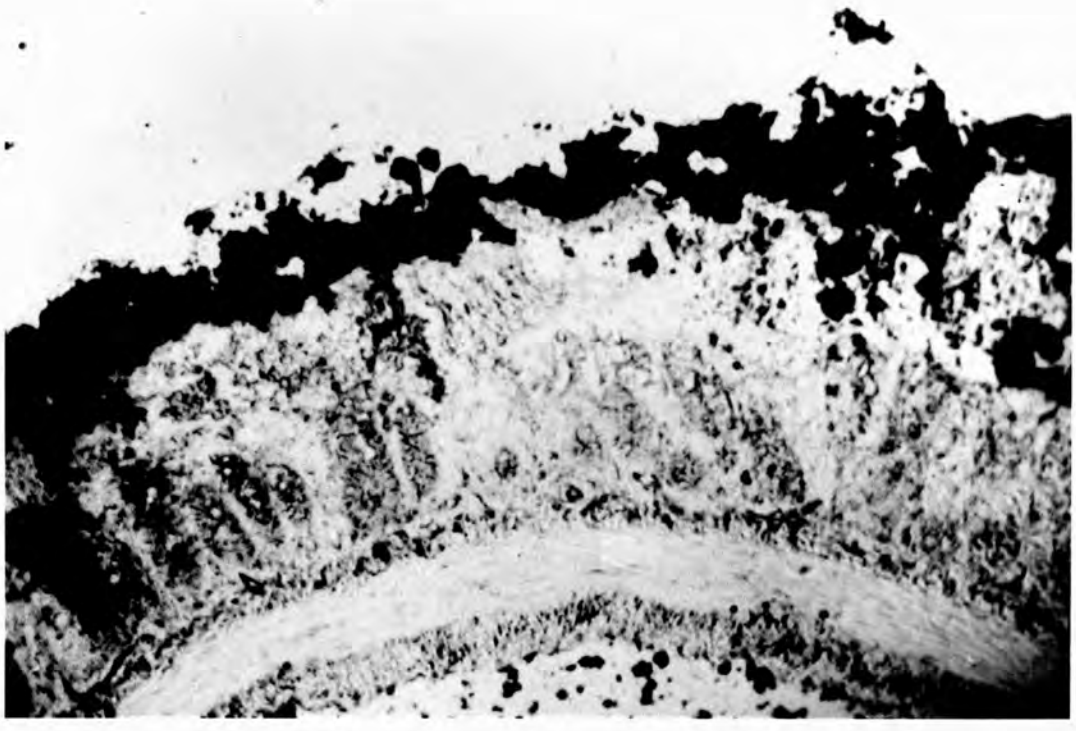


PLATE 4.4.

MICROPAQUE 12·5% w/v.

(× 133)

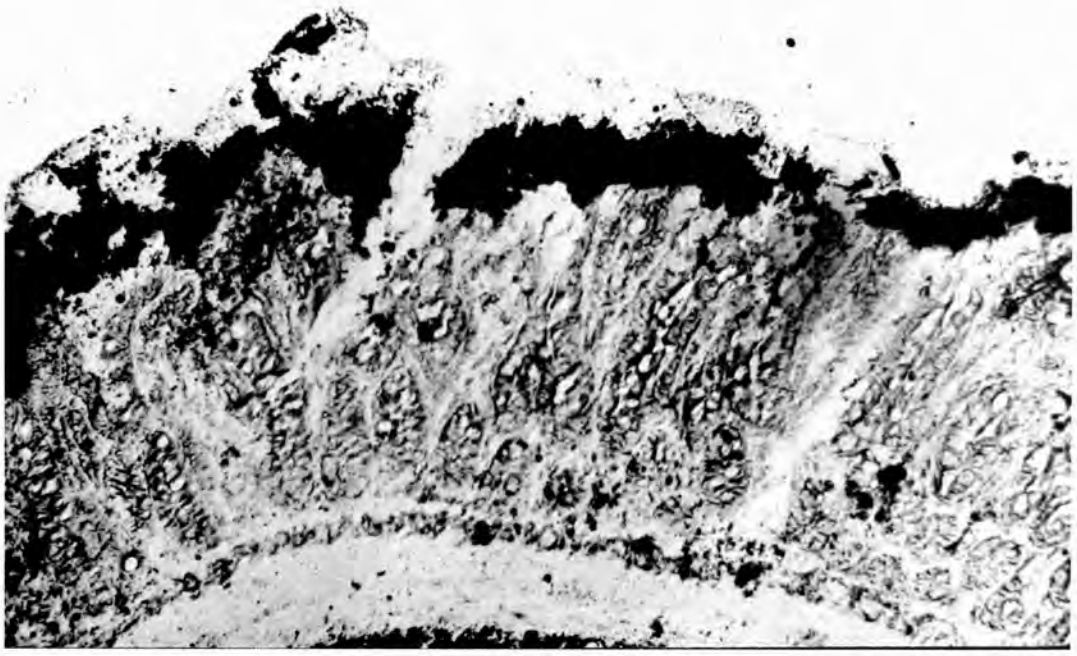


PLATE 4.5.

RAT SMALL INTESTINE COATED WITH UNSTABILISED BARIUM SULPHATE 50% w/v.

(x 133)

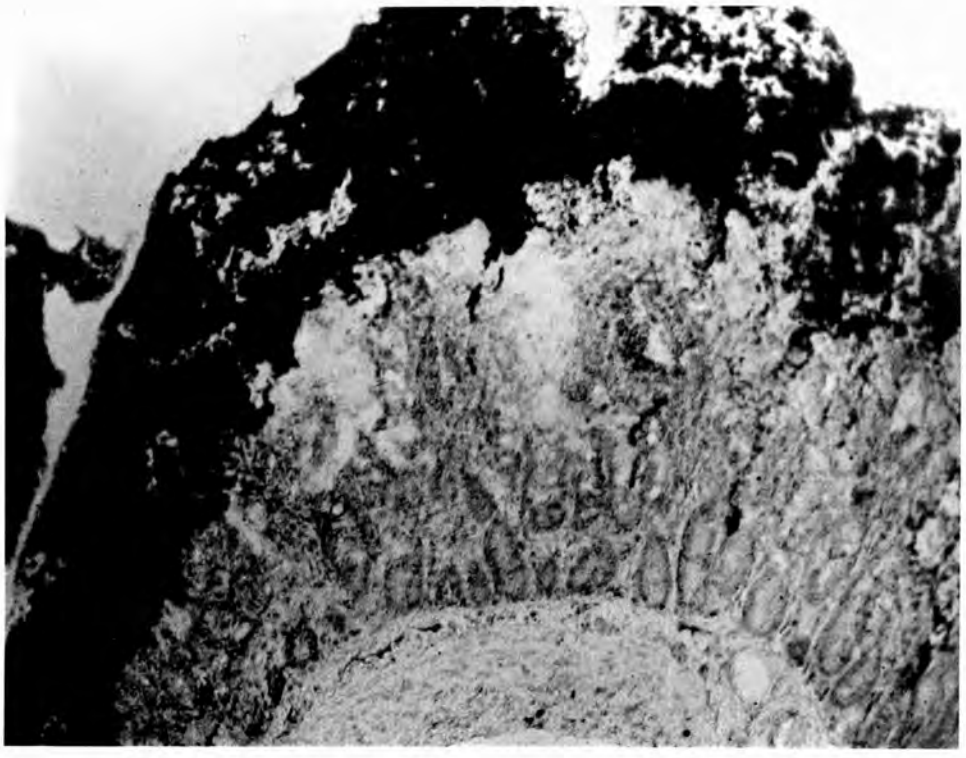


PLATE 4.6.

MICROPAQUE 12·5% w/v. pH 3

(x 133)

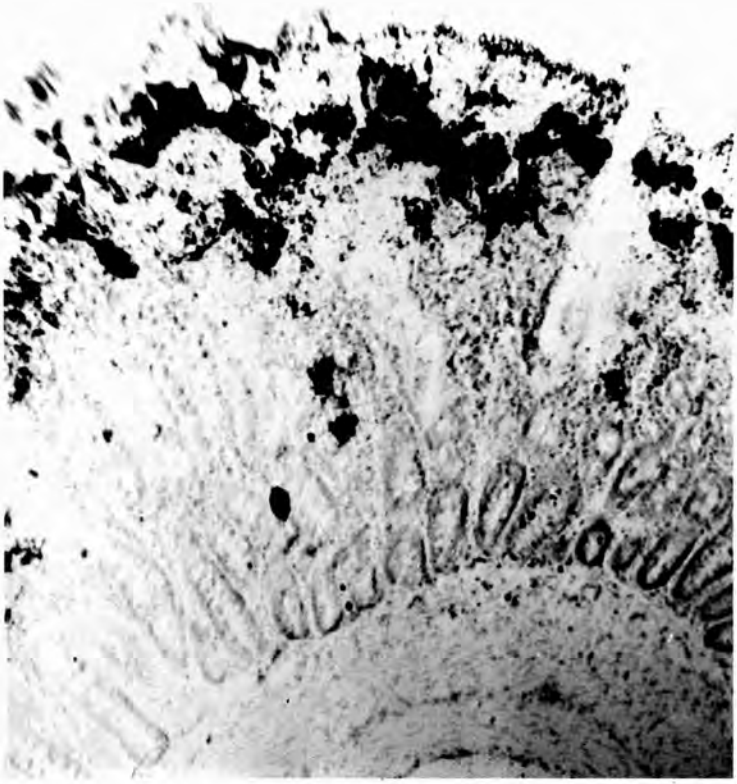
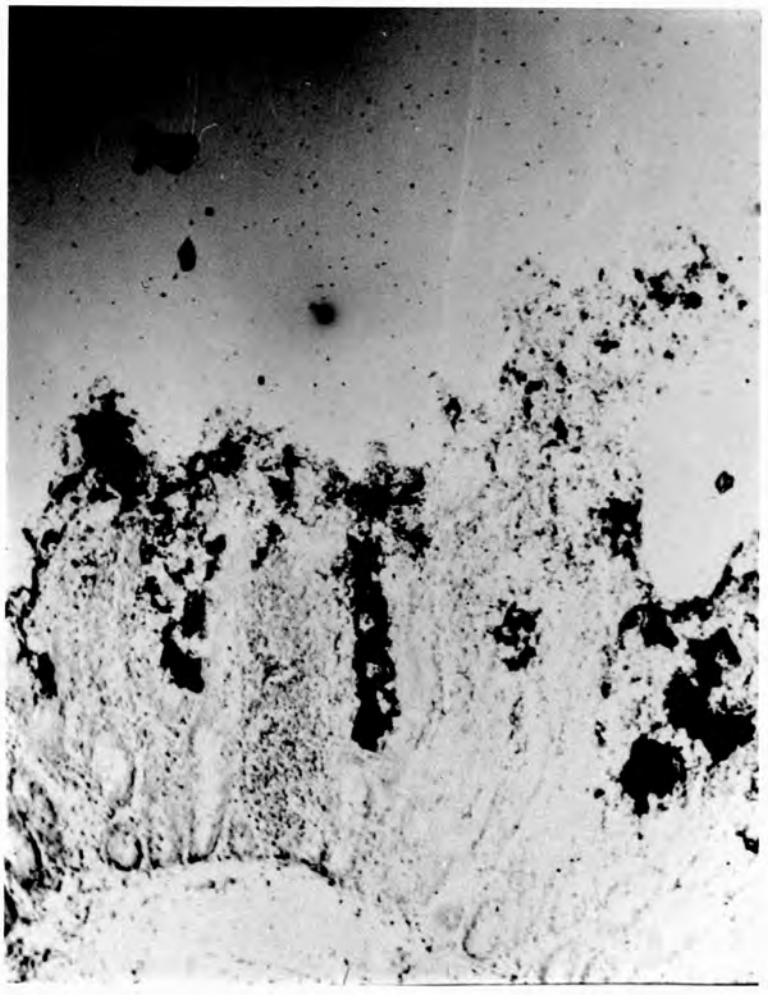


PLATE 4.7.

MICROPAQUE 12·5% w/v. pH 8

(x 133)



CHAPTER FIVE

DEVELOPMENT AND APPLICATION OF IN VIVO INVESTIGATION OF BARIUM MEALS

Chapter 5. Section 1. Introduction.

The *in vitro* testing of barium sulphate suspensions indicated that animal variation was not as great as had been thought. The particles of barium sulphate are held firmly to the mucosa, and adhesion is quite consistent along the length of the small intestine. The gravimetric analysis of samples of gut tissue with adhering barium sulphate particles was therefore thought possible and worthy of investigation. This method would be truly quantitative and allow more subtle criteria of the adhesive properties of mucosal barium sulphate suspensions to be investigated.

Chapter 5. Section 2. Experimental Technique.

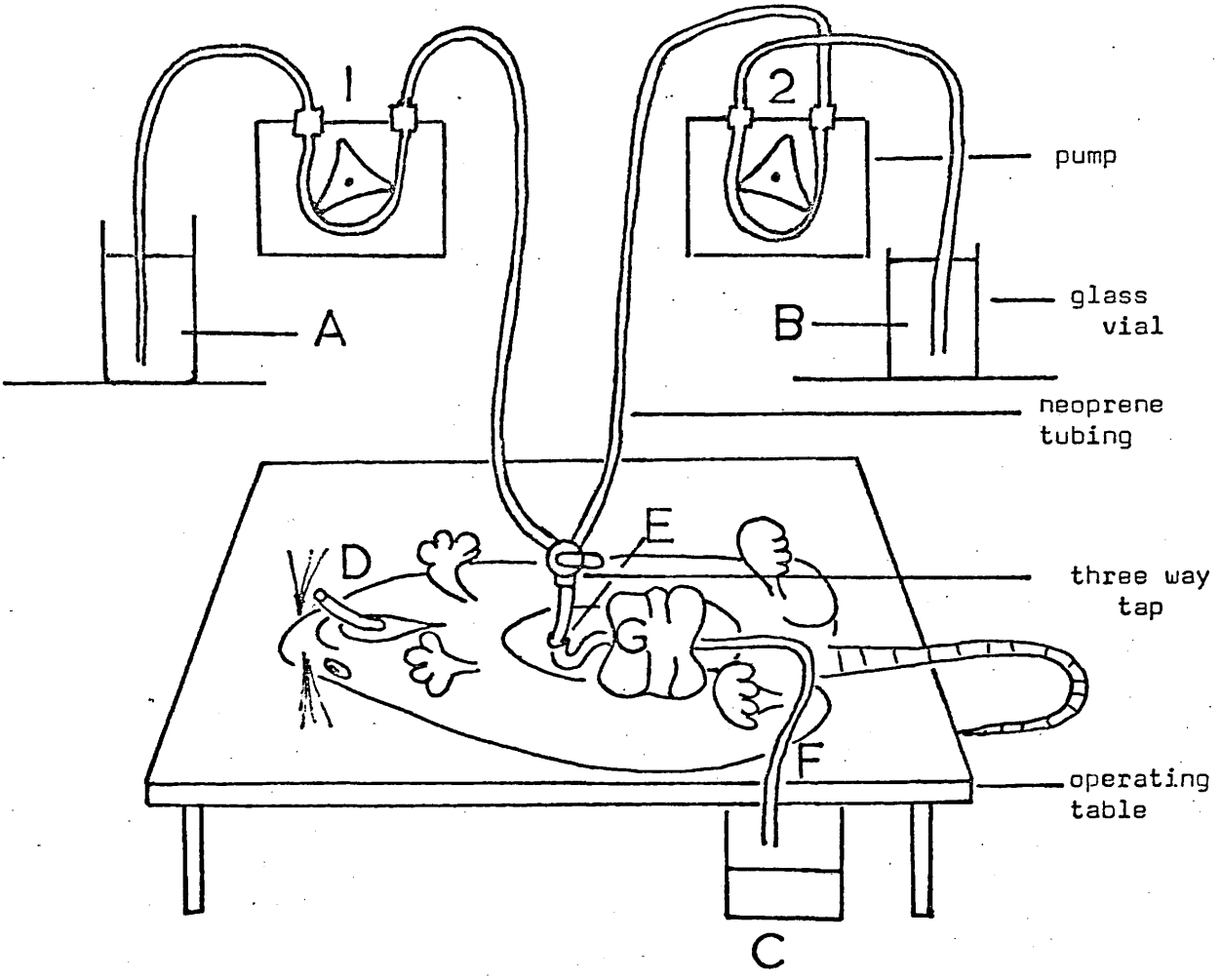
The experimental method finally developed depends upon the introduction of a suspension into the small intestine of a rat, the washing of the intestine, followed by the removal of the intestine and the gravimetric estimation of the amount of barium sulphate adhering to a known length of the intestine. Because of their availability rats were again used. The males ofasingle strain (CFHB) were used to minimise possible animal variation.

The animal was weighed and anaesthetised with urethane solution. This was injected as a 25% w/v. solution into the abdominal cavity. A dose of 5 cm^3 /kg. body weight was used. The rat was transferred to a small operating table and the trachea cannulated with neoprene tubing to ease the breathing of the animal. The body cavity was opened, and the stomach revealed. A catheter was inserted through the lower stomach wall to pass through the pyloric sphincter, and tied in place with cotton. A similar catheter was inserted at the end of the small intestine and secured as before. The anterior catheter was connected to a 3 - way nylon tap assembly, attached to two penstaltic pumps with neoprene tubing. The delivery rate of the two pumps was 3 $cm^3\ min^{-1}$, and these were fed by glass vials containing either isotonic (0·9% w/v.) saline

solution or the barium sulphate suspension under investigation. The posterior catheter drained into a similar vessel. (Fig. 5.1.) The exposed intestine was covered with cotton wool or tissue soaked in isotonic saline to prevent drying out the organ.

The small intestine was washed through with isotonic saline from one of the pumps. As it was not known what disruptive effect this would have upon the mucous barrier of the small intestine pumping was carried out for a specific time, so that the effect would be the same for all animals. The suspension of barium sulphate was then perfused through from the other pump, after a few minutes all isotonic saline was removed and the concentration of suspension was the same in the gut as in the vial. The suspension was left to equilibrate, for a specific time. The gut was then further washed with isotonic saline until the perfusate became quite clear. The two ends of the small intestine were tied off, the catheters removed, and finally the whole of the small intestine carefully removed, taking care not to disturb the adhering layer of barium sulphate. The intestine was laid on the operating table and moistened with isotonic saline; great care was taken not to stretch the gut. 5 cm. portions were then tied off with cotton from specific regions of the gut, which characteristically measured 30 - 35 cm. The pH of the various perfused liquids was recorded. The gut pieces were dried at 80 - 100° in weighed crucibles, and ignited under a bunsen. The carbon residue was removed in a muffle furnace at 600 - 800°. The crucibles were allowed to cool, and the contents treated with concentrated sulphuric acid to ensure the re-oxidation of any barium sulphate reduced by the carbon. Excess sulphuric acid was removed in the muffle furnace, and the crucibles heated to a constant weight. The weight of barium sulphate for each removed 5 cm. length of gut was recorded.

Fig.5.1. APPARATUS FOR IN VIVO PERFUSION OF SUSPENSIONS.



A Suspension

B Saline

C Perfusate

D Trachea catheter

E Anterior intestinal catheter

F Posterior intestinal catheter

G Cotton wool

Chapter 5. Section 3. Standardisation of Method.

(a) Variation due to animals.

Initially the times for which the small intestine was washed before perfusion of the barium meal, the perfusion of the meal, and the time it was allowed to remain undisturbed in the gut were quite arbitary, as was the time of the final washing of the gut. As these were the same for each animal results from different animals were comparable. The standard conditions adopted were:-

(1) The time of washing with saline solution - 5 min. (15 cm^3).

(2) The time of perfusion of suspension - 5 min. (15 cm^3), and this was left a further 5 min.

(3) The gut was washed for 10 min. (30 cm^3). The pH of the saline solution and suspension, was adjusted to pH 6 - 7 with 0·1 mol. dm^{-3} NaOH or HCl solution before the experiment.

(4) Two 5 cm. samples were removed from 10 - 15 cm. and 15 - 20 cm. along the small intestine, measured from the pyloric sphincter.

