NEURAMINIC ACID, FUCOSE AND SULPHATE CONTENT OF SPUTUM IN DISEASE AND DURING TREATMENT

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MARIA TERESA LOPEZ-VIDRIERO

L.M&S (Madrid), M.D.(Madrid)

Department of Experimental Pathology
Cardiothoracic Institute
Brompton Hospital
University of London

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ABSTRACT

Sputum is a mixture of epithelial glycoproteins and glycoproteins derived from serum. Bronchial glycoproteins contain large amounts of carbohydrate - particularly fucose and N-acetyl neuraminic acid - and sulphate groups have also been reported but mannose is not usually found. In contrast, serum glycoproteins contin a few carbohydrate units but are rich in mannose and N-acetyl neuraminic acid with little fucose or sulphate. Fucose and sulphate have, therefore, been used as marker substances of bronchial glycoprotein, mannose of serum glycoprotein and N-acetyl neuraminic acid of both bronchial and serum.

In the present work these marker substances have been studied in normal bronchial fluid and in sputum so that their natural variation, change in disease states and response to treatment can be established.

Sputum produced by normal subjects after inhalation of prostaglandin F2α represents stimulated secretion from a normal bronchial tree and is shown to have a negligible amount of serum transudate.

A diurnal variation in the marker substances is observed in sputum from patients with chronic bronchitis, but from year to year the sputum of a given patient is very similar. Each seems to have an individual chemical pattern.

Variation in marker substances, particularly N-acetyl neuraminic acid and mannose, is found to be greater between macroscopic types than between diseases.

The wide range of values of marker substances, previously reported in asthmatic sputum, has been elucidated since the various types of asthma are separately analysed.

Two types of bronchorrhoea are described according to response to steroid or atropine treatment. In diseases not associated with bronchorrhoea the effect of atropine is on salivary rather than on bronchial secretion.

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CHAPTER I

HISTORICAL INTRODUCTION

SPUTUM

"The examination of the sputum, by which is meant all the stuff which coughing and hawking mechanically eject from the respiratory passages is of importance, because through it pathological products, which have been formed in organs hidden from sight, are brought to view and indicate by their character and quality the existance of processes whose exact nature cannot perhaps be estimated, at least for the time, by other means."

This paragraph taken from Troup's "Sputum its Microscopy and Diagnostic and Prognostic Significations" (1886), summarises the complexity of the material to be examined, stresses the importance of examining such material whose chemical composition and physical properties, even in our days, are still a source of speculation.

Definition

Blancard (1684) defined sputum as a secretion "thicker than ordinary spittle", thus pointing to one of the physical properties of sputum. Hooper's definition of sputum was based on its constituents and their origin: "that which is cast out of the mouth by spitting or hawking as spittle, is

properly sputum; but it also applies to expectorated matter or that which comes from within the chest and it is spit out".

A more precise definition of the word sputum is given by the Shorter Oxford Dictionaire (1950): "saliva or spittle mixed with mucus or purulent matter, and expectorated in certain diseased states of the lungs, chest or throat, a mass or quantity of this". Three key words are mentioned in this definition: saliva, mucus and purulent matter; in earlier definitions these components have been included in vague terms such as "that" (Hooper 1831) or "stuff" (Troup 1886). It is interesting to note that the word mucus was not mentioned in the early definitions probably because of the confusion, at that time, on the use of the terms mucus, mucilage, gel, mucin, schleimige etc., and also to the poor knowledge of its chemical composition.

Bostock (1805) was the first to distinguish, on chemical basis, between albumin and mucus and from then on the study of the chemistry of mucus caught the interest of many leading biochemists of the XIXth century.

The presence of purulent matter in sputum is important since it may contribute to its physico-chemical properties.

Study of Sputum

The history of the study of sputum could be divided into three phases, each one being concerned particularly with one disease.

."Phthisis" was the primum movens of the first phase. It is difficult to know when this phase started but the earliest references go back to Hippocrates (460-345 B.C.). Hippocrates in The Prognostics and the Aphorisms described with detail not only the macroscopic appearance of sputum but also other characteristics such as smell and introduced two tests for assessing the prognosis of pulmonary tuberculosis. One was the heat test: "in persons affected with phthisis, if the fluid expectorated by the phthisical yield, when exposed to heat, a fetid odour, and the hairs fall off, death is denoted (Coar 1822). The second test, known as the floatation test, which states that "if expectorated matter sinks in sea-water, the disease will shortly be fatal"; this test was still being used by physicians in 1936 (Webb 1936).

The next milestone in the macroscopic examination of sputum was Christopher Bennet (1720) with his Theatrum Tabidorum. He described the different types of sputum: white and frothy, yellow colour, blackish, bluish or rust, dirty ash-coloured and purulent; according to its taste he also described the "salt spittle" and the "sweetish spittle" commonly expectorated by patients suffering from consumption.

Leeuwenhoeck (1674) opened a new era in the study of sputum; he was the pioneer of the microscopic examination and described for the first time the presence of globules and odd corpuscles in sputum. From Leeuwenhoeck until the XIXth century there seems to be a "dark period" for the microscopic examination of sputum, which ended when Pearson published his work in 1809. After Pearson, a remarkable interest was shown in sputum microscopy judging by the large number of monographs and traitises published (Henle 1838; Vogel 1838; Biermer 1855; Troup 1886; Mckenzie 1886; Bezancon and de Jong 1912; Hoesslin 1921).

In the last quarter of the XIXth century the cytological study of sputum declined and the bacteriological examination took over. The

principal aim of this period was to discover the cause of tuberculosis and other infectious diseases and it reached its peak with the discoveries of Koch, Frankel, Friedlander and Pfeiffer.

The disease responsible for the second phase in the study of sputum was lung cancer. Although the study of sputum for exfoliated tumour elements is of recent origin, the first reports in the literature date from the eighteen fifties (Walshe 1851, 1860; Lancereaux 1858).

The first systematic investigation on the cytological diagnosis of lung cancer by examination of sputum for exfoliated neoplastic cells was made by Dudgeon and his colleagues in 1935. A few years later, Papanicolaou (1942) opened a new field in the cytodiagnosis of cancer and ever since his technique has been proven to be a valuable tool.

The third phase - chemical and rheological - was triggered by a disease associated with dysfunction of all exocrine glands including those that are mucus producing. This generalised, inheritable disease of unknown cause is cystic fibrosis (Andersen 1938), one of the pathological findings being abnormally viscous secretions from

the gastro-intestinal and respiratory tracts. Ninety per cent of the patients suffer with chest symptoms which account for a high percentage of the mortality rate; therefore it is not surprising that the study of the chemical composition and rheological properties of the sputum produced by these patients is of great importance.

GLYCOPROTEINS

Definition

The term "glykoproteide" was first used by Hammarsten (1885) to define a carbohydrate-protein complex joined together by a firm chemical bond. Jeanloz (1960) described glycoproteins as those compounds containing a carbohydrate component attached to a polypeptide component through a strong covalent linkage, thus specifying the type of firm chemical bond mentioned by Hammarsten. Few years later Gottschalk (1966) introduced two new factors: the importance of the number of sugar residues and the lack of a serially repeating unit and he defined glycoproteins as "conjugated proteins containing as prosthetic group one or more heterosaccharides with a relatively low number of sugar residues, lacking a serially repeating unit

and bound covalently to the polypeptide chain"

Glycoproteins, as a group are therefore characterised by the occurrence of covalently attached carbohydrate units that have structural features in common, and require similar enzymatic mechanisms for their assembly and degradation. They are widely distributed in nature: animals (vertebrates and invertebrates), plants, unicellular organisms and even viruses.

Structure

The three main chemical features of glycoproteins were established in the XIXth century:

Eichwald (1865) working with material from ovarian cyst fluid, showed that it was a carbohydrate-protein complex.

Landwehr (1882): the carbohydrate of mucin was not a single monosaccharide.

Hammarsten (1885): carbohydrate and protein were linked by a firm chemical bond.

The development of new chemical and physical methods have widened our knowledge on the structure of glycoproteins particularly on the nature of the carbohydrate units, types of carbohydrate-peptide linkages and aminoacid sequences along the

polypeptide chain.

Nature of the carbohydrate units - Sequential enzymatic degradation with glycosidases, graded acid hydrolysis, alkaline borohydrate treatment, periodate oxidation or methylation have been used to isolate the carbohydrate units and to determine their size, number and position. Glycoproteins contain from less than 1% to 80% carbohydrate by weight (Spiro 1973), the sugars most commonly found being, galactose, fucose, galactosamine, glucosamine, mannose and sialic acids (particularly N- and O-acetyl and N-glycolyl derivatives of neuraminic acid).

According to the charge of the sugar units or presence of sulphate at the free ends of the carbohydrate side chains, the glycoproteins can be divided into: neutral (L-fucose, D-galactose or D-mannose) and acid (neuraminic acid or sulphate).

The size of the carbohydrate units range from 162 to 3500 and the number of carbohydrate units per molecule vary from 1 (ribonuclease B, ovalbumin) to 800 (ovine submaxillary glycoprotein). The ratio of the number of aminoacids per carbohydrate unit can be used as an index for

assessing the degree of carbohydration of a protein. Mucins tend to have a lower number of aminoacids per carbohydrate unit than serum glycoproteins. The ratio in Ig G is 779 aminoacid residues per carbohydrate unit, while in ovine submaxillary mucin the ratio is 6 per carbohydrate unit.

Carbohydrate-peptide linkages - Meyer (1938, 1945) based his classification of the carbohydrate-protein complex on the type of chemical union (ionic or covalent) between the carbohydrate and the peptide. Jeanloz (1960) distinguished the polysaccharide-protein complexes with a weak linkage (salt linkage or hydrogen bonding: condroitin sulphate) from the glycoprotein group with a strong covalent linkage (submaxillary mucin). The classification on the grounds that covalent linkages only occur in the glycoprotein group was critised by Gottschalk (1966) since covalent linkages, in fact, occur in both groups.

The type of linkage is given by the monosaccharide and aminoacid residues involved. This linkage always involves C1 of the most internal sugar residue and a functional group of an aminoacid in the peptide chain. In the last two decades three major types of covalent linkages

have been established.

1. Glycosylamine-asparagine linkage. The N-glycosidic linkage between C-1 of N-acetylglucosamine and the amide nitrogen of asparagine was first described in hen ovalbumin (Johansen et al., 1961). This type of linkage has also been isolated from other glycoproteins including bovine ribonuclease (Plummer et al., 1968) α1 acid glycoproteins (Yamashina et al., 1965: Wagh et al., 1969) calf and human thyroglobulin (Spiro 1965).

2. O-glycosidic linkage to serine. N-acetyl galactosamine is the sugar component involved and it has been shown that it is the commonest type of linkage in glycoproteins from bronchial secretions (Harbon et al 1964; Anderson et al. 1964) and glycoproteins with blood group activity (Lloyd et al., 1968). N-acetyl galactosamine can be linked to either serine or threonine.

3. O-glycosidic bond to hydroxylysine. It involves O-glycosidic linkage between a galactose residue and the hydroxyl group of hydroxylysine.

This type of linkage is mainly present in basement membranes (Spiro 1967, 1969) and in a large number of fibrillar collagens from vertebrate sources (Spiro, 1969).

Aminoacid sequences:- The three dimensional structure of the glycoprotein molecule is determined by the location of the carbohydrate units of the polypeptide chain. On the other hand the sequence of aminoacid around the carbohydrate linkage could be the recognition signal for the specificity of the enzymatic glycosylation (Neuberger et al., 1969).

The aminoacid sequences vary according to the type of carbohydrate-peptide linkage. In the type involving asparagine the commonest sequence is As-X-Ser (Thr), X being any aminoacid as it has been reported in α1 acid glycoprotein (Schmid et al, 1971), IgG immunoglobulin (Edelman et al., 1969).

In the O-glycosidic linkages to serine and threonine the aminoacid sequence is less well documented and so far the information available is restricted to glycoproteins in which N-acetyl galactosamine is the linkage sugar. Proline seems to be present in most of the glycoproteins studied, particularly in glycoproteins from mucous secretions (Hashimoto and Pigman, 1962). These authors suggested that proline may help to determine the glycosylation by N-acetyl galactosamine of serine and threonine.

The general pattern of aminoacid sequence in the hydroxylysine type of bond is: Glycine - X - Hyl - Gly - Y - Arg, in which X and Y may be any aminoacid (Spiro 1973).

Biosynthesis

Studies carried out with puromycin, an antibiotic inhibitor of protein synthesis, have shown that the synthesis of the peptide backbone takes place before the synthesis of the carbohydrate portion and that these two events are independent (Spiro and Spiro 1966).

The assembly of peptide chains is genetically controlled while the initiation, elongation and possibly termination of oligosaccharides appear to be controlled by substrate specificities of the transferases, every oligosaccharide prosthetic group being assembled by the concerted action of a multiglycosyltransferase system (Schachter and Roden 1973).

Once the protein chain has been synthesized by the ribosomes of the rough endoplasmic reticulum it enters the cisterna of the endoplasmic reticulum on its way to the Golgi apparatus. The assembly of the carbohydrate units involves a series of

enzymes, transferases, which seem to be located in the membranes of the rough and smooth endoplasmic reticulum such as those involved in the assembly of the more internal sugar residues (Whur et al., 1970; Spiro and Spiro, 1972); the membranes of the Golgi apparatus appear to be the site of a number of glycosyltransferases particularly involved in the attachment of the peripheral sugars (Bennett and Leblond, 1970).

The function of the glycosyltranferases is to transfer an activated sugar from its nucleotide derivative to a protein acceptor. The specificity of these enzymes depends on various factors: the base of the nucleotide, the activated sugar, nature of the terminal non reducing sugar of the carbohydrate chain to which the new sugar is to be attached, the penultimate sugar and the type of linkage between the terminal and penultimate sugars. Other important determinant factors are the ionic charge of the peptide chain to which the carbohydrate unit is attached and a defined aminoacid sequence (M.J. Spiro and Spiro 1968, 1971; Roseman 1968; Helting and Roden 1969; R.G. Spiro and Spiro 1971; Ginsburg et al., 1971).

Heterogeneity:- Purified preparations of

glycoproteins frequently differ from each other in structure, this is called heterogeneity and may be associated with modification of the peptide chain or of the carbohydrate portion,either internal or peripheral, of the molecule. When heterogeneity takes place in the peripheral residues at, or adjacent to, the non-reducing ends of the carbohydrate chains has been widely referred to as 'microheterogeneity".

Variations in the structure at genetic level, structural changes during biosynthesis or degradation may be responsible for heterogeneity, and it is possible that they may occur at more than one stage in the formation of a glycoprotein.

Variations in the primary sequence of aminoacids as a result of a genetic factor, has been established in a number of cases (Emura et al., 1971; Wiseman et al., 1972). Results of investigations of glycoproteins from individuals of a given blood group type seem to be against a genetic factor as the cause if microheterogeneity (Watkins 1972). Microheterogeneity may be due to a lack of completion of glycosylation, inhibition of further glycosylation due to the presence of a charge sugar (i.e. sialic acid) prior to completion

of the main saccharide chain or to partial enzymatic degradation of the carbohydrate units in their passage in or out of the cell (Spiro 1973; Beeley 1974).

Physical properties

The carbohydrate units of glycoproteins are responsible for the physical properties of these molecules.

Sialic acid residues seem to play an important role in the physical properties of glycoproteins, particularly when they occupy a terminal position. Spiro (1960) showed that the sialic acid influences the electrophoretic mobility; when sialic acid residues are removed by neuraminidase the isoelectric point increases from 3.3 to 5.2.

Many workers have demonstrated that sialic acid is responsible for the high viscosity of some glycoproteins such as ovine submaxillary glycoprotein (Gottschalk and Thomas 1961), bovine cervical mucus (Gibbons 1959; Gibbons and Glover 1959), ovarian cyst fluid (Odin 1959) and bronchial glycoprotein (Munies et al., 1968).

More recent studies carried out with sputum from patients with chronic bronchitis have shown that the rheological properties of sputum, in particular its apparent viscosity, are related to dry weight, NANA and fucose concentrations, these representing the concentration of bronchial glycoprotein in sputum (Keal 1970; Keal and Reid 1972; Lopez-Vidriero et al., 1973; Charman et al., 1974).

Biological functions:- Glycoproteins are widely distributed in nature and their biological activities are multiple and diverse. A list of human glycoproteins grouped in relation to suggested function is given below:

Transport	Transferrin
	Ceruloplasmin
	Thyroxin-binding globulin
	Corticosteroid binding globulin
	ß lipoprotein
	Lactotransferrin
	Intrinsic factor
Enzyme	Cholinesterase
	Atropinesterase
	Amine oxidase

	α amylase
	Ribonuclease
	Deoxyribonuclease
	Lipase
	Kallikrein
Immunoprotective	Immunoglobulins Ig G, Ig A, Ig M
	Clq complement
Hormone	Chorionic gonadotropin
	Follicle stimulating hormone
	Luteininzing hormone
	Thyroid stimulating hormone
	Thyroglobulins
Lubrication-protection	Submaxiallary glycoproteins
	Gastrointestinal mucous secretion
	Bronchial mucous secretion
Receptors cell adhesion	Red cell membrane glycoprotein

SOURCE OF BRONCHIAL MUCUS SECRETION

Gland/cell system. Mucus secreting cells

Two types of mucus secreting cells have been described: the goblet cells of the surface epithelium and the cells of the submucosal glands (Florey et al., 1932).

Goblet cells:- Goblet cells are non ciliated cells of the bronchial surface epithelium, the number varies from one in four epithelial cells in main bronchi to one in 200-300 in the periphery. The height of the goblet cells is 20 μ and the volume of all goblet cells has been estimated to be 0.5 ml. (Reid 1960). Electron microscopy studies have shown that these cells have an electron dense cytoplasm, an irregular nucleus and many secretory granules each measuring 1 μm. In normal human bronchi these granules are electron dense with a well defined limiting membrane (Jeffery and Reid 1973).

Submucosal glands:- Submucosal glands lie between the epithelium and cartilage, but occasionally they could be seen between the plates of cartilage or even external to them (Miller 1947; Reid 1968).

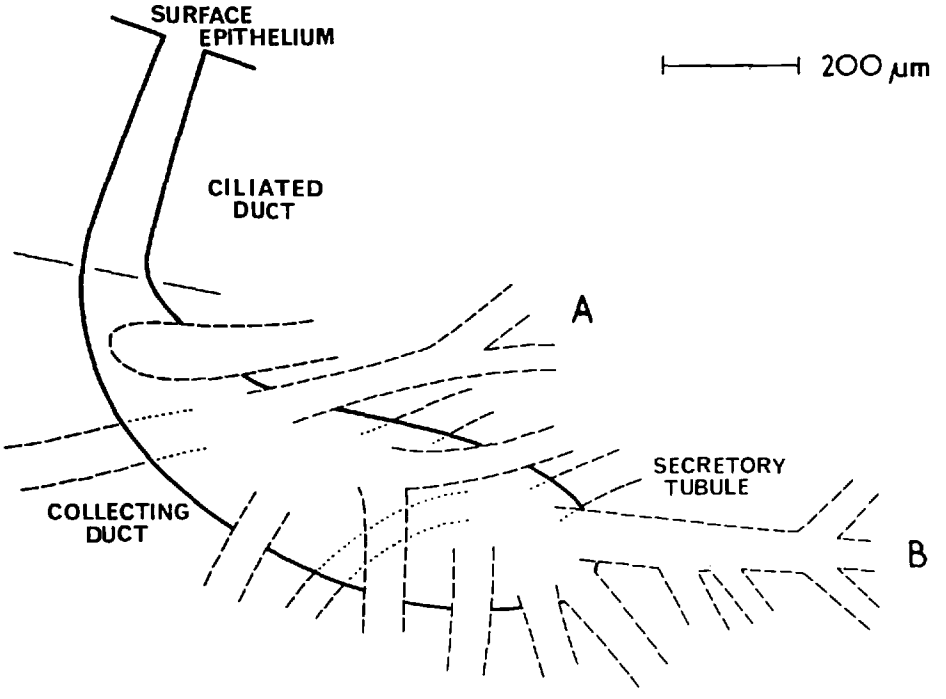
In the normal human bronchial tree the submucosal glands are found in those airways with

cartilage in the wall. The bronchial glands are branched tubular glands, two cell types have been described: the mucous and the serous or albuminous (Fuchs-Wolfring 1898), also known as distended and granular (de Haller and Reid 1965). The gland concentration is one gland per square millimetre (von Hayek 1953) and the volume has been estimated to be 6 ml., that is 40 times greater than that of goblet cells.

The submucosal gland consists of a ciliated duct which up to a distance of 200 μm is lined by a pseudostratified ciliated columnar epithelium, beyond this point the epithelium is replaced by one composed of non ciliated cells, rich in mitochondria, and it is called the collecting duct. This collecting duct gives rise to numerous branches known as secretory tubules which can be of two types: mucous and serous (Meyrick, Sturgess and Reid 1969). The mucous tubules always terminate in serous tubules, therefore the secretion from the serous cells passes over the mucus secreting cells on its way to the collecting and ciliated ducts. A graphic reconstruction of human bronchial submucosal gland is shown in Fig. I,1.

Figure I,1 Reconstruction of human submucosal gland showing region of collecting duct from which mucous tubules (A and B) open

Fig. I,1


SURFACE
EPITHELIUM
200 µm
CILIATED
DUCT
A
SECRETORY
TUBULE
COLLECTING
DUCT
B

Other cell types have been described with the light and electron microscopes: myoepithelial cells (Fuchs-Wolfring 1898; Meyrick and Reid 1970) have been found at the base of serous, mucous and collecting duct cells and it seems very likely that their action contributes to the passage of secretion along the tubules and collecting duct, since motor nerves have been seen between the basement membrane and the secretory cells (Jeffery and Reid 1973).

Collecting duct cells have been described by Meyrick et al. (1969) and Meyrick and Reid (1970), these cells are packed with mitochondria and may be responsible for regulating the water and ion balance of the gland secretion.

Clear cells are identified by their thin layer of cytoplasm, few organelles and mitochondria (Meyrick and Reid 1970). These cells resemble lymphocytes and therefore may be responsible for the production of immunoglobulins, particularly Ig M (Meyrick and Reid, 1970).

Changes in disease:-

Chronic bronchitis:- Reid (1954; 1960) described the basic structural changes in chronic

bronchitis: hypertrophy of mucus secreting structures and increase in number of goblet cells particularly in the peripheral airways. The gland/wall ratio (Reid index) has been proven to be a useful tool for assessing the degree of gland hypertrophy. The normal range being 0.14 - 0.36 with a mean of 0.26, while in chronic bronchitis was found to be 0.59 with a range of 0.41 - 0.79 (Reid 1960). De Haller and Reid (1965) found an increase in the number of mucous cells relative to the serous cells.

Asthma:- Houston, De Navasquez and Trounce (1953) studied bronchoscopy material from patients suffering from asthma without sputum production and reported no quantitative or qualitative abnormalities in the mucous glands and goblet cells. Similar findings have been reported by Glynn and Michaels (1960) although in some cases they found a moderate to marked increase in goblet cells.

Cystic fibrosis:- Reid and de Haller (1967) reported that newborn children with cystic fibrosis had a similar pattern of distribution of goblet cells and submucosal glands to that found in the foetus and normal newborn, that is there were no goblet cells at the periphery and the submucosal glands were of normal size. The histological changes seen in late stages of the disease, when

infection and hypersecretion are present, are similar to those found in chronic bronchitis.

Histochemistry: types of glycoprotein

Different histochemical techniques have been used to identify the acidic groups, sialic acid and sulphate of intracellular glycoproteins.

Alcian blue (AB) in combination with periodicacid Schiff (PAS) stain the acid glycoprotein blue and the neutral red. Further studies using enzymes such as neuraminidase which removed the sialic acid residues, followed by staining with AB/PAS have shown that the sialic acid in acid glycoproteins can be totally or partly susceptible to this enzyme (McCarthy and Reid 1964). Acid hydrolysis of the sialic acid resistant glycoprotein showed that some cells lost all the blue staining while others did not and these were found to be sulphomucin by using sulphate specific stains or uptake of radioactive sulphate in tissue culture (Lamb and Reid, 1969; 1970).

More recently Jones and Reid (1973a) have identified three sulphate groups by using AB at various pH together with high iron diamine (HID). Five types of acid glycoproteins have been described.

1) Neuraminidase susceptible neuraminic acid.
2) Neuraminidase resistant neuraminic acid but susceptible to acid hydrolysis.
3) Sulphate staining with HID and AB.
4) Sulphate not staining with HID but staining with AB at pH 2.6 and 1.
5) Sulphate staining with HID or AB at pH 2.6 but not staining with AB at pH 1.

Lamb and Reid (1969) studied the intracellular distribution of the several types of acid glycoproteins and they identified six different cominations of acidic groups in mucous cells. (Fig. I,2).

Comparison of the uptake of radioactive glucose and sulphate between adjacent mucous and serous cells has shown that the serous cells have a higher degree of sulphation per unit of radiactive glucose uptake (Lamb and Reid 1970).

Species and regional variation

Comparative studies of mucus secreting structures in the tracheobronchial tree of man and animals have shown that there is a species variation (Goco et al., 1963). The tracheobronchial tree of pig contains large numbers of glands but no goblet

Fig. I,2 Types of acid glycoprotein identifiable histochemically and their intracellular combinations.

Fig. I,2

Type of Acid Glycoprotein	Individual Cell Type					
	I	II	III	IV	V	VI
a) Sialomucin						
Sialidase susceptible	+	+	+			
b) Sialidase resistant		+	+	+		
c) Sulphomucin						
S 35 uptake +ve			+	+	+	
stains -ve						
d) S 35 uptake +ve				+	+	+
stains +ve						

cells, the albino rat has goblet cells but no glands and in the horse neither glands nor goblet cells have been demonstrated. (Goco et al., 1963).

Histochemical studies (Reid et al., 1962) have also shown a species variation, in the mouse all sialo-mucin of the surface epithelium and glands is neuramidase susceptible and there is no sulphated glycoprotein, while in the rat trachea most cells take up sulphate and all the acid glycoprotein is resistant to neuraminidase.

A regional variation was reported in the rat, the peripheral airways contain no sulphated glycoproteins and the acidic groups seen were neuraminidase susceptible, while in the trachea the acidic groups are sulphated and neuraminidase resistant.

Although there is a wide variation between individuals, the pattern found within one bronchial tree seems to be consistent (Lamb and Reid 1972a).

Variation with age:-

During fetal life, from the 14th week until birth, all mucous cells produce sulphated glycoprotein of which 85% stains with specific stains for sulphate but no neuraminidase susceptible sialic acid is produced (Reid and

de Haller 1967; Lamb and Reid 1972b). After birth and within a period of weeks there is a rapid fall in the percentage of area of mucous cells producing sulphated glycoprotein. Over the first 2 to 3 years of life the percentage of mucous cells producing sulphated glycoprotein falls and the percentage of sialoglycoprotein rises to the normal adult levels.

Variation in disease

No abnormal type of acid glycoprotein has been found in disease. The histochemical changes reported in chronic bronchitis, bronchiectasis and cystic fibrosis (older children when infection is present) seem to follow a similar pattern: there is an increase in neuraminidase resistant and sulphated glycoproteins (Reid 1968; Lamb and Reid 1969; 1972c).

Control of tracheobronchial mucus secretion

Fuchs-Wolfring (1898) was the first to describe the effect of the parasympathetic stimulation on the tracheal glands of rabbits, the duct lumen became di lated and the cells depleted of granules.

Direct nerve estimulation on decerebrated dogs (Kountz and Koenig 1929), show that vagal stimulation increased bronchial secretion and sympathetic stimulation either diminished secretion or had no effect. Florey et al. (1932) working with cats and dogs showed that the submucosal glands were principally under parasympathetic control since secretion increased after stimulation of the recurrent laryngeal nerve or treatment with pilocarpine and was inhibited by atropine. These authors reported that goblet cells respond to direct irritation but do not secrete after nervous stimulation.

Policard and Galy (1945) suggested that a dual control mechanism (parasympathetic and sympathetic) may be responsible for mucus secretion. Recently Richardson (personal communication) has shown that electrical stimulation of the stellate ganglia in cats (which carry the sympathetic nerves to the trachea) caused a 70% increase in mucus secretion and this effect was slightly inhibited by α adrenergic blockade (phenoxybenzamine) and substantially diminished by β adrenergic blockage (propanolol). These studies support the findings reported by Jeffery and Reid (1973).

These authors found large numbers of nerves, adrenergic and cholinergic, lying within the surface epithelium.

CONSTITUENTS OF BRONCHIAL FLUID

Bronchial fluid is a mixture of secretion from mucus-secreting structures, serum transudate, surfactant and other substances of cellular origin. Bronchial fluid, in absence of infection, consists almost entirely of water, about 95% while the total solids account for 5% distributed as follows: ash 1.13%, DNA 0.028%, carbohydrate 0.951%, protein 1% and lipids 0.84% (by weight of sputum) (Matthews et al., 1963).

pH - Steinmann (1956) claimed that different factors might influence the acid-base equilibrium of the bronchial fluid i.e. carbonic acid, rate of secretion and degree of inflammation. This author found that the pH varied depending on the region of the bronchial tree, being more alkaline in the trachea (6 - 6.7) and more acid in the main bronchi (5.7 - 6) probably due to a higher carbonic acid content in the latter.

Other workers (Guerrin et al, 1968) also measured the pH in situ, at bronchoscopy, and reported that in two patients the pH decreased with the degree of inflammation.

Ryley and Brogan (1968, 1971) measure the pH in the continuous phase of sputum from chronic bronchitic and asthmatic patients and found a wide range from 5.4 to 7.9. Higher values than these have been reported in bronchial fluid from laryngectomised patients 7.45 to 8.15 (Kwart, Moseley and Katz, 1963) and in sputum from bronchiectatic patients 7.8 to 8.2 (Basch, et al 1941). The contradictory reports on the effect of inflammation on pH could be because pH measurements were made in different materials.

Electrolytes:- The osmolality of the water soluble fraction of bronchial fluid from laryngectomised patients has been reported to be 359 mOsm/l, being hyperosmotic in respect to serum (Matthews et al, 1963).

Sodium, chloride and potassium are present in bronchial fluid in higher concentration than in plasma and studies carried out in the water soluble fraction of bronchial fluid and in whole sputum have shown that calcium and chloride, particularly

calcium, are bound to insoluble material (Potter et al., 1967).

Chemical constituents of bronchial mucus:-

Glycoproteins represent the major group of macromolecules in bronchial secretion, 70 to 80% of the dry weight of the non dialysable material (Masson et al., 1965; Havez et al., 1967).

Muller (1901) demonstrated for the first time the presence of a mucin, that is a glycoprotein, in human bronchial secretions. This mucin contained 30 - 35% of sugar residues and he identified hexosamine as one of the sugar residues. The identification and chemical characterization of bronchial glycoproteins did not attract much interest until the 1950s. Werner (1953) identified galactose, fucose and small amounts of mannose as constituents of bronchial glycoproteins; Gyorgi and Rose (1954) reported that bronchial mucus contained substances with blood group activity.

Havez et al. (1966, 1967, 1973) have been able to separate by preliminary treatment of sputum with

disulphide bond breaking agents followed by ion exchange chromatography and gel filtration chromatography, two major fractions: a neutral fraction containing blood group activity rich in fucose and an acidic fraction containing sulfo and sialoglycoproteins. Bronchial glycoproteins are similar in their sugar and aminoacid composition to other epithelial glycoproteins and differ from serum glycoproteins in the relatively high levels of hydroxyaminoacids and proline, the absence of mannose and the presence of large amounts of galactosamine.

Degand et al. (1973a)studied the sugar content of neutral glycoproteins with blood group activity in sputum from chronic bronchitics and found that glycoprotein with H blood group activity contained more sialic acid and sulphate than those with A or B activity; glycoprotein with B group activity showed the lowest levels of fucose and sialic acid. Neutral glycoproteins are characterised by their high fucose content, low sialic acid, negligible or no sulphate and equimolecular content of galacose and hexosamines. Similar results have been reported in purified glycoproteins from bronchogenic cysts (Degand et al., 1973 The

aminoacid composition of the neutral glycoprotein fraction is similar to that of the acid glycoprotein fraction, that is, is rich in threonine and serine, 30% and 15% respectively, and absence of cysteine.

Two types of acid glycoprotein have been separated in purified bronchial secretion, by preliminary treatment with thiol compounds followed by ion exchange and gel-filtration chromatography: a sialoglycoprotein and a sulfoglycoprotein (Havez et al., 1967; Degand et al., 1973). In fact these two fractions are rather similar apart from the degree of sialylation or sulphation.

Recently Roberts (1974) isolated a glycoprotein from the gel phase of sputum by solubilisation with 6M urea followed by fractional precipitation and considered that the glycoprotein components show a continuous variation with respect to their sugar and sulphate content rather than a discrete population of different glycoproteins (neutral, sialo and sulfoglycoproteins) as reported by Havez et al. (1967) and Degand et al. (1973a).

The type of carbohydrate-protein linkage in bronchial glycoprotein is an O-glycosidic linkage between threonine and serine in the polypeptide

chain and galactosamine in the carbohydrate side chains (Havez et al., 1968; Degand et al., 1973a; Roberts 1974). The majority of the sialic acid is linked by an α-ketosidic linkage to C_3 of a penultimate galactose. One half of the fucose residues are linked to C_2 of other penultimate galactose, one quarter to C_4 of glucosamine units already linked at C_3 and the remainder are linked to C_3 of glucosamine units already substituted at C_4 (Roberts 1974).