Suspensions of Micropaque, diluted with water to 25% w/v. were used in the initial experiments, because they did not sediment to any great extent in the neoprene tubing or the gut, as was the case for more dilute suspensions, and suspensions of different radiopaques.

The initial wash was quite sufficient to remove all food particles but left the surface mucus intact, and 5 min. of perfusion with Micropaque was long enough to ensure that its prepared concentration was maintained in the gut. The final perfused liquid was initially very cloudy, but became clear after 3 - 4 minutes. Gentle manipulation of the small intestine to remove any sedimented particles resulted in a slight turbidity of the perfusate; such manipulation was only done once for each animal during the final wash. The perfusate quickly cleared and remained so for the rest of the time of perfusion.

Controls where the Micropaque suspension was replaced with isotonic saline were always included. 16 animals, from the same batch were used. These had been kept on a standard holding diet for 14 days. All weighed

between 230 and 260 g. Under the standard conditions of test the amounts of barium sulphate recovered from a 5 cm. length of gut are recorded in Table 5.1.

Table 5.1.

The weight of Barium sulphate recovered from a 5 cm. length of gut, 25% w/v. Micropaque.

Rat Number	Weight of barium sulphate mg. (a) 10 – 15 cm.	(b) 15 – 20 cm.	Average mg.
1	97	65	86
2	79	80	79•5
3	75	74	74•5
4	100	63	81•5
5	76	91	83•5
6	98	63	80•5
7	74	89	81•5
8	112	58	85•0
		Average	82•1 mg.

The control rats which were simply subjected to perfusion with isotonic saline produced an average residue amounting to 7•25 mg. per a 5 cm. length of gut.

In all subsequent tables and figures the amount of barium sulphate recovered has been corrected for residue of 7•25 mg. from a control animal. Thus the average amount of barium sulphate retained for a 5 cm. length of gut was 74•95 (75 $\pm$ 7 mg.).

These results gave a standard figure, or base to which results from other experiments where the experimental conditions were changed, could be compared. The variation of sample (a) to sample (b) was due almost entirely to handling of the small intestine in its removal from the body and separation of 5 cm^3 portions. In later experiments the difference was reduced to less than the variation found from animal to animal.

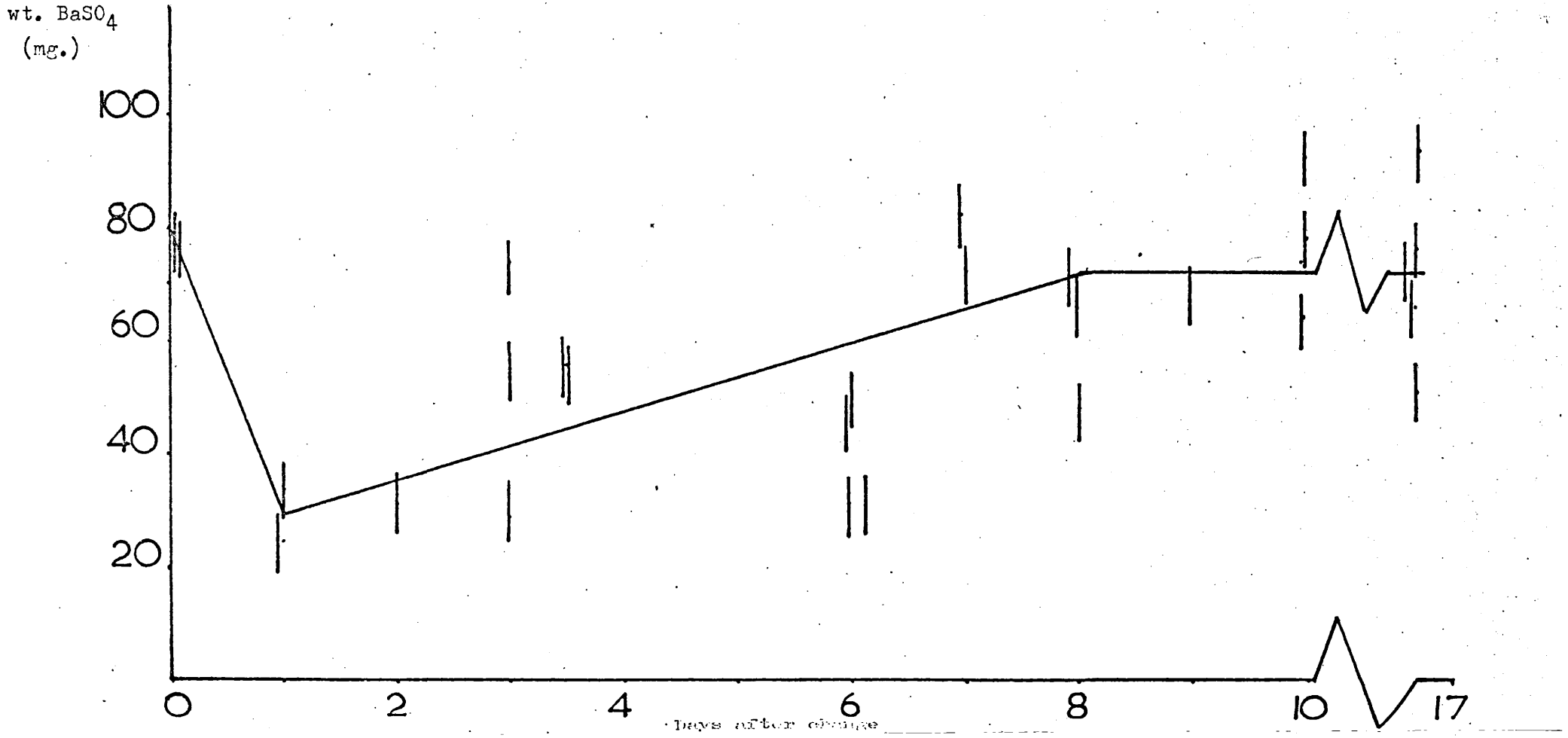
(b) Variation of barium sulphate recovered from the gut due to change in diet of animals.

When the rats were delivered to the animal house the pellet diet was automatically changed from a breeding diet to a holding diet. This change was found to have a great effect upon the adhering layer of barium sulphate, (Fig. 5.2.) measured under the standard conditions.

When the rats were used on the first day an amount of barium sulphate, comparable to 75 mg. was recorded. When used one day after, this amount was reduced to 25 - 30 mg. For the next 3 - 6 days, the amount recorded was in the range 31 - 75 mg., varying in a random manner. Only after 7 - 8 days following the change of diet did the amount of barium sulphate adsorbed return to a normal high figure (64 - 99 mg.). Even then some variation was found. The effect is not due to the size of the rats at the time of the experiment. After 8 days rats originally weighing 90 g. weighed 140 g. and a normal amount of barium sulphate was recovered. When rats of initially 140 g. were used erratic results were again recorded up to 8 - 10 days after the change of diet.

Fig. 5.2.

BARIUM SULPHATE RECOVERED AS A FUNCTION OF TIME FROM CHANGE OF DIET



As a result rats were kept for a minimum of 8 days before being used in the experiments. Their original weight was 140 - 160 g. and this rose to 200 - 220 after 8 days; after 17 days a weight of 260 - 280 g. was normal.

(c) Variation of adsorbed barium sulphate due to procedure.

(i) Time of final wash. This had no effect upon the weight of barium sulphate recorded, as long as the saline solution was finally clear.

(ii) Time of perfusing the suspension. The time of perfusion was a compromise between ensuring the correct concentration in the gut, and the abrasive effect which particles of barium sulphate, whether uncoated or coated, must have upon the mucus of the intestinal surface. As long as the distance between the anterior and posterior catheters was 25 - 30 cm. the weight recorded was not altered if the time of perfusing varied from 3 - 5 minutes.

(iii) Time suspension remained in gut. For a stable suspension, such as Micropaque varying this time did not have any effect. The procedure had to be altered for more unstable suspensions, but it appeared that any settled particles were quite readily removed in the final wash.

(iv) Time of initial wash. In the initial wash free mucus is removed as well as food. This was demonstrated by adding a sample of the perfusate to a solution of 0•2% w/v. Alcian blue in buffer (pH = 5•8) when a precipitate of mucus was formed (Piper, 1970). Removal of free mucus, and perhaps mucus from the ileal surface could well affect the adhesion of barium sulphate, and since the amount of food present in the gut varied from animal to animal, surface mucus could well also vary. Differences in the mucus layer could account for the animal variation found. It was assumed that as long as the wash was standard the results from the experiments would be strictly comparable.

(v) Variation of weight of barium sulphate retained with distance

along the small intestine. 5 cm. lengths were carefully removed from 0, 5, 10, 15, 20, 25 cm. from the pyloric sphincter. The variation along the gut was small and within the range of the variation from animal to animal.

(d) Discussion.

The method as developed gave reproducible results, and the weights of barium sulphate recovered from 5 cm. lengths of intestine from different animals treated with 25% w/v. Micropaque were comparable. The procedure, with a few additions according to the nature of the experiment was adhered to in all further experiments. It was convenient to remove only 5 cm. samples from the intestine since these could easily be dried and ignited and the amount of barium sulphate such samples gave could be weighed accurately. Had the whole of the small intestine been used analysis would have become a lengthy and tedious procedure. If two samples were taken for each animal the variation along the gut, a probable result of handling during the experiment could be judged. This gave an indication of possible disruption of the mucous layer, and such could be allowed for.

The animal variation was expected, and though minimised by control of the animal diet, it necessitated a large number of experiments (usually a minimum of 6) for each experimental parameter investigated. This was a lengthy procedure and reduced the number of parameters that could be investigated. Whenever possible Micropaque diluted to 25% w/v. was included in a day's experiments. This acted as a "control" or standard, to which results from the same day's experiments could be compared. If the control was grossly different from 75 mg. i.e. below 60 mg., the experiments were repeated. Results from experiments where a control was included are quoted in terms of % of the control of that day.

Chapter 5.Section 4. Experimental investigations.

(a) Effect of fasting of animals on the adsorption of barium sulphate.

It was apparent that diet had a great effect upon the amount of barium sulphate taken up by the ileal mucosa. This effect was further investigated by dieting of animals. These diets were used

(1) a total fast of water only.

(2) Carbohydrate only diet, in this instance sugar.

(3) A combination of (1) and (2).

The normal diet was replaced by the experimental diet the morning before the day of the experiment, so that animals were kept for a minimum of 24 hours upon the chosen diet. The effect of restoration of a normal diet 24 hours and 2 hours before experiments was also investigated. In all experiments a 25% w/v. suspension of Micropaque was used. (Table 5.2.).

(b) Effect of pH of suspension and saline solutions on the adsorption of barium sulphate.

Micropaque, 25% w/v. and isotonic saline solutions were adjusted to a wide range of pH values with 0·2 mol. dm^{-3} NaOH or HCl. In the gut the pH of both pH and saline solutions were subject to change, tending to pH 6 – 7. The pH of all fractions (initial perfusion, suspension, final perfusion)were therefore recorded and averaged after each experiment. Weights of all animals were also recorded. (Table 5.3. Fig. 5.3.).

(c) Effect of concentration of Micropaque on the adsorption of barium sulphate.

100% w/v. Micropaque was diluted with water to give suspensions of 75, 50, 25, and 12·5% w/v. The pH of each was adjusted to 6 – 7. The results are presented in Table 5.4. and Fig. 5.4.

(d) Effect of different meals on the weight of barium sulphate recovered from 5 cm. lengths of gut.

The following barium meals were studied:-

(i) Unstabilised barium sulphate suspension 50, 25, 12·5% w/v.

Table 5.2.

Effect of fasting on the weight of barium sulphate recovered from 5 cm. lengths of gut (25% w/v. Micropaque).

Description	Wt. of rat /g.	Wt. barium sulphate mg.		Average
		(a) 10 – 15 cm.	(b) 15 – 20 cm.	
Rats fasted 24 hrs.	187	34	38	31
	176	24	26	
	147	33	37	
	120	33	21	
Rats sugar diet only	155	35	44	36
	135	38	27	
Sugar diet after fasting 24 hrs.	140	8	6	14
	160	20	21	
Restoration of normal feed (24 hrs.)	163	49	66	69
	173	66	71	
Restoration of normal feed (2 hrs.)	170	26	26	26
Control	160	68	68	68

Table 5.3.

The effect of pH on the weight of barium sulphate recovered from 5 cm. lengths of gut (25% w/v. Micropaque).

Wt. rat/g.	pH (average)	wt. barium sulphate/mg.		
		10 - 15 cm.	15 - 20 cm.	Average
240	3•0	100	97	99
165	3•6	71	57	64
235	4•0	93	77	85
260	4•1	94	77	86
245	5•5	74	89	83
161	6•3	74	74	74
240	6•5	75	68	71
152 (control)	7•0	61	68	65
160	7•7	64	77	71
250	8•4	71	84	78
150	9•9	55	55	55

Fig. 5.3. THE EFFECT OF pH UPON THE WEIGHT OF $BaSO_4$ RECOVERED FROM 5 CM. LENGTHS OF GUT (MICROPAQUE 25% w/v.)

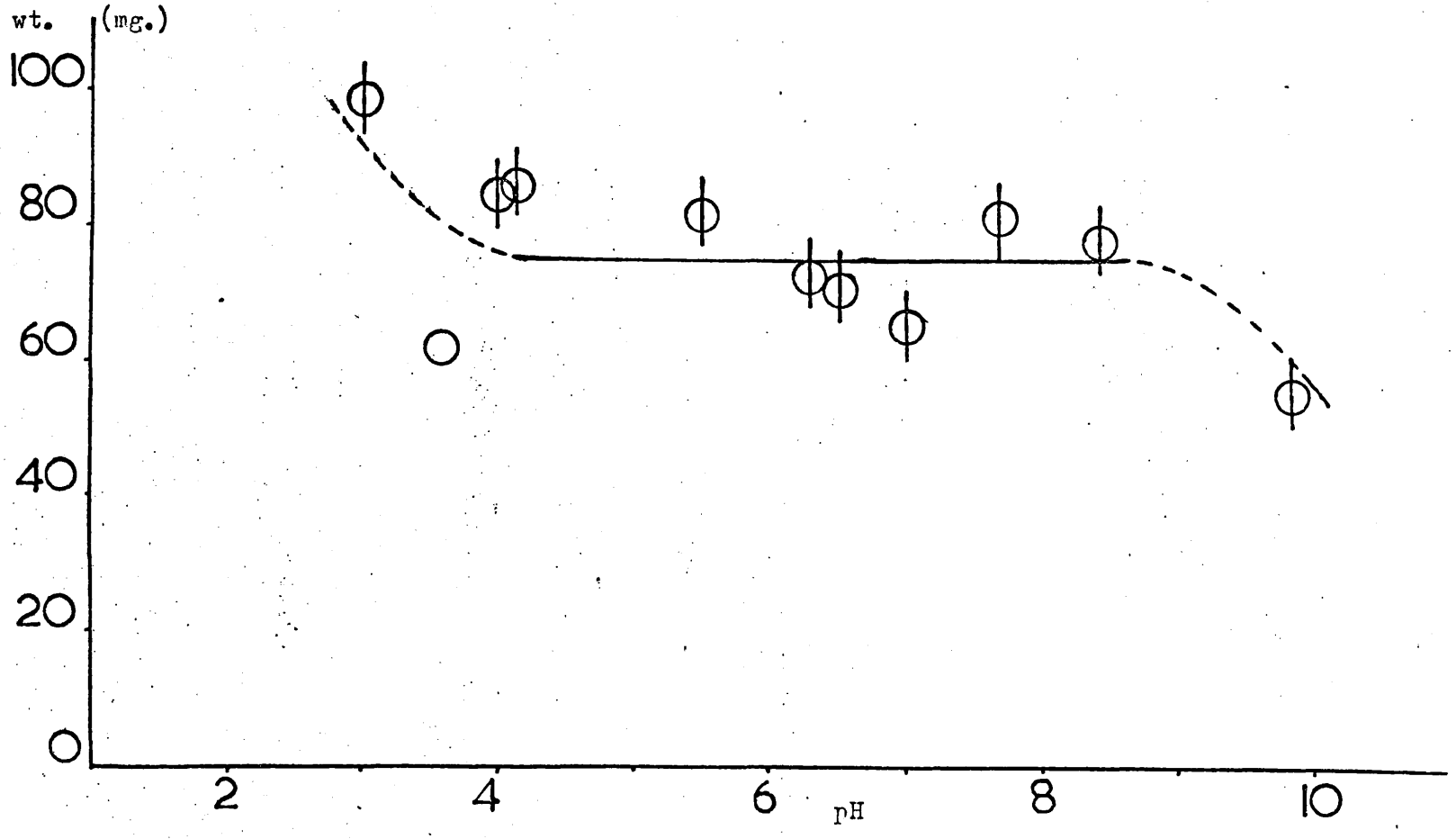
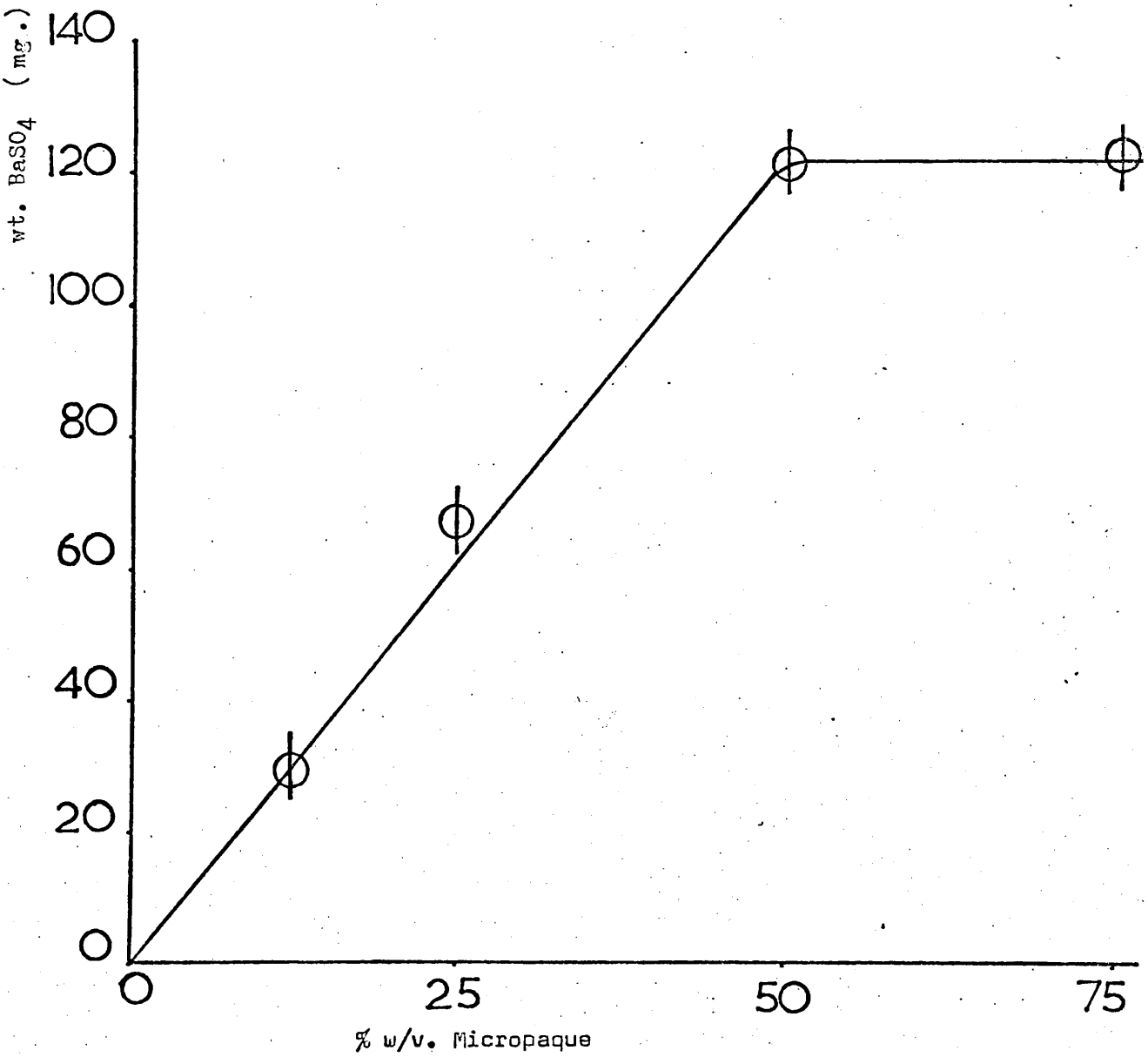


Table 5.4.

Effect of concentration of Micropaque on the weight of barium sulphate recovered from 5 cm. lengths of gut.

conc. % w/v.	wt. barium sulphate/mg.	Average
75	122 113 143	126
50	121 115 126 128 119	124
25 (control)	75	75
12•5	29 45 25 26 14	28

Fig.5.4. EFFECT OF CONCENTRATION OF MICROPAQUE ON THE WEIGHT OF BARIUM SULPHATE RECOVERED FROM 5 CM. LENGTHS OF GUT.



(Table 5.5. Fig. 5.5.).

(ii) Barospere at the same concentrations.

(iii) cmc. stabilised suspension (the same as used in the in vitro investigation).

(iv) Citrate stabilised suspension. Dilutions of a 100% w/v. suspensions have received good reports in the literature (Embring, Mattson, 1968) and are characterised by initial effervescence when prepared, very low viscosities and therefore low stabilities (Goddard & James, 1971).

The suspensions, especially at the lower concentrations, were much less stable than Micropaque at equivalent concentration. This resulted in sedimentation of the suspensions in the pumps, neoprene tubes and small intestine, so that the absolute concentration of suspensions in the gut was not known with certainty. To minimise this situation the pumps and tubes responsible for perfusion of the suspensions were prefilled with saline. The vials containing suspensions were stirred and the suspensions introduced into the tubes. Timing was started when the suspension reached the 3-way tap. The reservoirs were continuously stirred during perfusion. Whilst the suspension remained in the gut, the intestine was moved from lying on one side of the animal to lying upon the other side every minute. By this it was hoped to re-suspend sedimented particles. The time the suspension remained in the small intestine was reduced to 3 minutes. During the final wash the gut was manipulated as before; after each the perfusate became cloudy, but cleared after a few minutes. The manipulations were continued until they resulted in no cloudiness in the perfusate. For some suspensions, notably those stabilised with citrate, manipulation resulted in the appearance of flocculated particles in the saline solution, which never became completely clear after the "turning" over of the small intestine. In such cases the washing was terminated after 5 minutes.

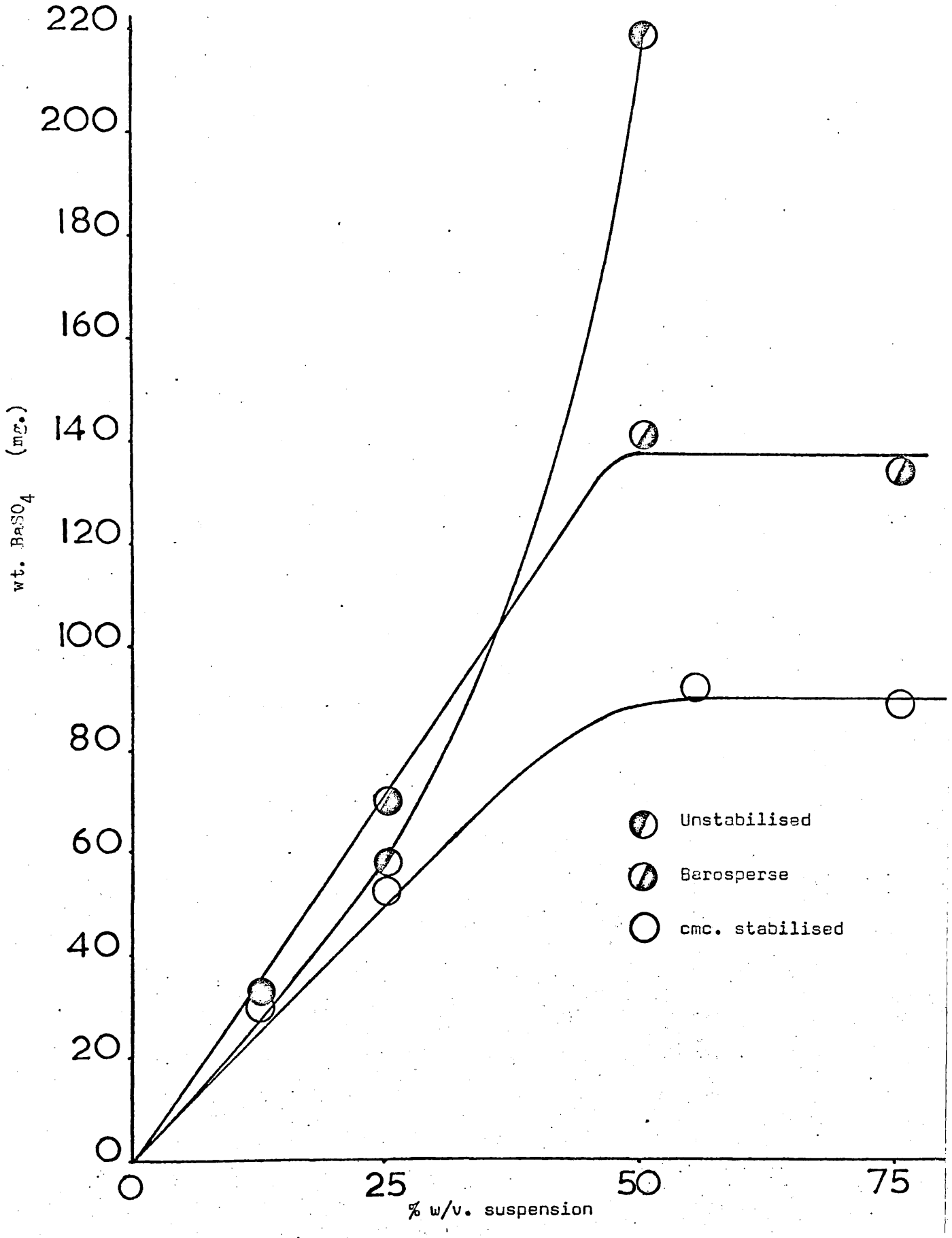
Table 5.5.

The effect of concentration of various meals on the weight of barium sulphate recovered from 5 cm. lengths of gut.

Meal	Concentration / % w/v.			
	12·5	25	50	75
i) unstabilised barium sulphate	25, 65, 38, 64, 30, 34 (34)	48, 49, 70, 60, 53 (56)	228, 181, 174, 189, 292, 242 (218)	—
ii) Barospere	33, 33, 24, 26 (34)	33, 56, 92, 25, 107 (70)	34, 170, 209, 140 (141)	157, 117, 130, 172 (134 mg.)
iii) cmc. stabilised	32, 39, 20 (30 mg.)	56, 61, 60, 30, 45, 72 (54 mg.)	141, 112, 49, 58, 86, 113 (93 mg.)	91, 96, 64, 96 (87 mg.)
iv) sodium citrate stabilised	—	80, 66, 79 (75 mg.)	69, 97, 81 (83 mg.)	109, 182 (146 mg.)
Micropaque (control)	(28 mg.)	(75 mg.)	(124 mg.)	(126 mg.)

Figures in brackets are "average" values.

Fig.5.5. EFFECT OF CONCENTRATION OF VARIOUS MEALS ON THE WEIGHT OF BARIUM SULPHATE RECOVERED FROM 5 CM. LENGTHS OF GUT.



(e) Other observations.

The small intestine was quite transparent, and it was possible to observe the coating of barium sulphate, and to estimate the uniformity and thickness. Generally the coating was uniform, and a concentration of 30 mg. barium sulphate per 5 cm. length of gut was sufficient to coat the gut surface uniformly under the best conditions. Above this the coating became denser and its uniformity difficult to judge during the experiment. Anomalous results, where areas of the ileum were bare, whilst other areas showed a thick layer, were more common with unstable preparations, and most severe with citrate stabilised suspensions which flocculated in the gut. Such results were also more common with fasted animals.

Sections of the coated ileum were prepared and investigated microscopically. These were essentially similar to sections from the previous experiments (chapter 4.) except that the coating was thicker, and more uniform.

The size of the gut was quite uniform in animals weighing from 140 - 300 g. after 20 minutes washing.

50% w/v. unstabilised barium sulphate was very viscous, and its introduction into the ileum distended the gut to a great extent. The washing tended to be slower, but the perfusate became clear, and remained so, after 8 minutes.

(f) Discussion

The investigations were of necessity in the nature of an initial study, and no attempt at statistical design of experiments was made. This was not so important for Micropaque, which gave more consistant results, but caution is needed in the interpretation of results for the more unstable preparations. Because of the spread of results obtained, especially at high concentration, no real differences between Barospere, Micropaque, and cmc. stabilised suspensions are readily apparent and assigned to such physical parameters as the charge carried by particles

(due to different stabilisers used), particle size, or viscosities of suspensions.

Nevertheless for all suspensions the shape of the concentration adsorption graph is clear and typical of a Langmuir adsorption isotherm. This would indicate that a mucus/particle reaction is occuring, dependant upon the relative concentrations of particles in suspension and the mucus upon the ileal wall. The anomolous result obtained for unstabilised barium sulphate at 50% w/v. may well be due to the high viscosity of the suspension and associated difficulties in washing. Results for sodium citrate stabilised suspensions are difficult to interpret because of their great instability in the gut.

In the range 3 - 8 the pH of the suspension of Micropaque has no effect upon the adhering layer. This points definitely to a non-electrical interaction between mucus and barium sulphate particles. Flocculations at extremes of pH may be due to differences in the stability of Micropaque at such pH values, or the topical action that suspensions of very high or low pH may have upon the ileal mucosa, in the inhibition or stimulation of secretion of mucus or pancreatic juice.

The greatest effect on this adsorption is the diet. Diet, and especially fasting, has been shown to effect the mucus secretion in mammals (Ballinger & Wise, 1969, Menguy, 1969). That fasting causes such a drop may be due to (a) less mucus present (b) change in nature of the mucus, possibly in viscosity, due to a change in the chemical makeup of the glycoprotein.

Results from these experiments confirm to an extent those previously obtained. The thickness of the adhering layer of barium sulphate is dependant upon the concentration of the suspension; it is independent of pH, and results for unstable preparations are difficult to interpret.

CHAPTER SIX

FURTHER IN VIVO INVESTIGATIONS.

Chapter 6. Section 1. Introduction.

Results of previous experiments indicated that the state of the mucus present in the gastro intestinal tract had a great effect upon the layer of barium sulphate adsorbed by the mucus barrier of the gut. It is possible to affect this barrier mechanically, chemically or physiologically:

(a) by perfusion of an abrasive suspension;

(b) by perfusion of a mucolytic agent or

(c) by injection of a drug such as pentagastrin.

The effect of abrasion upon the mucous barrier is unknown, and the effect of pentagastrin is uncertain and not well documented. A mucolytic agent would disrupt the mucous barrier, perhaps by the breakage of disulphide bridges, and subsequent removal of portions of the barrier, or by causing a conformational change at the surface, disrupting the viscosity and the surface tension of the outer layer of mucus present in the barrier.

Before such effects could be investigated a method of estimation of surface mucus had to be developed.

Chapter 6. Section 2. The estimation of mucus.

The method depends upon the estimation of dye binding activity of mucoproteins, and is developed from the method of Frankel-Conrat & Cooper (1944) and Piper et al.(1970) and is similar to that of Whiteman (1973).

Alcian blue is a soluble cationic dye which forms an insoluble complex with glycoproteins. The precipate can be removed, washed and made soluble. The bound dye can be estimated photometrically either by estimation of the resolubilised dye or measuring the dye precipitated by difference. Because of difficulties in solubilising precipitates the latter method was chosen.

Method.

A 0•2% w/v. solution of Alcian blue (BDH) in 0•1 mol. dm^{-3} citrate buffer solution, pH 5•8, was prepared. A rat (male CFHB, 190 g.) was anaesthetised and the small intestine washed by perfusion with isotonic saline solution. The small intestine was removed, and divided into 5•5 cm. portions.

Each portion was homogenised for 5 min. in citrate buffer solution (0.1 mol. dm^{-3}, pH = 5•8) in a Potter - type ground glass homogeniser, and the suspension made up to 50 cm^3 with buffer solution. The homogenising process removed and homogenised only surface mucus, and left the muscle and peritoneal layers of the intestine intact. The remains of the intestine were removed with forceps. A volume of the suspension, between 0 - 5 cm^3 was added to 5 cm^3 of the prepared Alcian blue solution in a centrifuge tube and made up to 10 cm^3 with buffer solution. This mixture was left for 24 hrs., and the precipitate of Alcian blue - glycoprotein complex removed by centrifuging and decantation of the supernatant. A portion of this was diluted x 2 with citrate buffer. A standard adsorbance - concentration curve for Alcian blue in 0•1 mol. dm^{-3} citrate buffer solution was prepared (Fig. 6.1.), this was linear over the concentration range 0 - 100 mg. dm^{-3}. It was thus possible to determine the concentration of Alcian blue in each solution and hence the weight of Alcian blue precipitated as a complex from the original dye - mucus suspension estimated. Fig. 6.2. shows the effect of the addition of various volumes of homogenate to the Alcian blue solution. This demonstrated that the precipitation of mucus as a complex was quantitative under the conditions of the experiment. Fig. 6.2. is in fact a calibration curve.

The variation of binding along the small intestine was investigated. Two rats (240, 215 g. CFHB) were anaesthetised and the small intestines washed with saline solution (0•9% w/v.). These were then removed, and four 5•5 cm. samples removed from each. These were homogenised after

Fig. 6.1. STANDARD CURVE FOR ALCIAN BLUE IN 0·1 MOL. DM^{-3} CITRATE BUFFER, pH 5·8 (at 620 nm.).

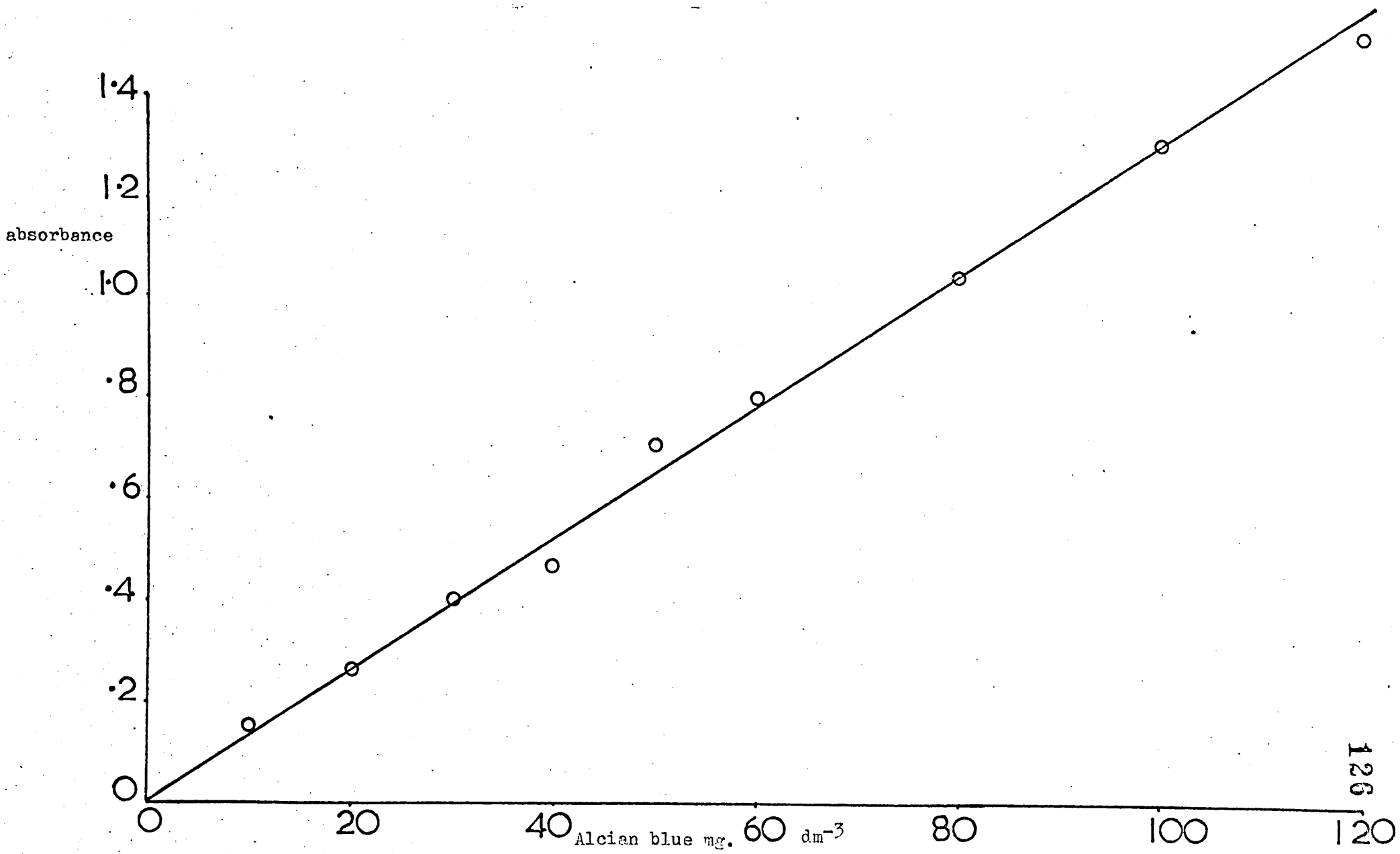
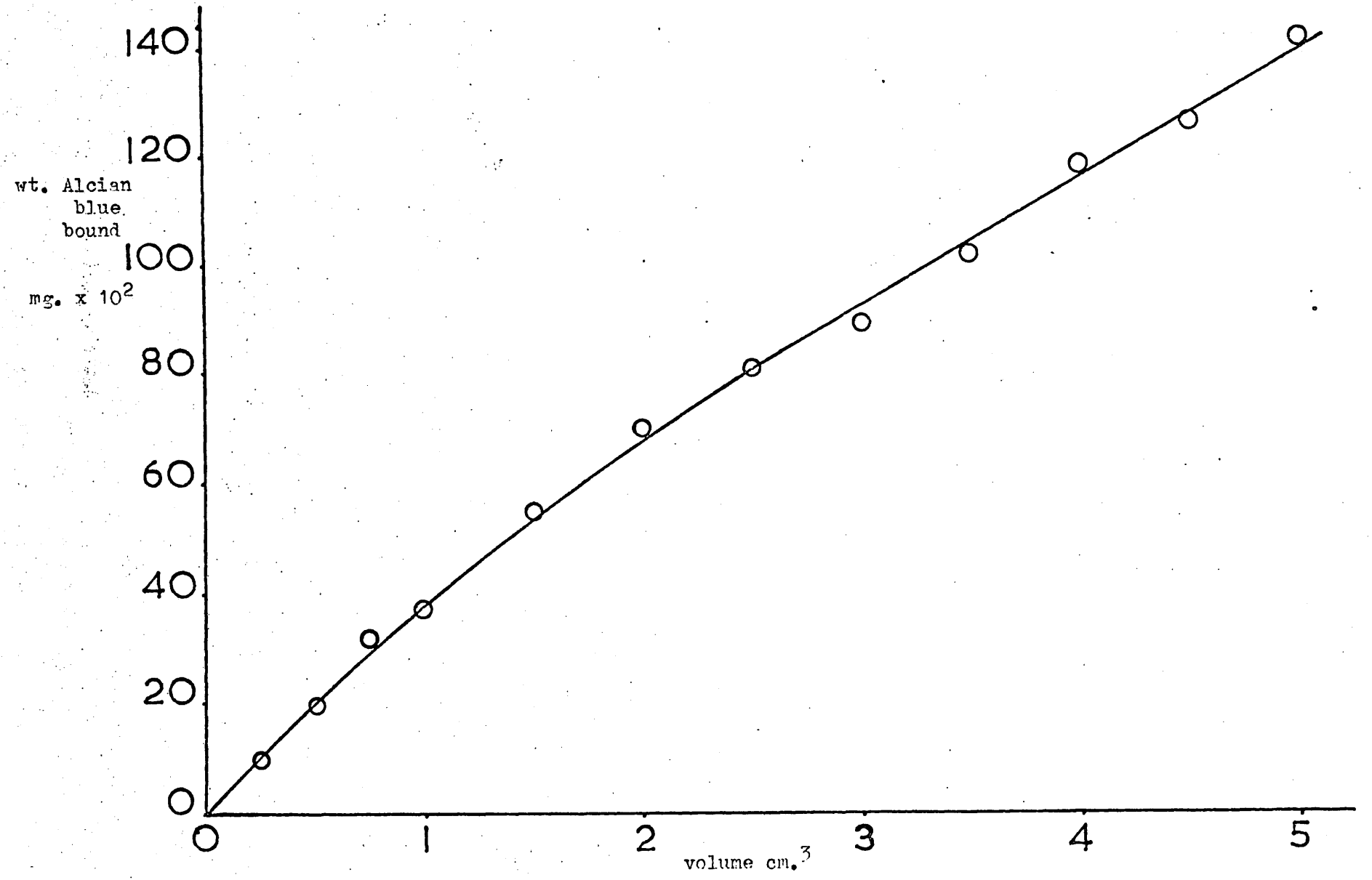


Fig. 6.2. EFFECT OF VOLUME OF HOMOGENATE UPON WEIGHT OF ALCIAN BLUE BOUND AS A COMPLEX



the mechanical removal of mucus. 3 cm^3 of each homogenate made up to 50 cm^3 was added to the Alcian blue solutions, and the resulting reaction made up to 10 cm^3 with buffer solution. The samples were left 24 hrs. and the Alcian blue bound by the mucus estimated as before.