Immunofluorescence and organ culture studies (Martinez-Tello et al, 1968; Boat et al., 1971) have shown that respiratory tract cells synthesize other glycoproteins such as lactoferrin, secretory Ig A, α 2-globulin and salivary ß1 globulin. Secretory Ig A is a dimer, two 7S Ig A molecules, joined by a polypeptide J component that contains a glycoprotein of low molecular weight called the secretory piece. Exocrine Ig A is synthesized by the plasma cells infiltrating the bronchial mucosa, the secretory piece being produced by glandular and epithelial cells (Tourville et al 1969; Martinez-Tello et al, 1968).

Lactoferrin was first reported in sputum by Biserte et al (1963), immunofluorescence studies

have shown that, in bronchial mucosa, lactoferrin is present in serous cells although it has also been demonstrated in mucous cells.

Fleming (1922) studied the histological distribution of lysozyme and was able to detect it in numerous human tissues and biological fluids including sputum. The presence of lysozyme in bronchial aspirate has also been reported by Lorenz et al. (1957). Under normal conditions bronchial lysozyme originates from alveolar macrophages (Heise and Myrvik 1967) and it has been estimated that its concentration in alveolar macrophages is 2 - 4 μg per 10^6 cells (Myrvik et al., 1961).

Chemical constituents of serum transudate component:-

Studies carried out in fetal tracheal fluid (Adams et al., 1963) have shown that part of the bronchial fluid is serum transudate. Steinmann (1956) observed that labelled albumin appears, within a few minutes of intravenous injection, in the surface of the bronchial mucosa and Bonomo and D'Addabo (1964) using intravenous radio-iodinated albumin were able to demonstrate that

0.5% of the labelled albumin was eliminated in protein bound form into the sputum over a period of ten days.

Electrophorectic and immunoelectrophoretic studies have shown the presence of different serum proteins in the sol phase of bronchial fluid (Biserte et al., 1963; Gernez-Rieux et al., 1963; Dennis et al., 1964; Keimowitz 1964; Masson et al., 1965; Ryley and Brogan 1968; Ryley 1970, 1972; Turgeon et al., 1971; Havez et al., 1973). The following serum proteins have been identified in the sol phase:

Albumin	Ceruloplasmin
Ig A	Fibrinogen
Ig G	α_2 macroglobulin
Transferrin	β_1Ac - globulin
α_1 antitrypsin	D_2A - globulin
Haptoglobin	
Hemopexin	

Other chemical substances of bronchial fluid

Free aminoacids have been detected in tracheobronchial secretions (Kohler et al., 1969) but the source of these aminoacids is at present unknown. Histamine has been identified in sputum

from both non-allergic and allergic individuals (Thomas and Simmons 1969), in sputum of asthmatic patients, serotonin and possibly SRSA are also present. (Harkavy, 1930; Levy et al., 1961).

Havez et al., (1966) isolated kallikrein in bronchial secretions. Kallikrein is an inactive preenzyme which released kallidin from plasma kininogen; kallidin has a strong activity on blood vessels and smooth muscle and is involved in the bronchoconstriction occurring during asthma (Herxheimer and Stresemann 1961; Melon and Lecomte 1962; Abe et al., 1967).

Little deoxyribonucleic acid (DNA) is present in mucoid (non infected) bronchiao fluid and it has been used as a marker of inflammatory reaction (Burgi et al., 1968). Traces of lactic dehydrogenase (LDH) have been detected in mucoid secretions (Potter et al., 1969; Burgi et al, 1968), the isoenzymes found in non infected secretion were 1 and 3, while in infected secretions the isoenzymes were 4 and 5 (Burgi et al., 1968).

The total amount of lipids in secretions from laryngectomised patients accounts for less than 1% of whole sputum (Matthews et al., 1963). Warembourg et al., (1968) estimated the lipid content of the frothy, the fluid and the mucus layer. The frothy layer contained more phospholipids, particularly lecithin, than the fluid or mucus layers, but no triglycerides or free fatty acids were found. These lipids corresponded to the surfactant since similar amounts have been reported in crushed lung (Abrams (1966) and in bronchial lavage fluid (Morgan, Finley and Falkow 1965).

Biological properties of bronchial fluid:-

The main biological functions of epithelial mucus are to protect mucosal surfaces and facilitate the propulsion of the contents of hollow viscera (Hoppe-Seyler 1877). The relative impermeability of mucus suggests a water-proofing action. Negus (1963) observed that removal of body mucus from eels, lampreys and toads impaired the osmotic regulation of their skin.

The mucus covering the surface of the respiratory tract is responsible for the collection, transport and elimination of foreign particles.

The efficacity of the mechanical removal of inhaled micro-organisms and other inhaled particles depends on the functional integrity of the ciliary system and the rheological properties of the mucus layer. Many factors can affect the mucociliary system, systemic and local dehydration, infection, vitamin A deficiency, trauma, temperature, pH, (Bang and Bang 1963; Cragg and Smith 1961; Baetjer 1967).

The antimicrobial activity of the bronchial fluid lies in certain substances that it contains such as immunoglobulins, lysozyme and lactoferrin. Secretory Ig A seems to be more important than serum Ig A in protecting against infection (Cate et al., 1966; Tourville et al., 1969; Small and Waldman 1969). Masson and Heremans (1966) reported that almost all the Ig A present in bronchial fluid was secretory Ig A (82%); studies carried out in children with immune deficient haematological syndromes have shown that secretory Ig A levels in sputum were normal or even higher than in sputum from patients with chronic bronchitis (Miszlai et al., 1973). Lysozyme acts by hydrolysing glycoproteins of bacterial cell walls, specially polymer of N-acetyl glucosamine residues having O-D-lactyl

groups attached (Chipman and Sharon 1969). Masson et al. (1966) reported the bacteriostatic effect of lactoferrin on iron dependent micro-organisms such as Staphylococcus albus.

Nungester and Jourdanais (1935, 1936) showed that laboratory animals resistant to many infective agents developed pneumonia after intratracheal introduction of Diplococcus pneumonia if mucin was administered simultaneously, that is they showed the resistance lowering properties of mucus. Olitzki (1948) postulated that the resistance lowering properties of mucus was due to the coating effect of mucin on bacteria with consequent inhibition of phagocytic digestions and inhibition of antibody uptake. In the respiratory tract in addition to the coating effect, mucus also contributes by occluding the smaller airways and impairing the mucociliary mechanism. Reid (1954) suggested that obstruction of peripheral airways by mucus may predispose to infection and can also provide a mechanism for spread of infection along the airways towards the alveolar spaces.

Physical properties of bronchial fluid:-

Mucus has been defined as a slimy, viscid tenacious substance of the animal body (Darwin, 1778,). Bronchial fluid is a mixture of bronchial mucus secretion and serum transudate, the macromolecular material accounts for 5% of the total weight, the remaining 95% being water.

The difference between fluids and solids is that solids do not flow but are deformed when a force is applied and they store energy. This property represents "elastic" behaviour. Fluids on the other hand flow when a force is applied, the rate of flow being proportional to the amount of force applied, this takes place without storing energy and represents "viscosity". Therefore, bronchial fluid has the physical properties of both a fluid and a solid that is, it is a viscoelastic material.

Sputum is a non Newtonian fluid with pseudoplastic or shear thinning behaviour since its viscosity is a function of the flow rate and an increase in applied shearing force produces a fall in apparent viscosity. Other characteristic

property of sputum is the shear-related and time-related increase in viscosity (Sturgess 1970), which is not thixotropy since the increase in viscosity is also related to the degree of shearing.

Viscosity has been defined as the tangential shearing force per unit area that will produce a unit velocity gradient between two parallel planes a unit distance apart. This can be expressed as:

$$= \frac{F/A}{V/D}$$

where F = required applied force (dynes)

A = area of the planes (cm^2)

V = constant velocity gradient ($cm.s^{-1}$)

D = distance between planes (cm)

$$\frac{F}{A} = \frac{\text{dynes}}{cm^2} = \text{shear stress } (dyn.cm^{-2})$$

$$\frac{V}{D} = \frac{cm.s^{-1}}{cm} = \text{shear rate } (s^{-1})$$

$$\text{(poise)} = \frac{\text{shear stress } (dyn.cm^{-2})}{\text{shear rate } (s^{-1})}$$

Several methods have been used for measuring the viscoelastic properties of sputum.

- Pourability index: although a very crude estimation, it has proved to be a useful method for assessing sputum viscosity. Keal (1970) found in mucoid chronic bronchitis sputum, a significant correlation between pourability grade and viscosity measured with a cone and plate viscometer.
- Capillary viscometers: the general principal of this method is to force a liquid through a fine bore tube and the viscosity of the liquid is determined from the measured volumetric flow rate, applied pressure and tube dimensions. Capillary viscometers can be divided into three main types: cylinder-piston or plunger, the glass capillary and orifice viscometers. These types of viscometers have been widely used for measuring viscosity of Newtonian fluids.
- Rotational viscometers: the basic principal of this method is that a rotating body immersed in a liquid experiences a viscous drag or retarding force; the amount of viscous drag being a function of the speed of rotation of the body. The main advantages of the rotational method is that continuous measurements at a given rate of shear can be made for extended periods of time.

Couette (1890) devised a concentric cylinder viscometer consisting of a rotating cup and an inner cylinder which was supported by a torsion wire and rested in a point bearing in the bottom of the cup. The torque developed in the suspending wire was a measure of viscosity. Based on this principal various rotational viscometers have been used for studying the viscoelastic properties of sputum: Rotovisko, Merrill-Brookfield, Ferranti-Shirley, Weissenberg rheogoniometer among many others.

- Other methods include rolling, falling and sliding spheres and nuclear magnetic - resonance method (NMR).

CHAPTER II

MATERIALS AND METHODS

MATERIALS

Sputum:- Bronchial secretion has been studied as sputum, that is bronchial fluid plus saliva (Fig. II,1). Although a normal human bronchial tree produces 100 ml. of mucus in 24 hours (Policard and Galy 1945), this is imperceptible and is swallowed without producing cough and expectoration. It is not even normal to find enough mucus to aspirate from the airways (Reid, 1970), and for large scale studies bronchial secretion collected at bronchoscopy is rarely possible and the bronchial fluid may be altered by premedication with hypnotics and sympathomimetic drugs.

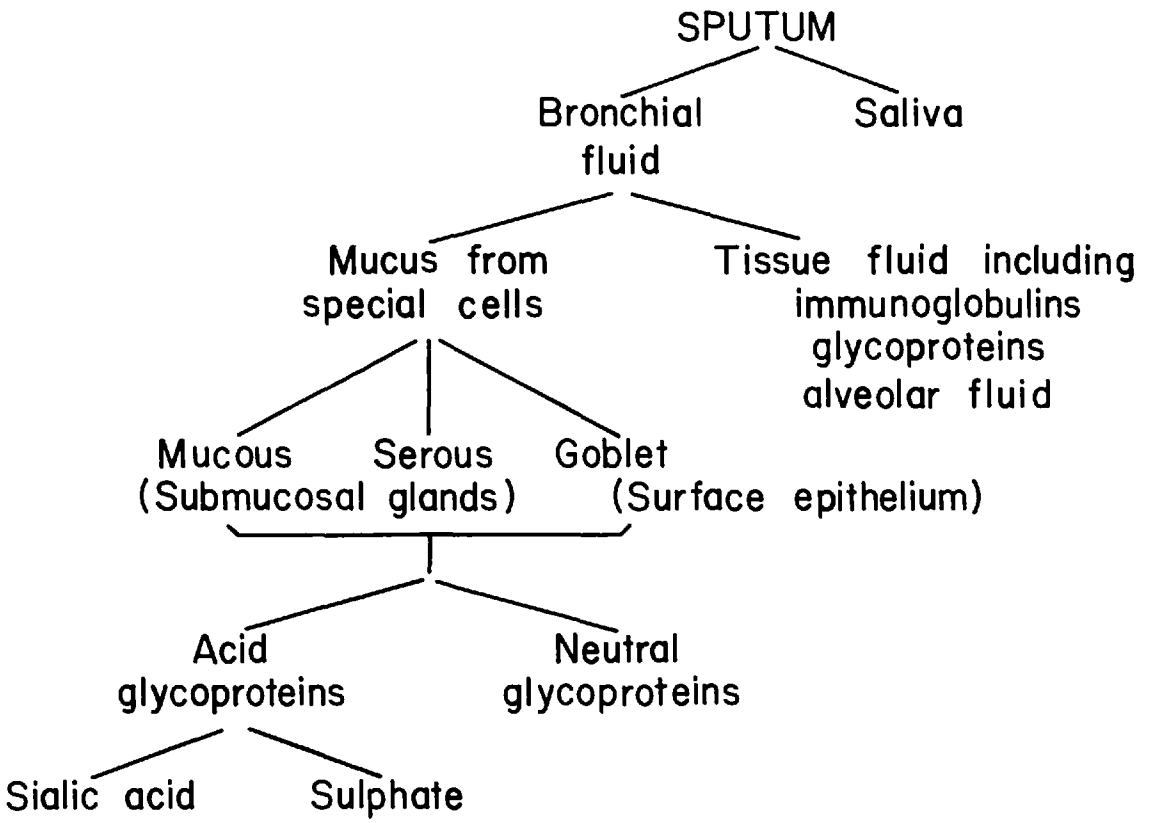
Diagnostic grouping of patients studied:- Sputum samples from the following disease groups were examined:

Chronic bronchitis
Asthma
Bronchiectasis
Cystic fibrosis
Bronchorrhoea

Chronic bronchitis: (MRC 1965) the criteria was hypersecretion of mucus sufficient to cause cough and expectoration on most days, for three months

Fig. II,1 Simplified scheme of some of the constituents of sputum.

Fig. II,1


SPUTUM
Bronchial fluid
Saliva
Mucus from special cells
Tissue fluid including immunoglobulins glycoproteins alveolar fluid
Mucous (Submucosal glands)
Serous
Goblet (Surface epithelium)
Acid glycoproteins
Neutral glycoproteins
Sialic acid
Sulphate

of the year over two successive years.

Asthma: (Ciba 1959, 1971): defined as the condition of subjects with widespread narrowing of the bronchial airways, wnich change its severity over short periods of time either spontaneously or under treatment. The patients were classified as suffering from extrinsic or intrinsic asthma according to the following criteria:

Extrinsic asthma: those cases triggered by known external allergens and supported by immediate, later dual skin responses to the relavant antigen.

Intrinsic asthma: refers to those cases where no allergen can be identified from the history and skin tests remain entirely negative.

Cystic fibrosis: the diagnosis was based on a high sweat sodium (Anderson and Freeman, 1958).

Bronchiectasis: this group includes patients with localised deformity of the bronchial tree demonstrated bronchographically and producing sputum.

Bronchorrhoea: the term bronchorrhoea is used to designate a condition characterised by a daily sputum volume in excess of 100 ml. (Keal 1970, 1971). This condition has been described in association with chronic bronchitis (Kourilsky 1960), asthma (Keal 1971), alveolar cell carcinoma (Wood 1943 ;

Kennamer 1951; Storey et al. 1953; Schools and Ray 1961) and idiopathic (Hartley and Davies, 1923).

Macroscopic examination:- Sputum samples were examined macroscopically before storage and divided into one of three groups according to the content of pus:

Mucoid: white or clear homogenous with no evidence of pus.

Purulent: almost homogenous, green or yellow, showing therefore gross signs of pus.

Mucopurulent: appearance between that of mucoid and purulent.

Other material to be examined:-

Sputum produced after inhalation of prostaglandin F2α: Smith (personal communication), found that inhalation of PGF2α was followed by cough and sputum production. This method was used for obtaining bronchial fluid from normal bronchial tree. Healthy volunteers inhaled 40µg of PGF2α which was followed by cough and sputum production.

Saliva: saliva samples were obtained from healthy volunteers and patients with bronchorrhoea. No artificial stimulation of salivation was used.

Serum: venous blood from healthy volunteers was obtained by venopuncture.

The purpose of the study was to estimate the concentration of marker substances of bronchial and serum glycoproteins in sputum. The study was divided into five sections:

1. Changes in chemical constituents of sputum with macroscopic type.

2. Changes in chemical constituents of sputum in disease states: chronic bronchitis, asthma, cystic fibrosis and bronchiectasis.

3. Chemical constituents of bronchial fluid from normal bronchial tree.

4. Natural variation of chemical constituents of sputum.

5. Changes in chemical constituents of sputum in response to drugs.

METHODS

Collection of samples:- Sputum samples were collected during the first hour after waking. When serial biochemical analysis were made over a period of several hours in the same day and studied, samples were collected at three hours interval.

Sputum from patients attending outpatients clinics or general practitioners surgeries were delivered to the Department within four hours of production.

Storage:- The samples for biochemical analysis were stored in a deep-freeze at -20°C or were transported in vacuum flasks containing dry ice (solid carbon dioxide - Cardice: Distillers Company Ltd.) at a temperature of -70°C and then stored at -20°C.

Preliminary treatment:- Specimens were removed from deep-freeze and thawed rapidly under tap water to prevent enzymatic degradation of glycoproteins (Woodcraft et al.). Specimens were then heated in a water bath at 100°C for ten minutes. Whenever the volume allowed 5 ml. aliquots were taken for dialysis. If the volume was less than 5 ml. the container was weighed before and after the specimen was transfered to the dialysing tube and the wet weight recorded.

Specimens were dialysed in Visking dialysis tubing (18/32") with a porosity of 24 Ångstrom, permeable to a molecular weight of 10,000. Dialysis was carried out against distilled water (2L) for 72 hours at 4°C, the water being changed twice a day.

Drying of specimens:- Dialysed specimens were then transfered to weighing bottles, previously weighed and the weight read to the 5th decimal place, and skin freezed in cardice and acetone. Drying was carried out overnight in a freeze drier (Chemlab). On removal from freeze drier the bottles were immediately transfered to a desiccator since the material is extremely hygroscopic. Bottles were stoppered and reweighed again to the 5th decimal place. The dry material could then be stored at $4^{o}C$ or treated with pronase in order to breakdown the protein molecules and make the solution more homogeneous. Distilled water and 0.1 ml. of pronase (5% solution. Koch and Light Ltd.) were added to the dry material and the specimens were left in an incubator at $37^{o}C$ for two hours. Specimens were then stored at $-20^{o}C$ until chemical analyses were carried out.

Chemical analysis

Estimation of N-acetyl neuraminic acid:- The thiobarbituric acid method of Warren (1959) was used for the estimation of N-acetyl neuraminic acid.

Reagents

1N sulphuric acid	S.G. 0.04904 Concentrated Volumetric Solution. British Drug Houses Ltd.
Neuraminic acid Standard	N-acetyl neuraminic acid L. Light and Co.
Sodium arsenite solution	Sodium arsenite 10% in a solution of 0.5M sodium sulphate -0.1N sulphuric acid. Sodium arsenite - General Purpose Reagent. Hopkin and Williams Ltd. Sodium sulphate-Anhydrous. Analar. Hopkins and Williams Sulphuric acid - S.G. 1.84 Hopkins and Williams
Thiobarbituric acid	Laboratory Reagent. British Drug Houses Ltd. Freshly prepared - 0.6 G. dissolved in 100 ml. distilled water.
Cyclohexanone	General Purpose Reagent. Hopkins and Williams Ltd.

Method

To 50 µl. of pronase treated samples were added 100 µl of 2N sulphuric acid and 50 µl of distilled water, 10 ml. centrifuge tubes were used. The resulting solution was heated in a water bath at 80°C for one hour to achieve complete hydrolysis.

Arsenite solution, 1 ml. was added and a brown colour developed, the tubes were agitated until this coloration had cleared.

The thiobarbituric acid solution, 3 ml. was added, tubes stoppered with ground glass stoppers and heated in a water bath at 100°C for 15 minutes. After cooling, 4.5 ml. of cyclohexanone was added, tubes shaken and centrifuged at 3,000 rpm for ten minutes, the colour was left in the supernatant cyclohexanone phase. Supernatant was pippeted and the optical density read at 549 nm and 532 nm using a 3 ml. cuvette with a 1 cm. light path in a Beckman spectrophotometer.

Duplicate tubes were set up for each specimen and with each batch duplicate standards and distilled water blanks were prepared:

Standard A: 0.1 ml. of 0.5% stock solution of N-acetyl neuraminic acid, was made up to 10 ml. with distilled water = 50 µg/ml.

Standard B: 3 ml. of standard A was made up to 5 ml. with distilled water = 30 µg/ml.

Standard C: 1 ml. of standard A was made up to 5 ml. with distilled water = 10 µg/ml.

The values at 532 x 0.033 were subtracted from the values at 549 x 0.090 and the results from the three standards solutions were used to prepare a graph from which the concentration of N-acetyl neuraminic acid (NANA) in the test solutions could be read directly.

Estimation of methyl-pentose-fucose:-

Fucose was estimated by the method of Gibbons (1955) using the pronase treated samples.

Reagents:-

Sulphuric Acid — Analar S.G. 1.84 British Drug Houses Diluted 6.1 with distilled water.

Thioglycollic acid	Laboratory reagent. British Drug Houses. 3.3 v/v. Dilute 1 ml of thioglycollic acid in 30 ml with distilled water. Stored at - 20°C.
L-Fucose	Koch and Light Laboratories. 1% solution. Stored at - 20°C.

To 14mm x 150mm Pyrex tubes containing 50 µg. of pronase treated material and 0.950 ml of distilled water, 4.5 ml. of the sulphuric acid solution was added slowly while the tube was agitated in ice-cool water to prevent rise in temperature. Tubes were shaken and stoppered with marbles and after warming at room temperature, were placed in a water bath at 100°C for ten minutes. The tubes were then cooled in tap water and 0.1 ml of the thioglycollic acid solution was added with constant shaking. The tubes were left in the dark for three hours after which the optical density was read at 400 nm and 430 nm in 3 ml glass cuvettes with a 1 cm. light path in a Beckman spectrophotometer.

Duplicate tubes were set up for each test solution and with each batch blanks and standards were prepared.. Standard fucose solutions were

prepared as follows:

Standard A: 250 µl of 1% stock solution was made up to 100 ml. with distilled water = 25 µg/ml.

Standard B: 15 ml. of standard A was made up to 25 ml. with distilled water = 15 µg/ml.

Standard C: = 5 ml. of standard A was made up to 25 ml. with distilled water = 5 µg/ml.

The values at 430 nm were substracted from the values obtained at 400 nm and the results from the three standards were used to prepare a graph from which the concentration of L-fucose in the test solutions could be read directly.

Estimation of sulphate:-

Sulphate was estimated by the method of Antonopoulos (1962), pronase treated preparation of macromolecular solids was used.

Reagents:-

Formic acid — Analytical Reagent-Analar. 90% - British Drug Houses Ltd.

Ethanol	Analar. 95% British Drug Houses Ltd.
Benzidine	Puriss. Analytical Reagent. Koch-Light Laboratories Ltd. 0.5% solution in 95% ethanol. 125 mg. in 25 ml. ethanol.
Amyl alcohol	Analar - British Drug Houses Ltd.
Acetone-ethanol solution (v/v)	Acetone: Analar British Drug Houses Ltd. Ethanol Analar 95% British Drug Houses Ltd.
1N Hydrochloric acid	Analar. S.G. 1.16 British Drug Houses Ltd.
0.1N Sodium nitrate	Analar. Hopkin and Williams. 1.725 G. disolved in 250 ml. of distilled water.
0.5% Thymol	Thymol - British Drug Houses Ltd. disolved in 2N sodium hydroxide - British Drug Houses Ltd.

To 10 ml. Sovirel tubes containing 100 µl of pronase treated material and 100 µl of distilled water, 100 µl of 25% formic acid was added; tubes were shaken, stoppered and heated in a thermoblock at 100°C for 24 hours until complete hydrolysis

was achieved.

To 300 µl of hydrolysate 0.5 ml. of 95% ethanol and 0.2 ml. of benzidine solution were added, the tubes were then kept in the dark at $0^{o}C$ for two hours, after which the tubes were centrifuged at 4,000 rpm for six minutes. The supernatant was carefully decanted and discarded: 1 ml. of acetone ethanol solution and 0.5 ml. of amyl alcohol were added, tubes were shaken and sonicated to ensure dispersion and then recentrifuged at 4,000 rpm for six minutes. The supernatant was again decanted and discarded and 1 ml. of acetone - ethanol solution was added. Tubes were shaken, sonicated and recentrifuged at the same speed and the supernatant decanted and discarded. The final precipitate was disolved in 1.5 ml. of 1N HCl and 0.5 ml. of 0.1N sodium nitrate was added. Tubes were shaken and left on the bench for three minutes, 2.5 ml. of thymol reagent was added and tubes were shaken again until a coloration appeared. The optical density was read at 505 nm in 3 ml. glass cuvettes with a 1 cm. light path in a Beckman spectrophotometer.

Triplicate tubes were set up for each test solution and blanks and standard solution were

prepared with each batch. Standards were prepared as follows:-

Standard A: 50 µl of 1% stock solution was made up to 25 ml of formic acid = 20 µg/ml

Standard B: 4 ml. of standard A made up to 5 ml. with 25% formic acid = 16 µg/ml.

Standard C: 3 ml. of Standard A made up to 5 ml. with 25% formic acid = 12 µg/ml.

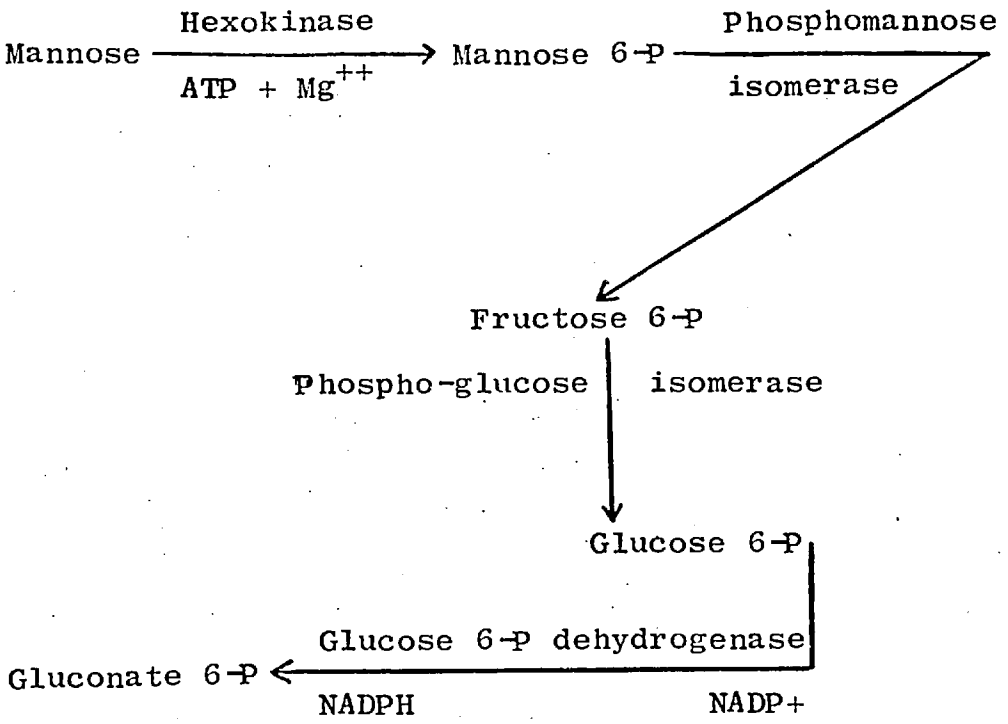
Standard D: 2 ml. of standard A made up to 5 ml. with 25% formic acid = 8 µg/ml.

Standard E: 1 ml. of standard A made up to 5 ml. with 25% formic acid = 4µg/ml.

The results of the five standard solution were used to prepare a graph from which the concentration of sulphate in the test solutions could be read directly.

Estimation of mannose:-

The enzymatic assay for mannose estimation in glycoproteins by Das et al. (1974) was used. The method is based on the following reaction sequence:



Reagents

Triethanolamine buffer pH 7.5

Magnesium sulphate

NADP

ATP

Phosphoglucose isomerase

Hexokinase

Glucose 6 phosphate dehydrogenase

Phosphomannose

All reagents were obtained from Boehringer Co. (England)

Method:-

To 2 ml. of pronase treated fraction 0.34 ml. of 12N hydrochloric acid was added (final concentration of HCl 2N), the solution was gassed with nitrogen, tubes stoppered and heated at 100°C in a water bath for six hours.

To 10 ml. Sovirel tubes containing 0.26 ml. of hydrolysate 0.3 M triethanolamine buffer containing:

4mM magnesium sulphate	2.50 ml
12 mM NADP	0.10 ml
16 mM ATP	0.10 ml
Phosphoglucose isomerase 1mg/ml	0.01 ml
Hexokinase 1mg/ml + Glucose 6 phosphate dehydrogenase 1mg/ml	0.02 ml

After mixing the optical density was read in a Unicam SP 500 spectrophotometer with 1 cm. light path using 3 ml. glass cuvettes. Zero reading was adjusted and 30 minutes later the optical density was read at 340 nm (ΔE_1). 0.01 ml. of phosphomannose isomerase (1mg/ml) was added to the solution contained in the cuvette. Cuvettes were shaken and 60 minutes later the optical density at 340 nm was

read. (ΔE_2). Substracting ΔE_1 from ΔE_2 the increase in optical density due to mannose was calculated.

Samples, blanks and standards were prepared in duplicate. The standards were used to prepare a graph from which the concentration of mannose in the test solutions could be read directly.

Estimation of deoxyribonucleic acid:-

The diphenylamine method of Croft and Lubran (1965) for the estimation of deoxyribonucleic acid in the presence of sialic acid was used.

Reagents

Deoxyribonucleic acid (DNA): stock standard solution was prepared from highly polymerised calf-thymus DNA (BDH Chemicals Ltd. Batch No. 0283210.)

Perchloric acid: 60% Analar Reagent (Hopkin and Williams)

Sodium hydroxide: 1N (BDH Chemicals Ltd.)

Sulphurid acid: 98% (BDH Chemicals Ltd.)

Acetic acid glacial: (BDH Chemicals Ltd.)

Acetaldehyde: (BDH Chemicals Ltd.)

Diphenylamine: Analar Reagent (Hopkin and Willams.

Method:-

To 250 µl of pronase treated sample 250 µl of 1N perchloric acid and 4.5 ml. of 0.5N perchloric acid were added. Test tubes and standard tubes were heated at 70°C for 20 minutes and then cooled in tap water. Test tubes were then centifruged at 2,500 g for 20 minutes in a MSE Centrifuge; 2 ml. of supernatant were pipetted and duplicates for each sample were prepared. Two millilitres of diphenylamine reagent (2G of diphenylamine A.R. disolved in 100 ml. of glacial acetic acid + 1.5 ml. of sulphuric acid + 0.5 ml. of 1.6% aqueous acetaldehyde solution were added to test and standard tubes. After mixing, tubes were heated at 30°C for 18 hours and the extinctions read against the blank (0.5N perchloric acid) at 550 nm and 600 nm in 3 ml. glass cuvettes with a 1 cm light path in a SP 500 Unicam Spectrophotometer. With each batch blanks and standards solutions were prepared as follows:-

Stock standard DNA solution 40 mg. of DNA/100 ml in 0.005M sodium hydroxide.
Standard A: to 250 µl of stock solution 250 µl of 1N perchloric acid and 1.5 ml of 0.5N perchloric acid were added = 50 µg/ml.

Standard B: to 100 µl of stock standard 100 µl of 1N perchloric acid and 1.8 ml of 0.5N perchloric acid were added = 20µg/ml.

Standard C: to 50µg of stock solution 50µg of 1N perchloric acid and 1.9 ml. of 0.5N perchloric acid were added = 10 ug/ml.

The values at 550 nm were substracted from the values obtained at 600 nm, the results from the three standards were used to prepare a graph from which the concentrations of DNA in the test solutions could be read directly.

Measurement of sputum viscosity

A Ferranti-Shirley viscometer was used for direct measurement of viscosity. The instrument consists of a control unit, a drive or measuring unit and a recorder. (Fig. II,2).

The Ferranti-Shirley viscometer is based on the measurement of the reaction torque due to viscous traction on a solid element rotating at a known rate in a fluid, the rotating element being a disc with a slightly conical face. The plate is rigidly constrained whilst the cone is driven through an electromechanical torque dynamometer by

an electronically controlled motor providing continuously variable cone speed up to 1,000 rpm. The maximum shear rate at 1,000 rpm for the standard cone-plate angle of 0.3^{o} is 1,850 s^{-1}. The cone is driven through the torque spring, the angular deflection of which is measured by a precision potentiometer and indicated on a voltmeter on the indicater unit. The recorder gives a continuous plot of shear rate versus shear stress on Y and X respectively. A typical rheogram is shown in Fig. II,3).

Method of testing:- The measuring and recording units were left switched on for at least 30 minutes before any test was carried out in order to allow the electronic apparatus to warm up. The gap between the cone and plate was set up with the micrometer screw to an accuracy of 0.0001 inch. The sputum aliquot 0.5 - 1 ml. was loaded on the centre of the lower platen which was then slowly raised into contact with the cone, ensuring even distribution and any excess of fluid was removed. The specimens were tested over two rotational speeds: 0 - 10 rpm (0 - 180 s^{-1}) and 0 - 100 rpm (0-1,800 s^{-1}) with a sweep time of 60 seconds. The tests were made at room temperature (range $18^{o}C$ to $22^{o}C$), viscosity was measured on two or

three aliquots of each specimen. The maximum values for shear rate and shear stress were calibrated in the recorder. The viscosity was calculated from the formula:

$$\text{Viscosity (Poise)} = \frac{\text{Shear stress } (\text{dyns/cm}^2)}{\text{shear rate } (\text{s}^{-1})} = \frac{\text{torque} \times C_S}{\text{rotational speed} \times C_R}$$

C_S and C_R being the constants for the cone and torque spring respectively.

Fig. II,2 The Ferranti-Shirley viscometer

A: Cone and plate

B: control unit with galvanometer

C: Bryans X - Y plotter

Fig. II,2

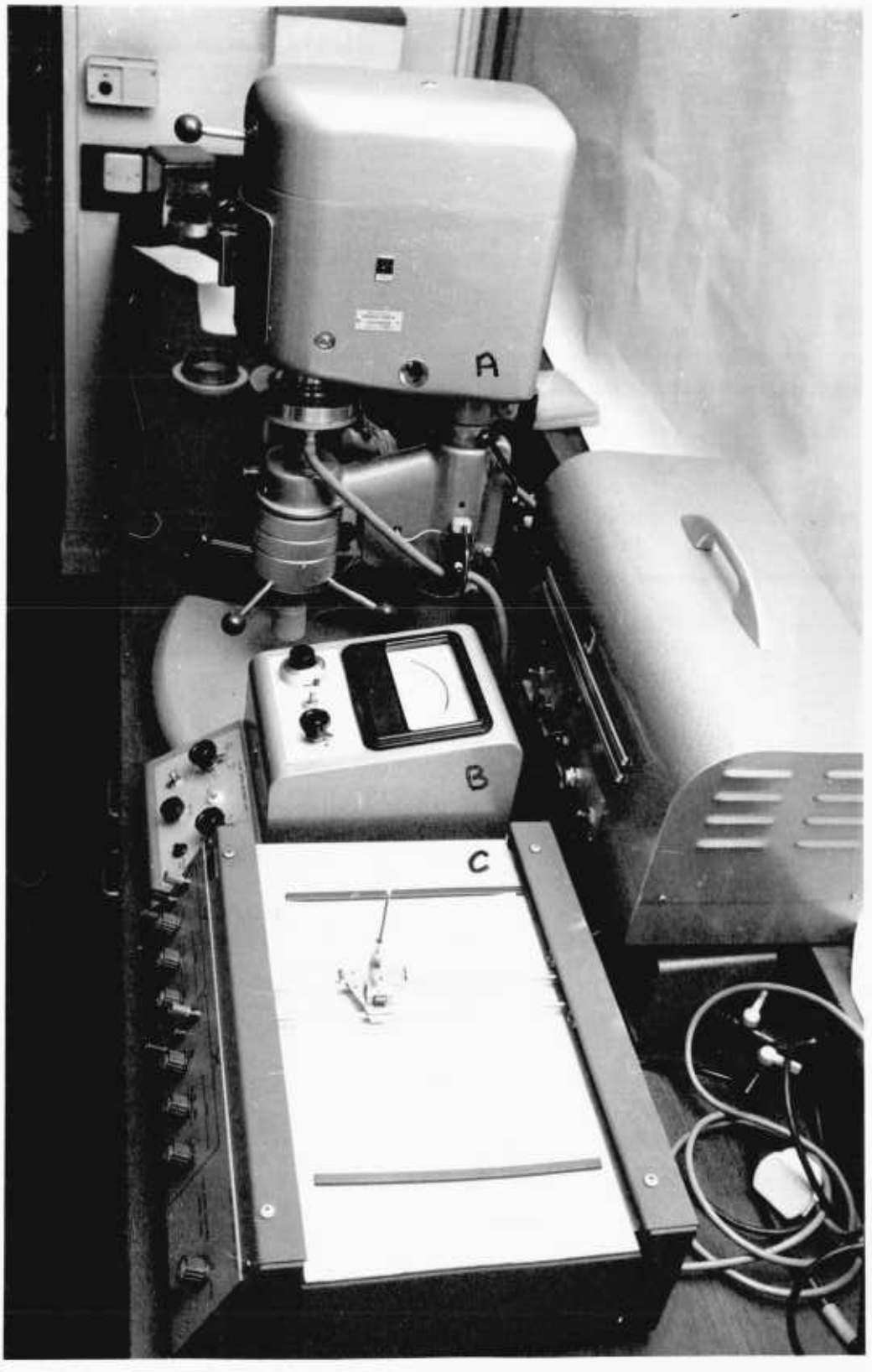
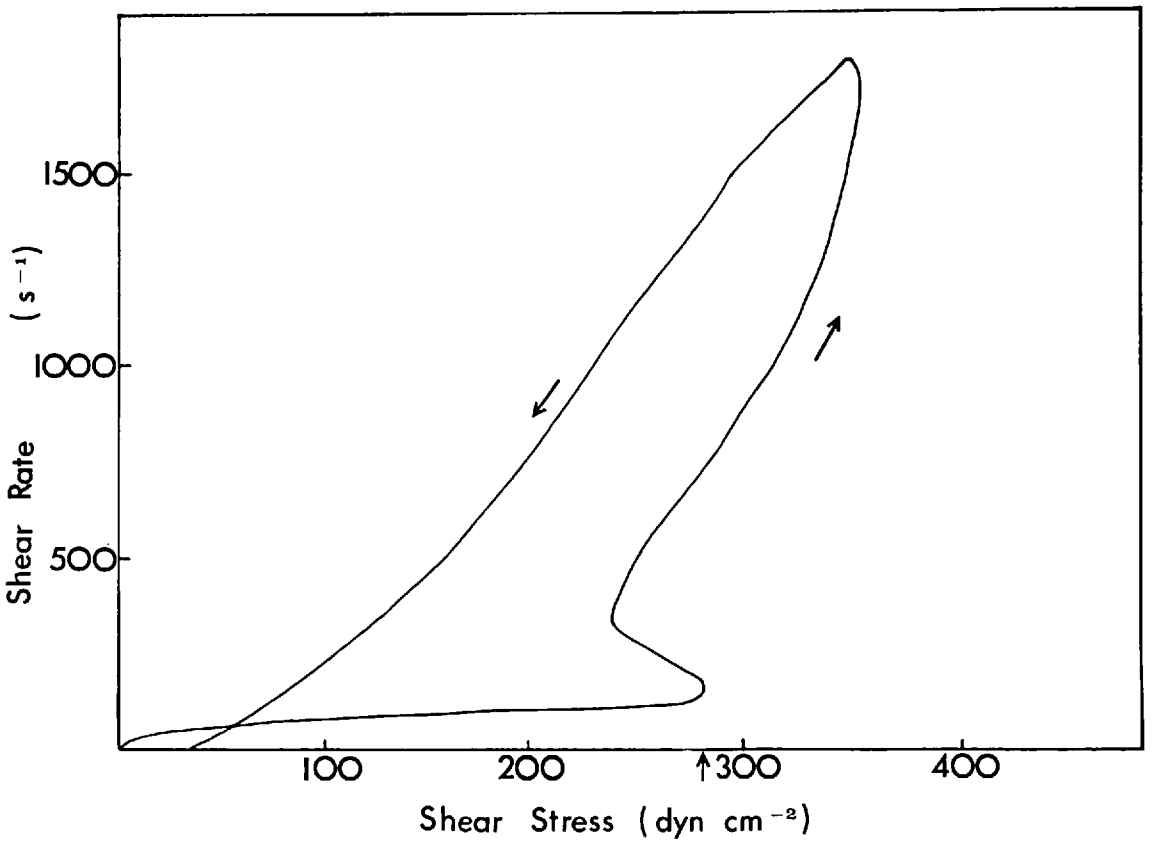


Fig. II,3 Rheogram recorded for sputum with the Ferranti-Shirley viscometer. Shear stress (dyn cm^{-2}) is plotted against shear rate (s^{-1}).

Fig. II,3


1500
1000
500
Shear Rate (s⁻¹)
100
200
300
400
Shear Stress (dyn cm⁻²)

CHAPTER III

CHEMICAL CONSTITUENTS OF SPUTUM: VARIATION WITH MACROSCOPIC TYPES

CHEMICAL CONSTITUENTS OF SPUTUM: VARIATION WITH MACROSCOPIC TYPES

Bronchial fluid is a mixture of secretion from the mucus secreting structures, serum fluid transudate, secretion from non specialised cells and cellular debris;- the presence of pus, therefore, will induce changes in the relative proportion of the different constituents, increasing particularly serum fluid transudate and exudate and cellular debris. These changes may influence the physical and chemical properties of the sputum irrespective of the primary disease.

Macroscopic types: classification

Sputum samples were examined macroscopically and graded as mucoid, mucopurulent or purulent according to the following criteria:

Mucoid: white or clear with no evidence of pus.

Purulent: almost homogeneous green or yellow showing therefore gross signs of pus.

Mucopurulent: appearance between that of mucoid and purulent.

Although macroscopic examination is a subjective method of assessing degree of infection, it has been shown by Miller and Jones (1963) that with

experience, it is accurate and correlates with sputum cellularity assessed by total white blood cell count in sputum and in particular with polymorphonuclear leucocytes. (Miller and Jones 1963).

Burgi et al. (1968) reported a direct relationship between degree of infection, based on clinical and bacteriological findings, and the number of deoxyribonucleoprotein fibres and concentrations of deoxyribonucleic acid (DNA) and lactodehydrogenase (LDH) in sputum. Although there was some overlap between degree of infection, particularly in those samples with large number of DNA fibres and high concentrations of DNA and LDH, these authors found that these indices could be used as reliable objective criteria for assessing the degree of infection.

DNA content of sputum and macroscopic type:-

DNA estimations were carried out in 44 sputum samples taken at random from a large series of specimens to assess the reliability of the macroscopic classification.

Number of samples, mean values, standard error of the mean and range for each macroscopic type

are given in Table III,1 and Fig. III,1.

Traces of DNA were detected in only two samples out of the 11 mucoid specimens examined while only one mucopurulent sample out of 18 contained DNA. All purulent samples contained DNA. Although there is some overlap between macroscopic types, a statistically significant difference was found between mucoid and purulent ($P<0.001$), mucoid and mucopurulent ($P<0.001$) and mucopurulent and purulent ($P<0.001$) Table III,2.

Comment:-

The DNA content of sputum increases with the degree of purulence as has been reported by various authors (Matthews et al. 1963; Potter et al. 1967; Burgi et al. 1968). Although mucupurulent and purulent samples showed a wide range of DNA content resulting in an overlap between macroscopic types, macroscopic examination seems a reliable method for assessing degree of purulence. The overlap between mucopurulent group included samples with various degrees of purulence: from only traces of pus to pus accounting for more than two thirds of the specimen.

Table III,1

Number of samples, mean values standard error of the mean and range of DNA content of sputum for each macroscopic type (mg/ml)

	Macroscopic types		
	M	MP	P
Number of samples	11	18	14
Mean value	0.004	0.360	1.060
Standard error	0.004	0.090	0.160
Range	0-0.046	0-1.406	0.114-2.380

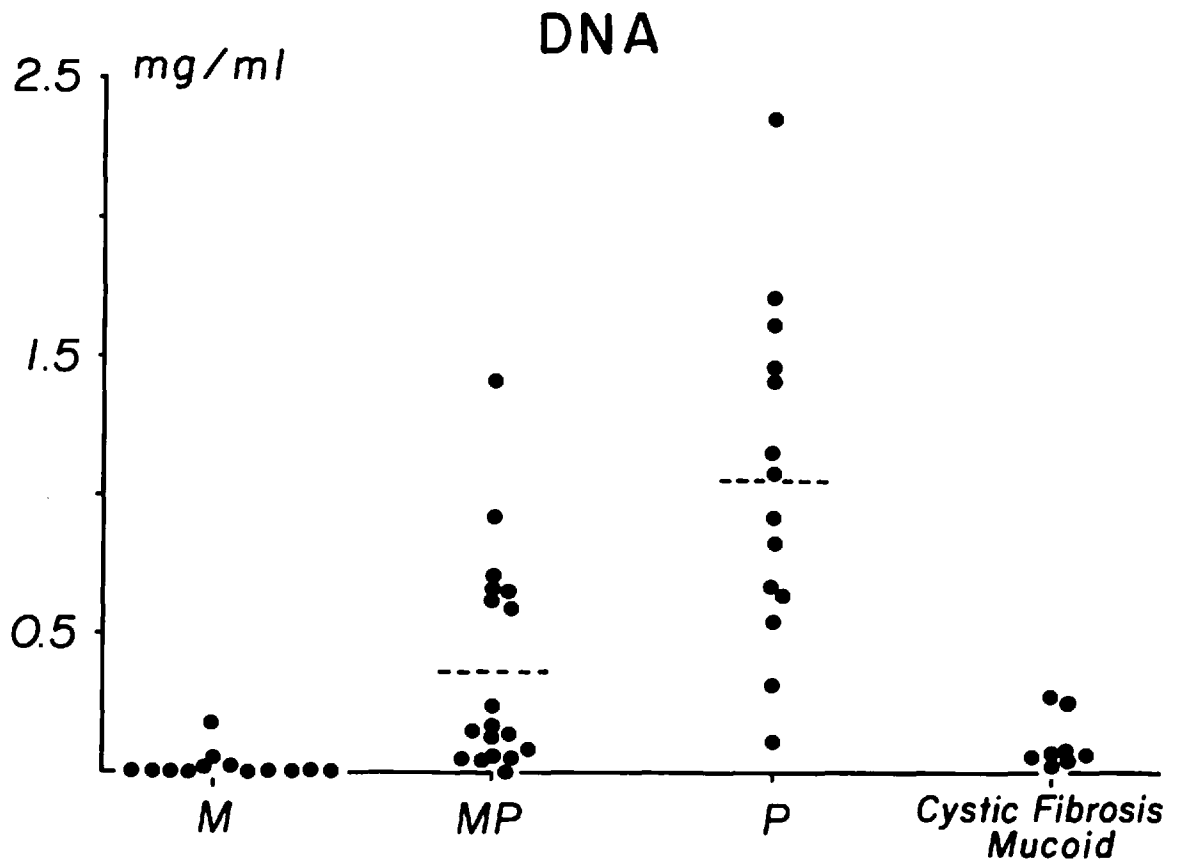
Table III,2

DNA content of sputum: comparison between macroscopic types

Mucoid/Purulent	- 6.4992	$P < 0.001$
Mucoid/Mucopurulent	- 3.9802	$P < 0.001$
Mucopurulent/Purulent	- 3.7632	$P < 0.001$

Fig. III,1 The range of values for deoxyribonucleic acid (DNA) in mucoid, mucopurulent and purulent sputum and in mucoidsputum from patients with cystic fibrosis.

Fig. III,1



Changes in dry weight and chemical constituents of sputum with degree of purulence:

All diseases studied showed a similar pattern of variation in dry weight and chemical constituents with the degree of purulence (see Chapter IV). As a result in this chapter the samples are not considered by disease but by macroscopic types.

The chemical constituents analysed include: NANA, fucose, mannose and sulphate.

Number of samples studied for dry weight and each chemical constituents for each macroscopic type are given in Table III,3.

Table III,3

Number of samples studied for dry weight and chemical constituents of sputum for each macroscopic type.

Chemical constituents	Macroscopic types		
	M	MP	P
Dry weight	49	56	52
NANA	49	56	52
Fucose	49	56	52
Sulphate	22	26	25
Mannose	19	18	13

Mean values and standard error of the mean of chemical constituents of sputum for each macroscopic type are given in Table III,4 Fig. 2,3,4,5, and 6.

Comparison between mucoid and purulent samples showed that absolute levels of dry weight and chemical constituents, except sulphate, were significantly higher in purulent sputa than in mucoid (Table III,5). When mucoid and mucopurulent samples were compared, mean values of dry weight and chemical constituents were found to be higher in mucopurulent than in mucoid but the difference only reached significant levels for dry weight, NANA and NANA/Fucose ratio (Table III,5). Mean values of dry weight and chemical constituents were also found to be higher in purulent than in mucopurulent, a statistically significant difference was seen for dry weight and NANA (Table III,5).

Comment:-

The increase in dry weight and chemical constituents of sputum with degree of purulence followed a similar pattern to that seen for the

Table III,4

Dry weight and chemical constituents of sputum for each macroscopic type.

Mean values and ( )* standard error of the mean.

Chemical constituents	Macroscopic types		
	M	MP	P
Dry weight (mg/ml)	16.7	24.1	49.8
	(1.2)*	(1.5)*	(2.9)*
NANA µmol/ml	2.6	3.3	4.2
	(0.2)*	(0.2)*	(0.2)*
Fucose µmol/ml	5.1	5.8	6.3
	(0.3)*	(0.5)*	(0.3)*
Sulphate µmol/ml	1.6	1.9	2.2
	(0.3)*	(0.3)*	(0.2)*
Mannose µmol/ml	0.7	1.1	1.4
	(0.1)*	(0.2)*	(0.2)*
NANA/Fucose ratio	0.5	0.6	0.7
	(0.03)*	(0.04)*	(0.06)*

Table III,5

Chemical constituents of sputum: comparison between macroscopic types (Student's test).

Chemical constituents	M/P	M/MP	MP/P
Dry weight (mg/ml)	-10.421***	-3.795***	-8.004***
NANA (µmol/ml)	-4.956***	-2.128*	-2.851**
Fucose (µmol/ml)	-2.747**	-1.218	-0.948
Sulphate (µmol/ml)	-1.819	-0.856	-0.791
Mannose (µmol/ml)	-3.235**	-1.845	-0.976
NANA/Fucose ratio	-3.367**	-2.278*	-1.873

*** $P < 0.001$

** $P < 0.01$

* $P < 0.05$

DNA content - the changes between mucoid and purulent are greater than those observed between mucoid and mucopurulent and between mucopurulent and purulent. The different degree of purulence within the mucopurulent group could explain the failure to achieve statistically significant differences between mucoid and mucopurulent samples and between mucopurulent and purulent samples for some of the chemical constituents analysed.

The yield of macromolecular material showed a significant increase with degree of purulence as it has been reported by Matthews et al. (1963).

Mannose, a marker substance of serum glycoproteins, increases with degree of purulence, and although it showed no significant difference between mucoid and mucopurulent sputum the percentage increase was higher than that of fucose and sulphate and even that of dry weight and NANA.

It is interesting to note that fucose and sulphate, marker substances of bronchial glycoprotein, also increased with degree of purulence. Potter et al. (1967) reported that purulent sputum from patients with bronchiectasis or cystic fibrosis contain more carbohydrate as a percentage of wet

weight of sputum than mucoid sputum but they made no attempt to analyse individual sugar components.

The increase in marker substances of serum glycoproteins, mannose and of bronchial glycoprotein, fucose and sulphate, could explain why NANA, a marker substance of both bronchial and serum glycoproteins, was the only chemical constituent which showed a significant increase from mucoid to mucopurulent and from mucopurulent to purulent.

Fig. III,2 The range of values for dry macromolecular weight in mucoid, mucopurulent and purulent sputum.

Fig. III,2

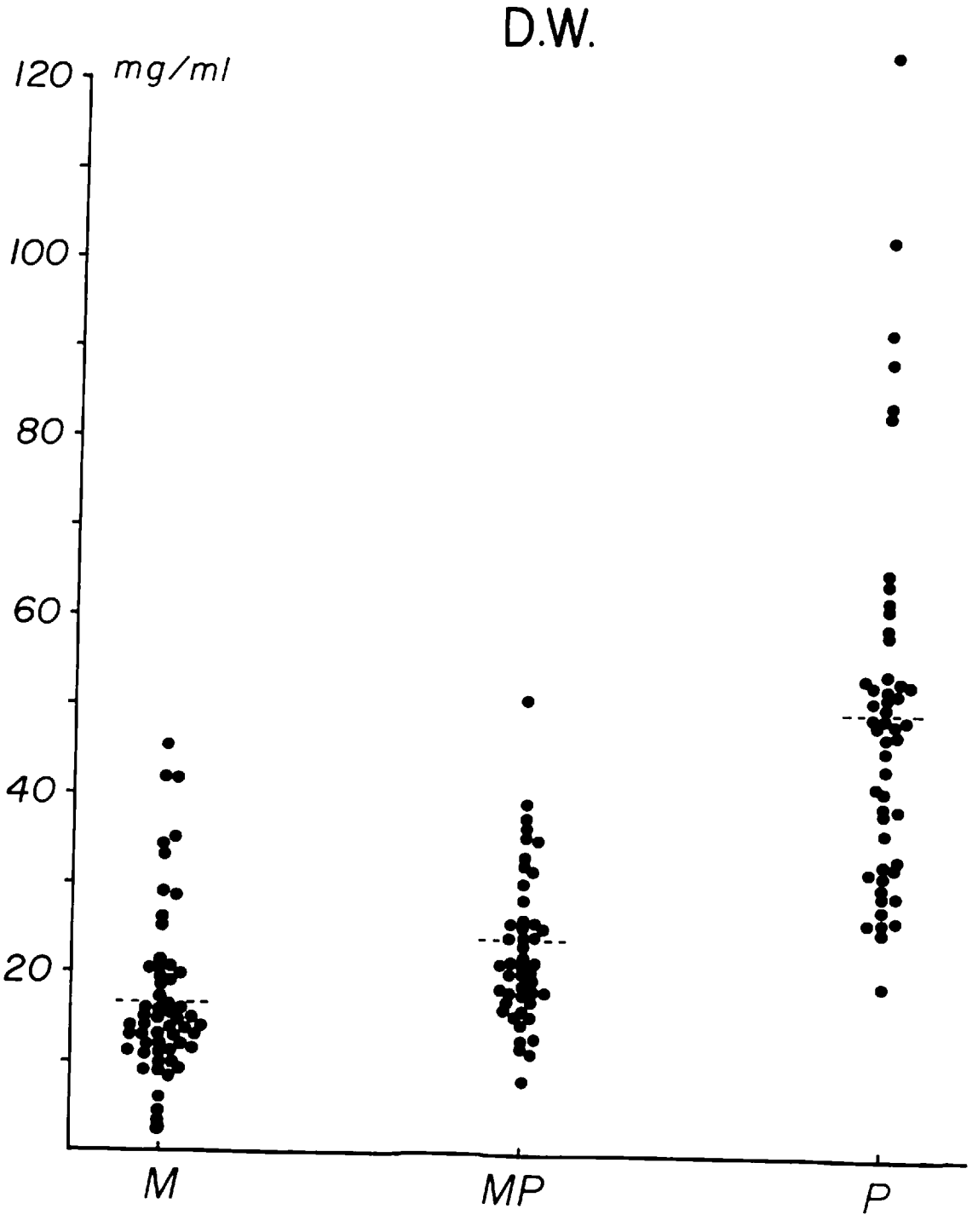


Fig. III,3 The range of values for N-acetyl neuraminic acid (NANA) in mucoid, mucopurulent and purulent sputum.

Fig. III,3

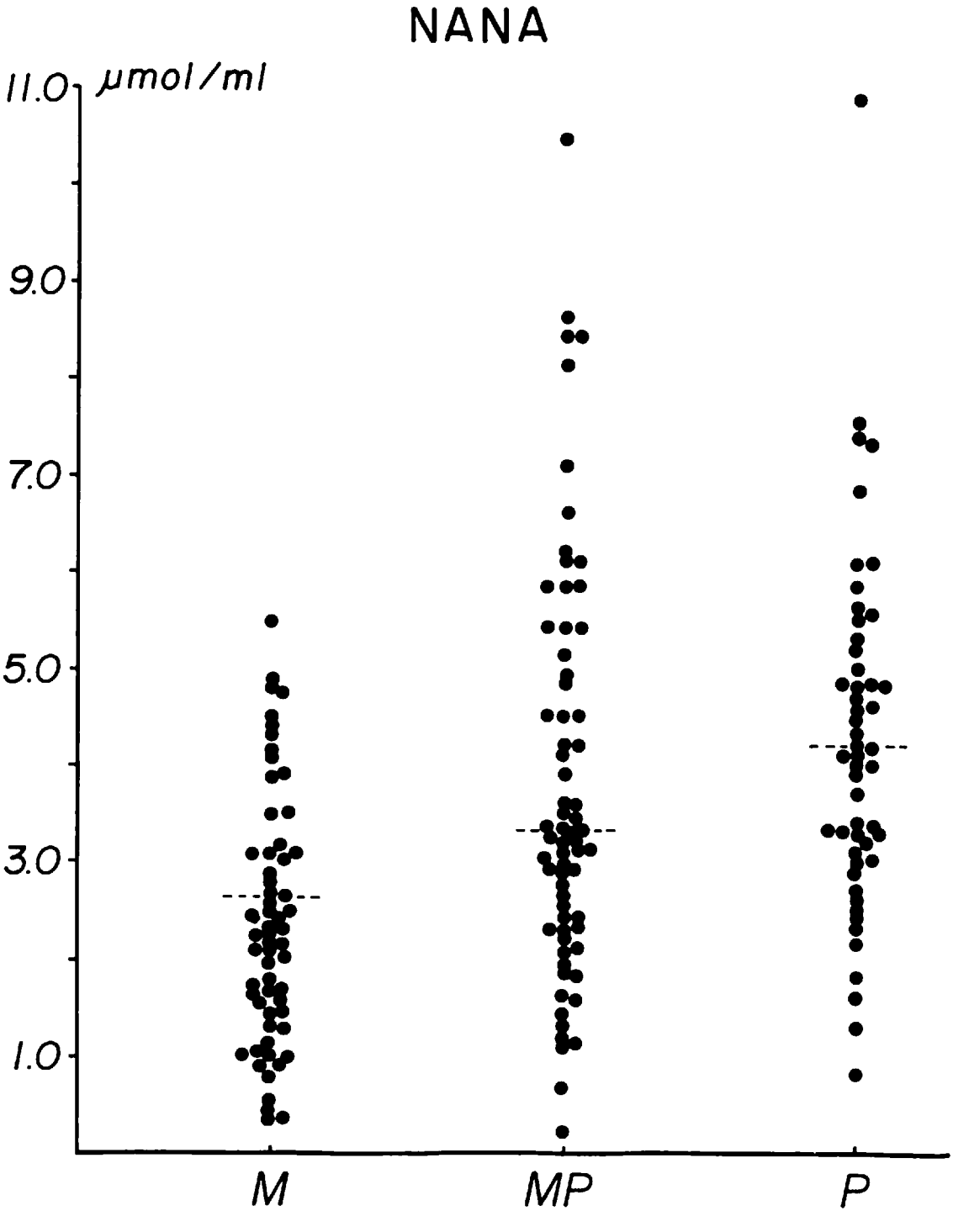


Fig. III,4 The range of values for fucose in mucoid, mucopurulent and purulent sputum.

Fig. III,4

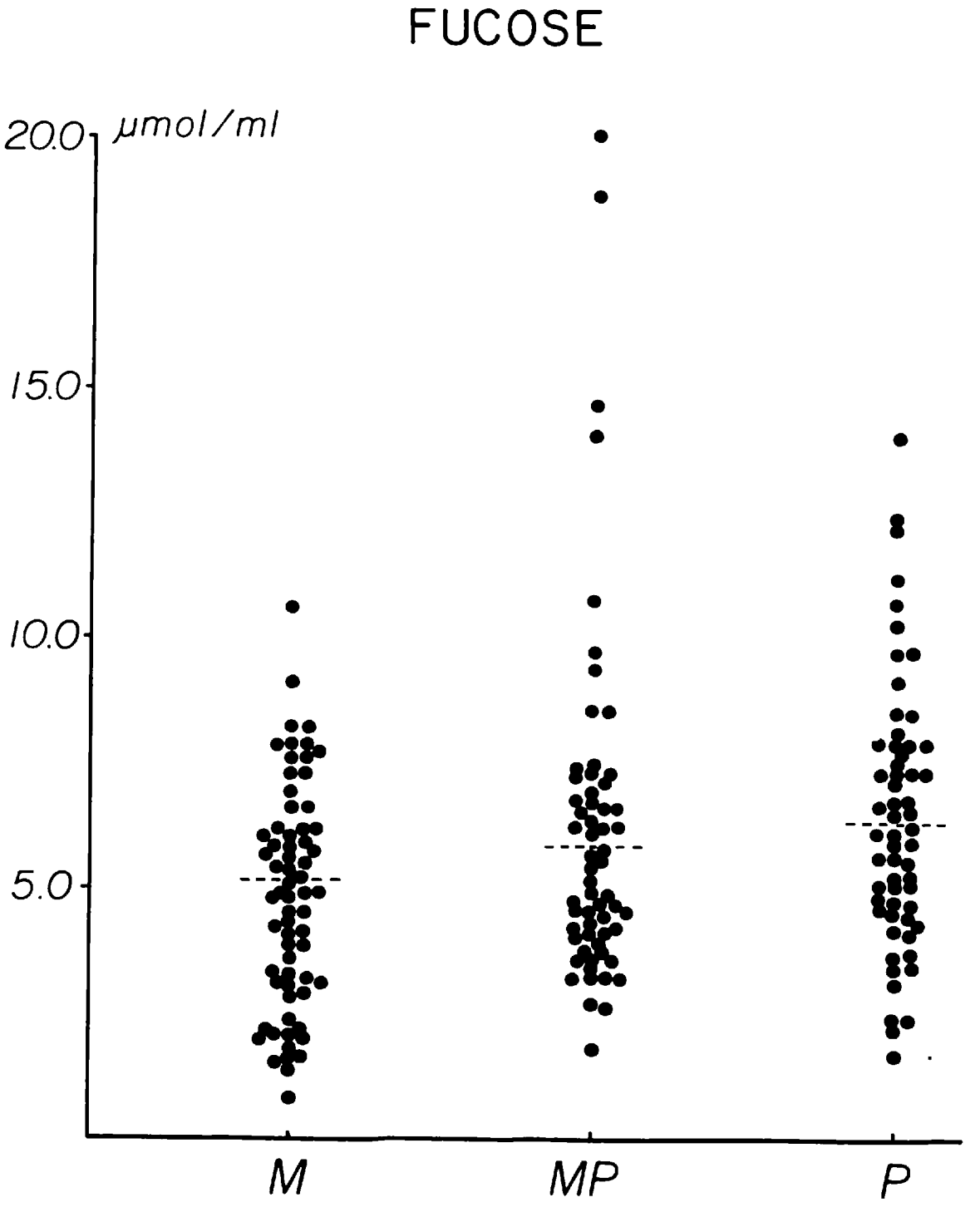


Fig. III,5 The range of values for sulphate in mucoid, mucopurulent and purulent sputum.

Fig. III,5

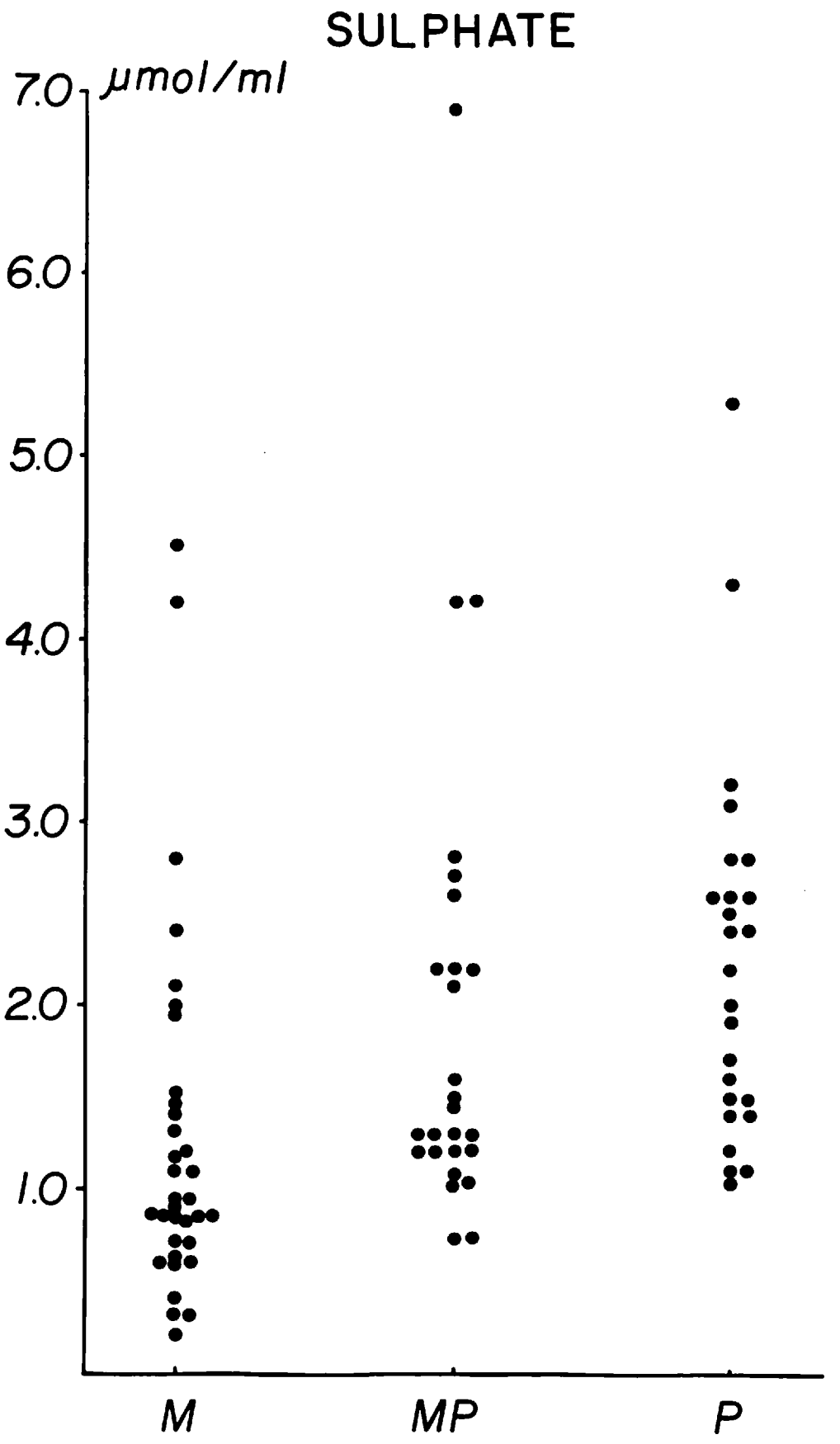


Fig. III,6 The range of values for mannose in mucoid, mucopurulent and purulent sputum.