The controls, where the mucus had been removed mechanically bound 0•17 – 0•30 mg. of Alcian blue for each 3 cm^3 of homogenate, whilst those untreated bound 0•50 – 0•70 mg. Alcian blue. (Fig.6.3.).

A similar experiment in which two 5•5 cm. samples were removed from six different rats was carried out, and eight blanks were also prepared. This revealed that variation from animal to animal was not great, and such variations as there were were within the variation along the length of the small intestine. The control samples all bound between 0 and •18 mg. of dye for 3 cm^3 of solution. Amount of Alcian blue bound for 5•5 cm. portion is obtained by multiplying the value for 3 cm^3 by 50/3.

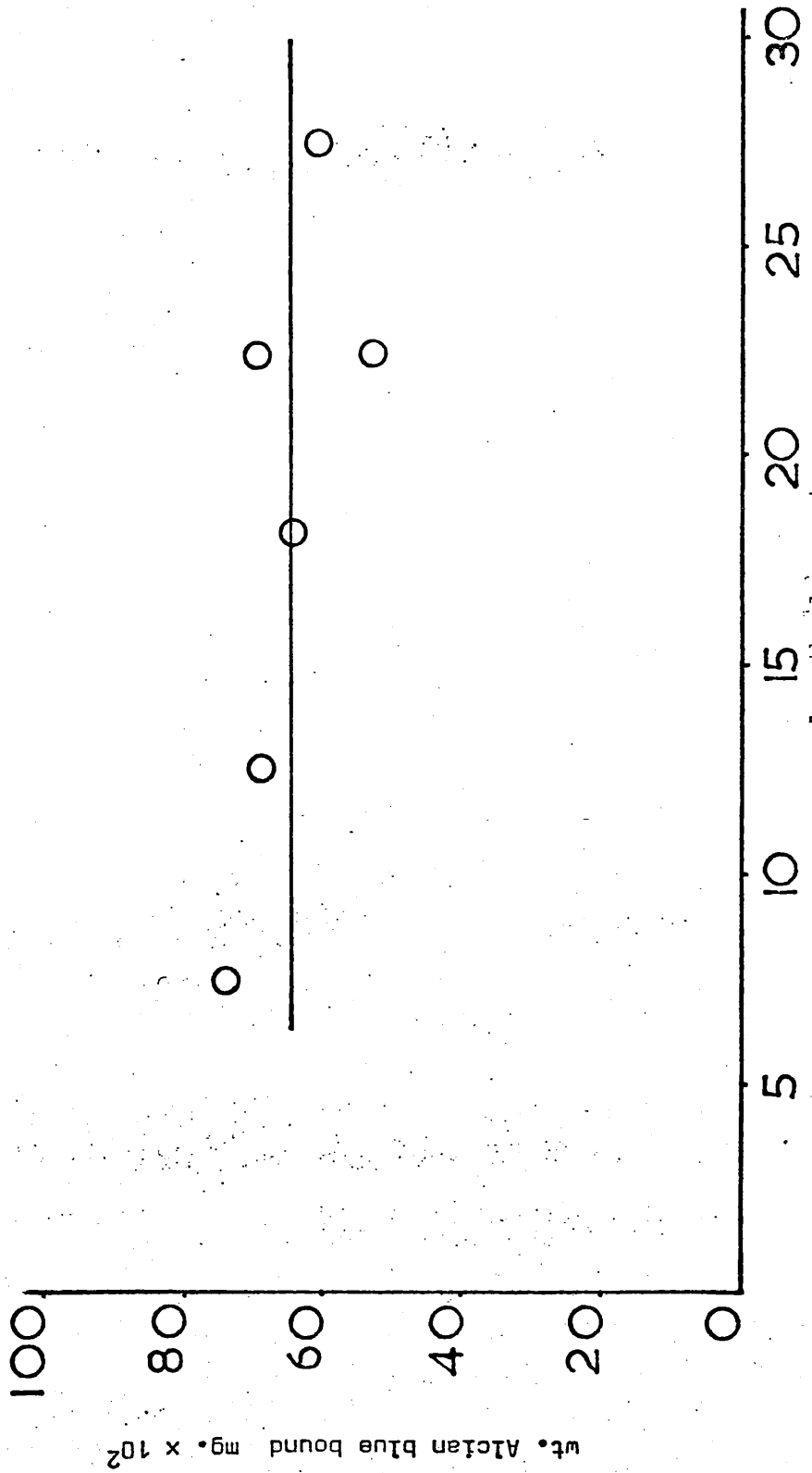
Chapter 6. Section 3. Experimental disruption of mucus from the small intestine.

N-acetyl cysteine (NAC) has been reported (Sheffner, 1963) as a mucolytic agent which reduces the viscosity of mucous solutions *in vitro*. It has its greatest effect at pH 7•4. A 2•0% w/v. solution in 0•1 mol. dm^{-3} "Tris" buffer pH 7•4 was prepared. This solution was incorporated in the experimental procedure previously developed (Chapter 5.) in the following way.

One of the pumps and associated neoprene tubes were filled with saline solution, the other with the NAC solution. The small intestine was washed with saline, and then perfused with the NAC solution, after specific time an air bubble was introduced in the feeder tube of the pump, and the vial containing the NAC solution changed to one containing saline solution. The bubble travelled along the tube and gave indication of the replacement of NAC solution with saline solution. On reaching the 3-way tap, timing of a second saline wash was begun, and the time

Fig. 6.3.

VARIATION OF ALCIAN BLUE BOUND ALONG SMALL INTESTINE FOR 3·0 CM^3 HOMOGENATE



of perfusion of the NAC solution recorded. The second wash was completed after three minutes. 5•5 cm. of the ileum was tied off and the anterior catheter reinserted posterior to the tied off sample. Micropaque, 25% w/v. was then introduced, and the experiment for the measurement of the uptake of the radiopaque, completed in the usual way. The sample not treated with Micropaque, but treated with the NAC solution was homogenised and its Alcian blue binding capacity estimated. The time of perfusion with mucolytic solution was varied from 2 - 10 minutes. 4 experiments were completed each day, including a control. Any effect time of day might have upon the adhesion was allowed for by distributing times of perfusion in the experiment in a systematic fashion over the day.

Results are depicted in Fig. 6.4. The adhesion of barium sulphate was greatly affected by pretreatment with the NAC solution, but the effect is only noticeable under these experimental conditions after 2 minutes of perfusion of NAC solution, after which there was a drastic reduction in the weight of barium sulphate recovered; this levelled off at 35% of the control (26 mg.).

Estimation of Alcian blue bound showed no significant difference between those samples not treated with the mucolytic solution and those treated for 10 minutes. Either the mucolytic was not removing any surface mucus, or it was removing too little to be estimated. The spread of results for the Alcian blue determination was large, even for a single time of perfusion of NAC solution. It would seem that treatment with NAC affected the Alcian blue binding capacity of the mucus, and this interfered with the estimation procedure.

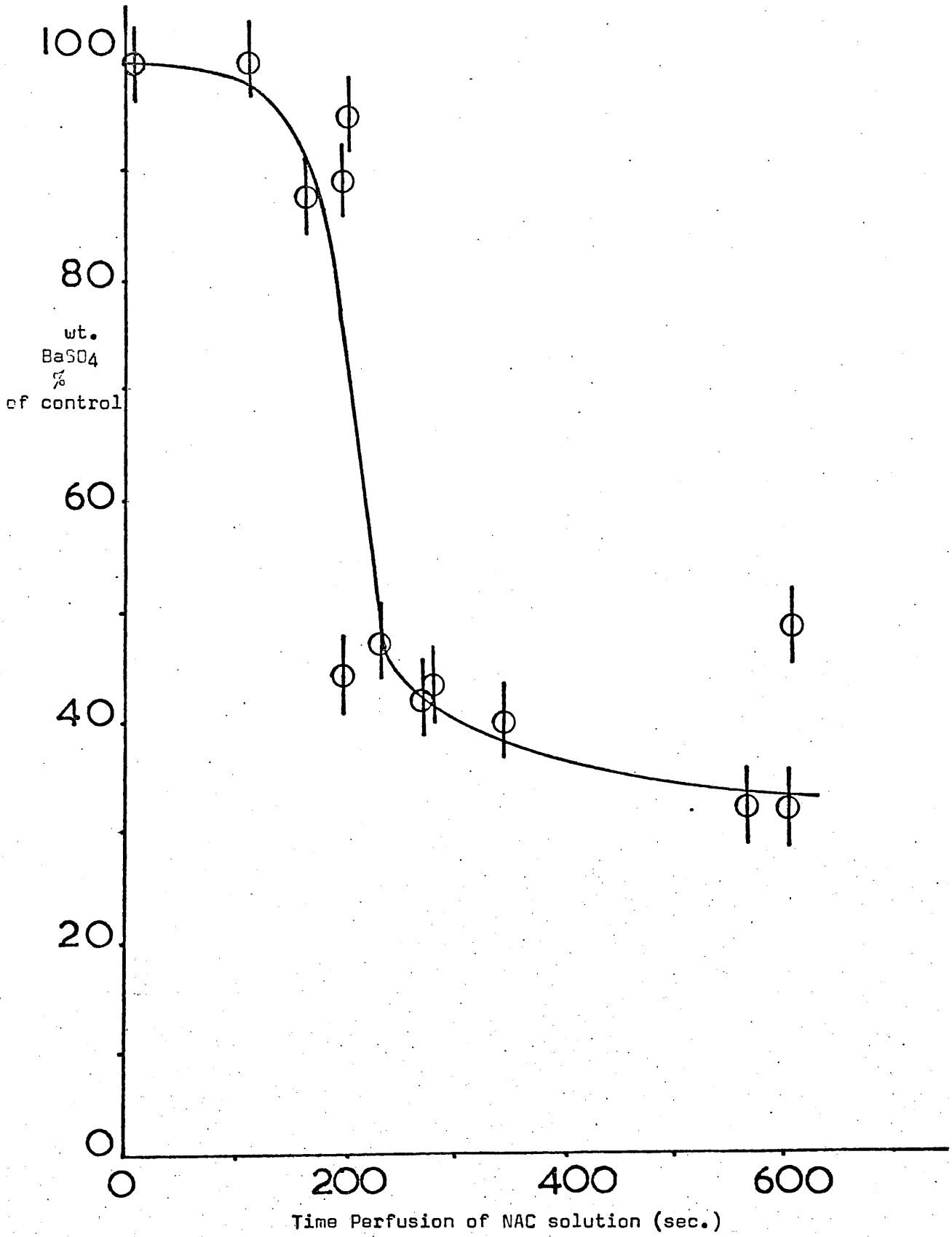
Dissection of samples that had been treated with the NAC solution for 10 minutes revealed the presence of copious amounts of surface mucus, but that there was only a sparse covering of barium sulphate. Accordingly the various perfused liquids were tested spectrophotometrically for the presence of removed mucus.

An animal (CFHB, 390 g.) was sacrificed, and the small intestine

Fig. 6.4.

VARIATION OF WEIGHT OF BARIUM SULPHATE RECOVERED FROM 5 CM. OF GUT AFTER TREATMENT WITH 2·0% w/v. NAC SOLUTION FOR VARIOUS TIMES

(MICROPAQUE = 25% w/v.)



removed. Surface mucus was squeezed out, taking care to keep desquamation to a minimum, and weighed. A suspension containing 2% w/v. mucus was prepared by homogenisation of the surface mucus in a solution of 2·0% w/v. NAC, 0·9% w/v. NaCl. Any food particles were removed by gentle centrifugation. A suspension of 2% w/v. surface mucus in 0·9% w/v. NaCl. was also prepared. This suspension became clear after centrifugation.

The spectra of these solutions were recorded in the uv region, a summary of the results is given in Table 6.1.

Table 6.1.

Spectra of Mucus Solutions.

Solution	blank	principle features
Mucus/0·9% w/v. NaCl/H_2O.	0·9% w/v. NaCl/H_2O.	Max. 208 nm. Shoulder 280 nm.
Mucus 0·9% w/v. NaCl. 2·0% w/v. NAC	0·9% w/v. NaCl.	shoulder 280 nm.
Mucus 0·9% w/v. NaCl. 2·0% w/v. NAC	0·9% w/v. NaCl. 2·0% NAC	Max. 280 nm.
2.0% NAC/0.9% w/v. NaCl.	0·9% NaCl.	Max. 215 nm.

NAC supresses the maximum adsorbance of mucus at 208 nm. and the shoulder at 280 nm. becomes well defined when NAC is present in the blank, and enabled the recognition of mucus in solution in the presence of NAC. The adsorbance at 280 is most probably due to tryptophan, or possibly tyrosine, in the protein moeity of the mucus. Both also adsorb strongly at lower wave lengths, 200 - 240 nm.

The previous animal experiments, incorporating NAC pretreatment were repeated with the following changes;

(1) The NAC solution, and the following perfusate of saline solution were collected after they had passed through the gut, and kept for spedtrometric analysis;

(2) Micropaque diluted to 50% w/v. was perfused instead of a 25% w/v. suspension.

(3) Final washing time was extended to 15 - 20 minutes to allow the saline solution to become completely clear. Results are shown in Fig. 6.5.

As before the perfusion of NAC solutions had great effect upon the weight of barium sulphate recovered from the small intestine. The effect was immediately apparent i.e. after 2 minutes perfusion, and again the amount recovered became constant at 27 - 28% of the control, (26 - 30 mg. barium sulphate) comparable to that for Micropaque at 25% w/v. (26 mg.).

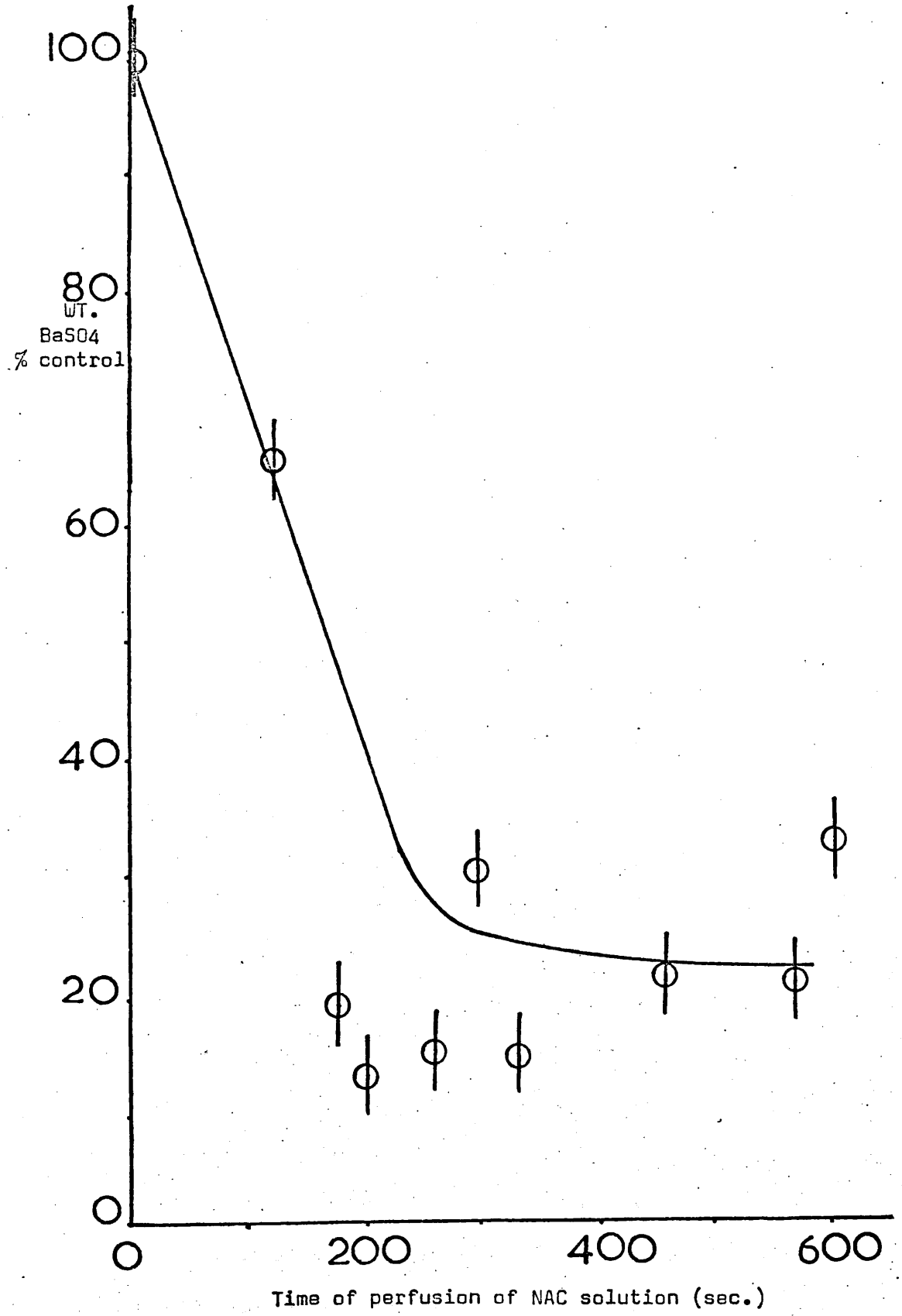
The spectra of the recovered saline and NAC solutions showed a maximum at 280 nm., when determined against a blank containing NAC. This indicated that mucus was removed from the intestinal surface.

In the case of rats not treated with NAC solutions, a sample of the first saline perfusate was collected after it had become free of food particles and mucus present initially in the lumen of the gut, the spectrum was determined in the uv. region. In no case was there any significant adsorbance at 208 or 280 nm., revealing that the surface mucus remained intact during the saline wash.

Fig. 6.5.

VARIATION OF WEIGHT OF BARIUM SULPHATE RECOVERED FROM 5 CM. OF GUT AFTER TREATMENT WITH 2·0% w/v. NAC SOLUTION FOR VARIOUS TIMES.

(MICROPAQUE = 50% w/v.)



Chapter 6. Section 4. Discussion.