Fig. III,6

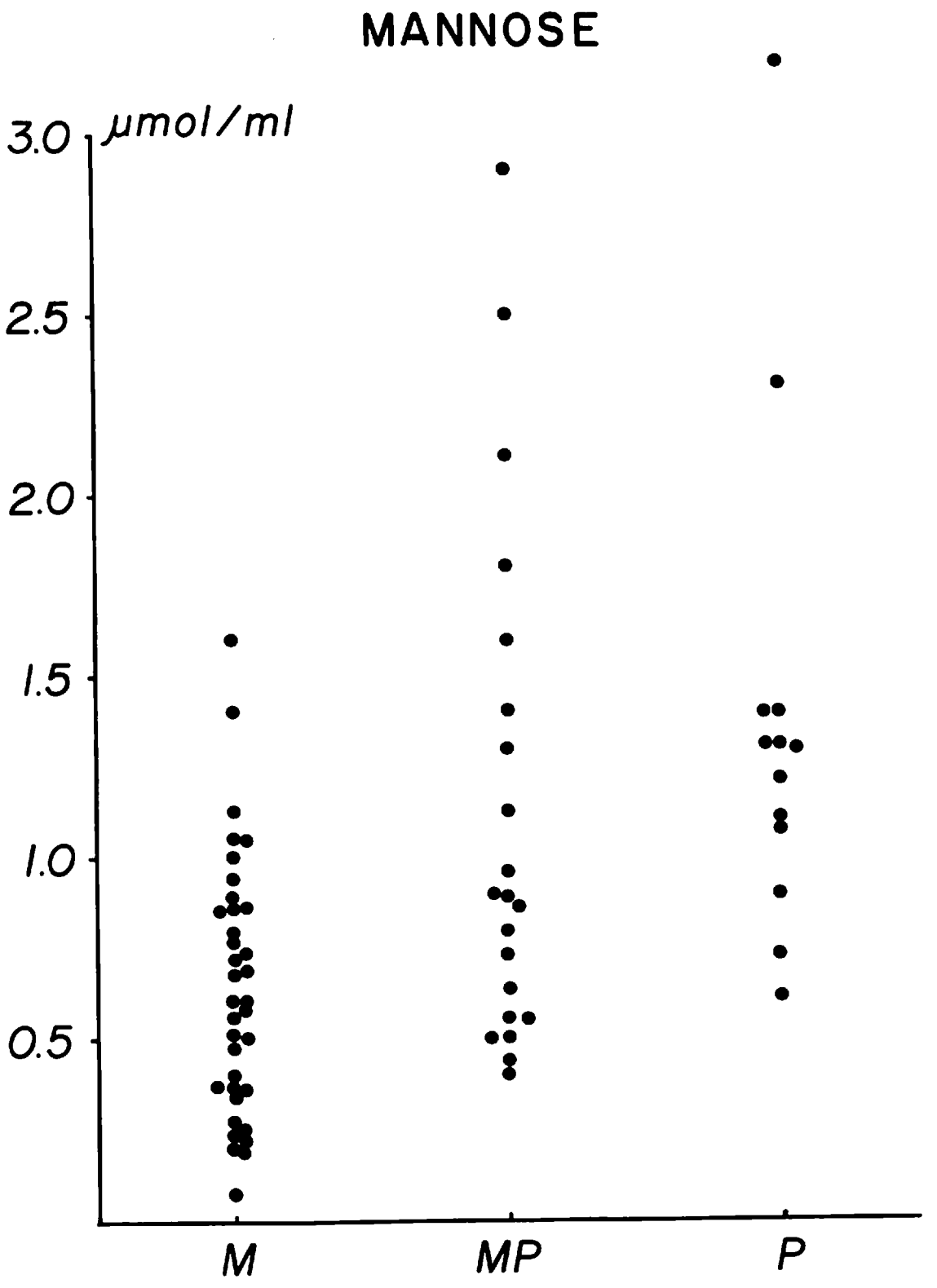
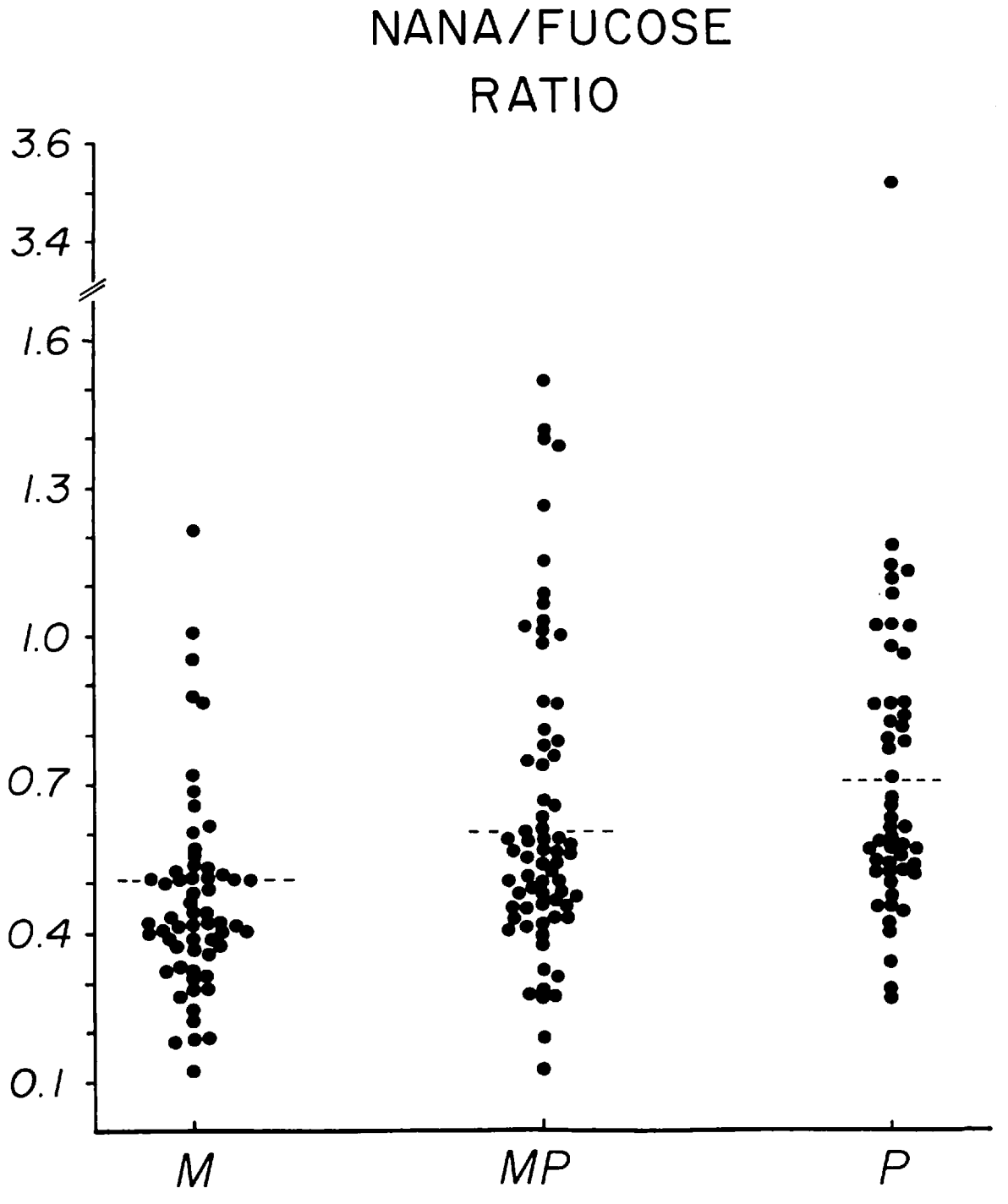


Fig. III,7 The range of values for NANA/Fucose ratio in mucoid, mucopurulent and purulent sputum.

Fig. III,7



CHAPTER IV

CHEMICAL CONSTITUENTS OF SPUTUM: COMPARISON BETWEEN DISEASES

Sputum is a mixture of saliva and bronchial fluid (Fig.II,1), bronchial fluid contains secretion from special cells - the mucous and serous cells of the submucosal glands and the goblet cells of the surface epithelium - and tissue fluid transudate. In disease states the contributions of mucus, serum transudate and/or inflammatory exudate will vary depending on the nature of the disease and whether infection is present.

The purpose of this work was to study the changes in absolute levels of marker substances of bronchial and serum glycoproteins in sputum from various diseases.

The diseases studied were: chronic bronchitis, asthma, cystic fibrosis and bronchiectasis. The diagnosis was based on:

Chronic bronchitis: MRC definition (1965)

Asthma: Ciba symposium (1971)

Cystic fibrosis: clinical and high sweat sodium levels

Bronchiectasis: bronchographic findings: airway dilatation and obliteration associated with persistent sputum production.

All the sputum samples represented the early morning production to avoid an increase in variation of the chemical constituents analysed. Chemical analyses of sputum included: N-acetyl neuraminic acid (NANA), fucose, sulphate and mannose; dry macromolecular weight was also estimated. Estimations of dry weight, NANA and fucose were carried out in all samples, sulphate and mannose only in some.

Sputum samples were macroscopically classified as: mucoid, mucopurulent or purulent.

In order to simplify the presentation of the results I have divided these into two sections:

Section 1: includes statistical analyses of the results.

Section 2: description of chemical features found for each disease.

Diagrams representing individual values of dry weight and chemical constituents of mucoid, mucopurulent and purulent sputum for each disease are included at the end of the chapter.

STATISTICAL ANALYSIS OF THE RESULTS

Table 1: Number of patients included in the study for each disease and number of mucoid, mucopurulent and purulent samples analysed for each disease.

Table 2: Mean values, standard error of the mean and range of dry weight and chemical constituents in mucoid sputum from each disease.

Table 3: Comparison between diseases

Table 4: Mean values, standard error of the mean and range of dry weight and chemical constituents for mucopurulent sputum for each disease.

Table 5: Comparison between diseases

Table 6: Mean values standard error of the mean and range of dry weight, and chemical constituents of purulent sputum for each disease.

Table 7: Comparison between diseases

Table 8: Comparison between macroscopic types for each disease group.

TABLE IV,1

Number of patients and number of samples for each macroscopic type for each disease group.

	Chronic bronchitis	Asthma	Bronchiectasis	Cystic fibrosis
No. of patients	26	18	16	18
No. of samples	74	59	36	45
Mucoid	85	37	0	18
Mucopurulent	26	22	11	10
Purulent	13	0	23	16

TABLE IV,2

Dry weight and chemical constituents of mucoid sputum from chronic bronchitis, asthma and cystic fibrosis. Mean values, standard error the the mean and range.

	Chronic bronchitis	Asthma	Cystic fibrosis
Dry weight mg/ml	15.04 0.84 26.10-5.00	17.96 1.84 45.5-2.70	13.4 1.4 25.7-3.60
NANA µmol/ml	2.17 0.21 3.60-0.40	2.49 0.22 5.50-0.30	1.90 0.20 3.40-0.40
Fucose µmol/ml	5.11 0.39 10.70-1.20	5.08 0.39 10.60-0.80	3.00 0.40 7.90-0.90
Sulphate µmol/ml	1.80 0.40 4.50-0.30	1.30 0.30 4.20-0.30	0.90 0.10 1.50-0.20
Mannose µmol/ml	0.90 0.20 1.60-0.40	0.70 0.10 1.40-0.10	0.50 0.06 1.10-0.20
NANA/Fucose ratio	0.42 0.03 0.80-0.08	0.51 0.04 1.20-0.20	0.60 0.10 0.90-0.40

TABLE IV,3

Dry weight and chemical constituents of mucoid sputum. Comparison between diseases; Chronic bronchitis (CB), Asthma (A), and Cystic Fibrosis (CF). STUDENT'S T TEST

	CB/A	CB/CF	A/CF
Dry weight	-1.4464	1.0111	1.9785
NANA (μmol/ml)	-1.0683	0.9446	2.0697*
Fucose (μmol/ml)	0.0400	9.1405*	9.1304**
Sulphate (μmol/ml)	0.9530	1.9932	1.1451
Mannose (μmol/ml)	0.7255	1.9398	1.6131
NANA/Fucose ratio	-1.8034	-1.7162	-0.3575

* $P < 0.05$

** $P < 0.001$

TABLE IV,4

Dry weight and chemical constituents of muco-purulent sputum from chronic bronchitis, asthma, cystic fibrosis and bronchiectasis. Mean value, standard error of the mean and range.

Chemical constituents	Chronic Bronchitis	Asthma	Cystic Fibrosis	Bronchiectasis
Dry weight (mg/ml)	21.40 1.500 51.1 -11.80	34.38 3.30 62.80-9.20	22.80 2.30 35.90-12.20	28.94 3.00 39.60-8.15
NANA µmol/ml	3.60 0.2 6.70- 1.80	5.69 0.57 12.20-0.20	2.20 0.30 4.20-1.10	2.13 0.27 3.20-0.67
Fucose µmol/ml	5.80 0.40 10.80- 3.20	8.64 0.99 20.10-1.70	4.60 0.70 7.30-2.70	3.92 0.42 6.80-1.80
Sulphate µmol/ml	2.03 0.35	3.26 0.53	1.96 0.29	1.37 0.32
Mannose µmol/ml	0.70 0.10 1.30- 0.40	2.10 0.40 2.90-1.60	1.20 0.20 2.10-0.80	0.90 0.30 2.50-0.40
NANA/Fucose ratio	0.70 0.10 1.40-0.30	0.70 0.07 1.50-0.10	0.50 0.10 0.60-0.03	0.57 0.07 1.02-0.19

TABLE IV,5

Dry weight and chemical constituents of mucopurulent sputum:- Comparison between diseases : Chronic bronchitis (CB), Asthma (A), Bronchiectasis (BR) and Cystic Fibrosis (CB)

	Chron. Bronch. Asthma	Chron. Bronch. Cystic Fibro.	Chron. Bronch. Brctsis	Asthma Cystic Fib.	Asthma Brctsis	Cystic Fib. Brctsis
Dry weight(mg/ml)	- 3.8310****	-0.5592	-2.3030*	2.8840***	1.2192	-1.6322
NANA (µmol/ml)	- 3.6143****	3.7662****	4.2559****	5.5346****	5.6751****	0.0914
Fucose (µmol/ml)	- 2.8751***	1.7061	3.2617***	3.8067****	4,3825****	1.1492
Sulphate (µmol/ml)	- 2.3683	0.1655	0.9454	2.7382**	3.8195***	1.1047
Mannose (µmol/ml)	- 3.8730****	-1.8779	-0.6261	2.2550**	2.3913*	0.7993
NANA/Fucose ratio	- 0.1224	2.0832*	1.2554	2.8370***	1.3112	-1.0001

* $P < 0.05$ STUDENT'S T TEST

** $P < 0.02$

*** $P < 0.01$

**** $P < 0.001$

TABLE IV,6

Dry weight and chemical constituents of purulent sputum from chronic bronchitis (CB), cystic fibrosis (CF), and bronchiectasis (Bctsis).

Chemical constituents	CB	CF	Bctsis
Dry weight mg/ml			
Mean	51.2	62.6	39.9
S.E.	4.5	6.9	2.2
Range	84.1-19.0	122.4-31.9	58.4-25.3
NANA µmol/ml	4.8	4.2	3.9
	0.4	0.5	0.4
	7.3-0.8	10.0-1.8	7.9-1.3
Fucose µmol/ml	5.7	5.9	6.9
	0.7	0.4	0.6
	12.4-3.4	8.5-3.1	14.0-2.2
Sulphate µmol/ml	2.5	2.0	2.3
	0.5	0.4	0.3
	7.3-0.8	1.1-5.3	1.4-3.1
Mannose µmol/ml	1.6	1.5	1.0
	0.5	0.2	0.2
	4.7-0.6	0.9-3.2	0.6-1.3
NANA/Fucose ratio	0.9	0.8	0.6
	0.1	0.1	0.1
	1.1-0.3	3.5-0.4	1.2-0.3

TABLE IV,7

Dry weight and chemical constituents of purulent sputum:- Comparison between diseases (Student's T test).

Diseases	Dry weight mg/ml	NANA µmol/ml	Fucose µmol/ml	Sulphate µmol/ml	Mannose µmol/ml	NANA/Fucose
Chronic bronchitis	-1.32074	0.92894	-0.25964	0.81882	0.14987	0.36594
Cystic fibrosis						
Chronic bronchitis	2.4883*	1.58558	-1.22104	0.20000	0.82222	3.53632**
Bronchiectasis						
Cystic fibrosis	3.60047***	0.46982	-1.22628	-0.50353	1.30175	1.21518
Bronchiectasis						

* $p < 0.02$
** $p < 0.01$
*** $p < 0.001$

TABLE IV,8

Dry weight and chemical constituents of sputum. Comparison between macroscopic types in chronic bronchitis, asthma, cystic fibrosis and bronchiectasis.

		Dry weight mg/ml	NANA µmol/ml	Fucose µmol/ml	Sulphate µmol/ml	Mannose µmol/ml	NANA/Fucose ratio
Chronic bronchitis	M/P	-7.8285****	-5.2895****	-0.7564	-0.9072	-1.3769	-8.5743****
	M/MP	-3.6657****	-4.7336****	-1.2117	-0.3326	0.7437	-3.7442****
	MP/P	-6.2752****	-2.4233**	0.0237	-0.7034	-1.7337	-2.0434*
Asthma	M/MP	-4.3449****	-5.2232****	-3.3414***	-3.9074***	-3.2827***	-2.4500**
Cystic Fibrosis	M/P	-7.0335**	-4.2236****	-10.2905****	-3.6216***	-3.9314****	-0.9908
	M/MP	-3.4729***	-0.7813	-7.5645****	-3.4066***	-3.1861***	1.2019
	MP/P	-5.5809****	-3.4611***	-2.1113*	-0.1648	-0.9995	-1.7817
Bronchiectasis	MP/P	-3.0645***	-4.0717****	-4.1625****	-4.3591***	1.9013	-0.5618

* P<0.05 ** P <0.02 *** P <0.01 **** P <0.001

CHEMICAL FEATURES OF SPUTUM IN DISEASE

A wide range of values of dry weight and chemical constituents - NANA, fucose, sulphate, mannose and NANA/Fucose ratio - is found in mucoid, mucopurulent and purulent sputum from each disease and also there is a great overlap between diseases, meaning that no single value of dry weight or chemical constituents is characteristic of a particular disease (Fig. IV,1 to Fig. IV,18). In spite of the overlap between diseases, some interesting features emerged.

Chemical features of chronic bronchitis sputum: -

The lower part of the range of dry weight, NANA, fucose and sulphate in mucoid chronic bronchitis sputum fell within the levels found in sputum produced after inhalation of prostaglandin F2α (see Chapter V, Fig. V,2,3,4 and5) Mannose concentrations were found to be always higher than in PGF2α suggesting that in mucoid chronic bronchitis sputum the serum transudate contribution is greater than in bronchial fluid from normal airways.

Of all chemical constituents in mucoid chronic bronchitis sputum, the NANA concentration showed the greatest variation: this could be due to

either a different degree of sialylation of the bronchial glycoprotein and/or to an increase in tissue fluid transudate component. All the patients studied were "late" chronic bronchitics, as assessed by disability - all had irreversible and marked airways obstruction, all the samples were macroscopically mucoid and the patients were studied in a stable phase of the disease; it is, therefore, probable that the relative contribution of NANA from serum glycoprotein was fairly constant and hence the variation could be due to a different degree of sialylation of the bronchial glycoprotein for each individual patient or a variation in the percentage of mucous cells producing sialomucin. This is supported by the fact that in patients with chronic bronchitis studied over a long period (two consecutive years) the chemical profile of the sputum changed very little within a patient (see Chapter VI).

When infection is present there is an increase in macromolecular material accompanied by an increase in NANA and mannose concentrations and the NANA/Fucose ratio, that is the proportion of serum glycoprotein components is increased. One would expect that with an increase in macromolecular

material from other sources than bronchial mucus, particularly DNA, the concentration of marker substances of bronchial glycoprotein - fucose and sulphate - should be lower in purulent sputum than in mucoid. The fact that fucose and sulphate levels, particularly sulphate, were found to be higher in purulent sputum suggests that infection increases mucus secretion as well as inflammatory exudate, the acid glycoprotein secreted being more sulphated than in mucoid sputum.

Chemical features of cystic fibrosis sputum:-

Absolute levels of dry weight and chemical constituents (NANA, fucose, sulphate and mannose) in mucoid cystic fibrosis sputum fell within the range found for mucoid chronic bronchitis and asthma sputa and yet some significant differences were observed.

Fucose concentration in mucoid cystic fibrosis sputum was found to be lower than in mucoid chronic bronchitis and asthma suggesting that the relative proportion of bronchial secretion is smaller, probably due to a relative increase in serum fluid transudate. This is supported by the fact that although the NANA concentration was low, the NANA/

Fucose ratio was found to be higher than in chronic bronchitis and even higher than that of asthmatic sputum. If the serum transudate component is relatively higher than the bronchial secretion, one would expect a very low dry weight as seen in bronchorrhoea sputum (Lopez-Vidriero et al. 1975), but the dry weight of cystic fibrosis sputum was within chronic bronchitis range, suggesting that the macromolecular material in cystic fibrosis sputum may come from other sources.

The commonest complication of cystic fibrosis being respiratory infection, total DNA estimations were carried out and it was found that all samples contained DNA and the levels fell within the range found for mucopurulent chronic bronchitis sputum. In only 2 out of 11 mucoid chronic bronchitis sputa were traces of DNA detected. The presence of DNA in mucoid cystic fibrosis sputum explains the relatively high dry weight with low fucose and NANA concentrations and high NANA/fucose ratio.

When mucopurulent and purulent cystic fibrosis sputa were examined, absolute levels of dry weight, NANA, fucose, sulphate and mannose fell within the range found in chronic bronchitis, and no

statistically significant difference was observed, suggesting that cystic fibrosis patients with gross signs of infection may have some degree of gland hypertrophy. Purulent cystic fibrosis sputum tended to have higher dry weight than chronic bronchitis or bronchiectasis sputa, probably due to a higher DNA content (Picot et al. in preparation).

Chemical features of bronchiectasis sputum:-

The first striking difference seen in bronchiectasis was that no mucoid sputum was available for study; pus was macroscopically present in all specimens.

Absolute levels of dry weight and chemical constituents - NANA, fucose, sulphate and mannose - in mucopurulent and purulent bronchiectasis sputa fell within the range found for same macroscopic types in chronic bronchitis and cystic fibrosis. This is not surprising since some of the bronchiectatic patients were also chronic bronchitics.

Chemical features of asthma sputum:-

Mucoid sputum from asthma tended to have a higher yield of macromolecular material and NANA

and fucose concentrations than mucoid chronic bronchitis sputum and showed a wide range of values (Fig. IV,1.2.3.). When mucopurulent sputa were examined these differences were found to be even greater than in mucoid asthmatic sputum, not only for dry weight, NANA and fucose but also for sulphate and mannose concentrations (Figs.IV,7,8,9,10,11).

Keal (1970) and Charman and Reid (1972) reported that sputum viscosity of asthmatic patients reached higher levels than in other diseases and the variation within samples was also greater.

It was felt that these findings needed further investigations; the clinical data were carefully examined and the patients were divided into four groups:

Group 1: patients with extrinsic asthma

Group 2: patients with intrinsic asthma

Group 3: patients with intrinsic asthma who fulfilled criteria of chronic bronchitis

Group 4: patients with extrinsic asthma who fulfilled criteria of chronic bronchitis.

The number of patients included in each group and number of mucoid and mucopurulent sputa examined for each group are given in Table IV,9.

Mean values, standard error of the mean and range of dry macromolecular weight and chemical constituents of sputum for each group are given in Table IV,10 for mucoid samples and in Table IV,12 for mucopurulent samples. Comparison between groups (Student's T test) are summarised in Tables IV,11; IV,13.

Although no statistically significant differences were found between the four groups for mucoid sputum, some interesting features emerged.

The intrinsic asthma and intrinsic asthma + chronic bronchitis groups showed a wider range of values, particularly for dry weight and NANA, than extrinsic asthma and extrinsic asthma + chronic bronchitis (Fig. IV,19,20). The variation of fucose concentration was similar in all four groups although extrinsic asthma + chronic bronchitis sputum tended to have higher levels (Fig. IV,21).

Extrinsic asthma sputum showed less variation of chemical constituents and the NANA/Fucose ratio was higher than in other groups suggesting that

these patients represent a more homogeneous group, as far as bronchial secretion is concerned, and that the serum transudate contribution is increased in relation to the other groups of asthma. (Fig. IV, 24).

Absolute levels (mean and range) of dry weight and chemical constituents of intrinsic and intrinsic + chronic bronchitis sputa were similar and were found to be closer to mucoid chronic bronchitis sputum suggesting that in both intrinsic asthma and intrinsic asthma + chronic bronchitis there is some gland hypertrophy, as seen in chronic bronchitis.

Although the number of patients and number of samples in the extrinsic asthma + chronic bronchitis group were too small, it seems that this group shows less variation in dry weight and chemical constituents than the other groups. Fucose concentration was found to be higher than in the other three groups, and the NANA/Fucose ratio lower, therefore it suggests that the relative proportion of bronchial secretion is increased.

Also when infection was present did asthmatic sputum show the greatest variation in dry weight, NANA and fucose concentrations and all chemical constituents, including sulphate and mannose, were found to be significantly higher than in muco-purulent chronic bronchitis, cystic fibrosis or bronchiectasis sputa (Table IV,5).

Number of samples, mean values, standard error of the mean and range of dry weight, NANA, fucose and NANA fucose ratio for the three groups studied are given in Table IV,12. No mucopurulent extrinsic asthma sputum was examined.

Comparison (Student's T test) between groups are summarised in Table IV,13.

Although the statistical analyses reached significance for differences in dry weight, NANA and NANA/fucose ratio between groups, particularly for extrinsic asthma + chronic bronchitis sputum, the number of samples (from a patient) was too small to draw a firm conclusion, but it seemed that intrinsic asthma and intrinsic asthma + chronic bronchitis were closer to mucopurulent chronic bronchitis sputum than to the extrinsic asthma + chronic bronchitis. (Fig. IV, 25,26,27,28.).

TABLE IV,9

ASTHMA Number of patients, sex, number of samples according to macroscopic type of sputum and total number of samples for each group.

	Extrinsic	Intrinsic	Extrinsic + Chronic bronchitis	Intrinsic + Chronic bronchitis
No. patients	6	4	3	5
Male	1	2	3	2
Female	5	2	0	3
Mucoid	12	8	3	10
Mucopurulent	0	8	4	14
Total No. of samples	12	16	7	24

TABLE IV,10

Dry weight chemical constituents of mucoid sputum from extrinsic asthma, intrinsic asthma, intrinsic asthma + chronic bronchitis and extrinsic asthma + chronic bronchitis. Mean values, standard error of the mean and range.

	E.A. n=12	I.A. n=8	I.A.+C.B. n=10	E.A.+C.B. n=3
Dry weight mg/ml	14.30 1.70 28.80-5.00	20.10 5.20 45.50-2.70	20.50 3.20 42.20-4.80	15.00 1.80 17.40-11.40
NANA µmol/ml	2.50 0.20 5.50-1.00	2.50 0.70 5.50-0.30	2.40 0.40 4.80-0.80	2.50 0.70 3.90-1.70
Fucose µmol/ml	5.00 0.60 7.90-2.10	4.30 0.80 7.60-0.80	5.20 0.60 9.10-1.40	7.30 1.70 10.60-4.80
NANA/ Fucose ratio	0.60 0.10 1.20-0.30	0.50 0.10 0.95-0.20	0.40 0.03 0.70-0.20	0.30 0.04 0.40-0.20

TABLE IV,11

Dry weight and chemical constituents of mucoid sputum from extrinsic asthma, intrinsic asthma, intrinsic asthma + chronic bronchitis and extrinsic asthma + chronic bronchitis. Comparison between groups (Student's T test).

	EA/IA n=20	EA/IA+CB n=26	EA/EA+CB n=15	IA/IA+CB n=22	IA/EA+CB n=11	IA+CB/EA+CB n=17
Dry weight mg/ml	-1.1643	-1.5814	0.1991	-0.9576	0.5351	0.7443
NANA umol/ml	0.2103	0.3089	0.2180	0.0207	-0.0049	-0.0232
Fucose umol/ml	0.7157	-0.1935	-1.6595	-0.8193	-1.7981	-1.3571
NANA/Fucose ratio	0.4395	1.5477	1.4298	1.1405	1.5624	1.5864

TABLE IV,12

Dry weight and chemical constituents of mucopurulent sputum from intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB), and extrinsic asthma + chronic bronchitis (EA+CB). Mean values, standard error of the mean and range.

	IA n=8	IA+CB n=14	EA+CB n=4
Dry weight mg/ml	32.20 6.90 57.40-16.00	31.30 3.60 47.70-9.20	46.40 6.10 62.80-42.50
NANA µmol/ml	5.40 0.80 8.60-2.40	4.80 0.80 8.40-0.20	8.40 1.30 12.20-5.80
Fucose µmol/ml	10.30 2.28 20.10-4.70	6.80 0.90 11.10-1.70	9.80 1.40 14.00-7.30
NANA/Fucose ratio	0.60 0.10 0.80-0.40	0.70 0.10 1.50-0.10	0.90 0.10 1.00-0.60

TABLE IV,13

Chemical constituents of mucopurulent sputum from intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) and extrinsic asthma + chronic bronchitis (EA+CB). Comparison between groups (Student's t test).

	IA/IA+CB n=18	IA/EA+CB n=12	IA+CB/EA+CB n=14
Dry weight mg/ml	0.1303	-1.3043	-2.1902*
NANA µmol/ml	0.5847	-2.0805	-2.4103*
Fucose µmol/ml	1.5144	0.1431	-1.7280
NANA/Fucose ratio	-0.7350	-2.7070*	-0.6245

* P 0.05

Fig. IV,1 The range of values for dry macromolecular weight in mucoid sputum from patients with chronic bronchitis (CB), asthma (A) or cystic fibrosis (CF).

Fig. IV,1

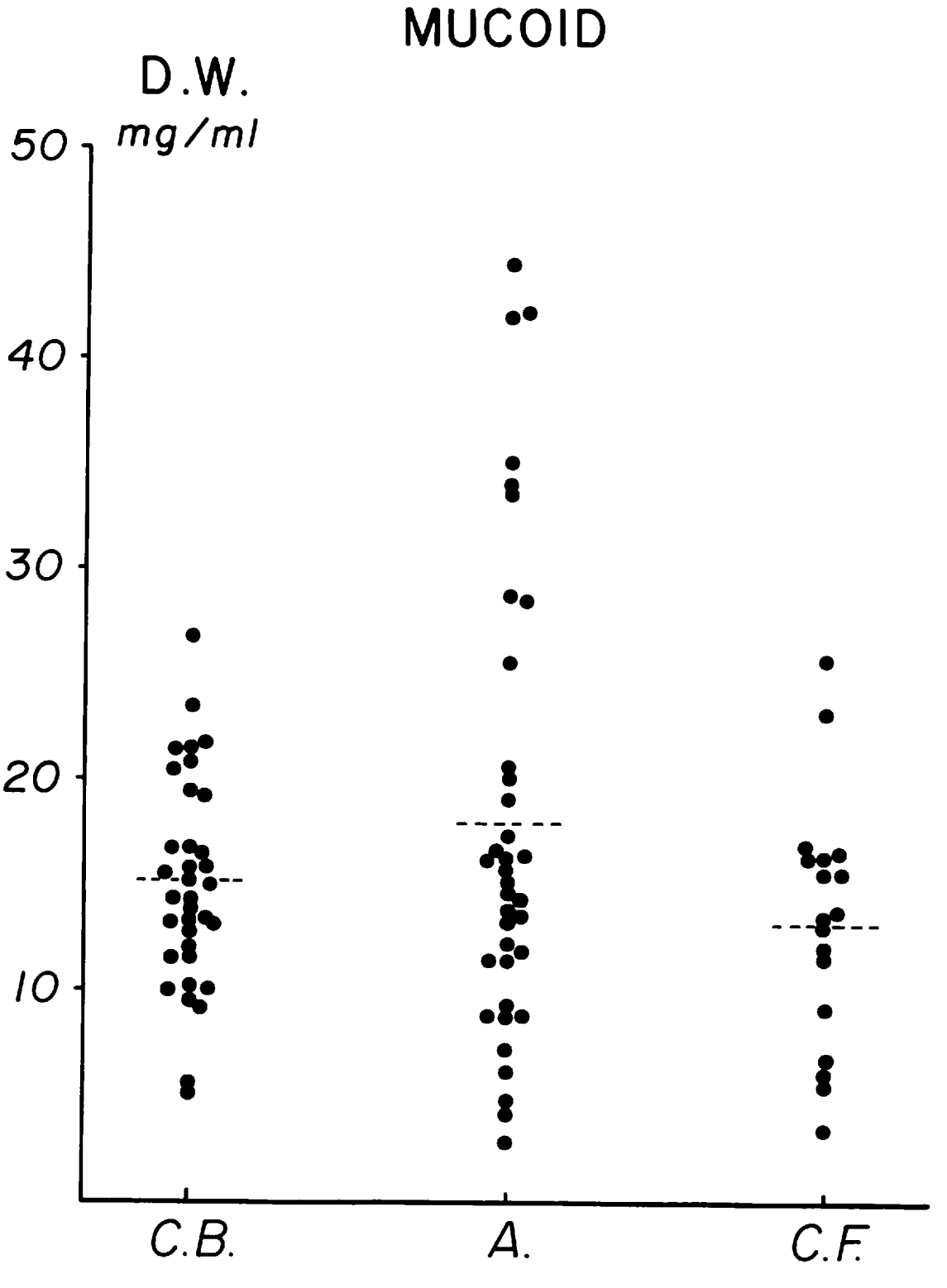


Fig. IV,2 The range of values for N-acetyl neuraminic acid (NANA) in mucoid sputum from patients with chronic bronchitis (CB), asthma, (A) or cystic fibrosis (CF).

Fig. IV,2

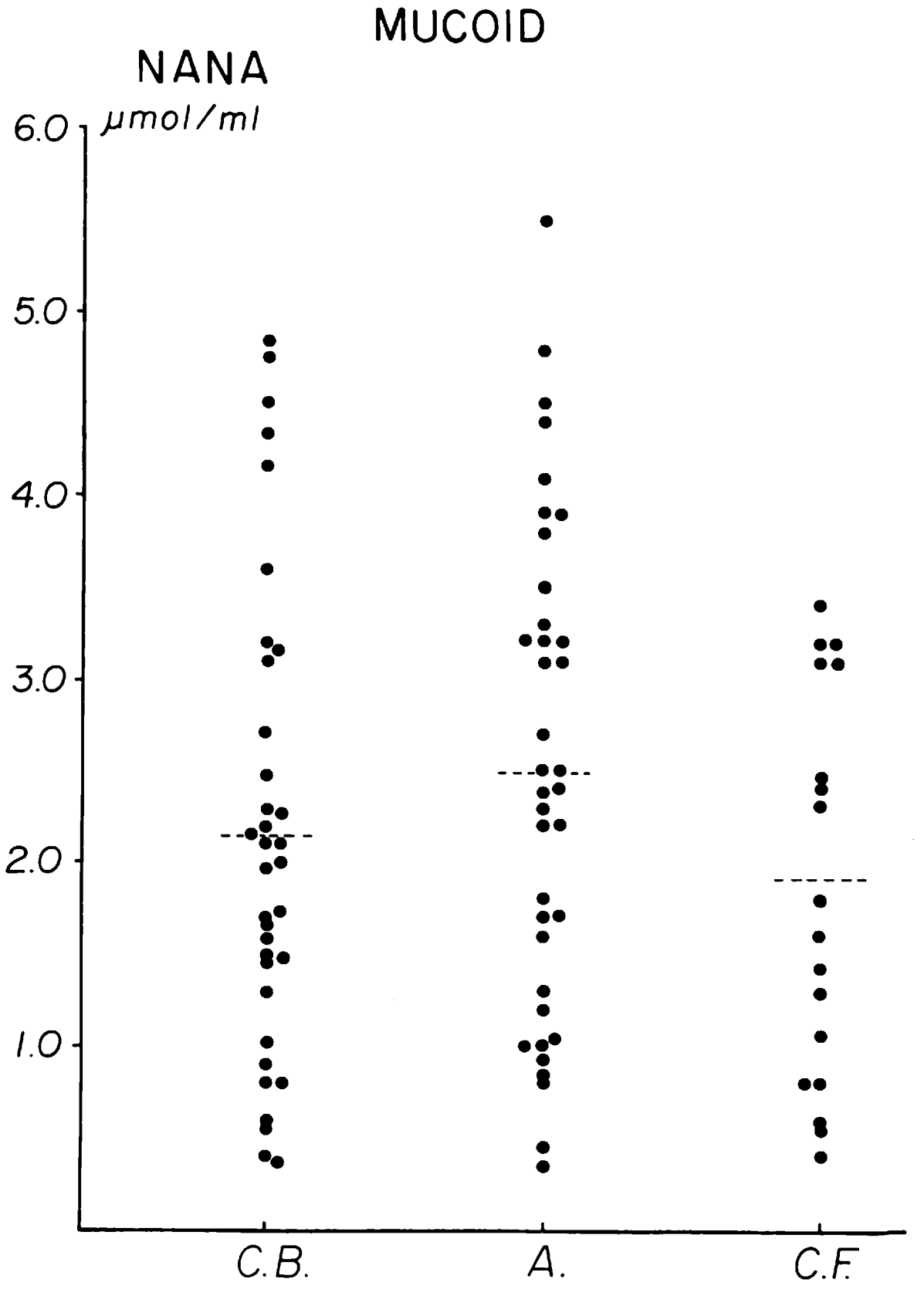


Fig. IV,3 The range of values for fucose in sputum from patients with chronic bronchitis (CB), asthma (A), or cystic fibrosis (CF).

Fig. IV,3

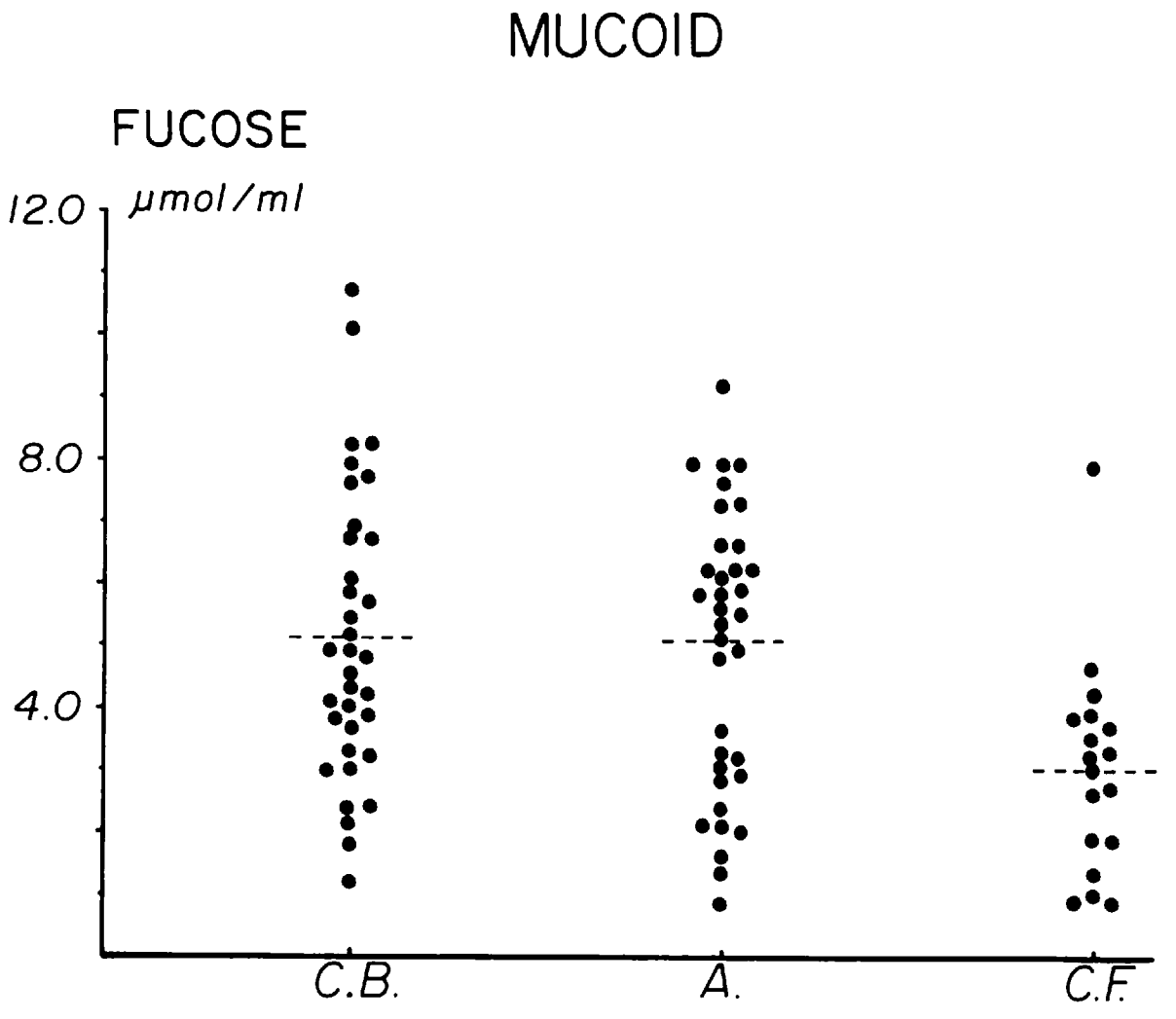


Fig. IV,4 The range of values for sulphate in mucoid sputum from patients with chronic bronchitis (CB), asthma (A), or cystic fibrosis (CF).

Fig. IV,4

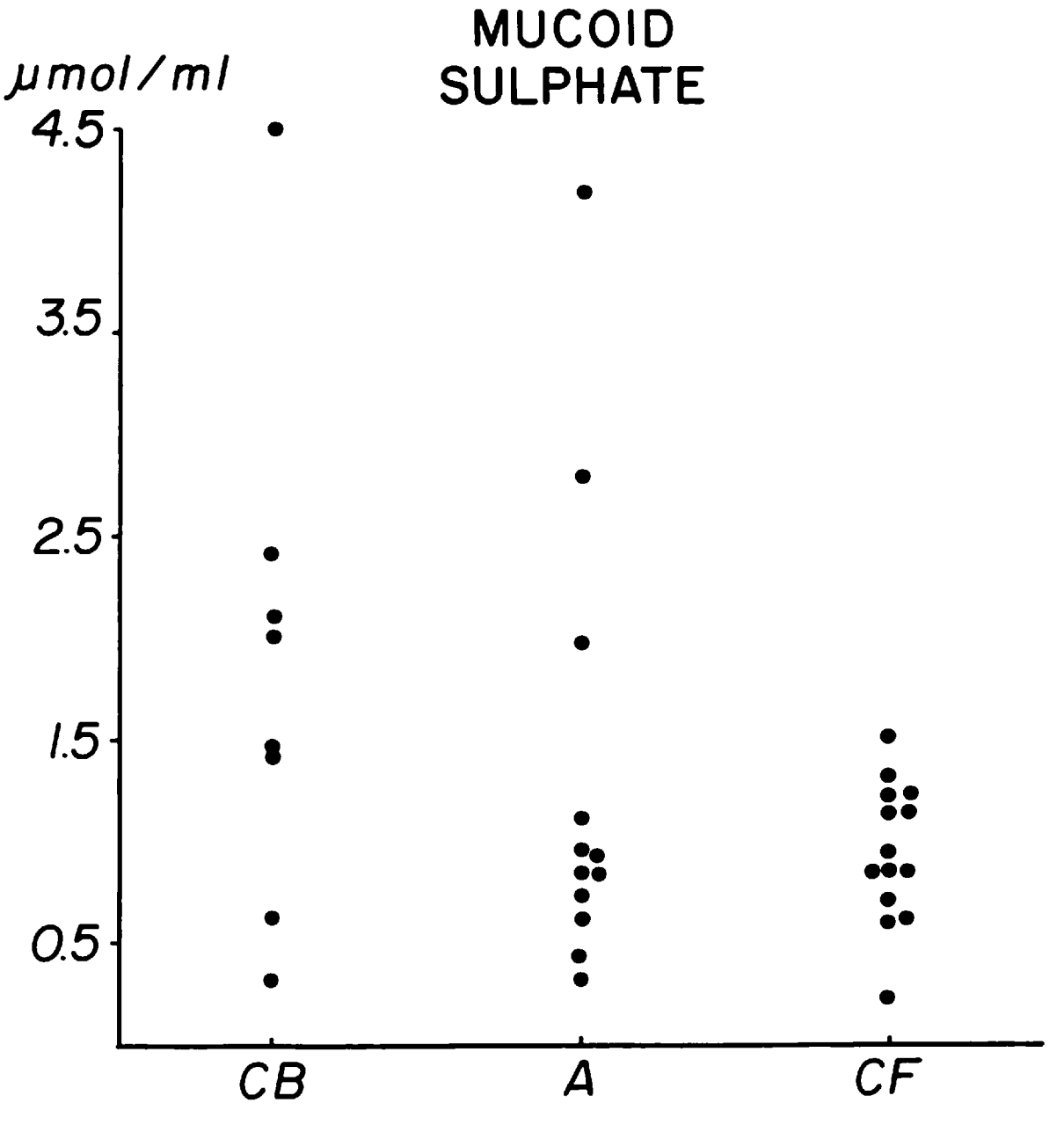


Fig. IV,5 The range of values for mannose in mucoid sputum from patients with chronic bronchitis (CB), asthma (A), or cystic fibrosis (CF).

Fig. IV,5

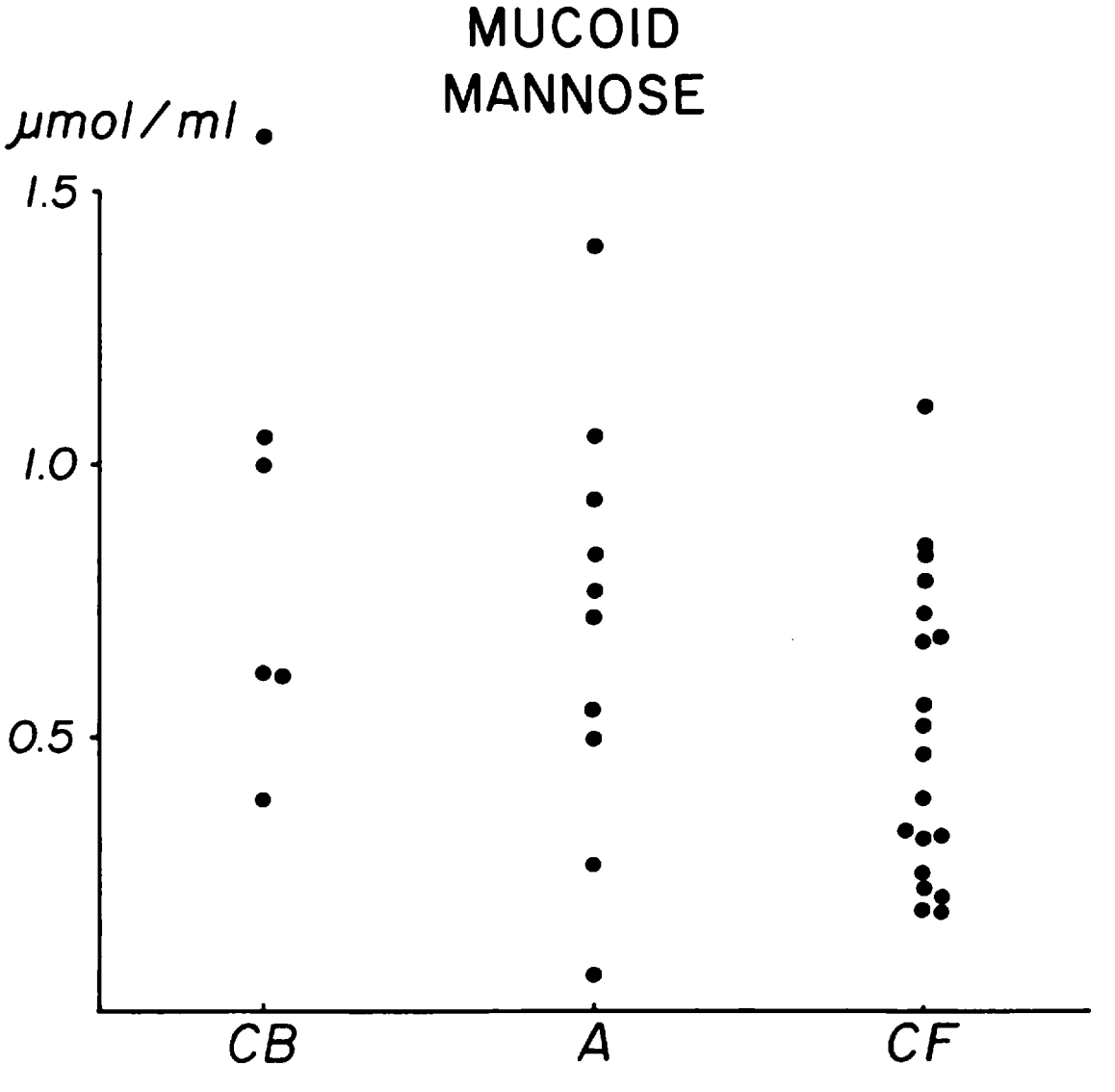


Fig. IV,6 The range of values for NANA/Fucose ratio in mucoid sputum from patients with chronic bronchitis (CB), asthma (A), or cystic fibrosis (CF).

Fig. IV,6

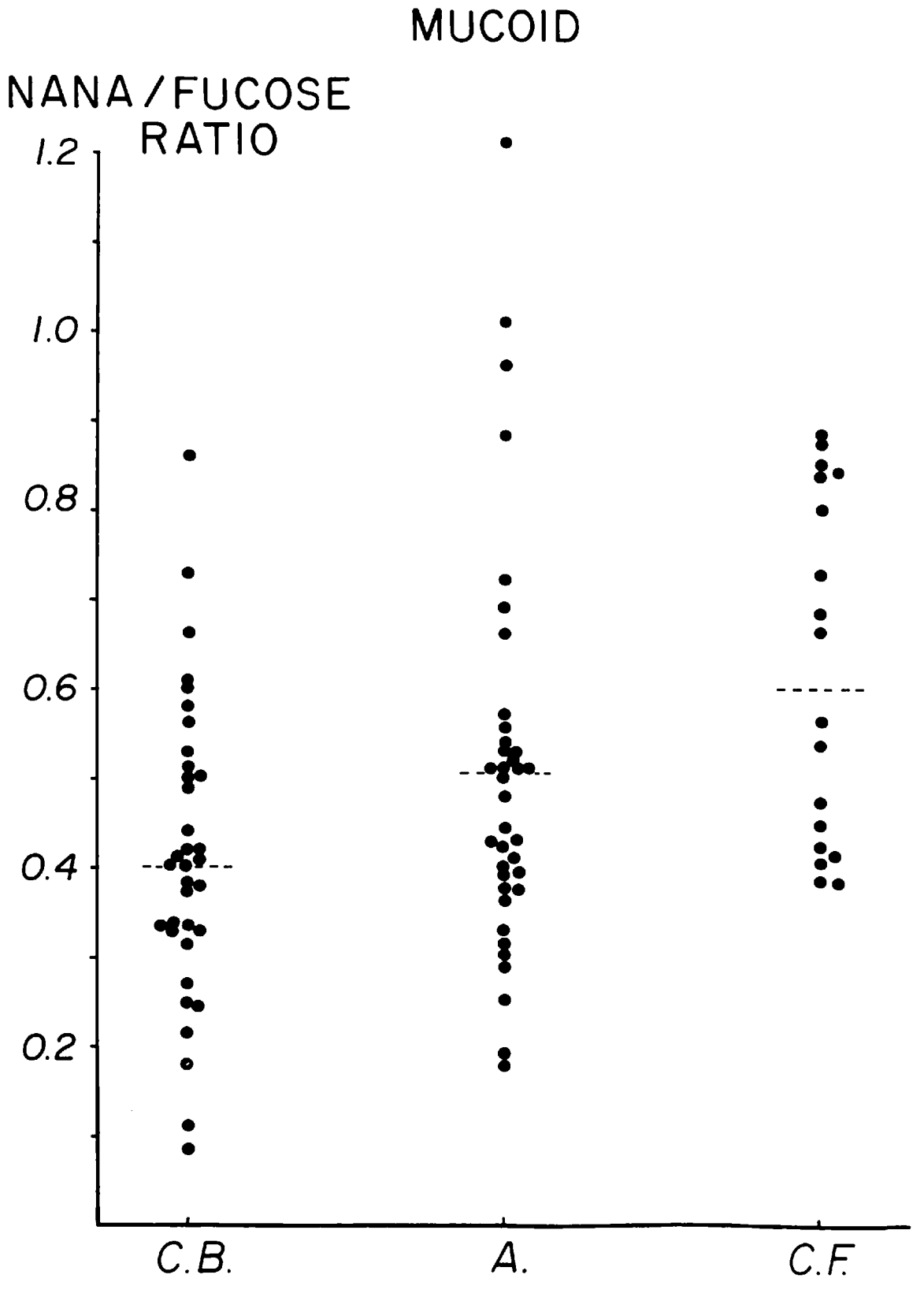


Fig. IV,7 The range of values for dry macromolecular weight in mucopurulent sputum from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF), or bronchiectasis (Bstsis).

Fig. IV,7

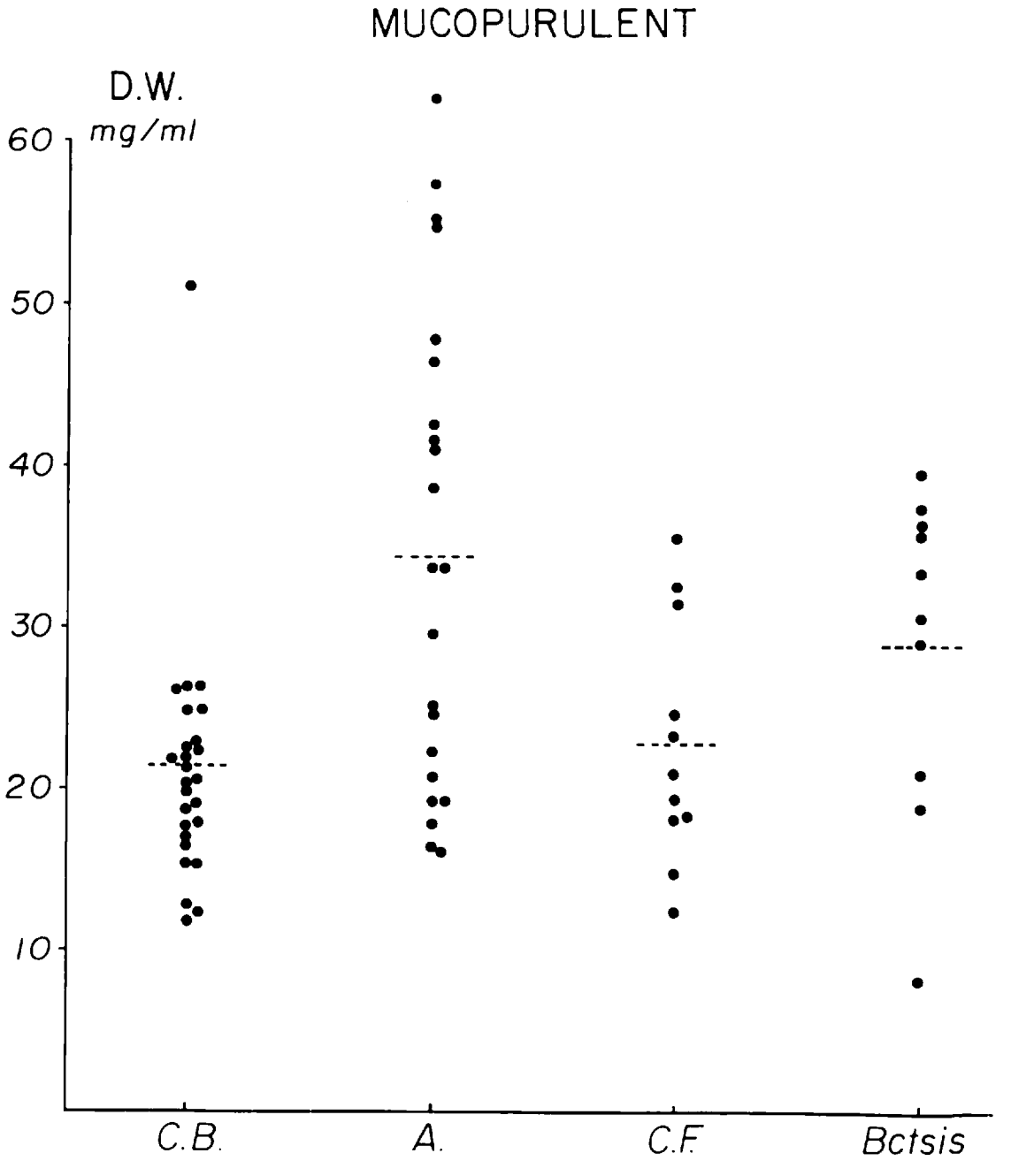


Fig. IV,8 The range of values for N-acetyl neuraminic acid in mucopurulent sputum from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,8

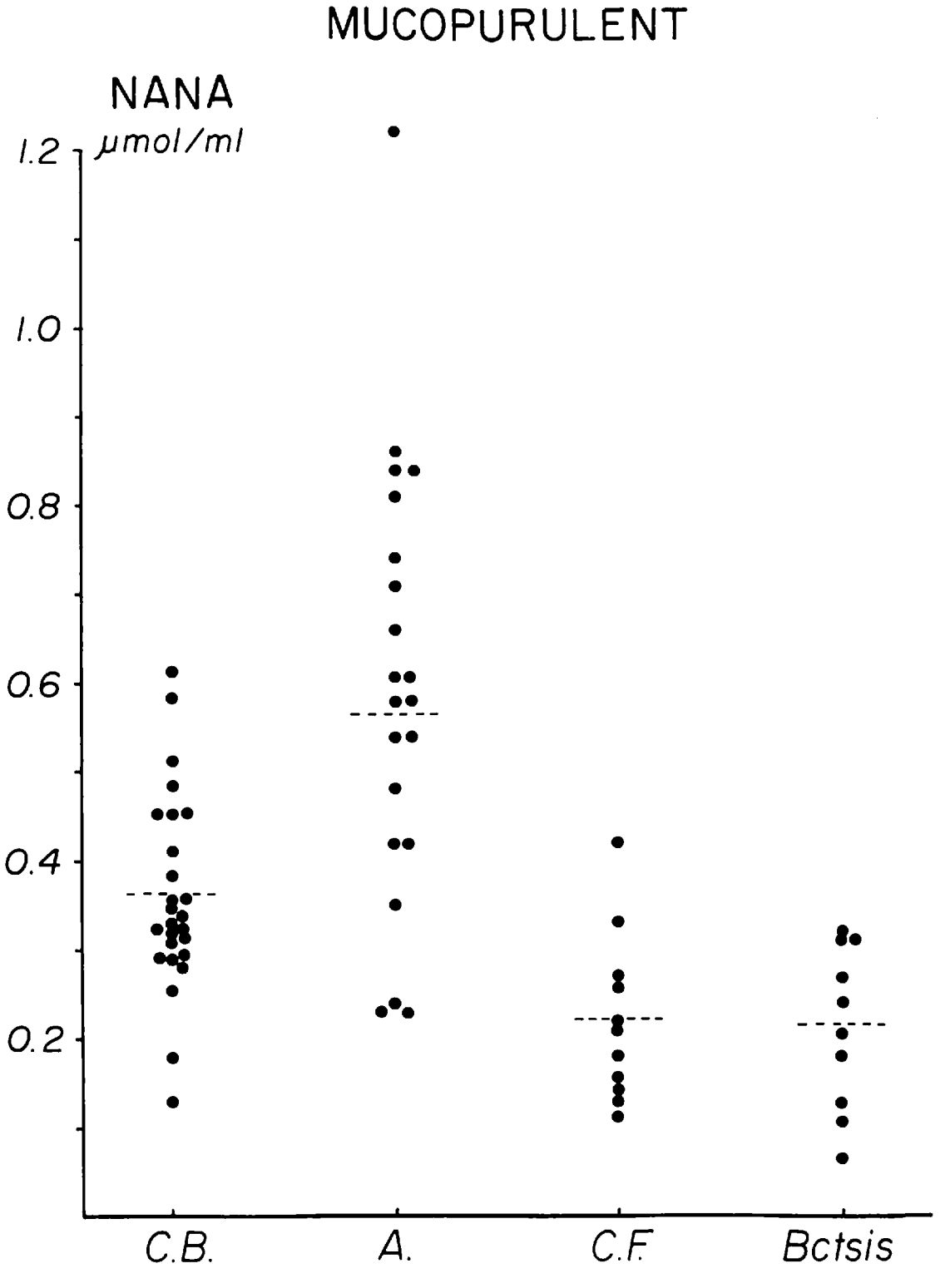


Fig. IV, 9 The range of values for fucose in mucopurulent sputum from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,9

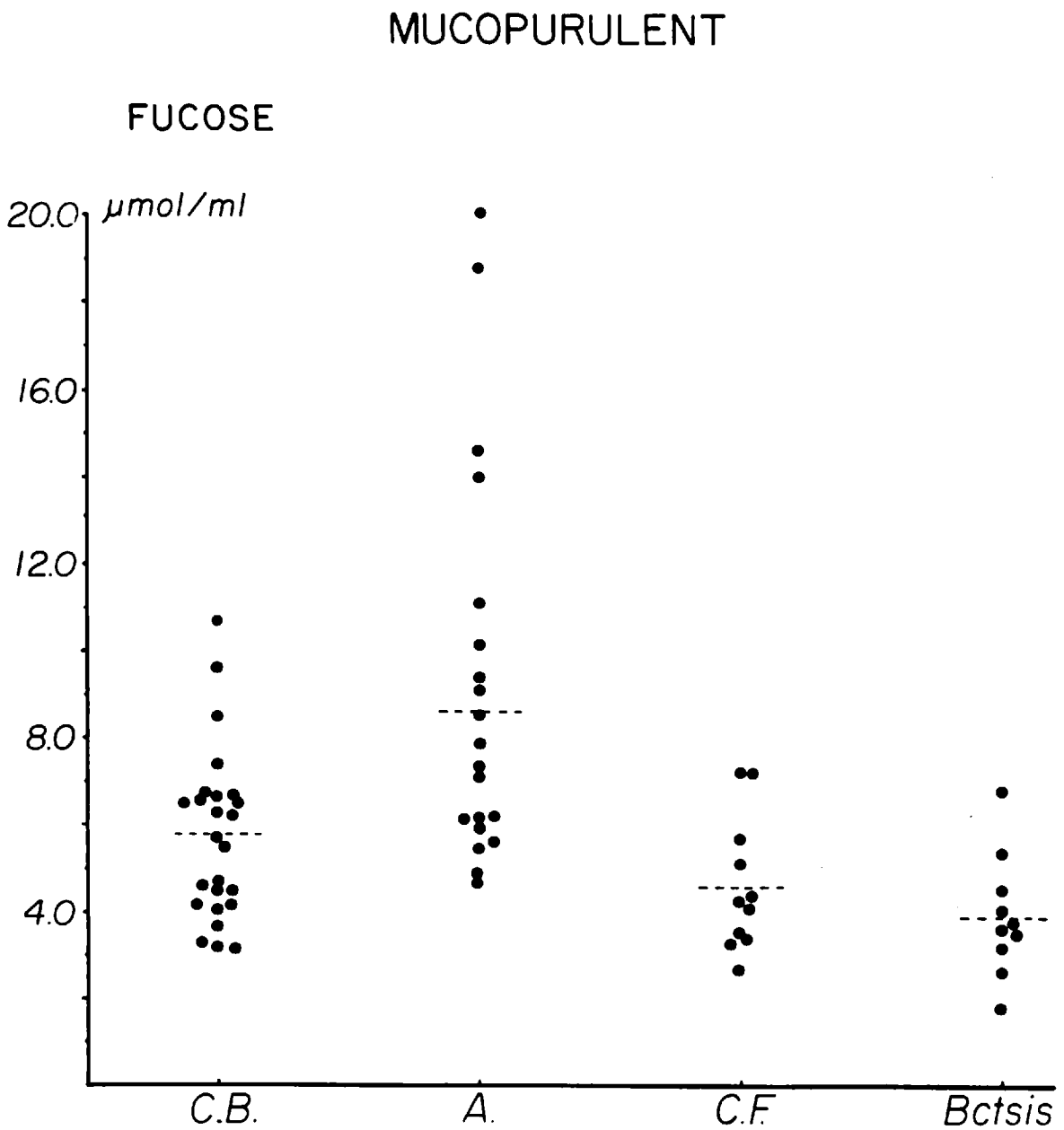


Fig. IV,10 The range of values for sulphate in mucopurulent sputum from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF), or bronchiectasis (Bctsis).