Despite the experimental difficulties encountered, and the spread of results, especially in Fig. 6.5., the effect of perfusion solutions of NAC upon the weight of barium sulphate recovered from 5 cm. lengths of the small intestine, and therefore upon the mucus barrier of the ileum has been demonstrated. This effect is not entirely due to removal of mucus from the intestinal surface, as revealed by macroscopic examination of the gastro intestinal tract after completion of an experiment, and the results of Alcian blue determination. Mucus is removed, but not to any great extent. The main effect of the NAC must be in disrupting the tertiary structure of mucus at the surface, or the presence of different surface groups revealed by removal of mucus sub units.

For Micropaque at both 50 and 25% w/v. concentrations, NAC solution and times of perfusion of 6 - 10 minutes the weight of barium sulphate recovered attains a constant value of 25 - 30 mg. for 5 cm. length. This indicates that a true interaction between mucus and Micropaque is occuring. Such an amount is also comparable to that recovered from fasted rats.

There is a correlation between results obtained from the two experiments (Table 6.2.)

Differences between weights recovered for 50% and 25% Micropaque suspensions may be due to:

(a) the greater abrasive nature of Micropaque at 50% w/v. and

(b) differences in time of final wash.

It would seem that the mucosal surface of the intestine is capable of adsorbing only a specific amount of mucus, and this is decreased by perfusion of NAC solutions. This will be a function of surface tension, viscosity, or the number of hydrogen bonds available at the surface, as the result of a conformational change. Increasing the concentration of stabilised suspension above a critical concentration does not result in any more barium sulphate being adsorbed, and thus recovered under the conditions of the experiment.

Table 6.2.

Weight of barium sulphate recovered from 5 cm. lengths of gut after perfusion of NAC solution.

Time (sec.) of perfusion	weight barium sulphate recovered	
	50% w/v.	25% w/v.
0	120	75
120		68
140	68	
180	22	
195	15	36
195		68
235		
255	18	34
270		31
275		31
303	44	
325	18	
340		30
450	33	
455	26	
565	26	24
600	44	24
605		37

Chapter 6 Section 5. In vitro and coating of barium sulphate particles.

The results from previous experiments indicated the possibility of a definate interaction between mucus upon the intestinal surface and particles of barium sulphate. The effect of Salivary mucus upon unstabilised and stabilised barium sulphate suspensions has been reported (Astley & French, 1951) and mucin (from an undefined source) has been used as an additive in barium enemas (Alexander & Alexander, 1949); Knoefel, Davis and Pilla (1955) demonstrated a correlation between visualisation of the gastric mucosa and the volume of gastric juice in the stomach. They also showed that the sedimentation rate of unstabilised suspensions increased upon addition of mucin, and that the barium sulphate particles removed a specific fraction from their mucin solution.

(a) In vitro coating of barium sulphate particles.

Three sources of mucus were used, two commercial and the other from living rat intestine. BDH and the Nutritional Biochemicals corporation supply spray dried porcine gastric mucus. Due to their method of preparation these are of doubtful composition, and could contain dried cellular fractions such as other proteins, glycoproteins and DNA.

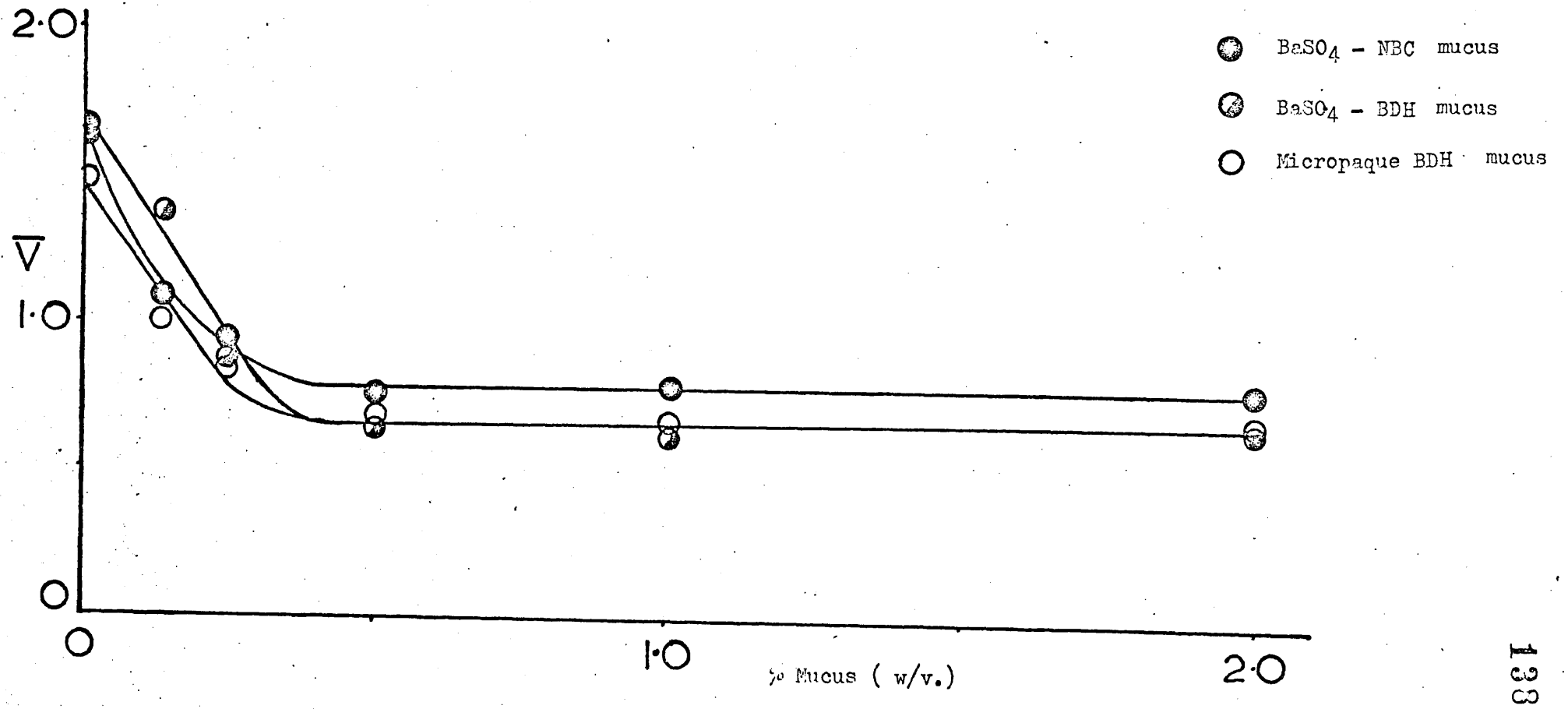
A 2·0% w/v. solution of each commercial preparation was prepared by adding the powder to water in a 500 cm^3. polythene bottle, the mixture was blended for 30 minutes, and allowed to stand overnight. The fine spray dried particles inbibed water, resulting in a clear colloidal suspension. Dilutions of these were prepared, and added to 5 cm^3. samples of (i) a 50% w/v. suspension of unstabilised barium sulphate (ii) 50% w/v. suspension of Micropaque from which the excess gum in solution had never been removed (Chapter 4. Section 4(d).).

The suspensions were immersed in an ultra sonic bath for 15 minutes and left to equilibrate for 24 hrs. 0·2% w/v. suspensions of all particles were prepared in barbiturate buffer and the mobility determined at pH 7·0 (Fig. 6.5.).

Fig. 6.6.

VARIATION OF MOBILITY OF BARIUM SULPHATE AND MICROPAQUE PARTICLES WITH CONCENTRATION OF MUCUS.

pH = 7, I = 0.02 mol. dm^{-3}



The particles, whether derived from Micropaque or unstabilised suspensions were coated with mucin. These results must be interpreted with care since the mucin was possibly denatured in the manufacturing process.

The mobilities of particles fully coated with mucus, i.e. taken from suspension containing 1·0% w/v. mucin were determined at different pH values in barbiturate buffer. Results are shown in Fig. 6.7.

The particles coated with the BDH preparation have a pH – $\bar{v}$ curve characteristic of a surface carrying carboxyl groups, and show no evidence of amino groups which would result in a positive mobility at low pH, which would be expected for a glycoprotein. Particles coated with the NBC preparation appear to have a non-iogenic surface. The increasing negative mobility at high pH is due to adsorption of hydroxyl ions.

In a subsequent experiment three rats were anaethetised, the small intestine removed, and the surface mucus squeezed out into "tris" buffer (pH 7·0) in which it was homogenised. The suspension was gently homogenised to remove particles of food, dilutions were made of the resulting clear suspension, and 1 g. of dry Barospere powder added to 10 cm^3. portions of these. Barosperse particles were more convenient to use in this experiment than those derived from Micropaque, since although stabilised they require no washing and also have high mobility. The suspensions were shaken and left 24 hours befors a small sample was transferred to barbiturate buffer solution, pH 7·0 for the measurement of their mobility values. The concentration of mucus in the original homogenate was determined gravimetrically, by drying a measured volume to a constant weight.

Again it was demonstrated that the mucus had coated the Barosperse particles (Fig. 6.8.) and, as with the commercial preparations a plateau was reached at 0·5% w/v. mucus. The limiting mobility value was 1·7.

These results indicated that stabilised and unstabilised barium

Fig. 6.7.

pH MOBILITY CURVE OF MUCUS COATED BARIUM SULPHATE PARTICLES (I = 0·02 MOL. DM.$^{-3}$)

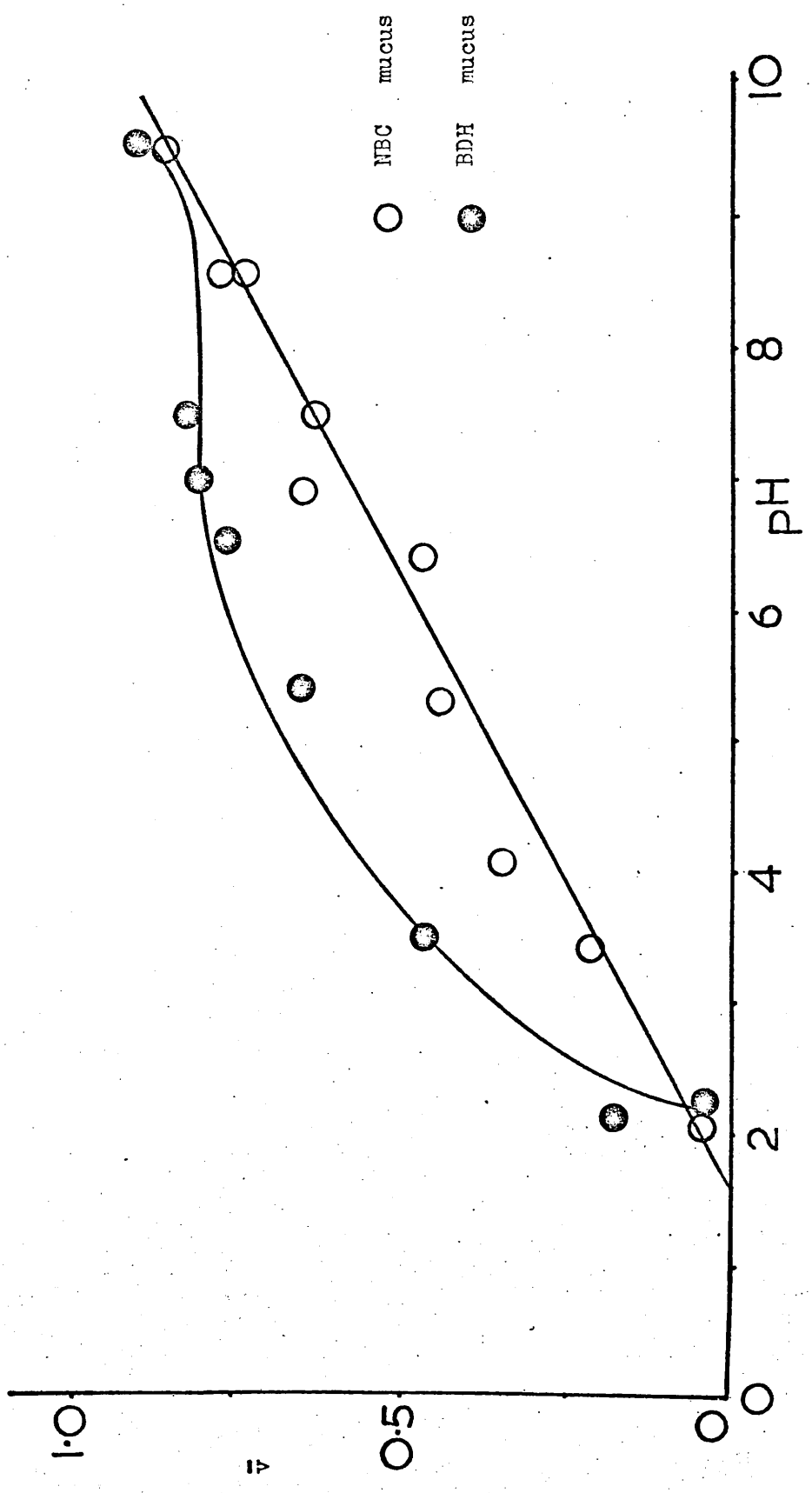
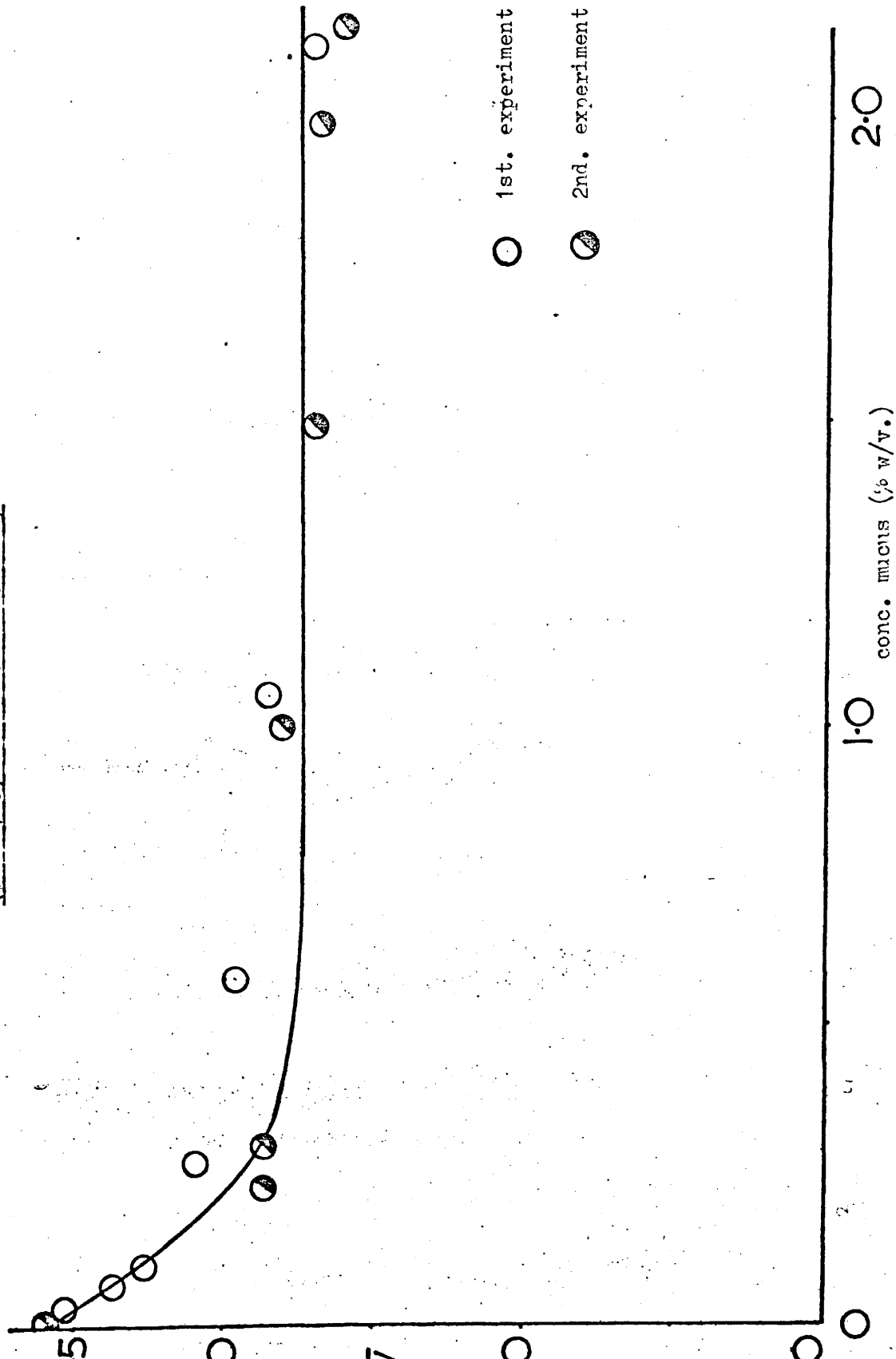


Fig. 6.8.

VARIATION OF MOBILITY WITH CONCENTRATION OF RAT INTESTINAL MUCUS

(pH = 7·0, I = 0·02 mol. dm^{-3})



sulphate preparations could be coated *in vivo* when passing through the gastro-intestinal tract.

Chapter 6. Section 6. In vivo coating of barium sulphate suspensions.

Micropaque suspensions, g. 25, 12•5, 6•25% w/v. were prepared by dilution of the original 100% w/v. suspension with water, and a Barosperse suspension of 12•5% w/v. was also prepared. These suspensions were then used in the perfusion of rat intestine, using the method previously described (Chapter 5. Section 1.). The 5 cm. lengths of intestine were immediately flash frozen at -60° and kept for the measurement of the electrophoretic mobility values of the barium sulphate suspensions they contained. Freezing was necessary because of the delay between the *in vivo* coating and electrophoretic examination; it ensured that coating of particles occurred during the experiment, when the barium sulphate particles became attached to the intestinal mucosa of the living animal, and not during the interval between the experiment and determination of the mobility, when denatuation of the mucus layer was possible. This did not occur upon freezing, since it was so rapid, and ice crystals were not formed.

The samples were allowed to thaw, and the intestine divided into 0•5 cm. lengths. These were immersed in barbiturate buffer solution of the appropriate pH,and opened. Dispersion of the surface mucus with a spatula rapidly gave a suspension of particles concentrated enough to be used for the determination of the mobility. Aggregations of particles did occur, and these were clearly visible in the apparatus, where they settled quickly. Only individual particles were timed for the mobility determination.

The mobility values of particles from all suspensions, independent of source (i.e. Micropaque, or Barosperse) and concentration were the same within the limits of experimental error (Table 6.3.) and quite different from the values of the controls.

Table 6.3.

Mobility of particles of Micropaque and Barospere recovered from rat intestine (pH = 7, I = 0•02 mol. dm^{-3}).

	conc. %	$\bar{v}$	$\bar{v}$ (control) (the absence of radiopaque)
Micropaque	6•25	1•35	1•48
	12•5	1•35	
	25	1•34 (1•35)	
	25	1•31	
	25	1•41	
Barospere	12•5	1•37	2•70
	25	1•33 (1•34)	
	25	1•31	

The difference between the mobility value of particles obtained after *in vivo* coating (1•35) and *in vitro* coating (1•7) could, amongst other unknown factors, be due to (a) freezing of the samples before electrophoretic measurements; (b) the far greater concentration of mucus *in vivo* (approximately 50% w/v. at the surface of the intestine).

To obtain further evidence on the cause of the *in vivo* and *in vitro* difference, a rat was perfused with 12•5% w/v. Barospere, and the whole of the small intestine was removed, and frozen. Upon thawing it was divided into portions, and the adhering particles removed and dispersed in barbiturate buffer solution. The suspensions so obtained were centrifuged and the particles resuspended in an ultra sonic bath in fresh barbiturate buffer solutions. This washing procedure was repeated four times, the electrophoretic mobility of the particles was determined after each washing (Table 6.4.).

Table 6.4.

Mobilities of particles coated with mucus in vivo.

Number of washings	$\bar{v}$ (av.) at pH 7·0 I = 0·02
0	1·36
1	1·61
2	1·60
3	1·60
4	1·59

It is evident that after one washing the mobility of the particles approaches the value reached after in vitro coating (1·7).