Fig. IV,10

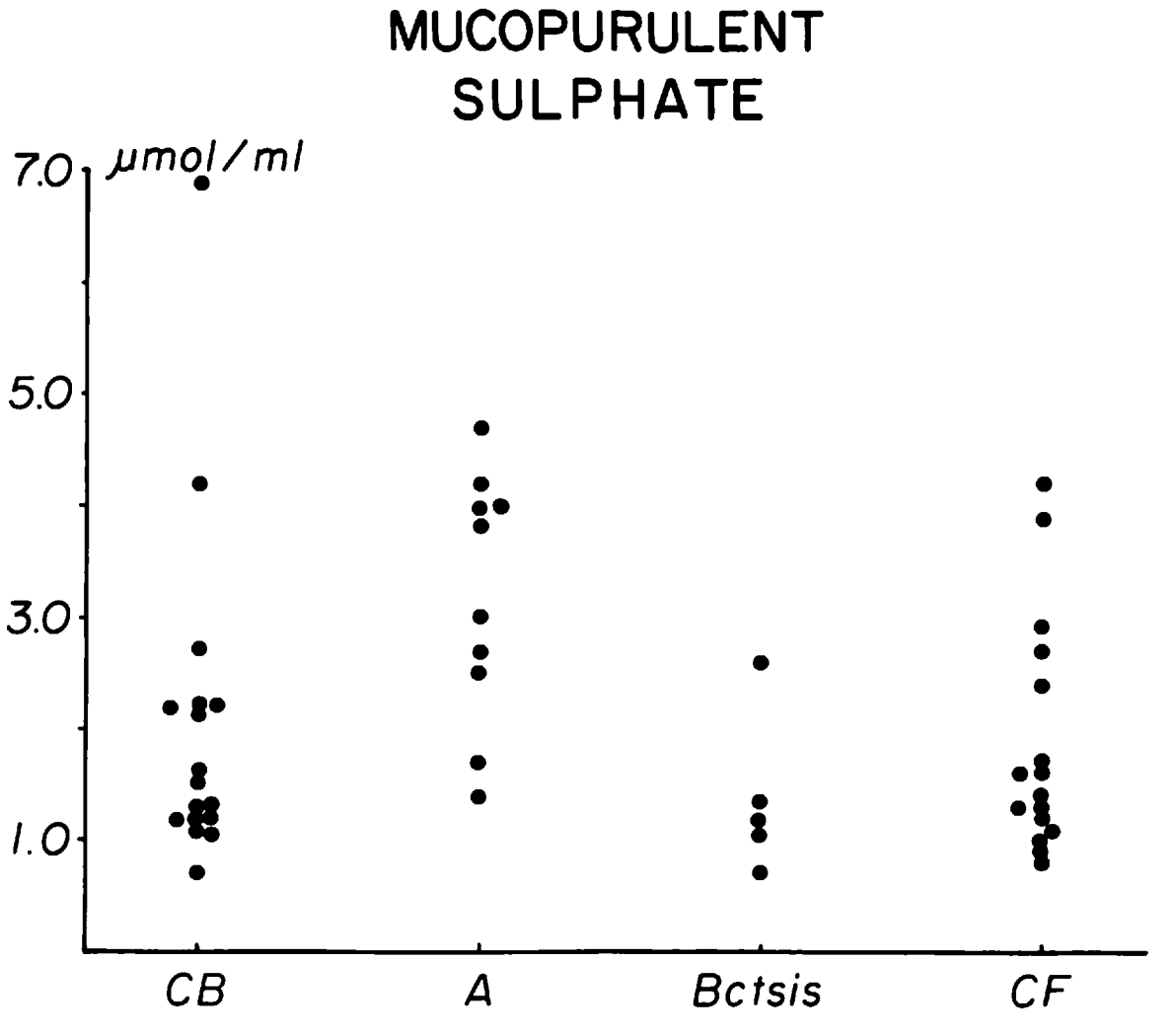


Fig. IV,11 The range of values for mannose in mucopurulent sputum from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,11

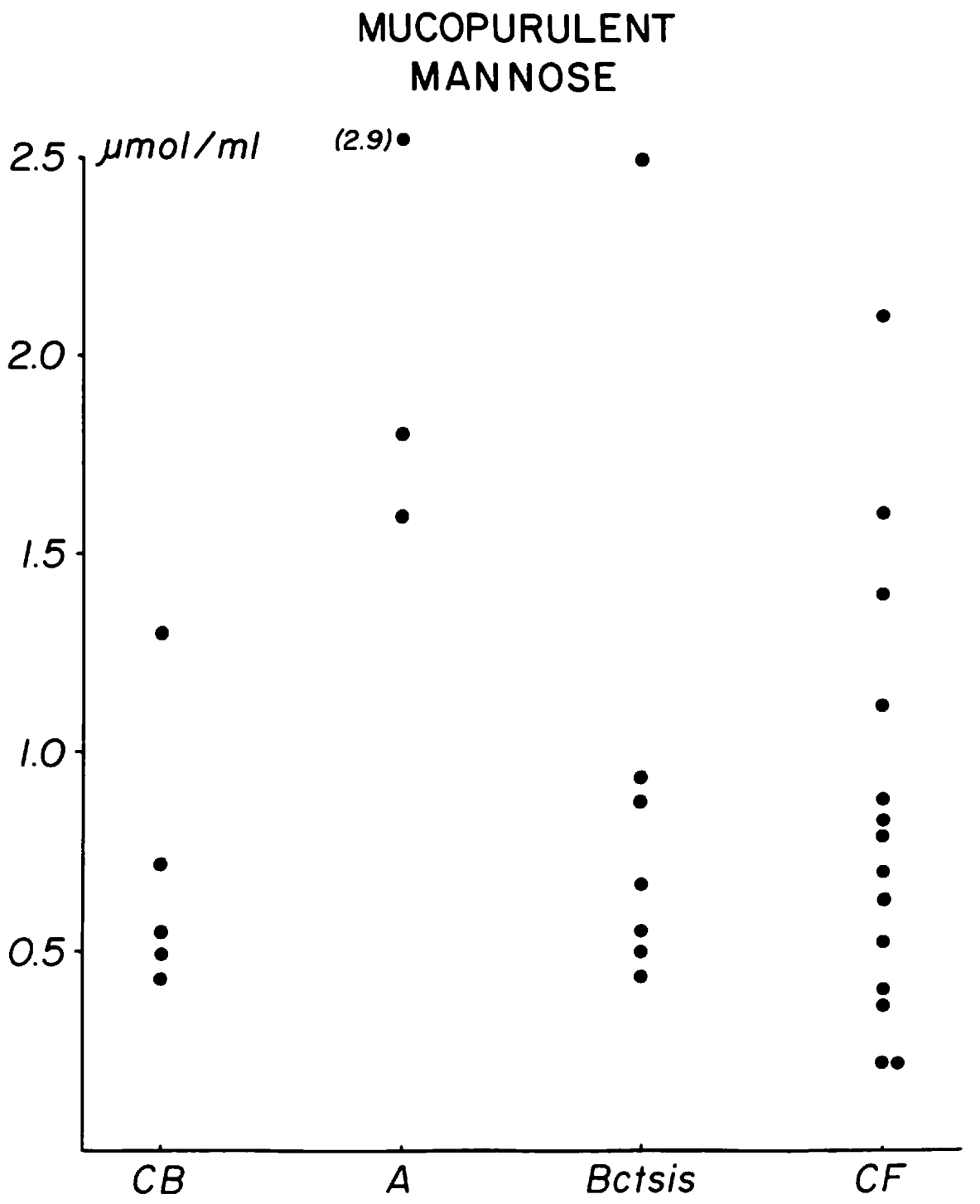


Fig. IV,12 The range of values for NANA/ Fucose ratio in mucopurulent sputum from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,12

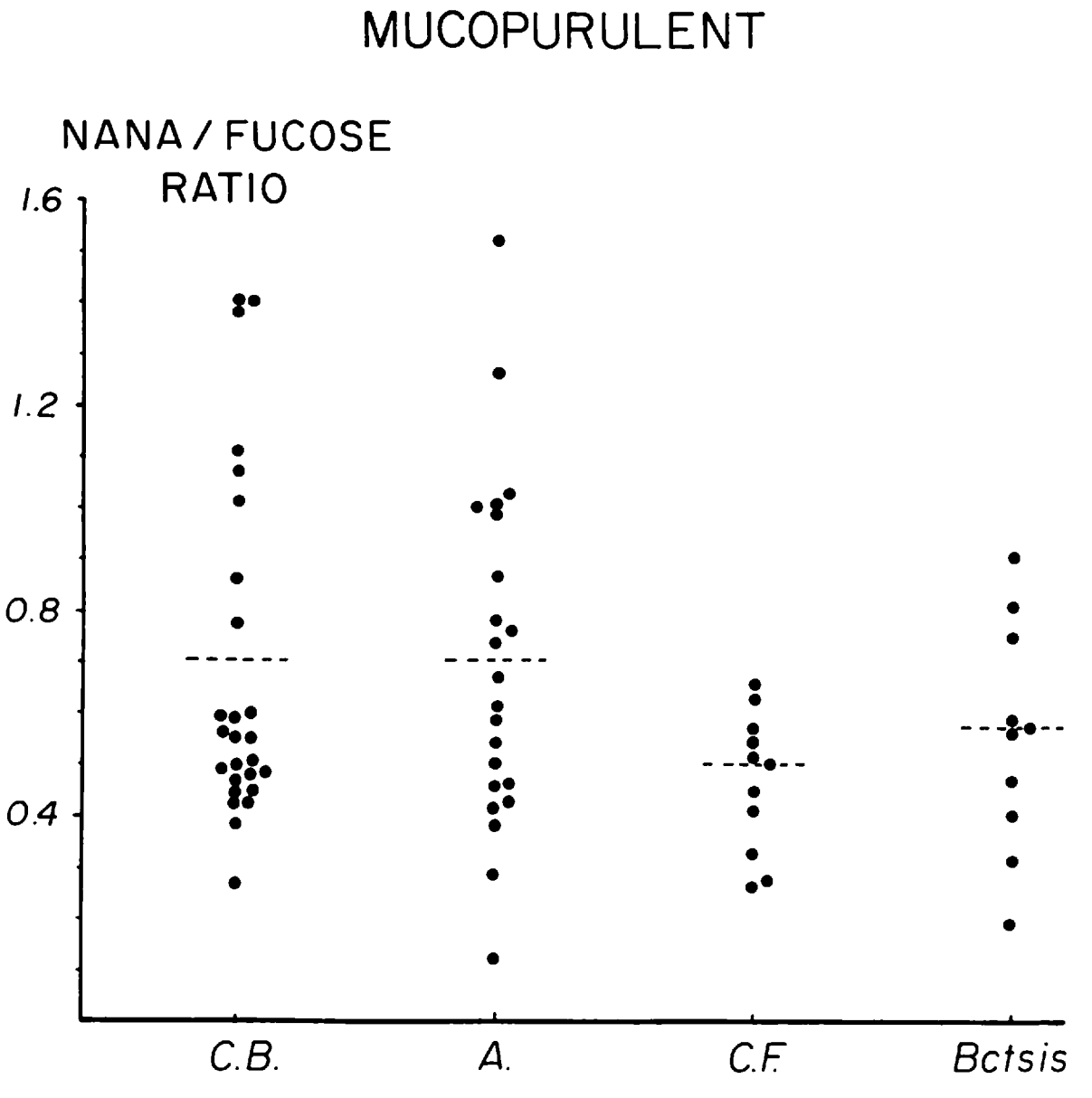


Fig. IV,13 The range of values for dry macromolecular weight in purulent sputum from patients with chronic bronchitis (CB), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,13

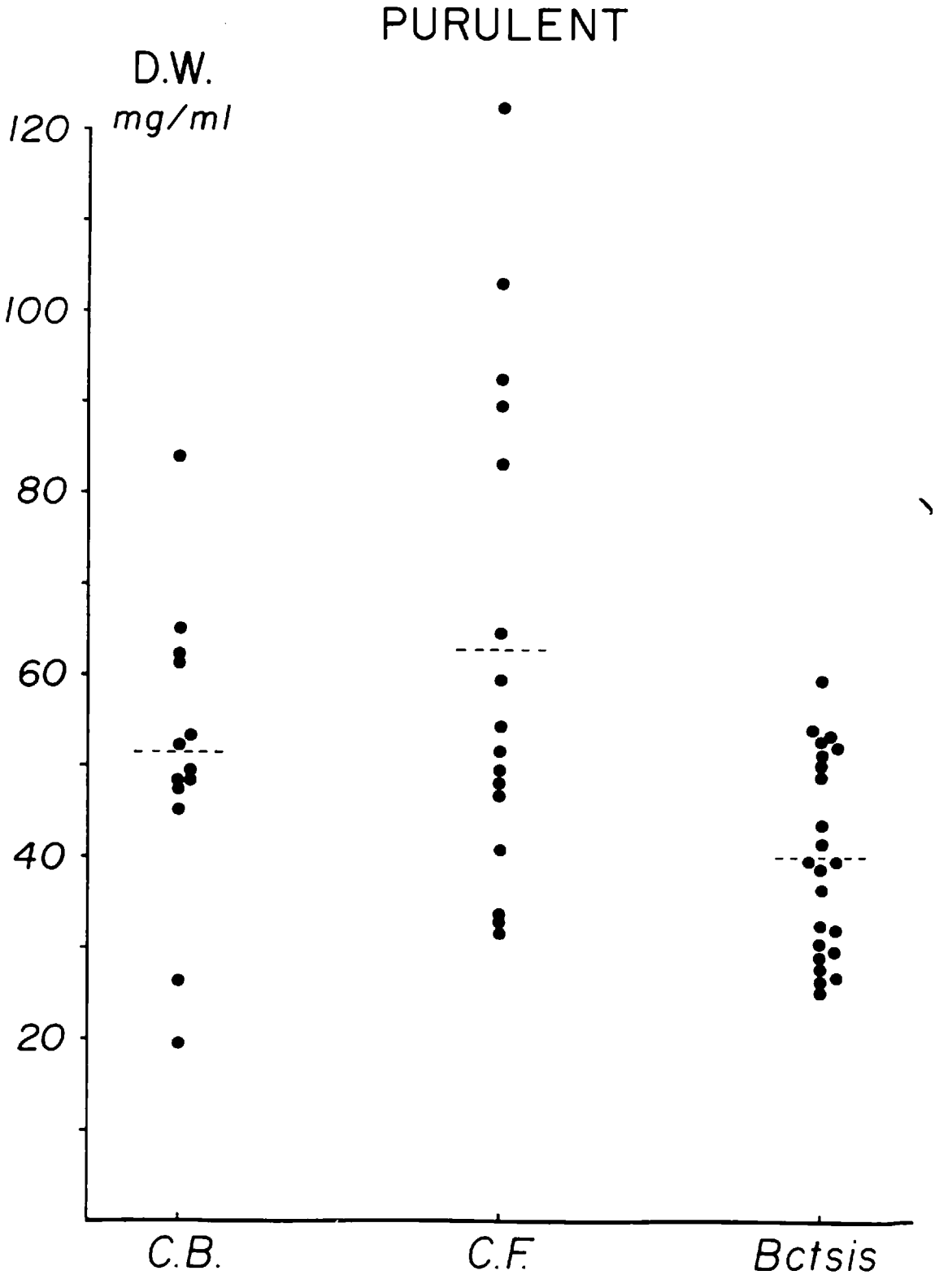


Fig. IV,14 The range of values for N-acetyl neuraminic acid (NANA) in purulent sputum from patients with chronic bronchitis (CB), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,14

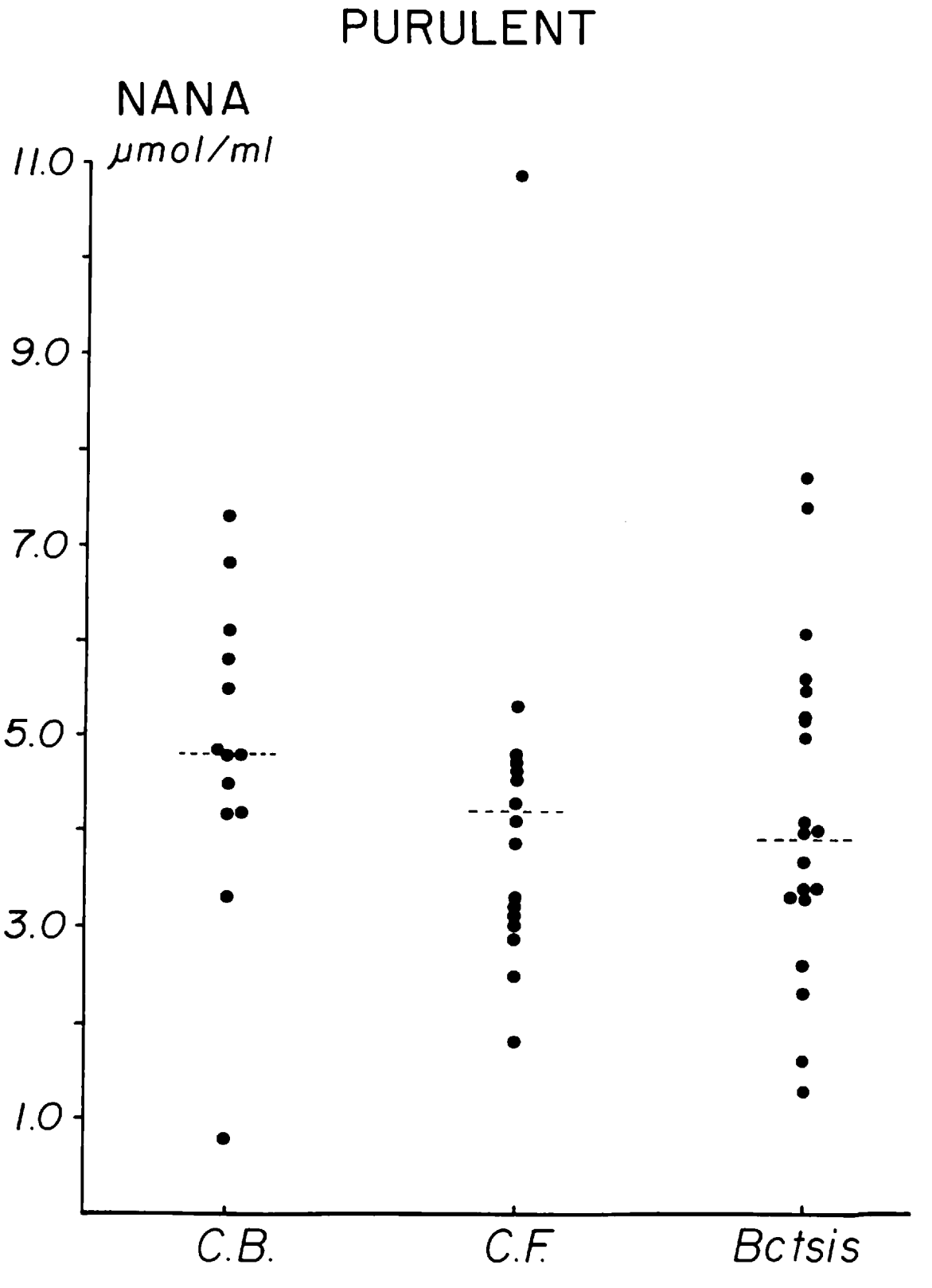


Fig. IV,15 The range of values for fucose in purulent sputum from patients with chronic bronchitis, (CB), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,15

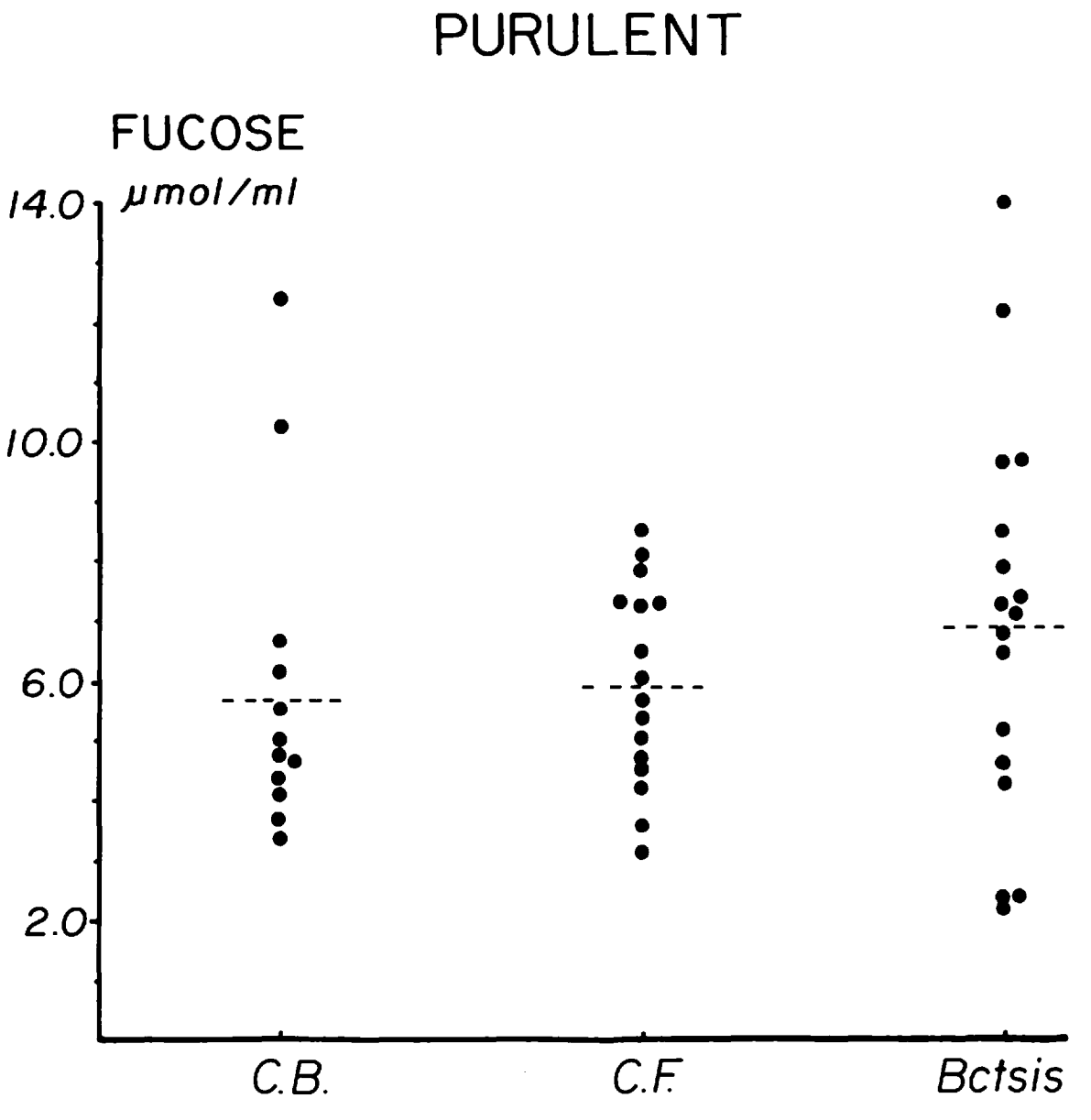


Fig. IV,16 The range of values for sulphate in purulent sputum from patients with chronic bronchitis (CB), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,16

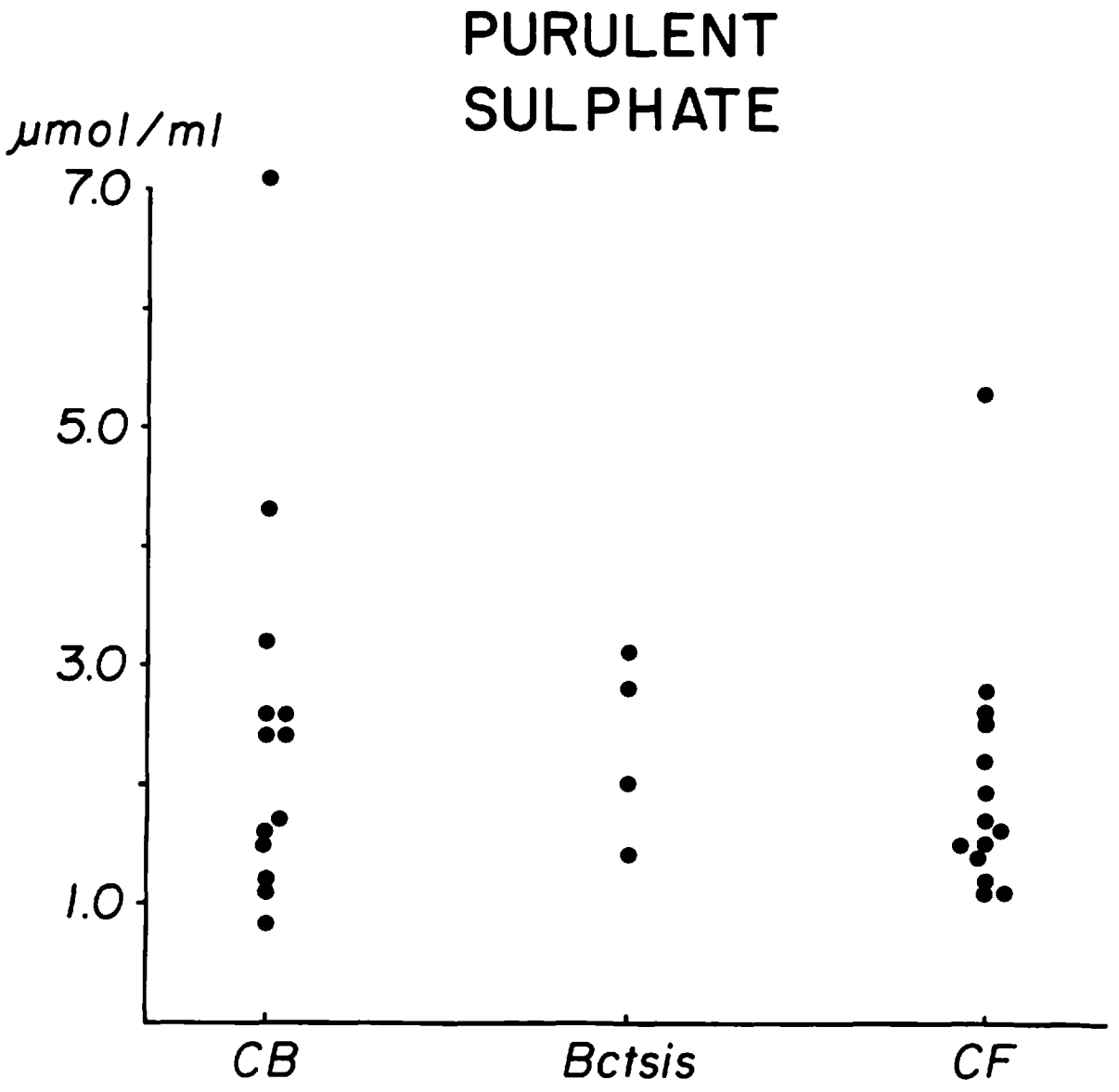


Fig. IV,17 The range of values for mannose in purulent sputum from patients with chronic bronchitis (CB), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,17

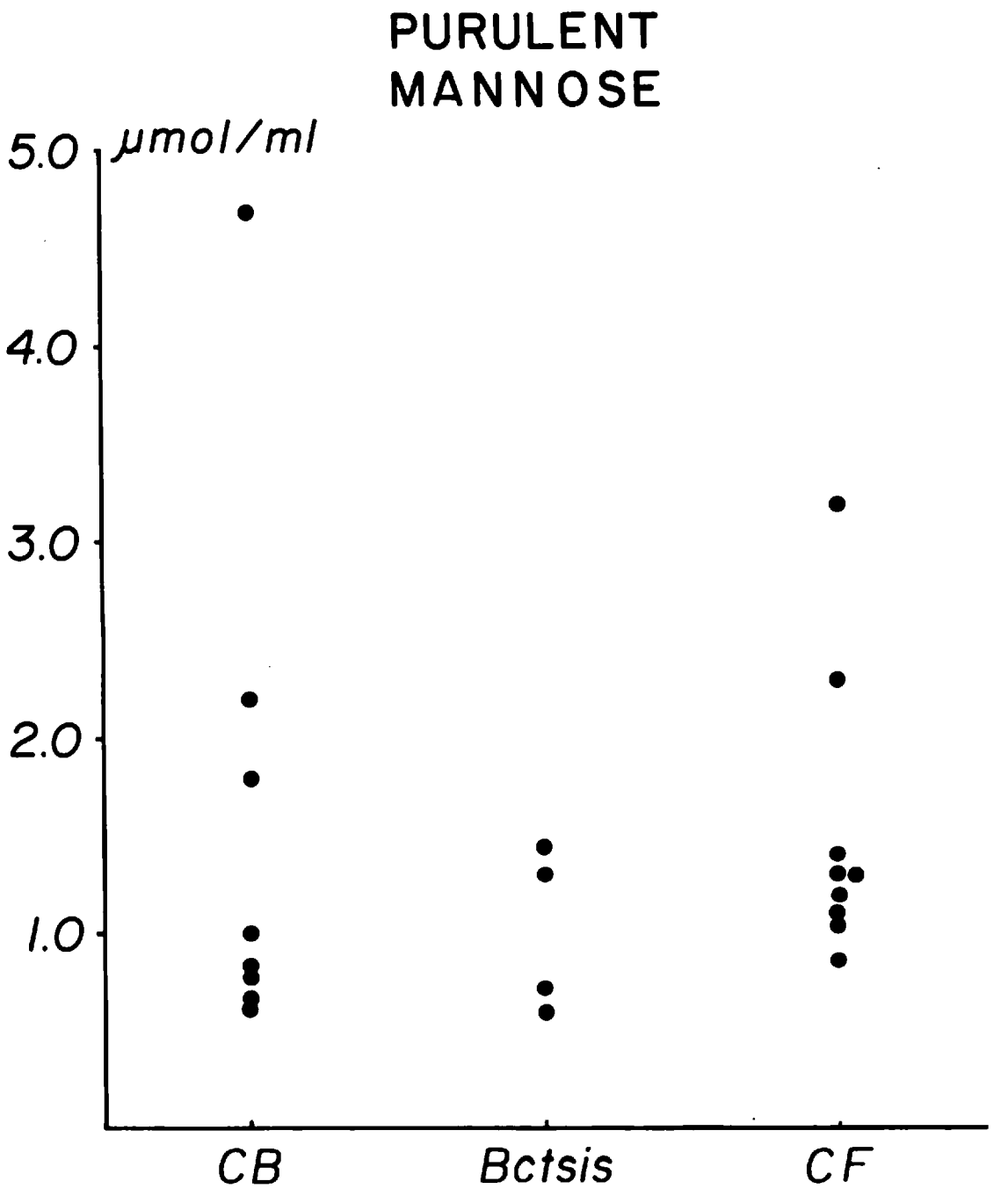


Fig. IV,18 The range of values for NANA/Fucose ratio in purulent sputum from patients with chronic bronchitis (CB), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. IV,18

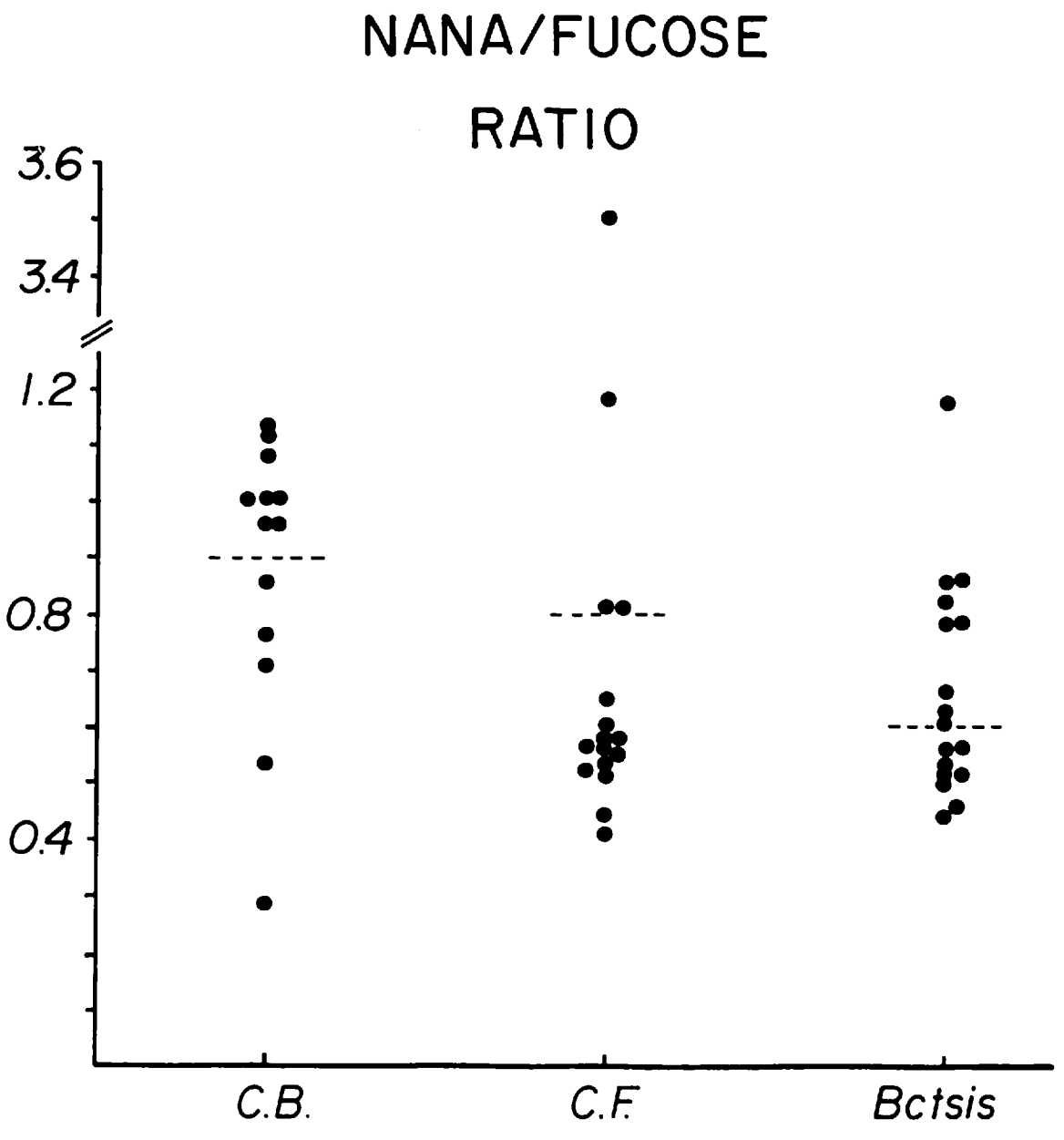


Fig. IV,19 The range of values for dry macromolecular weight in mucoid sputum from patients with extrinsic asthma (EA), intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,19

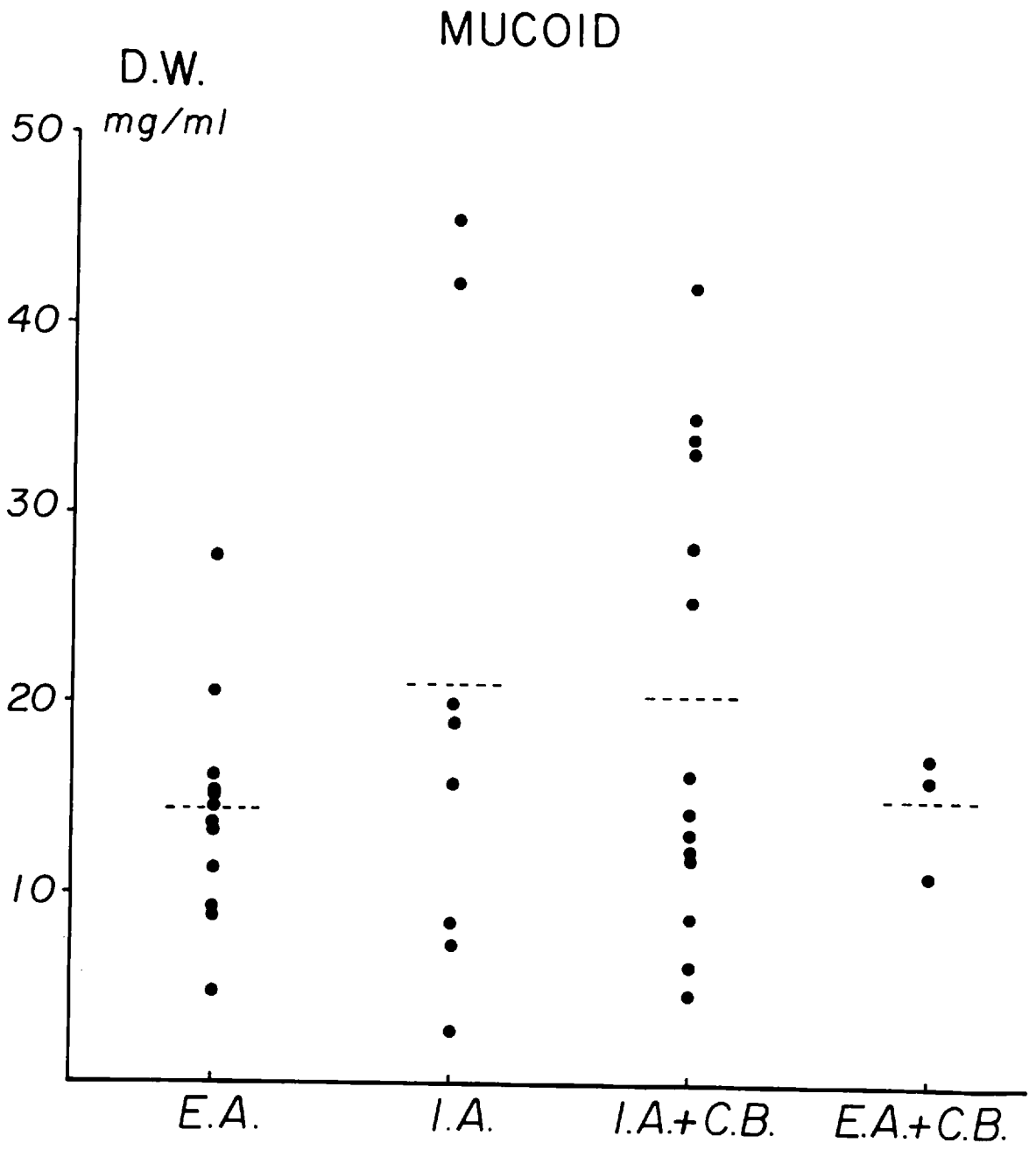


Fig. IV,20 The range of values for N-acetyl neuraminic acid (NANA) in mucoid sputum from patients with extrinsic asthma (EA), intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,20

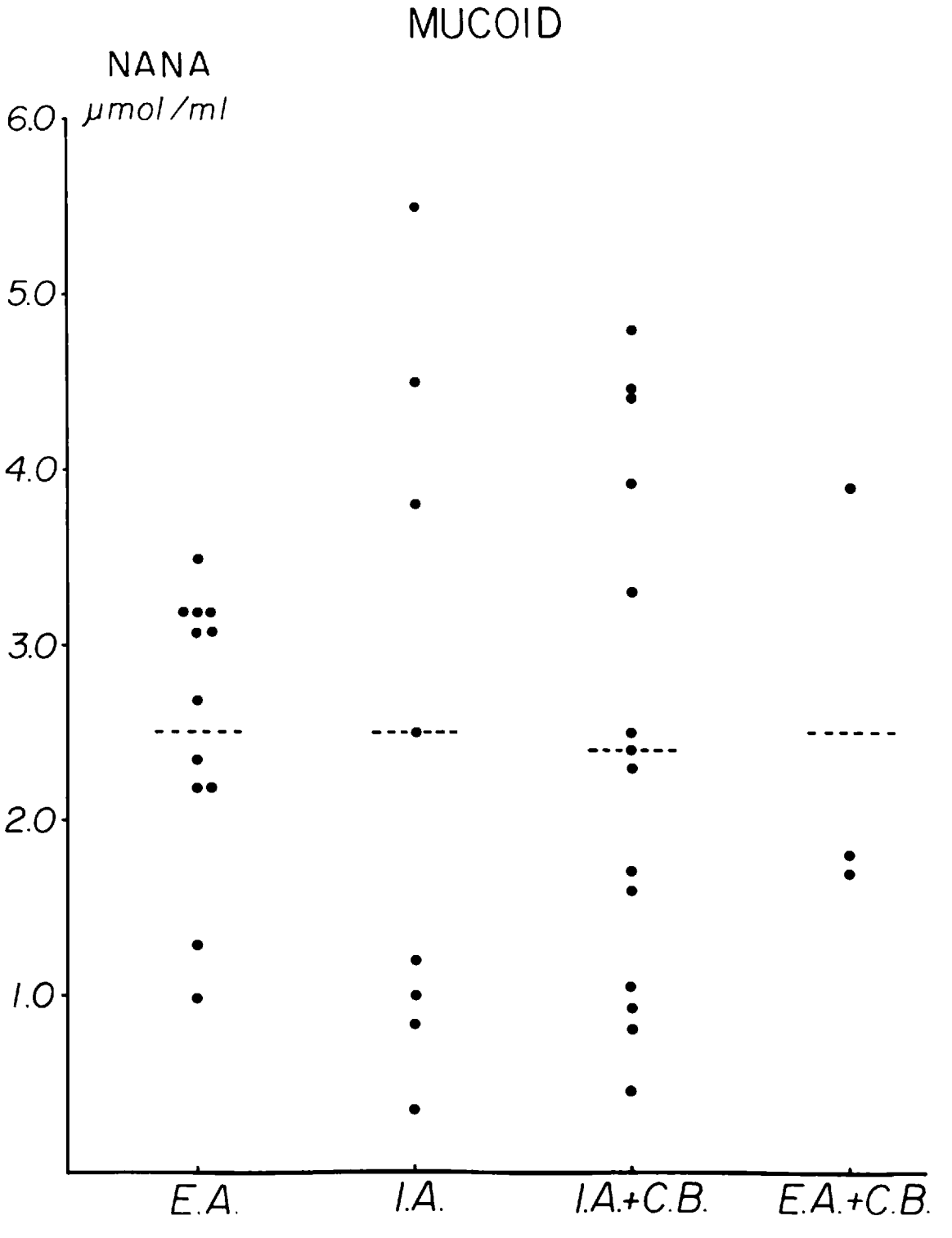


Fig. IV,21 The range of values for fucose in mucoid sputum from patients with extrinsic asthma (EA), intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,21

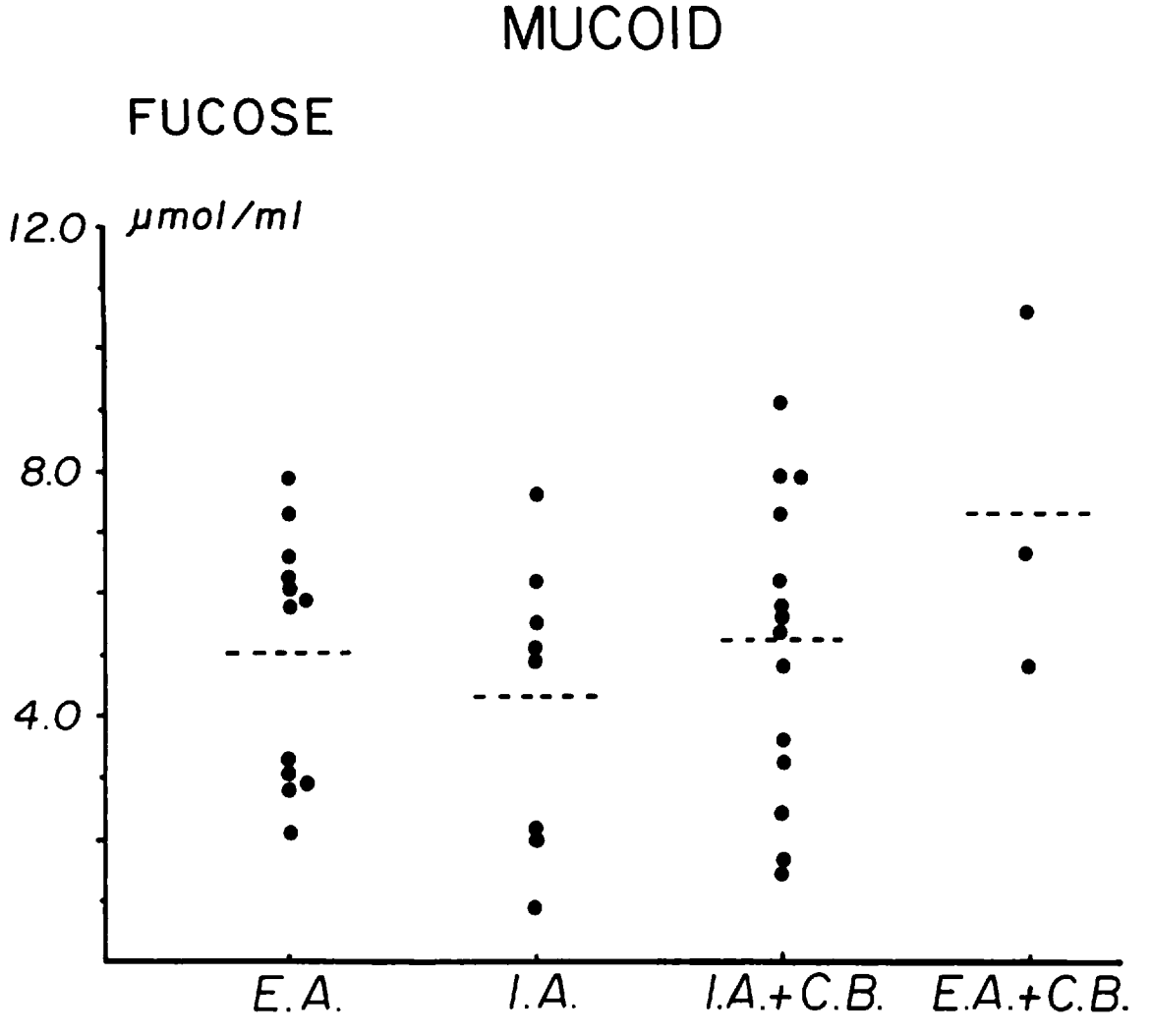


Fig. IV,22 The range of values for sulphate in mucoid sputum from patients with extrinsic asthma (EA), intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,22

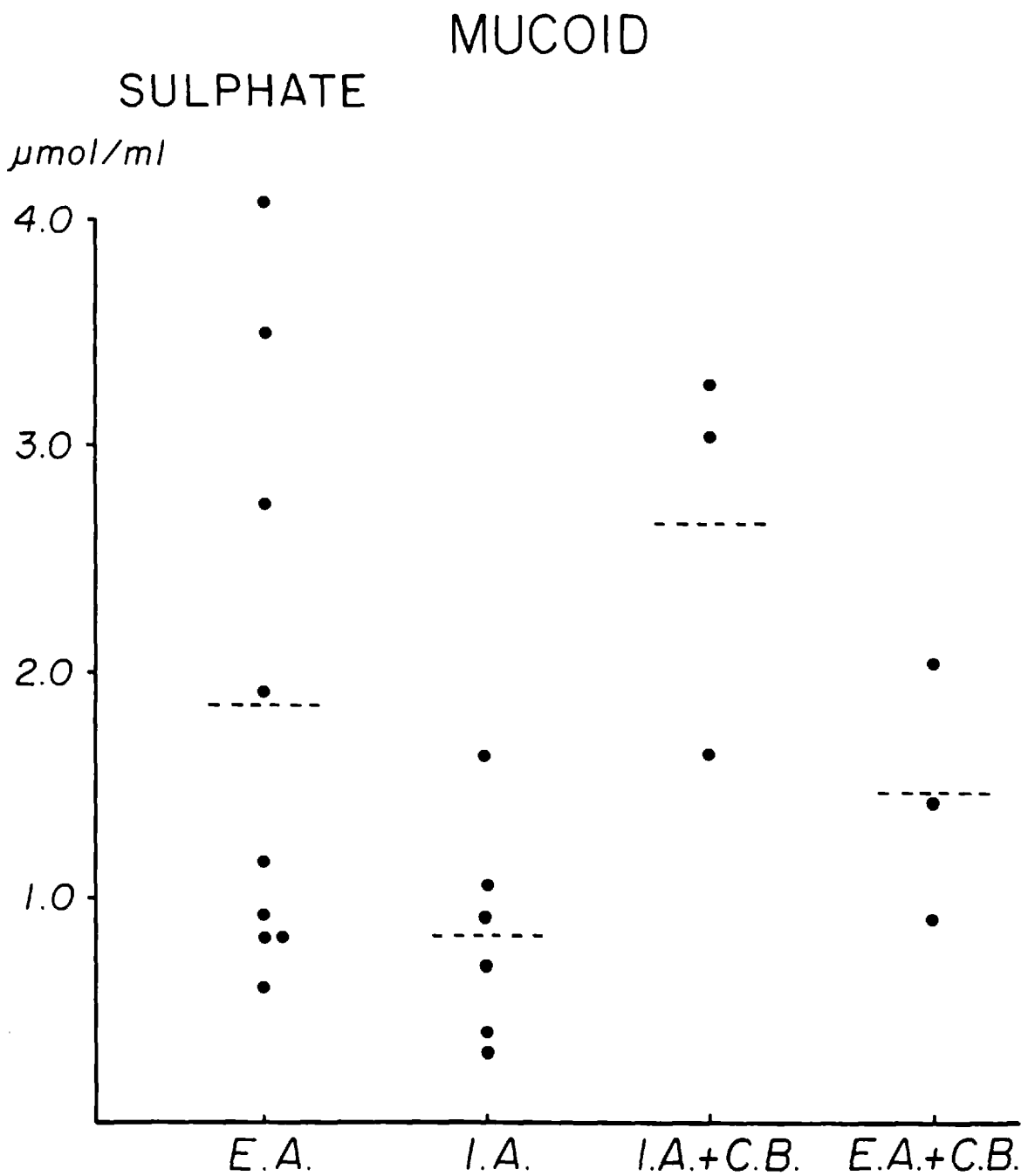


Fig. IV,23 The range of values for mannose in mucoid sputum from patients with extrinsic asthma (EA) intrinsic asthma (IA) or intrinsic asthma + chronic bronchitis (IA+CB).

Fig. IV,23

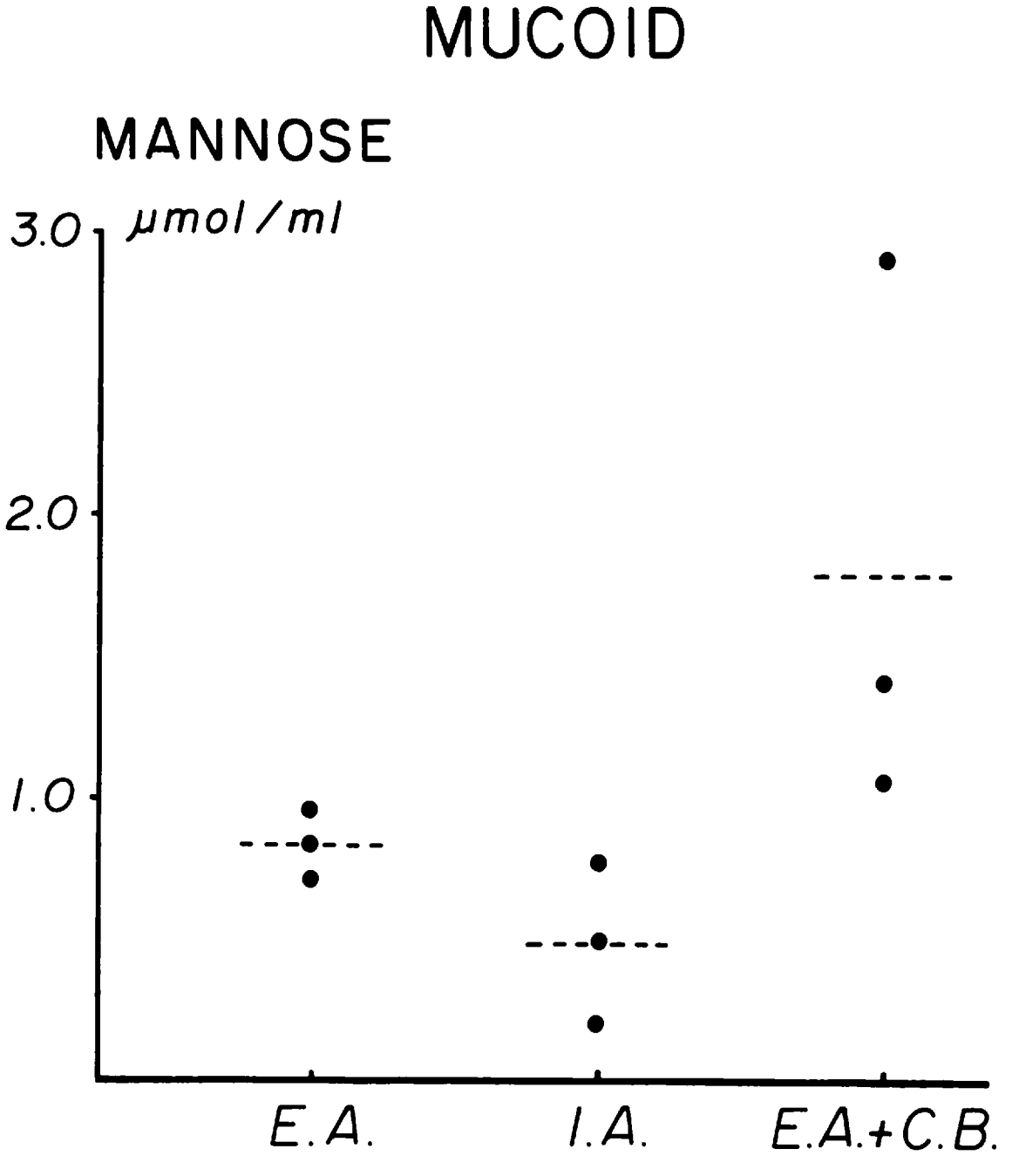


Fig. IV,24 The range of values for NANA/Fucose in mucoid sputum from patients with extrinsic asthma (EA), intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,24

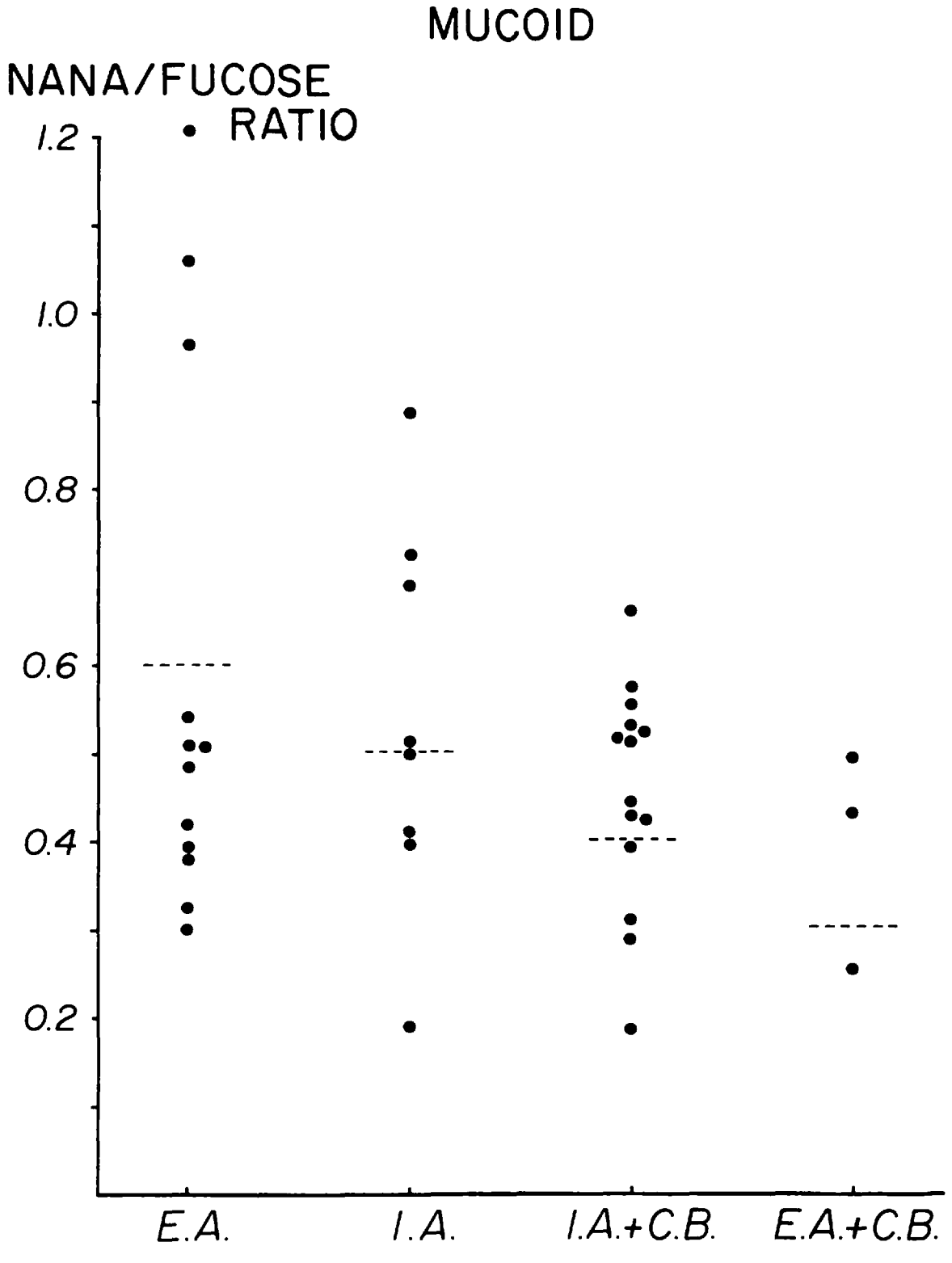


Fig. IV,25 The range of values for dry macromolecular weight in muco-purulent sputum from patients with intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,25

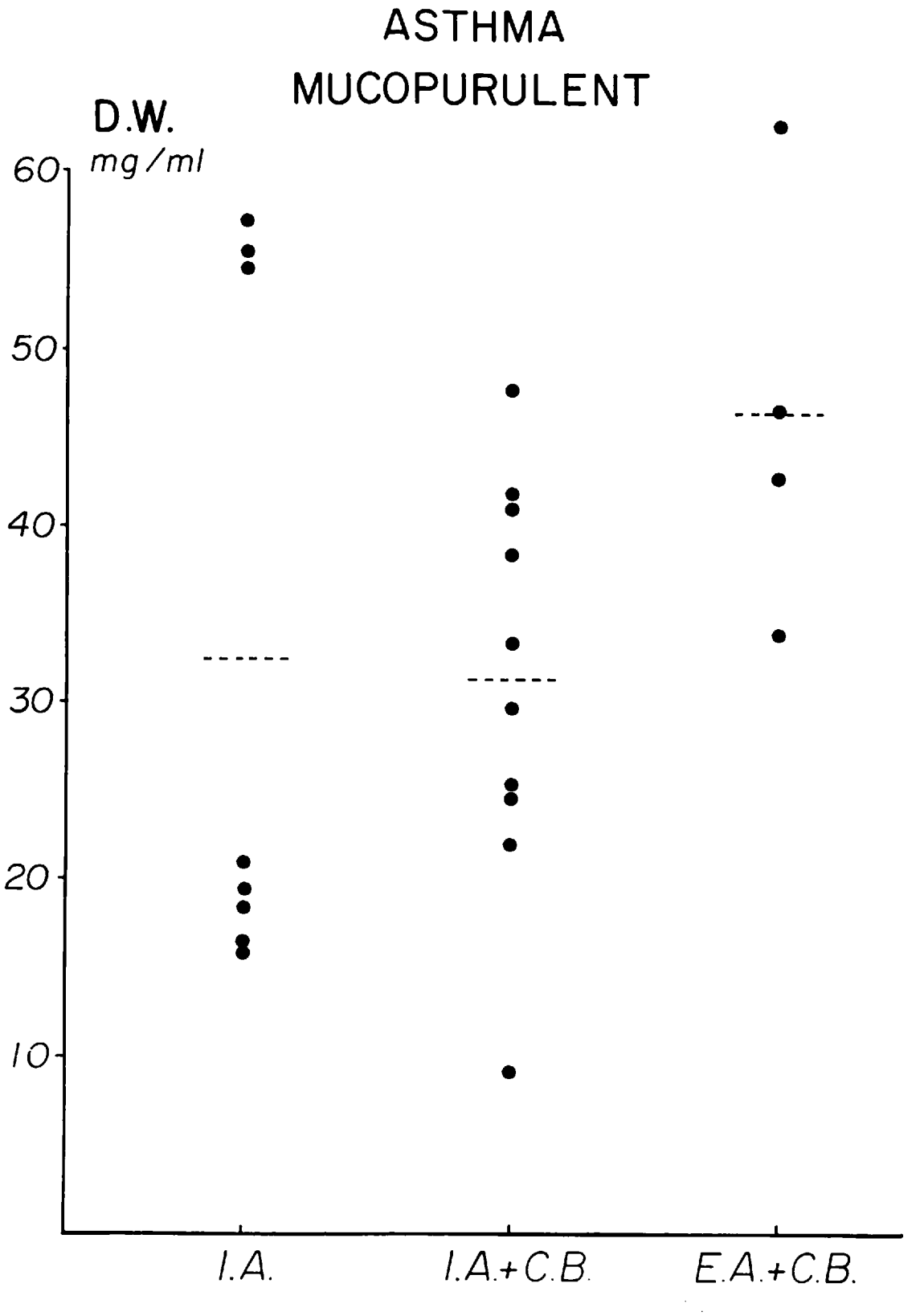


Fig. IV,26 The range of values for N-acetyl neuramic acid in mucopurulent sputum from patients with intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,26

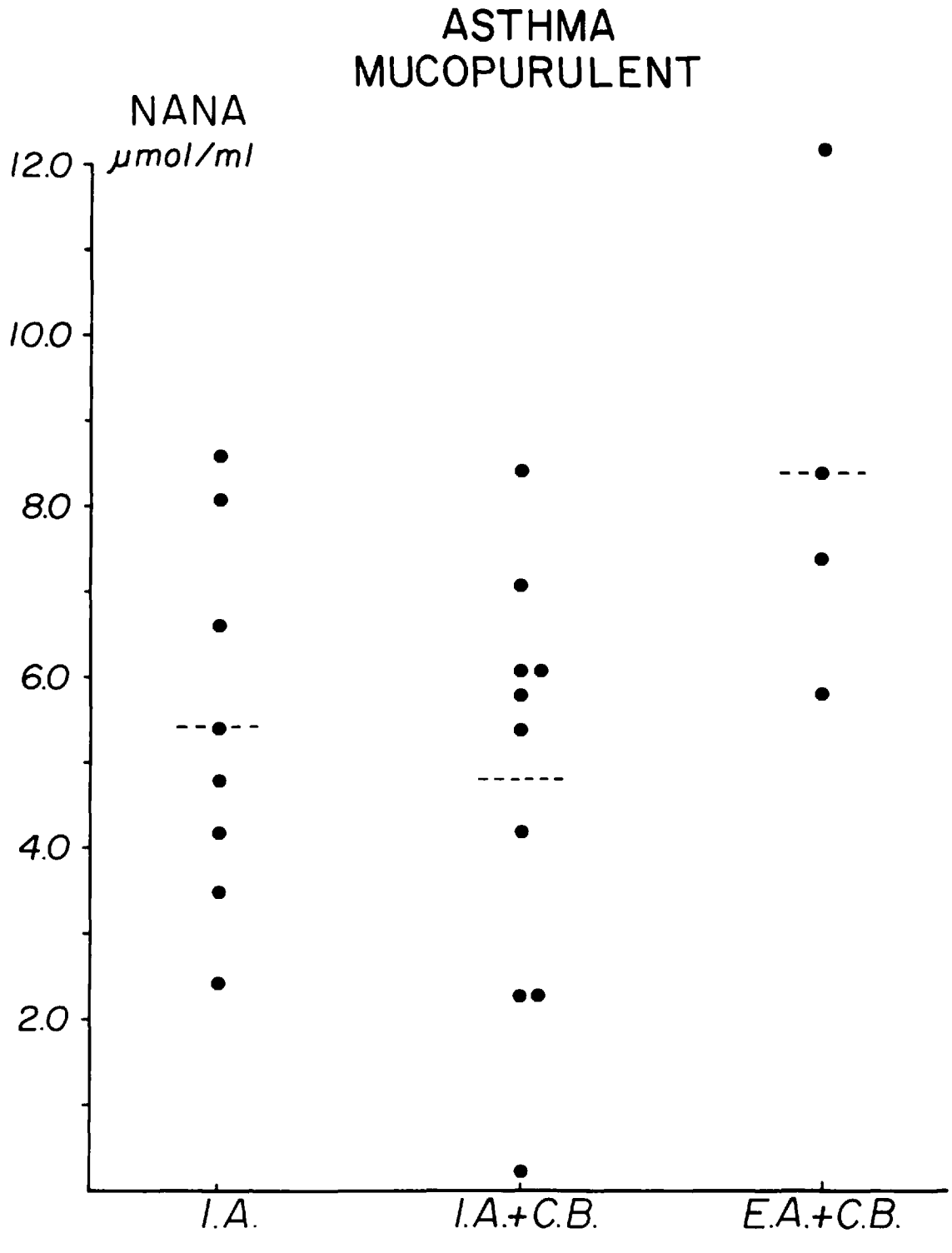

ASTHMA
MUCOPURULENT
NANA
µmol/ml
12.0
10.0
8.0
6.0
4.0
2.0
I.A.
I.A.+C.B.
E.A.+C.B.