In a further control experiment the intestinal tissue with adhering particles was not flash frozen; instead the mobility of recovered particles was determined very soon after perfusion. The mobility of particles suspended in barbiturate buffer was 1·35. This is no different to the mobility value obtained from frozen tissue, and therefore the freezing process did not cause denatuation of the mucus. (b) is therefore more likely, and it may be that washing the particles removed only a specific fraction of mucus from the surface; the other mucous fractions remain on the surface.

The pH mobility of in vivo coated particles suspended in barbiturate buffer without washing (Fig. 6.9.) is typical of a glycoprotein. The positive mobility exhibited at low pH (below 3·8) is due to protonated amino groups present in the protein moety of the molecule.

The negative mobility, acheiving a plateau at pH 6·5 is most probably due to sialic acid residues of the carbohydrate back bone of the molecule (Schultz, 1962, Oncley, 1962). The inflection at pH 9 is

due to the loss of a proton from the amino groups. Results were consistent from animal to animal, variation was within the accepted limits of experimental error.

Chapter 6. Section 7. The effect of diet upon in vivo coating of barium sulphate particles.

The possibility of a different mechanism taking place *in vivo* in fasted rats resulting in much less barium sulphate being recovered from the small intestine was investigated. Those fasts whose effects have been demonstrated (Chapter 5. Section 3.) were again used, i.e. a low residue sugar diet, total fast, both for 24 hrs. and a combination of the two. The restoration of normal feed was also investigated.

The previously described technique was used (Chapter 6. Section 6.), the barium meal used was 12•5% Barosperse. Particles were not washed before the mobility determination.

Results are listed in Table 6.6., Figs. 6.10 - 6.11.

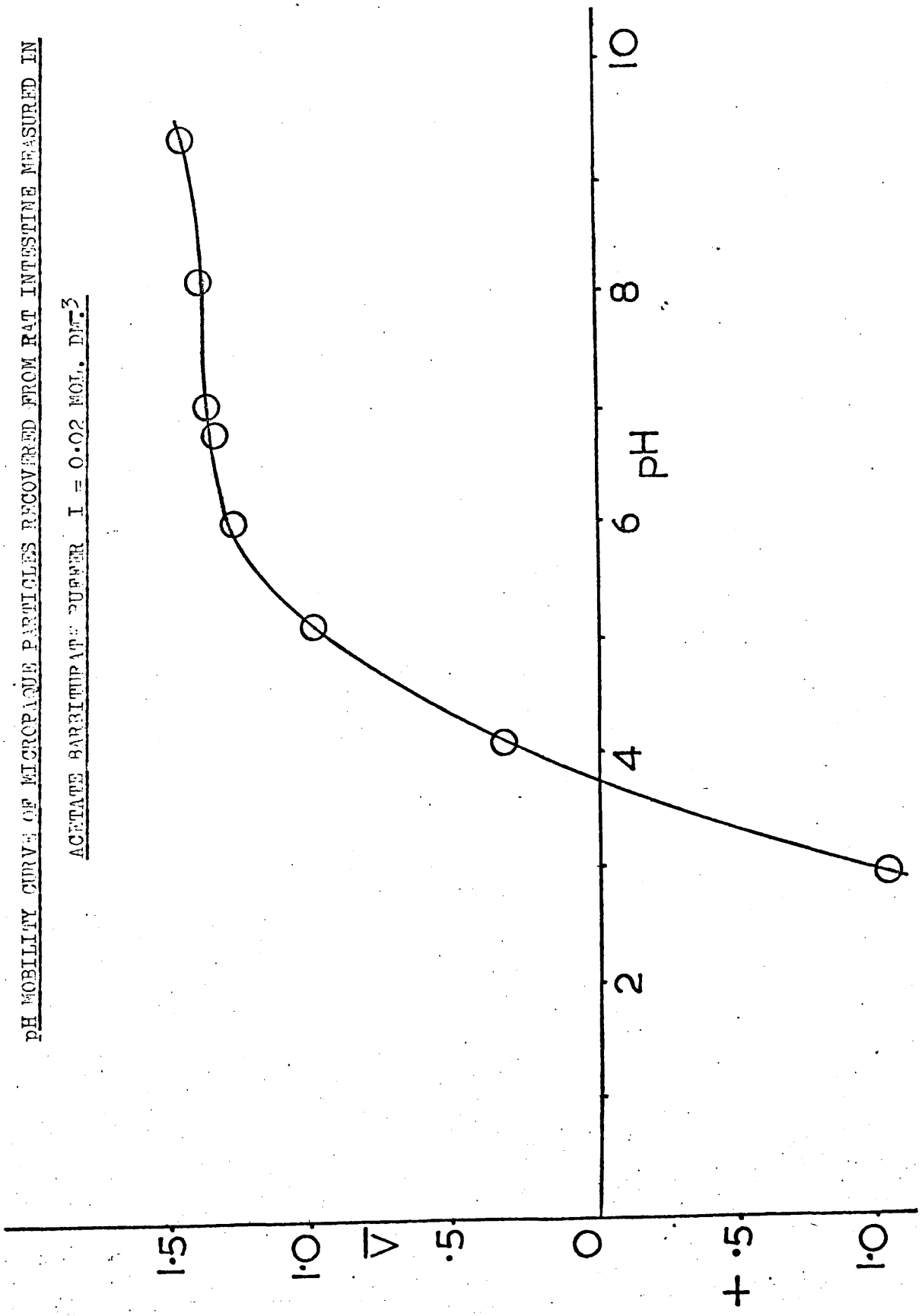
The results from all animals were consistent, and the difference in mobility between particles recovered from a normally fed animal and a fasted animal is significant. The change can be accounted for by differences in the mucus.

Chapter 6. Section 8. Effect of NAC solutions upon in vivo coating of barium sulphate particles.

The small intestine of the anaesthetised rat was treated with 2•0% w/v. NAC in tris buffer solution (Chapter 6. Section 3.) before the perfusion of 12•5% w/v. Barospere. The intestine was removed and frozen, and the Barospere particles recovered and resuspended in barbiturate buffer as previously described (Chapter 6. Section 6.). The times of perfusion of the NAC solution were all about 5 minutes. The average mobilities of the recovered particles was 1•31. This represents only a 4% change from the mobility value associated with particles recovered from untreated animals and cannot be taken as significant.

Fig. 6.9.

pH MOBILITY CURVE OF MICROPAQUE PARTICLES RECOVERED FROM RAT INTESTINE MEASURED IN ACETATE BARBITURATE BUFFER I = 0.02 MOL. DM^{-3}



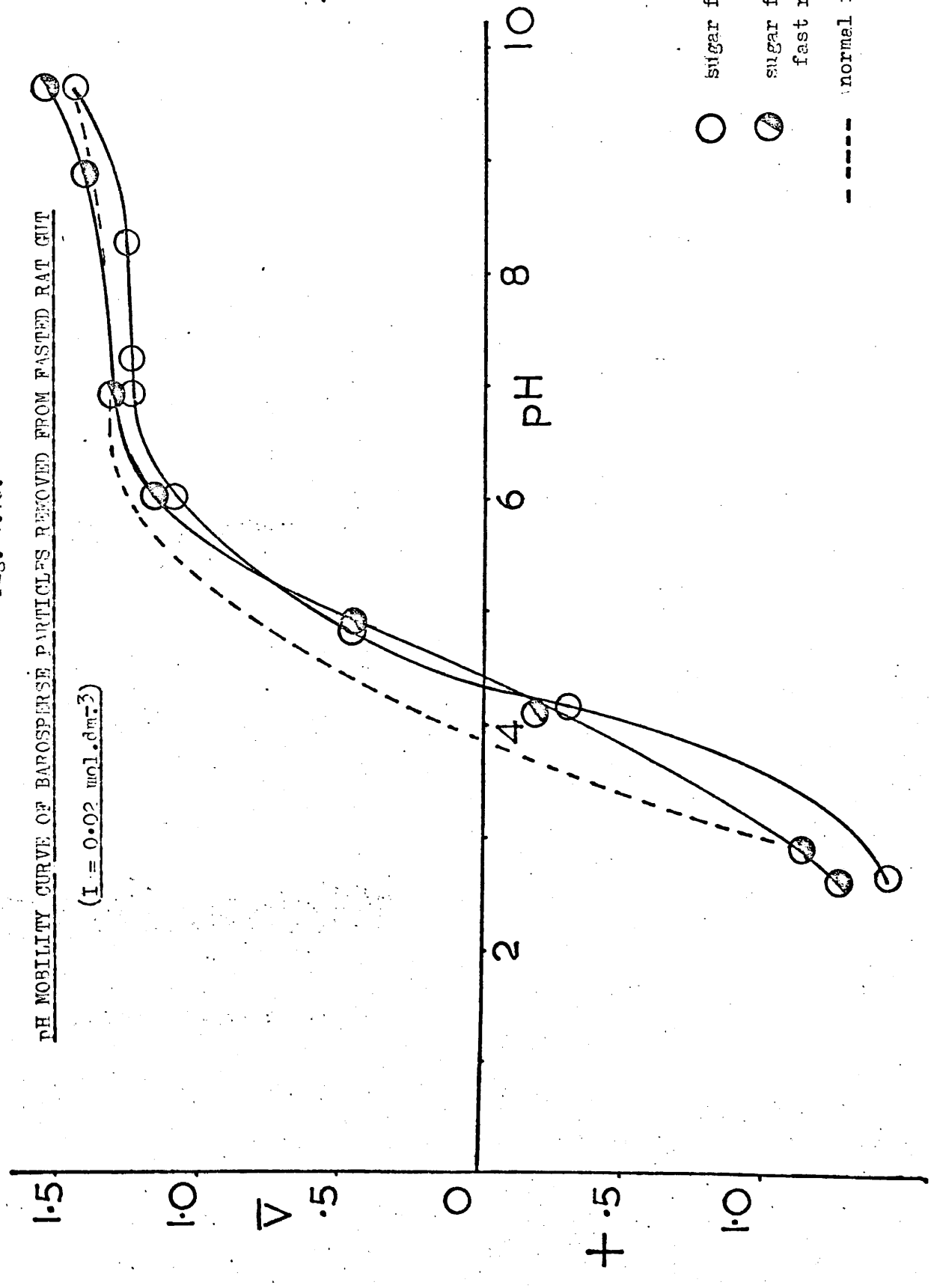


Fig. 6.10.

pH MOBILITY CURVE OF BAROSPERSE PARTICLES REMOVED FROM FASTED RAT GUT

($I = 0.02$ mol.dm^{-3})

Table 6.6.

The effect of fasting upon the mobility value of Barospere particles recovered from rat intestine.

Diet	$\bar{v}$ (pH 7·0) ($I = 0{\cdot}02$ mol. dm^{-3})	
Total fast 24 hrs. (i.e. water only)	1·22 1·28 1·26 1·29	(1·26)
sugar fast 24 hrs.	1·23 1·21 1·23 1·23	(1·22)
sugar fast followed by total fast (24 hrs.)	1·25 1·26	(1·26)
Total fast, Restoration normal feed 24 hrs.	1·20 1·20	(1·20)
normal feed		(1·33)

Pretreatment of the ileum with NAC solutions did not therefore prevent *in vivo* coating of barium sulphate particles, though the amount taken up by the mucus was so greatly reduced.

Chapter 6. Section 9. Observations.

During the experiments concerned with fasted animals the state of the ileal mucus was investigated macroscopically. Whereas mucus derived from a pellet fed animal was a firm gel, containing about 50% glycoprotein (by dry weight) and adhered strongly to glass, that derived from rats fasted, whether on sugar, or total, was a much less firm gel, water content was greater (60 - 80% by weight). Further, the mucus would not adhere to glass, and generally more of it was present in the ileum.

Chapter 6. Section 10. Discussion.

Coating of particles of barium sulphate, whether stabilised or unstabilised occurs *in vitro* and *in vivo*, and could well account for the flocculation of barium meals that occurs in the gut if large amounts of gastric juice are present. Coating of the particles with stabiliser whether CD75 (as in the case of Micropaque) or cmc. (as in Barospere) does not prevent this coating, and full coating occurs in all cases at 0·5% w/v. mucus, (at pH 7). *In vivo* coating with mucus occurs in all cases with fasted animals, and the mobility value of such recovered particles gave an indication of the rapid response of intestinal mucus to changes in diet. Ballinger and Wise (1969) have reported a drop in the carbohydrate/protein ratio in human patients in response to fasting. Such a chemical change in the mucus could well explain the change in viscosity and adhesive properties of the mucus observed. Despite this, the mechanism of adsorption appears to be the same in both normal and fasted animals and for those treated with NAC solutions, but because of the nature of the mucus less is held into the mucus, and removed by washing, or normal peristaltic movements of the gut.

CHAPTER SEVEN

DISCUSSION

Before any evaluation or interpretation of the experimental results is attempted it is essential that the accuracy and limitations of the various techniques be fully considered.

The accuracy with which the electrophoretic mobility of a particle can be determined, for many determinations of a single particle, is $\pm$ 3%. In the present study the average mobility value of at least 50 particles in suspension were determined on at least two different samples at the required pH. In no cases were great fluctuations between replicate determinations found. The shapes of the pH mobility curves for all particles were well defined, with very little scatter of points. In the vast majority of cases the shapes of the graphs were characteristic of particles with carboxyl, or carboxyl and amino groups at the surface (Chapter 1. Section 4.). Generally the absolute value of the mobility in the plateau region was not required with great accuracy, as the shape of the pH mobility curve provides a more important indication of the nature of the particle surface. All measurements of mobility were made under strictly controlled conditions of pH and ionic strength of the suspension medium and therefore any comparison of mobility values between different particles in suspension is valid. Values of the zeta potential have not been calculated because of the uncertain values of the relative permitivity and coefficient of viscosity within the electrical double layer.

When differences in limiting mobility values were important, for example for mucus-coated particles derived from normal and fasted rat intestine, a greater number of determinations were made. The small variation (4 - 5%) in the values obtained in replicate determinations were due not only to the variations in the electrophoretic technique, but also to animal variation. The difference in particle mobility of Micropaque particles isolated from fasted animals to those derived from normal rats is 10% can thus be taken as a significant indication of

differences in the particle surfaces (Figs. 6.9. & 6.10.).

If the mobility for Micropaque particles isolated from fasted animals is less than that found for similar particles from normally fed animals, this may be due to :

(a) Mucus from fasted animals has a different composition,

(b) a different orientation of the mucus at the surface.

If there was insufficient mucus present in the gut to fully coat the particles (this is unlikely as particles become fully coated at 0·5% w/v. mucus, see Fig. 6.8.) particles isolated from fasted animals would have a greater mobility than those particles isolated from normal animals.

In the determination of absorption isotherms, using electrophoretic techniques, some variation in mobility values was found, this was especially apparent before particles were fully coated. However the shapes of the isotherms were clear and the limiting value of mobility could be accurately assessed.

The accuracy with which the coefficient of viscosity at any given shear rate could be determined was limited by the accuracy of the instrument, and by the nature of the suspensions. The instrument scale of 100 could be read at best to $\mp$ 2 due to fluctuations in the motor speed. As the deflection was not full scale in all cases, the accuracy of readings varied. For the less stable suspensions (RS. $\ll$ 6) particles which sedimented between the top of the inner cylinder and the outer cylinder might well have an effect upon the shear rate applied to the suspension.

Values of apparent viscosity, and the yield value were determined from graphs where shear stress was plotted against shear rate (Chapter 2. Section 8.). The errors involved in determination of η_{App} were less

than errors in a given value of η since they were obtained from the best lines drawn through the points. Errors involved can be estimated graphically and are less than 5%.

Determinations of yv., especially when yv. was small, were subject to greater errors, since a 5% alteration in the slope of the graph results in a larger change in yv. The estimated error for yield values $\leqslant$ 100 cp. is about 10 - 15%; this reduces to 5 - 10% for values of yv. $\geqslant$ 200. Values of relative stability were also determined graphically (Fig.2.6.) from the plot of fractional height against time. The graphs obtained were all smooth monotonous curves exhibiting no significant deviations. Errors in individual timings, in measurement of the height of the sediment (read to $\mp$ 0·5 mm.) amount to an error of about 4 - 5%. Since all values are relative to the barium sulphate suspension used as a control, errors in the applied centrifuged force, and due disruption of the sedimented layer are thus automatically minimised. Repeated determinations of the relative stability of a single suspension of low RS. showed deviations of 10 - 15% and this figure should therefore be taken as the probable limit of error for such suspensions. For more stable suspensions (RS $\geqslant$ 6) less deviation was found and the error in RS. is about 5%.

A possible source of errors involved in many of these determinations lies in the accuracy with which the absolute concentration of barium sulphate in suspension is known. Many suspensions give an appreciable sediment repidly - in a matter of 30 - 60 minutes (when RS. $\leqslant$ 1) and transferance from one vessel to another, and even stirring with the colloid mixer involved the possibility of a change in concentration.

As in practically any other physiological technique, there were many possible sources of error in all the <u>in vitro</u> and <u>in vivo</u> experiments.

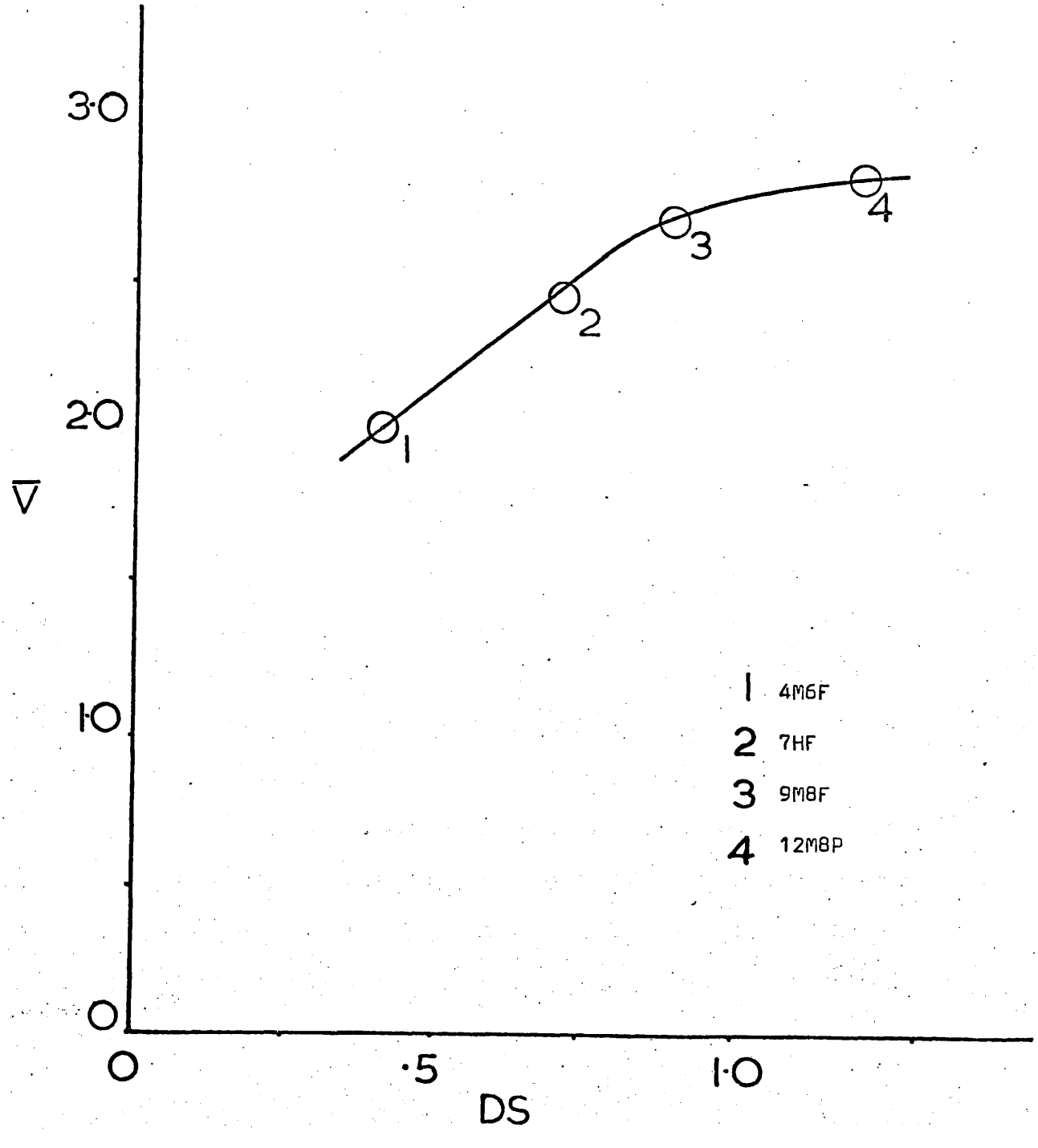
These may be summarised as (i) animal variation, (ii) variation due to technique e.g. variation of time of washing of gut, (iii) method of assessment of results (iv) physical properties of suspensions. The cumulative error involved is thus possibly very large, and the interpretation of the results would have been easier and more certain if the experiments for investigating the various parameters had been designed statistically.

The original aims of the experiments involving model systems were (a) to establish the techniques for the study of model systems (b) to determine the feasibility and usefulness of such techniques from the point of view of predicting in vivo clinical behaviour. (c) to determine those factors which grossly affected the adsorption of barium sulphate by the gut. For these purposes it was decided that results from a small number of experiments investigating a number of parameters would be more useful than investigating a fewer number of variables with statistically designed experiments involving a great number of individual experiments.

The in vivo technique developed was lengthy, and needed much preparation, and strictly statistical design of experiments was thus very difficult to achieve on this factor alone. In defence of the techniques established, in a limited number of experiments, however, it was shown that Micropaque could give very consistant results under controlled conditions. Observed changes in the weight of barium sulphate recovered from the lengths of gut, as a result of changes in diet etc: were very large (30 - 60%); such changes are obviously significant and can readily be detected even with a limited number of experiments. Because Micropaque was better in this respect than any other suspension it was used as a control in all techniques. For the in vitro techniques animal variation was allowed for by use of an internal control. The method of microscopic assessment, a subjective procedure, was unsatisfactory, but did allow some indication of the eveness of the adsorbed layer to be obtained, which was very difficult in the in vivo method.

Fig. 7.1.

VARIATION OF MOBILITY OF CMC. COATED PARTICLES (I = 0·02 MOL. DM^{-3}, pH = 7·0) WITH DEGREE OF SUBSTITUTION OF CMC. USED AS STABILISER.



The instability of suspensions necessitated greater handling of the small intestine, as every effort was made to prevent sedimentation and maintain the prepared concentration in the gut. This caused some disruption of the adsorbed layer and resulted in the much greater spread of results for suspensions which were less stable than Micropaque. The disruptive effect of handling was greater when the animal had been fasted; this might be due to the more delicate nature of the mucous membrane, since the mucus was definitely less viscous. Thus in vivo experiments with fasted animals, or where the mucous membrane was treated with N-acetyl cysteine solutions were restricted to Micropaque, and unstable suspensions, such as Barosperse were never used. Not only was Micropaque more consistent and here produced quite reproducible results, but also the control value was well established; thus smaller changes in the amount of barium sulphate adsorbed by the gut could be detected, and the values obtained were more significant.

Sodium carboxy methyl cellulose proved to be a very efficient stabiliser, and suspensions with widely differing properties were prepared. Cmc. was chosen because it is available commercially as a pure synthetic material, of varying degrees of substitution and polymerisation, and hence of varying viscosity. Synthetic materials do not suffer from the many drawbacks of naturally occuring products. Cmc. fully coated the barium sulphate particles at a concentration above 0·4 - 0·5% w/v. cmc., and the surface charge carried by the cmc.-coated particle is dependent upon the degree of substitution of the cmc. sample used (Fig.7.1.). The value of mobility increases linearly as a function of DS. for values of DS. below 1. Thereafter a 30% increase in DS. resulted in only a 5% change in $\bar{v}$. This may be due to the arrangement of the carboxy methyl groups in the molecule. For all values of DS. $\leqslant$ 1 there is only one or

less carboxy methyl groups for each anhydro-glucose unit. At low DS. it is apparent that most, if not all charged groups are orientated at the surface such that they contribute to the total surface charge in the same way, and the effect of added charged groups is additive. At higher degrees of substitution (DS. > 1) substitution will also occur at the 2 or 3 position of the molecule. On account of their orientation any free carboxyl group in these positions will not contribute so much to the total surface charge of the particle. Thus for particles whose charge is due only to a cmc. coating a mobility value greater than 3•0 would not be expected. Values of $\bar{v}$ greater than this have,however been encountered in commercial preparations, e.g. Baritop, which has a limiting mobility value of 3•2 (at I = 0•02 mol. dm^{-3}). This may be evidence of other additives in this particular preparation.

Suspensions prepared with cmc. of 12M8P grade were significantly more stable than those prepared with 9M8F, for concentrations of cmc. between 0•6 and 1•0% w/v. (Figs. 3.6. and 3.7.). A 60% w/v. barium sulphate suspension stabilised with 1% w/v. 12M8P has RS. = 17, in contrast the suspension stabilised with 1% w/v. 9M8F has RS. = 12. The difference in the mobility of particles coated with the two stabilisers is about 5% and would not account for such a difference in RS. Suspensions stabilised with cmc. of grade 9M8F exhibit non-Newtonian behaviour, whereas suspensions with cmc. of 12M8P grade showed Newtonian behaviour. This would suggest that inter-particulate forces are greater for 9M8F coated particles, resulting in the suspension having some degree of structure. The yield value gives an indication of the force required to break this structure and cause the suspension to flow. One would expect that such suspensions would be more stable than an equivelant suspension exhibiting Newtonian behaviour. However, this is not the case. Evidence that interparticulate forces do exist in the suspensions, whether Newtonian of non-Newtonian is given by the claying of particles upon sedimenting, this phenomenon of claying occured in both 12M8P and 9M8F stabilised suspensions at higher

concentrations of stabiliser, but not below concentrations of cmc. <0•6% w/v. Claying, therefore, seems to be associated with excess stabiliser in the liquid phase, rather than the degree of substitution of the stabiliser and hence the surface charge carried by particles.

Suspensions varied in their dispersive properties (Table 3.3.). From this it is apparent that to exhibit good properties in both acid and water (a) the barium sulphate particles must be fully coated (cmc. 0•4 – 0•6% w/v.) (b) the degree of substitution must be greater than 0•7 (c) suspensions must not be too viscous, and (d) better suspensions are obtained with suspensions exhibiting Newtonian behaviour.

A high degree of substitution of the hydrocolloid results in the particles being hydrated in suspension. The stability of suspensions has been related to the degree of hydration of particles in suspension (Goddard, 1970). The hydrated particles show some degree of hydrophilic behaviour, and this results in greater stability with respect to dilution, and to the addition of electrolytes.

The shapes of the pH mobility curves of particles of all commercial preparations, determined in acetate barbiturate buffer solutions are very similar, and generally typical of a carboxyl surface. The actual position of the curve depends upon the number of charged surface groups. The shape of all curves are quite unlike those for uncoated barium sulphate particles, which cannot therefore contribute to the surface charge, and the stabiliser used must coat the particles fully. This not only results in suspensions which are resistant, to an extent, to dilution and flocculation in the gut, but also prevents barium sulphate being attacked by the various physiological solutions in the gut, thus Ba^{++} ions will not readily pass into solution. Such a situation is not found in suspensions "stabilised" with sodium citrate, and doubts as to the safety of using this agent as

an additive have been expressed.

A typical adsorption isotherm for the uptake of barium sulphate by the gut was not found for either unstabilised or citrate stabilised suspensions. As particles from neither suspension can be considered as having a protective coating, the coating possessed by particles in other stabilised suspensions may in some way be responsible for the isotherms encountered *in vivo*.

After particles become fully coated with stabiliser further addition merely results in stabiliser accumulating in the liquid phase. One effect of this is that the wetting properties of the suspensions are altered, due to a change in surface tension of the suspension. Further, this excess stabiliser can compete with any free mucus present in the lumen of the gut, preventing particles from becoming coated with mucus and "precipitating" Since this process is more one of particles becoming associated with free mucus, rather than association of particles with each other, agglomeration is a better term for this effect. Thus the stabiliser increases suspension stability both *in vivo* and *in vitro*. Pirk et al (1967) did not take into account any excess stabiliser present in solution when they added gastric juice to various commercial barium meals, and assumed that different responses by the suspensions to such treatment was due to particle size and the nature of the stabiliser.

Because agglomeration *in vivo* is reduced by free stabiliser in solution, it might be expected that those suspensions containing excess hydrocolloid, such as Micropaque, to give more consistent results than those containing little or no excess stabiliser, such as Barosperse, or citrate stabilised suspensions, and this has been demonstrated experimentally.

The nature of the particle surface, if it is fully coated, will not directly affect the adhesion of a particle to the gut surface, since the charge carried by the particle plays little or no part in adhesion. Gross differences in *in vivo* behaviour of suspensions containing different stabilisers would not therefore be expected, provided there is sufficient stabiliser to fully coat the particles.

The study of particle -particle interaction has been extended by the work of Verwey-Overbeck (1948) who derived various equations for the calculation of interparticulate forces in suspensions of different ionic strengths and for different surface potentials. The equation for the potential V in terms of forces of attraction V_A and repulsion V_R is

$$V = V_A + V_R$$

$$= \frac{-A}{12\pi d^2} + \frac{4ne^2 \varphi_o^2}{Kkt} e^{-Kd}$$

where the Hamaker constant A is assumed to be 10^{-21}J. d is the distance (in A^o) between the particles of surface potential φ_o, e the electronic charge, k the Boltzmann constant and K the reciprocal thickness of the ionic atmosphere defined by

$$k = \sqrt{\frac{4e^2 \sum nizi^2}{DKT}}$$

where D is the dielectric constant in the ionic atmosphere.

Figs. 7.2. - 7.5. show the potential energy curves for two particles in a colloidal suspension, calculated for different ionic strengths, and different electronic charges carried by particles. The curves are relevant to all suspensions studied at physiological pH and were calculated for surface potentials of 10 - 40 mv. since it is a good approximation that the surface potential is 2 - 3 times ζ. Experimental values of mobility were determined only at I = 0·02 mol. dm^{-3} but even at I = 0·1 mol. dm^{-3} the limiting mobility of Micropaque particles in barbiturate buffer is greater than 1, i.e. $\zeta > 12{\cdot}5$ mv. For all ionic strengths, 0·02 mol. dm^{-3} (the ionic strength of acetate - barbiturate buffer) - 0·15 mol. dm^{-3} (isotonic with body fluids) the curves are essentially similar in shape,

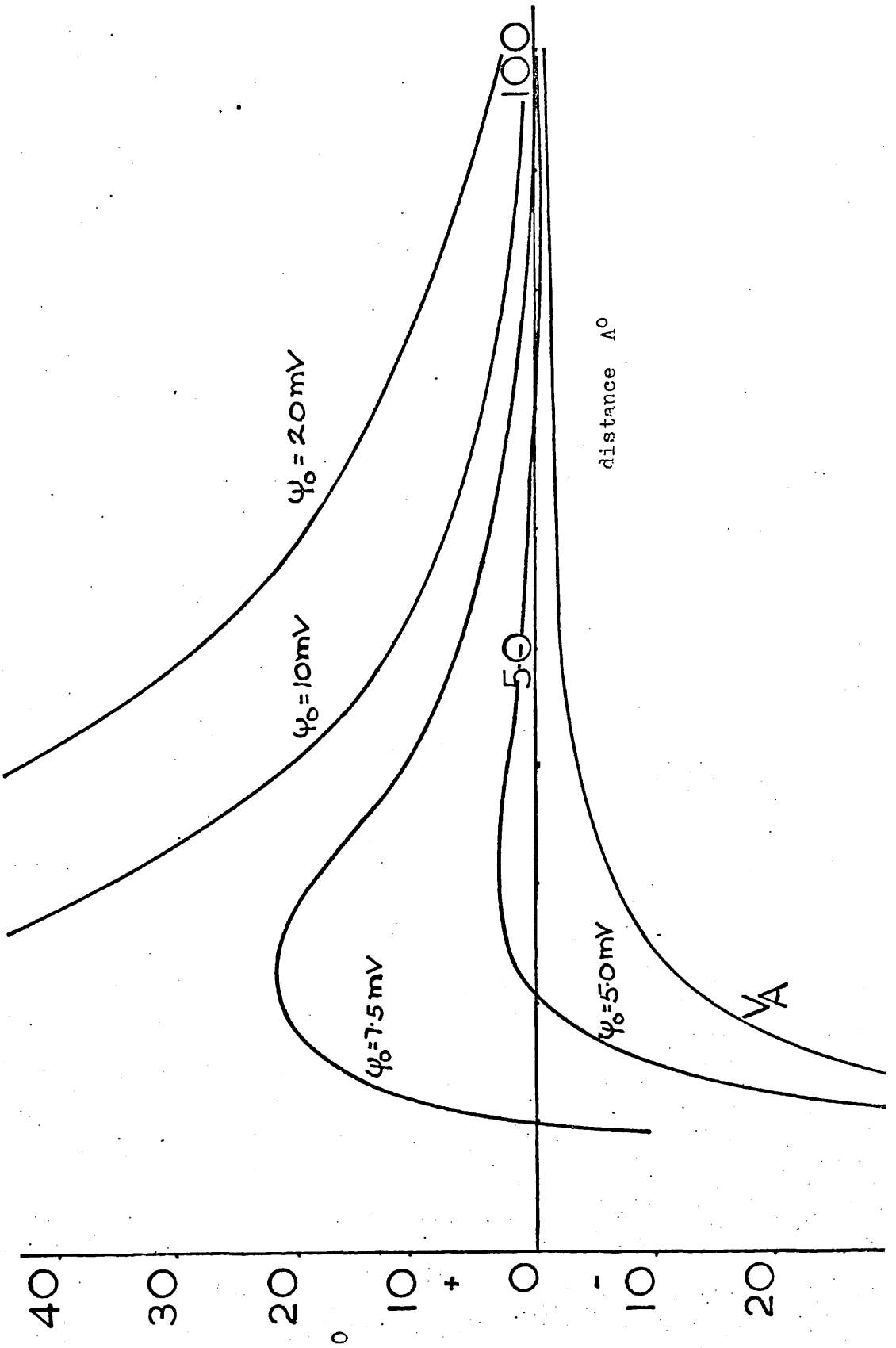


FIG. 7.2. POTENTIAL ENERGY - SEPARATION CURVES FOR TWO CHARGED PARTICLES (I = 0.02 mol. dm^{-3}).

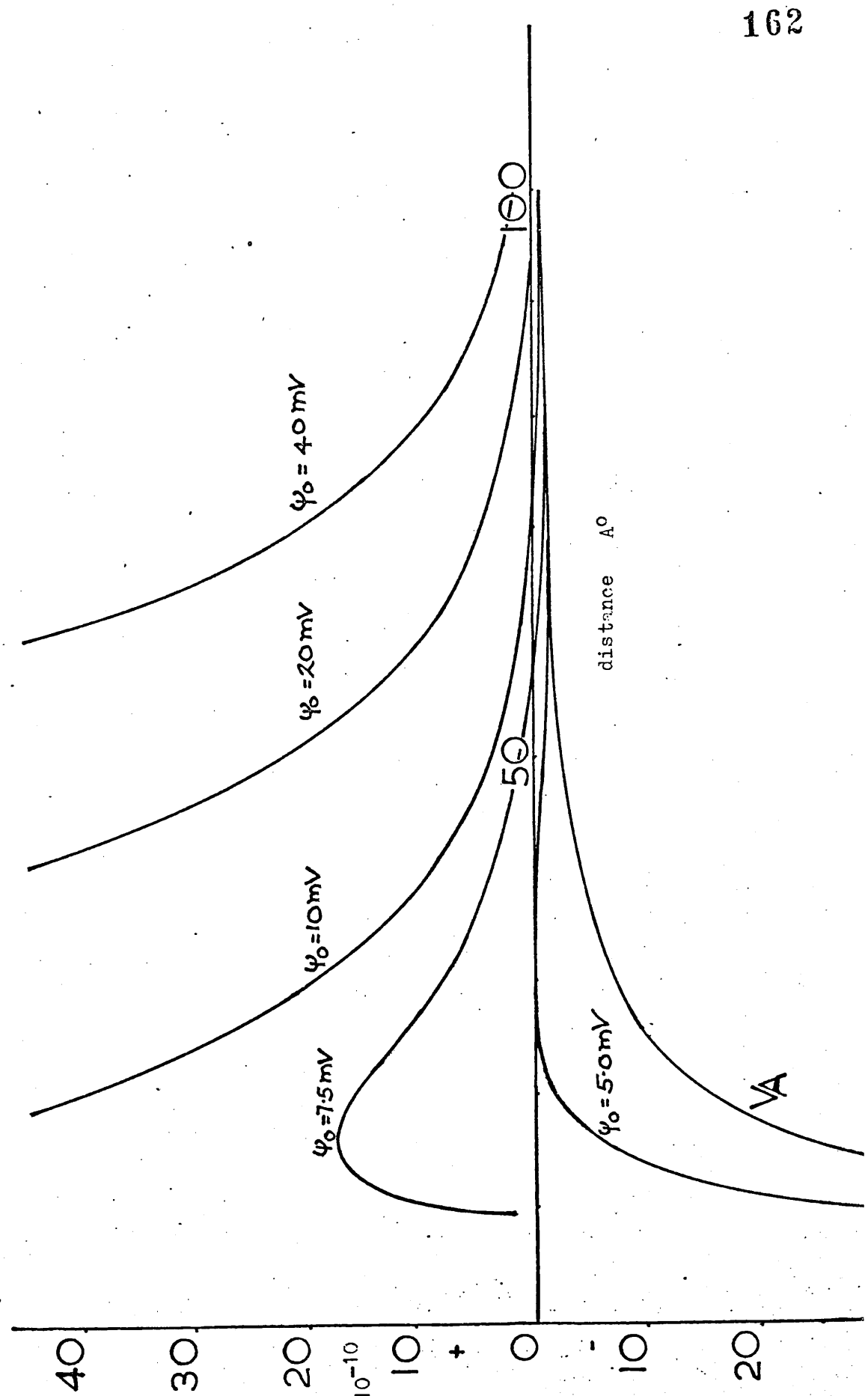


FIG. 7.3. POTENTIAL ENERGY - SEPARATION CURVES FOR TWO CHARGED PARTICLES (I = 0·05 mol. dm^{-3}).

FIG. 7.4. POTENTIAL ENERGY - SEPARATION CURVES FOR TWO CHARGED PARTICLES (I = 0·1 mol. dm^{-3})

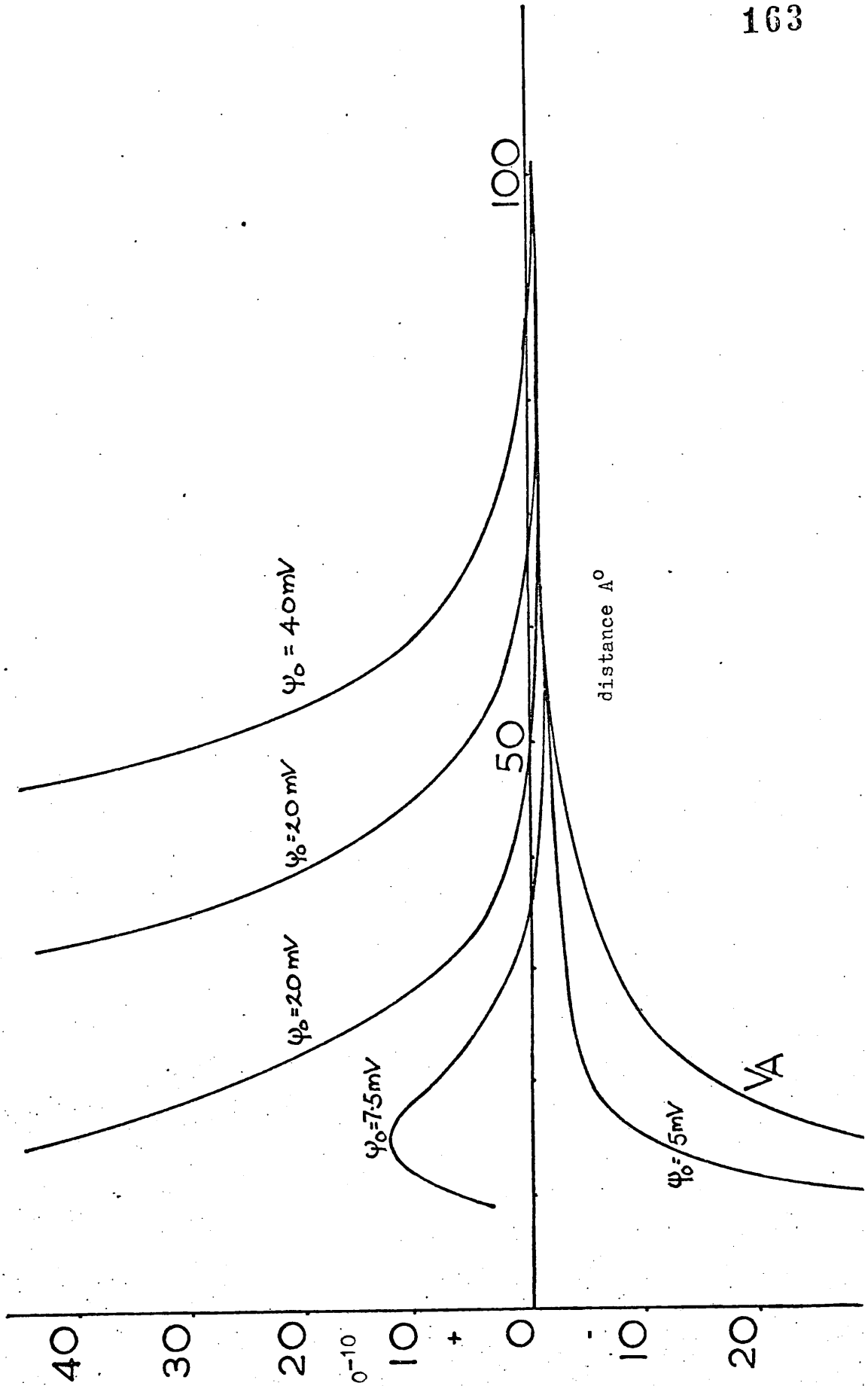
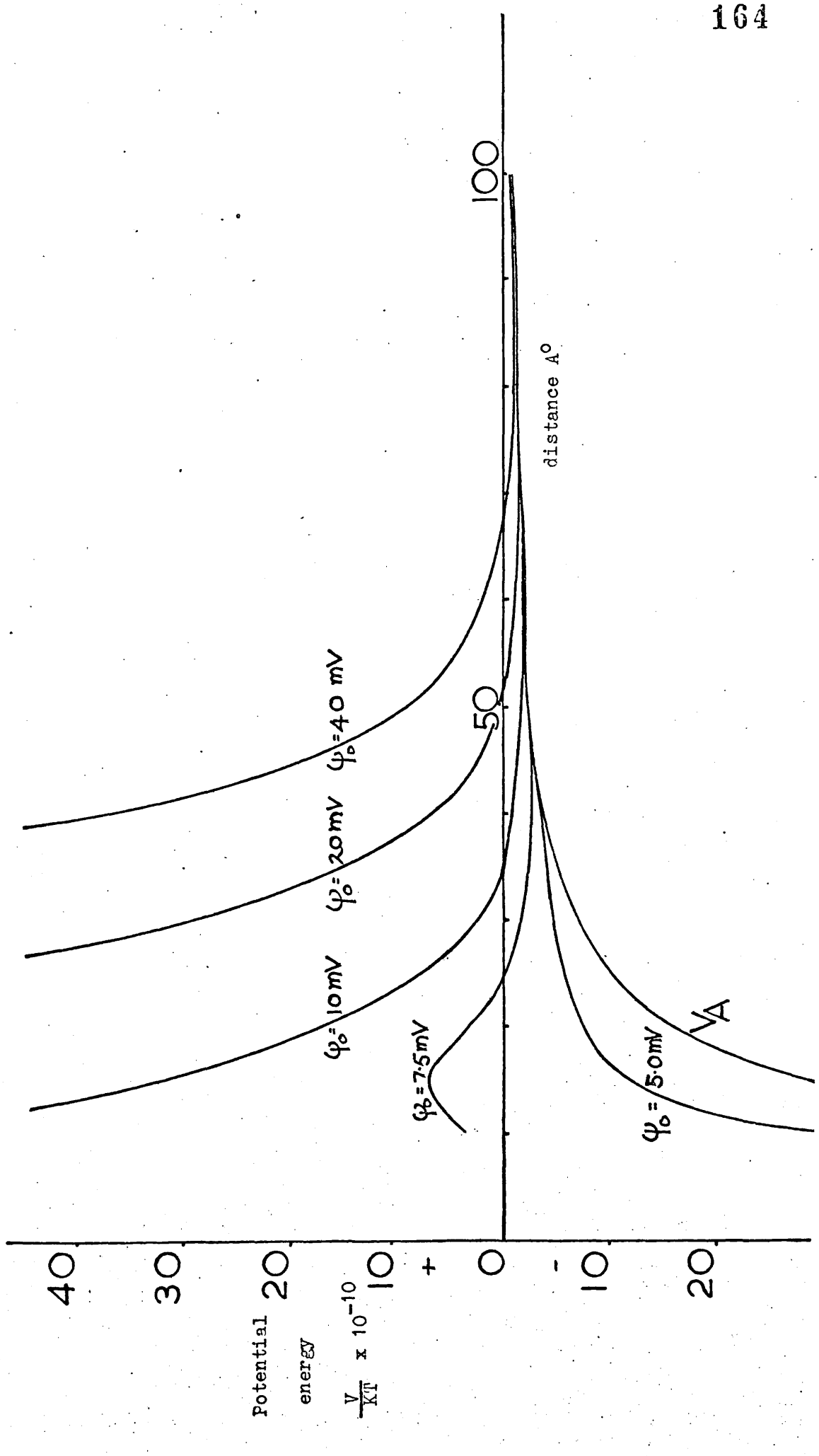


FIG. 7.5. POTENTIAL ENERGY - SEPARATION CURVES FOR TWO CHARGED PARTICLES (I = 0.15 mol. dm^{-3}).



their positioning however, changes with ionic strength.

The colloidal systems are obviously very stable under the experimental conditions used in this work; thus any instability of the suspensions is not due to a low surface charge on the particles, so that the collision of particles does not result in adhesion and flocculation.

Some approximation of the potential at the mucosal surface can be made. Mucous coated particles have a limiting mobility value of between 1•35 (normal rats) and 1•20 (fasted animals) corresponding to potentials of 16•9 and 15•0 mv. respectively. Thus the potential between two mucus coated particles, or between a mucus coated and a stabiliser coated particle will be essentially similar and the variation of potential with separation of the particles will certainly be covered by the graphs shown. There is a very large potential energy barrier to be surmounted if particles are to adhere at the primary minimum (Fig. 1.5.) but weak association at the secondary minimum may be possible, but unlikely. The surface potential, ψ_o of particles coated with mucus derived from fasted animals will be slightly different from those coated with mucus derived from normally fed animals, but the difference is by no means sufficient to explain the smaller affinity of the mucous lining in fasted animals for the coated barium sulphate particles.

Further, if the mucous coated micro villi are considered to behave as particles, similar potential energy curves for the interaction of the micro villi and mucus or stabiliser coated particles will be similar to those plotted, though the ionic strength in the glycocalyx is uncertain. What is clear is that on a purely physical chemical basis there is a large potential energy barrier to be overcome if particles are to approach the mucous surface and adhere at the primary minimum. Association at the secondary minimum, at distances of 60 - 100 A^o is not likely as the minimum is so shallow. Interaction between particles and the mucosal surface cannot thus be considered as only electronic, and it is doubtful if electronic forces are in fact involved to any great extent.

It is pertinent to ask whether the intentions(Chapter 1. Section 7.) of the model systems have been fulfilled, and how far the results obtained from the study of the model systems can be used to predict the behaviour of barium meals in the clinical situation. The advantages of the two model systems have already been discussed (Chapter 4. Section 3., Chapter 5. Section 2.); neither model is fully satisfactory, primarily because of the method of evaluation *in vitro* and animal variation *in vivo*, uncertainties in the knowledge of the exact conditions in the gut (e.g. suspension concentration) *in vivo*.

In retrospect the use of intestinal tissue from fasted animals *in vitro*, to minimise washing before the experiment, can be seen as a mistake. Although less washing was required, the mucous membrane was more delicate, and much more easily disrupted so that even the reduced handling had a marked effect. The affinity of the mucus for barium sulphate, if the situation *in vivo* obtains *in vitro*, is reduced, and this could account for the relative thinness of coatings observed, when compared to those obtained *in vivo*.

It can be asked whether the results using rats, however interpreted can be applied to the situation in man. Since the full structure of mucus for any species has not been elucidated, differences in the mucus cannot be evaluated. One would not expect, however, any great differences in either composition or structure, since the function of the mucus, viz. protection of the villi from an adverse physical and chemical enviroment, and lubrication, in rat and man is the same. The effect of dieting and fasting upon the mucous membrane is also thought to be the same. Other animals, notably the dog, have been used in clinical trials (Root & Morgan, 1969). These workers compared a commercial product with unstabilised

suspensions, these being administered to fasted animals. They concluded that small volumes of commercial preparations gave a better visualisation of the gastro intestinal tract, but made no attempt to apply their results to the human clinical situation.

The main aim of the present study, however, was not to mimmic precisely the chemical in vivo situation, but to detect and examine factors which affected the adhesion of barium sulphate to the mucosal surface. The effect of changing these parameters could then be investigated.

Unstabilised barium sulphate suspensions are widely reported as being unsatisfactory in vivo; these showed peculiar characteristics when used in the model systems, giving thick, uneven covering of the surface in vitro, and no limiting adsorption concentration in in vivo tests. Any preparation which gave similar results under the conditions of the tests would not be expected to behave satisfactorily in a clinical trial. Only suspensions with citrate as an additive showed similar characteristics, but no systematic clinical trials of such suspensions have been reported.

A stabilised suspension containing excess stabiliser has been investigated clinically (Bircher et al, 1971) and gave generally satisfactory and consistant results. The barium meal described by these authors is similar to Micropaque. If a suspension gave similar results to Micropaque when used in the model systems, one would expect satisfactory results in a clinical trial. Most suspensions did in fact show similar characteristics to Micropaque. Presumably all commercial preparations have proved satisfactory in clinical trials before marketing? Results from the model systems therefore reflect to some extent the clinical behaviour of a barium meal. The model systems may therefore be useful not only in investigating specific parameters affecting the adhesion of barium sulphate to the mucous membrane, but also in the initial development of a new preparation, and the investigation of unsatisfactory commercial preparations.

Some general observations and conclusions of the study of model systems can now be attempted. For all stabilised suspensions an increase of the concentration of particulate barium sulphate above 50% w/v. did not result in any increase in the amount adsorbed by the gut surface. There would, therefore, seem to be little point in going to great lengths to achieve a concentration greater than this at the gut surface. Further a concentration of 12·5% w/v. Micropaque suspension gave an even and thin coat, under the best conditions. The situation is complicated in the clinical situation in that a suspension has to pass through the stomach before entering the small and large intestines; it is, indeed, in the lower gut that difficulties are commonly encountered. The radiopaque has to withstand the physiological conditions in the stomach, and must not ogglomerate or precipitate if there is to be any possibility of an even coat on the mucousal surface of the small intestine. If agglomeration does take place in the stomach increasing the effective concentration will not improve the visualisation of the small intestine. The use of unstable preparations causes an initial non-uniform concentration at the gut surface because of sedimentation, according to the position of the patient, but since the greater concentration of barium sulphate does not result in greater adsorption in the surface, peristalsis, or manipulations by the examiner should result in sedimented particles passing along the gut. As more free mucus is present near the gut surface the use of unstable preparations can result in more barium sulphate particles becoming coated with mucus, with adverse effects upon their stability in the lower regions of the gut. A higher concentration of stabiliser in the preparation will result in a higher proportion of the suspension passing unchanged through the stomach, and thus improve the chances of good visualization of the small intestine.

Of all parameters affecting the adsorbed layer the state of the mucus was the most important. To ensure that a patient's gastro intestinal tract is clear of food, it is normal for him to be fasted 24 hours before examination. It has been shown that this procedure could drastically affect the adsorption characteristics of the mucous membrane, i.e. there is a smaller up-take of the barium meal in the fasted animals. This may be a result of the experimental technique only. Since the mucus is less viscous, it, and its associated barium sulphate particles are more easily removed by the subsequent washing procedures. The fact, however, that the same amount of barium sulphate is adsorbed for both 50% and 75% w/v. suspensions, despite the different abrasive effects that such suspensions must have, and the longer washing time necessary for the more concentrated suspension, suggests that the decrease in adsorbed barium sulphate is a real effect and is due to a change in the nature of the mucus.

If the situation is similar for human examinations, it would be useful to investigate the effect of diet upon the thickness and uniformity of the adsorbed barium sulphate layer. Diets could be low residue - and need not be carbohydrate only, since this affected the barium sulphate layer as much as total fasting - but could be high protein/carbohydrate with fat. The results from the model system suggest that investigation of patient preparation before examination would be worth while.

The site of adsorption for <u>in vitro</u> systems has been demonstrated (Plate 4.1.). Penetration of the mucus membrane by barium sulphate is deep, and it is not surprising that suspensions can remain in the gut for several hours, or even days. It will only be removed when the mucus is sloughed off and replaced, although even then interaction between the old mucuos coated particles and the newly secreted mucus is possible. The claim that particles are "mucophilic" which has been brought into question, would seem to be justified, since several photographs revealed barium sulphate present in the lumen of the gut, obviously attached to free mucus. Raybar, prescribed for examination of the lower

bowel in cases of steatorrhea, when there are copious amounts of free mucus present, gave the poorest coating of the mucus membrane. This is perhaps due to the very high concentration of hydroxy ethyl cellulose (around 5% w/v.) present preventing the coating of particles with mucus, and their subsequent attachment to free mucus, or mucus at the surface. The preparation is efficient for such conditions.

Increasing the speed with which a suspension travels through the gut would seem to decrease the possibility of particles becoming agglomerated before becoming usefully attached to the gut wall. However, the additions which seem to achieve an increase in speed by increasing peristalsis, also increase the rate of secretion of the various juices and mucus. When food additives, such as oil and sorbitol, are present, which not only increase secretion, especially if the gut is in a fasted condition, but do little to decrease the transit time of a suspension, a decrease in radiograph quality is to be expected, since peristalsis increased, and there is a possibility of large areas of surface mucus being sloughed off. This would result in flakiness, and greater agglomeration of the barium meal.

Ulcers, whether gastric, duodenal or intestinal, are thought to result from attack of the gut wall by digestive juices. This can occur when the mucous layer in a certain area is damaged, or non existant. It is this condition, amongst others, that the barium meal is required to reveal. However, it has been shown that the barium sulphate particles take up mucus. If a large concentration of barium sulphate is present in the gut, a great amount of mucus can be adsorbed by the particles, especially if additives are used to precipitate the suspension in the gut, and the gut is further manipulated. The removal of surface mucus, even though it can be quickly replaced can only complicate such conditions, conditions which the barium meal is supposed to detect. This, again is another reason for using as low a concentration of meal in the gut as possible.

It has been shown that an electronic interaction between particles and the mucous surface is unlikely to occur. The pH in the gut does not affect adhesion, and particles coated with different stabilisers all behaved similarly, and particles, when adsorbed on the gut surface became fully coated with mucus. General theories of adhesion are very few, and the reason that certain surfaces feel "sticky" is uncertain. The orientation of molecules to the surface, increasing or decreasing the number of groups available for hydrogen bonding with an adjacent surface, has been put forward. It is a well known phenomenon that a gum, when dry, does not exhibit surface "tackiness". If the gum imbibes water it becomes sticky, but as more water is taken up this decreases, and adhesive properties diminish. The viscosity of the gum also decreases as water is added to the gum, and after a maximum in "tackiness" this property decreases with the decrease in viscosity. Thus it is possible that some relationship exists between the adhesiveness of a gum and its viscosity. Surface mucus derived from fasted animals is both less tacky (since it will not adhere to glass) and contains a greater proportion of water. Attempts to measure the viscosity of such samples, with a rheogoniometer were unsuccessful, due to experimental difficulties, but measurements are feasible and are definitely a possible line of investigation.

In the gut the barium sulphate particles become associated with the mucous membrane by a process of "capture", whereby they become fully coated with mucus. The process continues until the surface is saturated; corresponding to a definite concentration of suspension (50% w/v.). The thickness and coherence of the adsorbed layer is controlled by the rate at which this layer is sloughed off, either due to peristalsis, or the secretion of fresh mucus. Peristalsis will disrupt the layer most if the mucous membrane is delicate, as is the case in fasted animals and

in certain disease conditions. This could be due to the greater water content of the mucus, and the suspensions,especially if unstabilised,could disrupt the membrane by an abrasive effect alone.

At this junction it is worth discussing the essential criteria for a barium meal. The perfect meal, it would seem, cannot be preparaed, since it would have to incorporate different, and indeed, incompatible physical characteristics. (1)The barium sulphate must be stabilised, not only to give the suspension some characteristics of a hydrophilic colloid, but also to prevent barium ions passing into the gut. (2) The optimal concentration of barium sulphate required per unit surface area of the gut, under specific conditions, is not known, The present study suggests that concentrations higher than 50% w/v. in the gut are not required, and that much less than this can coat the gut firmly and evenly. (3) The stability of the suspension, i.e. shelf life, has featured too prominently in the evaluation of meals. Stability is not over important, as long as dispersive properties are adequate. (4) Excess stabiliser must be present to minimise ogglomeration of suspensions in the gut. If the stabiliser concentration is too high then dispersive properties suffer, due to the increase in viscosity, and yield value. (5) As far as the nature of the stabiliser is concerned, no one stabiliser in the commercial preparations seemed superior to others. Carboxy methyl cellulose, as a stabiliser, has all the required properties, since solutions of high cmc. concentration but low viscosity can be prepared. If the cmc. is highly substituted the particles will be greatly hydrated and disperse readily. Claying upon storage can be prevented if the preparation is spray dried and the meal provided as a powder to be suspended in water.

Thus a suspension should have a high concentration of cmc. as stabiliser ($\geqslant$ 0·8% w/v. for 60% w/v. barium sulphate suspensions, but have a low viscosity, and thus stability (RS. $\leqslant$ 10) so that low concentrations, of suspension can be administered, but sediment in the gut to produce the required surface concentration.

The two model systems developed have proved valuable, and a great improvement upon previously laboratory testing techniques. The in vivo method, in which the coating of barium sulphate on the gut is quantitatively determined is truly objective. However, with the limited number of animals used for the experiments some of the several conclusions are not as clear cut as could be wished. Now that the technique has been established statistically designed experiments can be planned.

The results from the in vivo and in vitro experiments would have been easier to interpret if X-rays of the animals had been taken at the same time. The gross amount of barium sulphate taken up by the gut and the subjectively assessed radiograph could then be correlated. This would result in the laboratory behaviour of suspensions being more easily evaluated and their clinical performance more accurately predicted.

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E R R A T A

P.18, line 3 should read: confusion arises because no clear distinction is made between the sizes of individual particles, and aggregations thereof. The diameter of individual particles, as determined by electron microscopy is generally under 0.5 μm.

P.30, line 10 should read: when finely divided material is dispersed in such a phase, the energy is modified by interactions between the surface of the particles and the medium in which they are dispersed. Depending on the energy associated with such interactions the dispersed colloid may remain unstable (or metastable) with respect to the bulk phase (lyophobic dispersion) or may become thermodynamically stable (lyophilic dispersion).