Fig. IV,27 The range of values for fucose in mucopurulent sputum from patients with intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB) or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,27

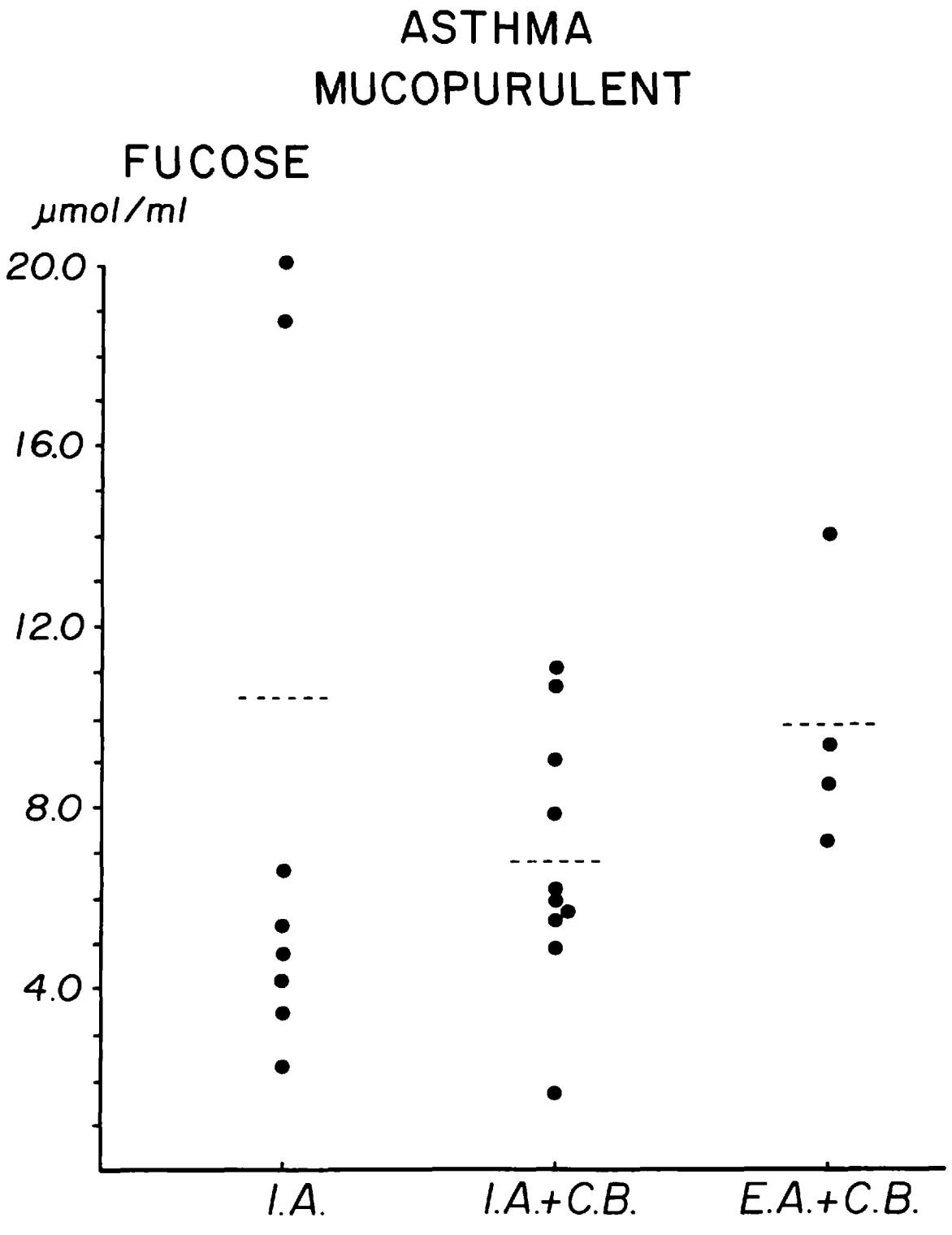
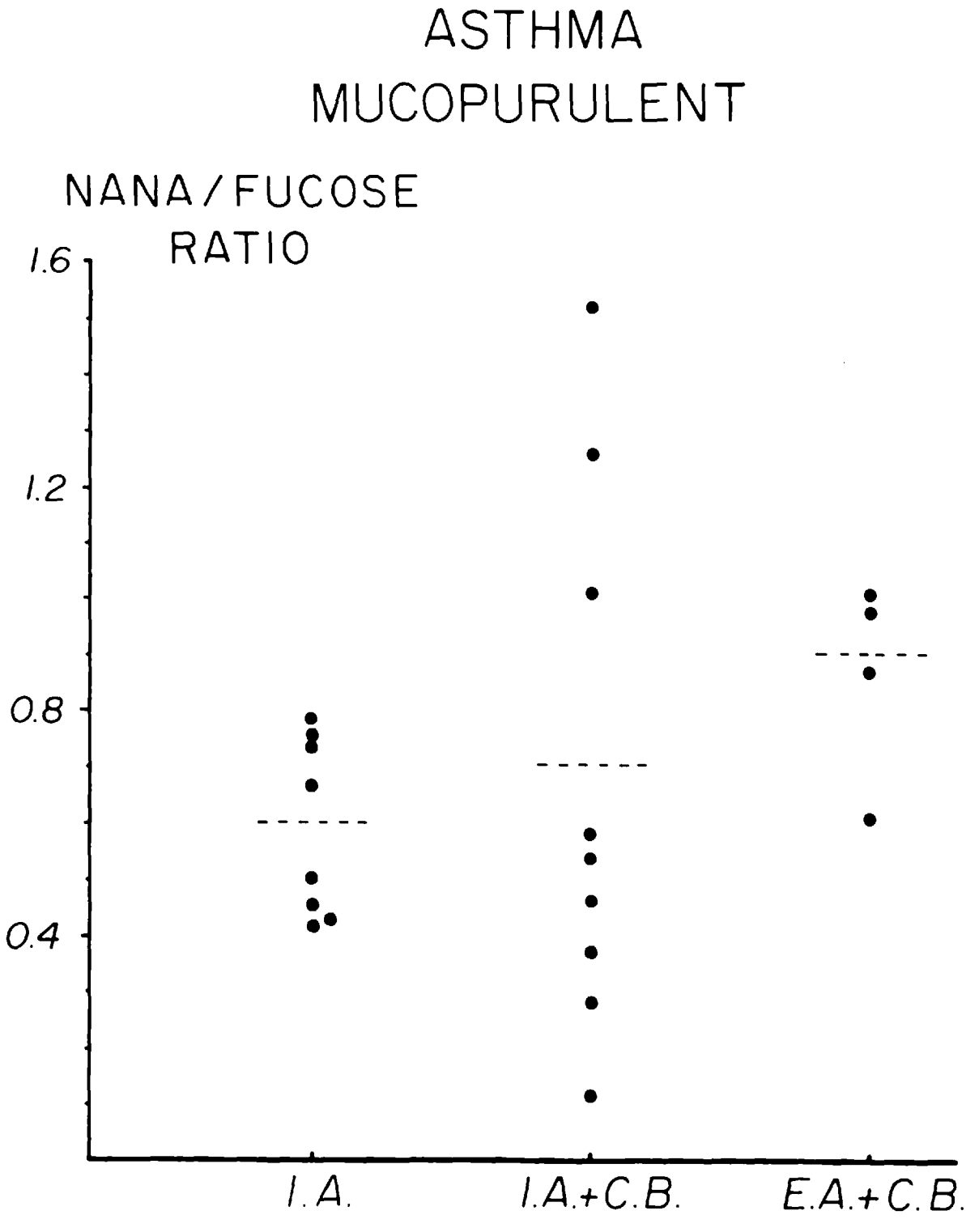

ASTHMA
MUCOPURULENT
FUCOSE
µmol/ml
20.0
16.0
12.0
8.0
4.0
I.A.
I.A.+C.B.
E.A.+C.B.

Fig. IV,28 The range of values for NANA/Fucose in mucopurulent sputum from patients with intrinsic asthma (IA), intrinsic asthma + chronic bronchitis (IA+CB), or extrinsic asthma + chronic bronchitis (EA+CB).

Fig. IV,28


ASTHMA
MUCOPURULENT
NANA / FUCOSE
RATIO
1.6
1.2
0.8
0.4
I.A.
I.A.+C.B.
E.A.+C.B.

CHAPTER V

BRONCHIAL FLUID FROM NORMAL BRONCHIAL TREE

BRONCHIAL FLUID FROM NORMAL BRONCHIAL TREE

Various methods are being used for obtaining normal bronchial fluid in man. Bronchoscopy has several disadvantages, first of all a normal bronchial tree normally does not contain enough secretion to be aspirated; the bronchial fluid has to be flushed out thus altering the concentration of any bronchial material. Furthermore, bronchoscopy usually requires premedication with anticholinergic drugs, such as atropine or hyoscine, that may affect bronchial secretion.

Other methods such as administration of an hypertonic saline solution (Boyd 1940; Bickerman et al. 1958) increases the serum transudate component of bronchial fluid therefore producing changes in the relative proportions of chemical constituents. Matthews et al (1963) have used laryngectomised patients for obtaining normal bronchial secretion but in these circumstances the respiratory tract is probably abnormal anatomically as well as physiologically.

Inhalation of prostaglandin F2α: a method to obtain sputum from normal subjects:-

During experiments carried out on healthy volunteers to study the bronchoconstrictor effect

of prostaglandin F2α (PGF2α), it was noticed that after inhaling the drug the subjects coughed and expectorated for about 20 minutes. This finding was investigated further.

Twelve normal subjects, 10 male and 2 female, took part in the study; their ages ranged from 52 to 21 with a mean of 35. Four subjects inhaled PGF2α on two separate occasions, 12 months apart.

A solution of PGF2α (500 μg/ml of the tromethamine salt) was administered by a pressure cycled ventilator (Bennet) adjusted to nebulize on inspiration only. The subjects were asked to take 10 maximal inspirations. The total amount of PGF2α delivered to each subject was between 40-60 μg.

The peak expiratory flow rate (PEFR) was measured with a Wright's peak flow meter and the measurement was recorded for each subject before and after inhaling the drug to assess the degree of bronchial constriction.

The sputum produced after inhalation of PGF2α was expectorated into a cardboard container and its viscosity measured immediately with a cone and

plate viscometer (Ferranti-Shirley). The remainder of the specimen was frozen and stored at -20°C for chemical analysis. The small amount of sputum produced only sufficed for measurements in all samples of dry weight, NANA, fucose and mannose and in seven samples, in addition, sulphate, albumin, IgA, IgG and transferrin.

Before inhaling the drug each subject was asked to produce 5-10 ml of saliva. Viscosity measurements, storage and chemical analysis were carried out on this in the same way as on sputum.

Bronchoconstrictor effect:- The percentage reduction in PEFR for each subject is given in Table V,1; the range varied from nil to -32% with a mean of 10.5%. Two subjects (Nos. 4 and 7) showed a marked bronchoconstrictor effect.

Rheological properties of sputum produced after inhalation of prostaglandin F2α:-

Levels of apparent viscosity of saliva and of sputum produced after inhalation of PGF2α are given for each subject in Table V,2. (Fig.V,1).

In all subjects except one (No. 12) the apparent viscosity of the material expectorated

after inhaling PGF2α was above saliva levels and fell within the range found in mucoid sputum from chronic bronchitis or asthma. Specimens No. 10(a) and 11 had very high viscosity, these two subjects were recovering from a cold and were therefore considered separately from the others in this study. Subject No. 12 was excluded since his sputum fell within his saliva level.

Mean values and standard error of the mean are given in Table V,2. The sputum produced after inhalation of prostaglandin F2α was found to be more viscous than saliva ($P < 0.02$).

Chemical constituents of sputum produced after inhalation of prostaglandin F2α:-

Absolute levels, mean, standard error of the mean and Student's t test for dry weight, NANA, fucose, sulphate, mannose, NANA/fucose ratio, albumin, IgG, IgA and transferrin, are given in Tables V,3,4,5,6,7,8 and 9 respectively. Samples 10(a), 11 and 12 were excluded from the study.

Levels of dry weight and concentrations of NANA, fucose and sulphate were found to be significantly higher in the sputum produced after inhalation of PGF2α than in saliva and fell within the lower range

of mucoid chronic bronchitis or asthma sputum. (Fig. V, 2,3,4, 5 and 7). Mannose concentration was slightly higher than in saliva but the difference did not reach significance, and the levels found in sputum produced after inhaling PGF2α were lower than those found in mucoid sputum from chronic bronchitis or asthma (Fig.V,6).

Levels of albumin in saliva were found to be within the range reported by Salvaggio et al. (1971) and showed a wide range of values. Albumin concentration in sputum produced after inhaling PGF2α were within the levels found in bronchial washings from normal subjects (Falk et al 1972) except in two subjects (Nos. 4 and 7) in whom albumin levels were found to be very high and fell within the range reported in mucoid sputum from chronic bronchitis or asthma (Brogan et al. 1971). It is interesting to note that the two subjects with high albumin levels were those who had the maximal bronchoconstrictor response to the drug.

Albumin levels were found to be significantly higher in sputum after inhaling PGF2α than in saliva ($P < 0.05$), but when the two high values were excluded the difference did not reach significance.

Levels of IgA and IgG of saliva and sputum produced after inhalation of PGF2α were within the range reported in saliva by Salvaggio et al. (1971) and in bronchial washings from normal subjects (Falk et al 1972). No significant difference was found in concentrations of IgA, IgG and transferrin between saliva and sputum produced after inhaling PGF2α.

In subjects Nos. 7,8,9 and 10 the experiment was repeated, under similar conditions, a year later. The sputum produced by subjects 8 and 9 was similar to that produced after the first inhalation of PGF2α. That of subject No. 7 differed slightly and in subject No. 10 there was a marked difference. This subject was recovering from a cold in the first experiment and this could explain the high values obtained in the first sputum.

Comment

Inhalation of PGF2α seems to be a good method for obtaining sputum from normal subjects and it has some advantages over other methods. The material obtained is sputum with little bronchial fluid and it is therefore a better control for comparison with sputum in disease states. No premedication or major procedure is involved as in bronchoscopy or bronchial washings. Aerosols with hypertonic saline

solutions are known to increase serum transudate (Bickerman et al 1958), while inhalation of PGF2α, except in those subjects with bronchial hyper-reactivity does not seem to cause a major tissue fluid transudate.

The material obtained after inhalation of PGF2α is of special importance in indicating the nature of normal secretion from the bronchial tree. The NANA/Fucose ratio in sputum produced after inhalation of PGF2α is lower than in most of the mucoid chronic bronchitis and asthma sputa studied, suggesting that in disease the bronchial glycoprotein is more sialylated. This shift to a more acid glycoprotein has been observed in experimental work done in rats (Jones, Bolduc and Reid 1972) and in histochemical studies carried out in humans (Lamb and Reid 1968; Lamb and Reid 1969).

Levels of mannose in PGF2α sputum were found to be significantly lower than in mucoid sputum suggesting that the serum glycoproteins in fluid from normal bronchial tree is negligible. This is supported by the fact that the two subjects recovering from a cold had the highest mannose concentration of the group and in one subject who

repeated the experiment one year later the mannose level had fallen to those levels found in the other normal subjects.

The levels of viscosity, dry weight and chemical constituents, including NANA, fucose and sulphate are within the lower part of the mucoid chronic bronchitis range. Thus, it seems that the hypersecretion and hence hypertrophied gland may give rise to a secretion resembling that of the normal size. Often however, hypersecretion is associated with a concentration of macromolecules above that found in what one might call normal secretion.

TABLE V,1

Subjects inhaling Prostaglandin F2α: peak expiratory flow rate (PEFR).

Subjects	Sex	Age	PEFR(l/m) resting	PEFR(l/m) after PGF2α	% change
1	F	50	420	375	- 11
2	M	25	510	460	- 10
3	M	21	530	500	- 6
4	M	23	545	430	- 21
5	M	22	630	625	- 1
6	M	27	560	490	- 12
7(a)	M	31	570	390	- 32
(b)			550	420	- 24
8(a)	M	34	570	540	- 6
(b)			540	500	- 7
9(a)	F	33	460	420	- 8
(b)			420	380	- 10
10(a)	M	28	520	520	0
(b)			545	495	- 9
11	M	52	550	525	- 5
12	M	25	660	600	- 6

TABLE V,2

Apparent viscosity (at 1350 s^{-1}) of saliva and of sputum after inhalation of Prostaglandin F2α. Mean value and standard error of the mean.

Subjects	Viscosity at 1350 sec $^{-1}$ (poise)	
	Saliva	Sputum
No. 1	0.011	0.840
2	0.013	0.090
3	0.025	0.080
4	0.016	0.080
5	0.028	0.090
6	0.006	0.033
7(a)	0.017	0.210
(b)	0.034	0.050
8(a)	0.022	0.070
(b)	0.016	0.050
9(a)	0.025	0.260
(b)	0.023	0.260
10(a)	0.022	0.670*
(b)	0.022	0.110
11	0.017	0.410*
12	0.025	0.037*
Mean	0.019	0.171
s.e.	0.002	0.057

t test 2.6460 $P < 0.02$ * excluded

(a) first experiment (b) second experiment

Fig. V,1 Apparent viscosity (at 1350 s^{-1}) of mucoid chronic bronchitis (CB) and asthma sputum (A) produced after inhalation of prostaglandin F2α (PGF2α)

Fig. V,1.

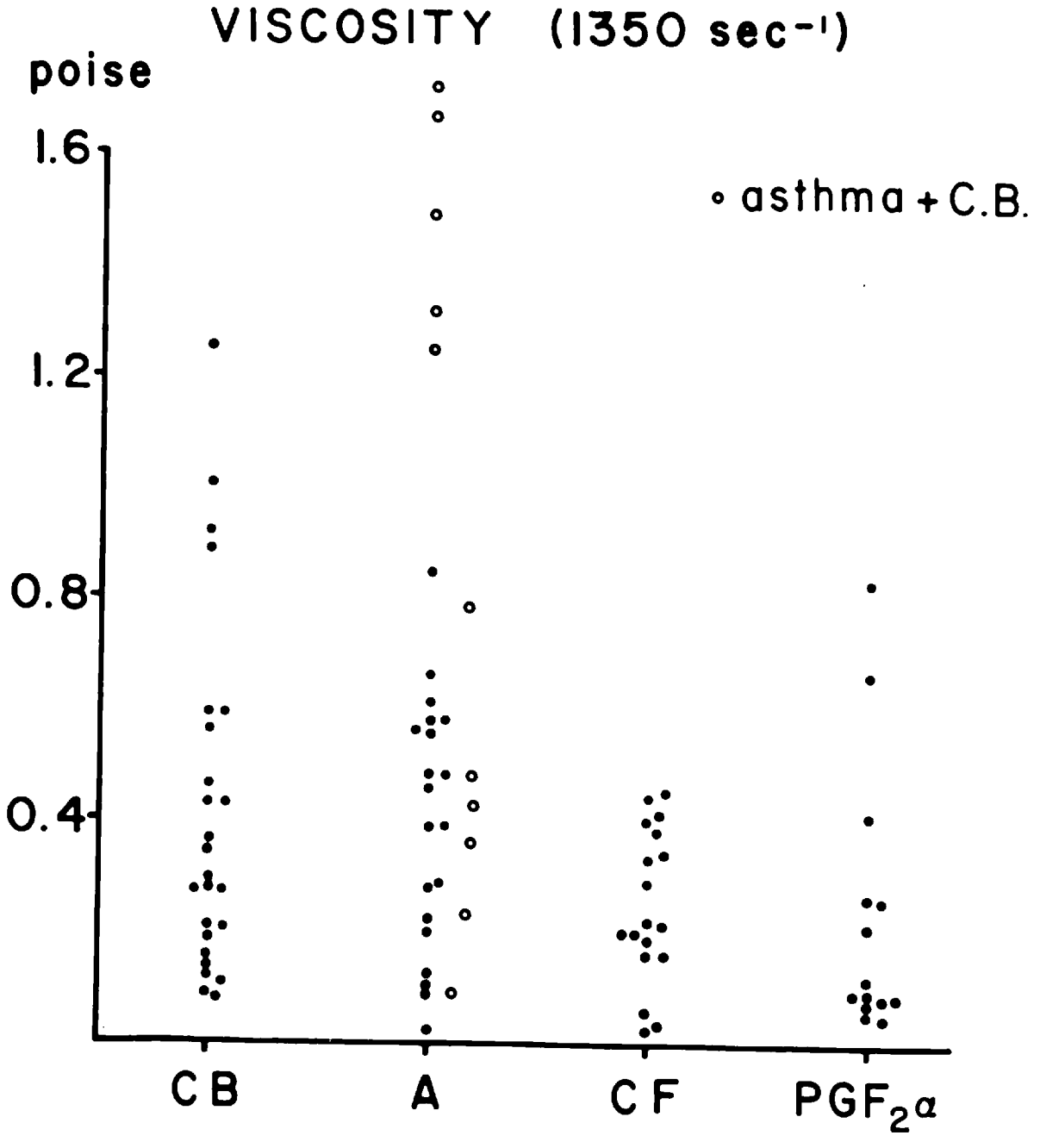


TABLE V,3

Dry weight concentration in saliva and in sputum after inhalation of prostaglandin F2α.

Subjects	Dry macromolecular weight (mg/ml)	
	Saliva	Sputum
1	6.8	7.4
2	2.1	2.4
3	4.6	7.5
4	4.1	7.5
5	4.6	12.0
6	1.7	4.7
7(a)	4.6	4.19
(b)	5.6	11.9
8(a)	2.1	5.4
(b)	2.2	4.4
9(a)	3.5	7.2
(b)	4.7	8.2
10(a)	4.9	12.5*
(b)	5.4	7.2
11	3.0	11.8*
12	3.1	3.1*
Mean	4.0	6.9
s.e.	0.02	0.7

t test 3.4737 $P < 0.01$ * excluded

(a) first experiment (b) second experiment

Fig. V,2 Absolute levels of dry weight (DW mg/ml) of mucoid chronic bronchitis sputum (CB), sputum produced after inhalation of prostaglandin F2α (PGF2α) and of saliva.

Fig. V,2

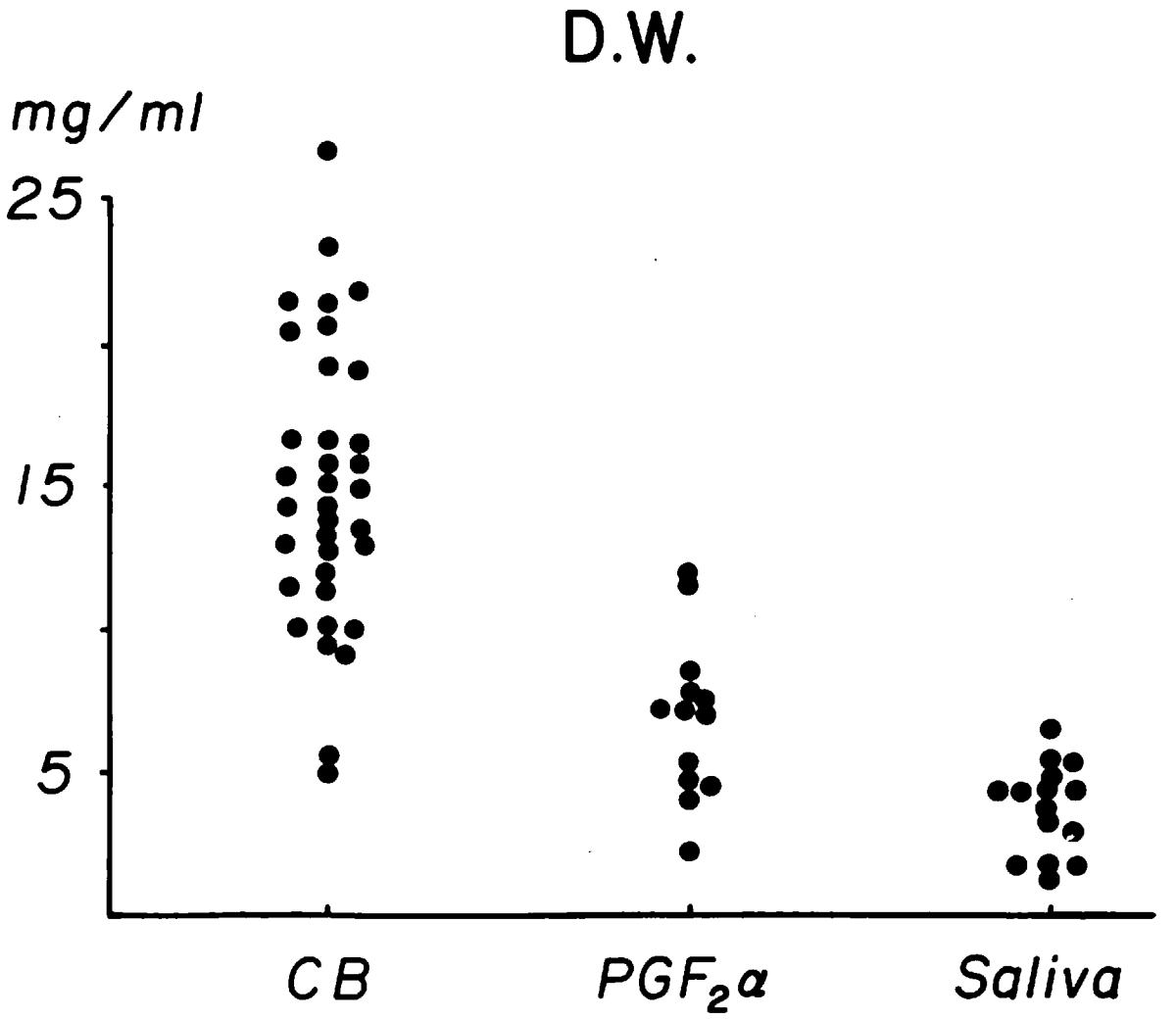


TABLE V,4

N-acetyl neuraminic acid concentration in saliva and in sputum after inhalation of Prostaglandin F2α.

Subjects	N-acetyl neuraminic acid (µmol/ml)	
	Saliva	Sputum
1	0.38	0.87
2	0.06	0.16
3	0.19	0.60
4	0.10	1.00
5	0.20	0.90
6	0.19	0.55
7(a)	0.19	0.51
(b)	0.26	0.30
8(a)	0.19	0.61
(b)	0.13	0.40
9(a)	0.16	0.58
(b)	0.10	0.50
10(a)	0.32	2.81*
(b)	0.19	0.90
11	0.12	2.77*
12	0.29	0.30*
Mean	0.18	0.61
s.e.	0.02	0.07

t test 6.1429 $P < 0.001$ * excluded

(a) first experiment (b) second experiment

Fig. V ,3 Absolute levels of N acetyl neuraminic acid (NANA μmol/ml) of mucoid chronic bronchitis sputum (CB) sputum produced after inhalation of prostaglandin F2α (PGF2α) and of saliva.

Fig. V,3.

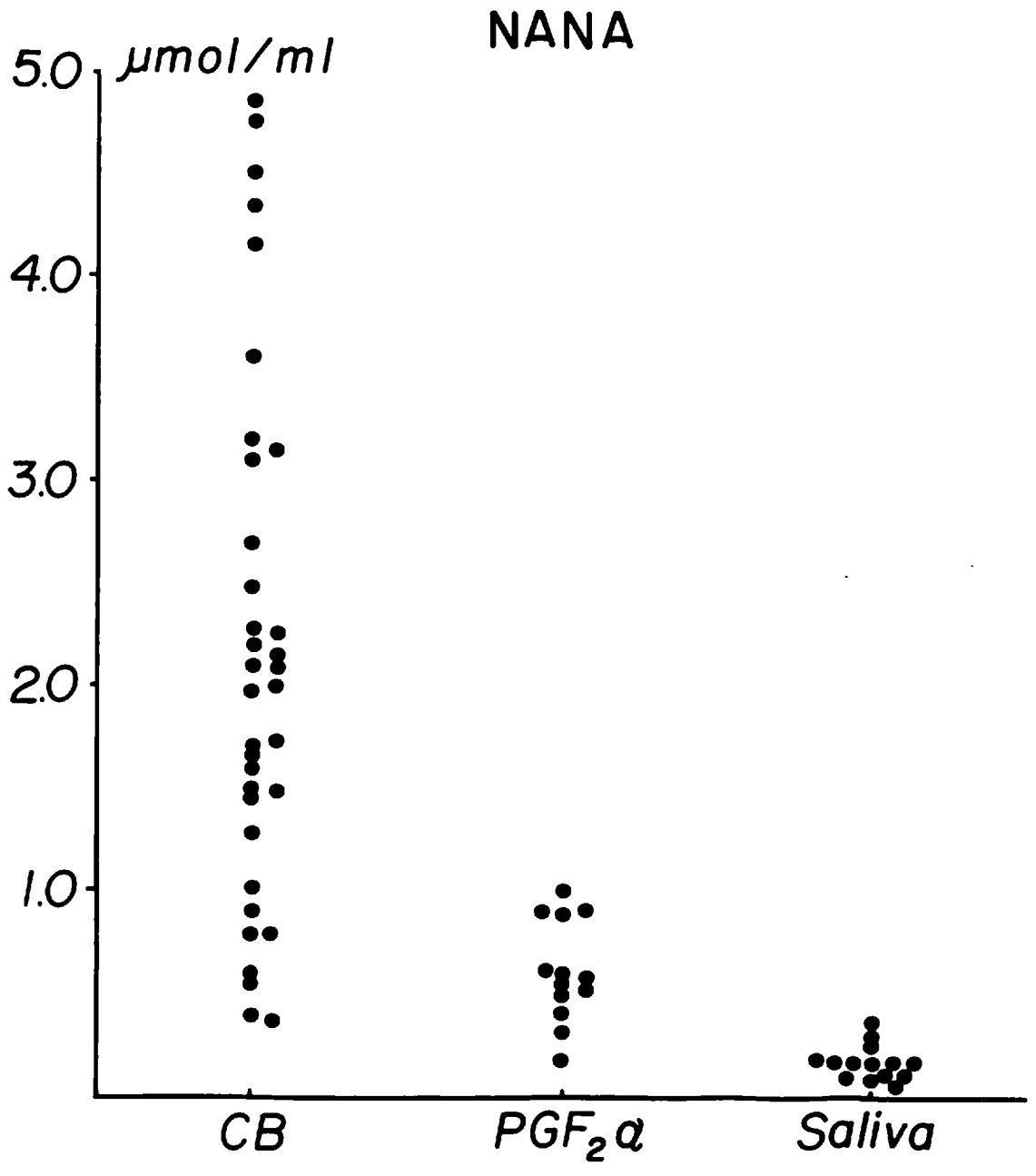


TABLE V,5

Fucose concentration in saliva and in sputum after inhalation of prostaglandin F2α.

Subjects	FUCOSE (µmol/ml)	
	Saliva	Sputum
1	1.52	3.57
2	0.48	1.09
3	0.36	3.30
4	0.91	3.40
5	0.70	4.10
6	0.42	1.90
7(a)	1.10	2.92
(b)	1.03	1.90
8(a)	0.36	1.70
(b)	0.26	1.20
9(a)	0.66	2.92
(b)	0.73	4.00
10(a)	1.03	3.53*
(b)	0.67	2.30
11	0.73	2.49*
12	0.91	1.20*
Mean	0.75	2.64
s.e.	0.08	0.27

t test 6.6507 $P < 0.001$

(a) first experiment (b) second experiment

* excluded

Fig. V ,4 Absolute levels of fucose (µmol/ml) of mucoid chronic bronchitis sputum (CB), sputum produced after inhalation of prostaglandin F2α (PGF2α) and of saliva

Fig. V,4.

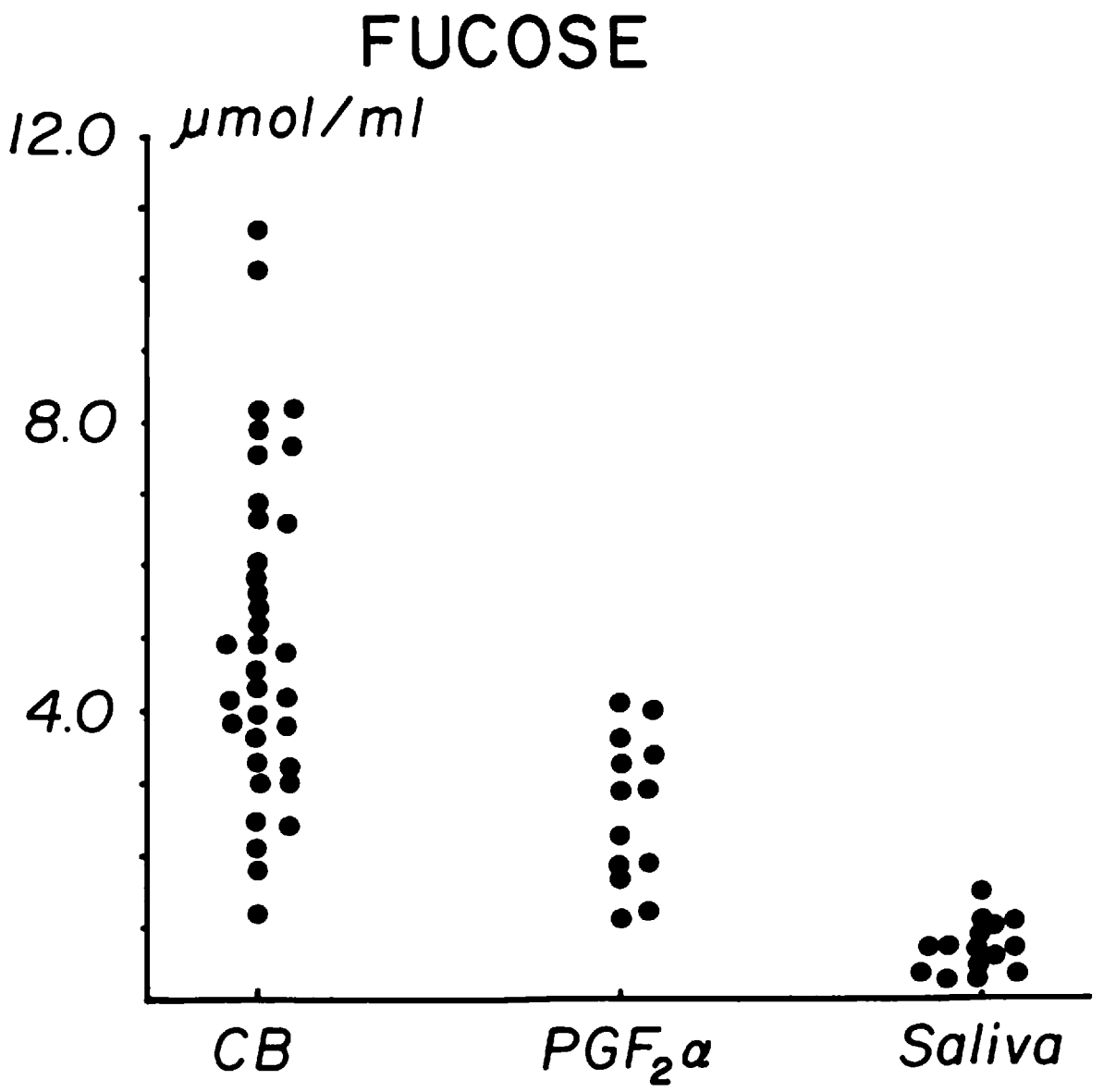


TABLE V,6

Sulphate concentration in saliva and in sputum produced after inhalation of prostaglandin F2α.

Subjects	SULPHATE (µmol/ml)	
	Saliva	Sputum
1	0.31	0.82
2	0.31	0.21
7(a)	0.57	0.60
8(a)	0.21	1.14
9(a)	0.21	0.89
10(a)	0.41	1.56*
12	0.31	2.08*
Mean	0.33	0.73
s.e.	0.05	0.14

Student's t test 5.8571 $P < 0.001$

(a) first experiment (see footnote Table V,2)

* excluded

Fig. V,5 Absolute levels of sulphate (µmol/ml) of mucoid chronic bronchitis sputum (CB), sputum produced after inhalation of prostaglandin F2α (PGF2α) and of saliva.

Fig. V,5.

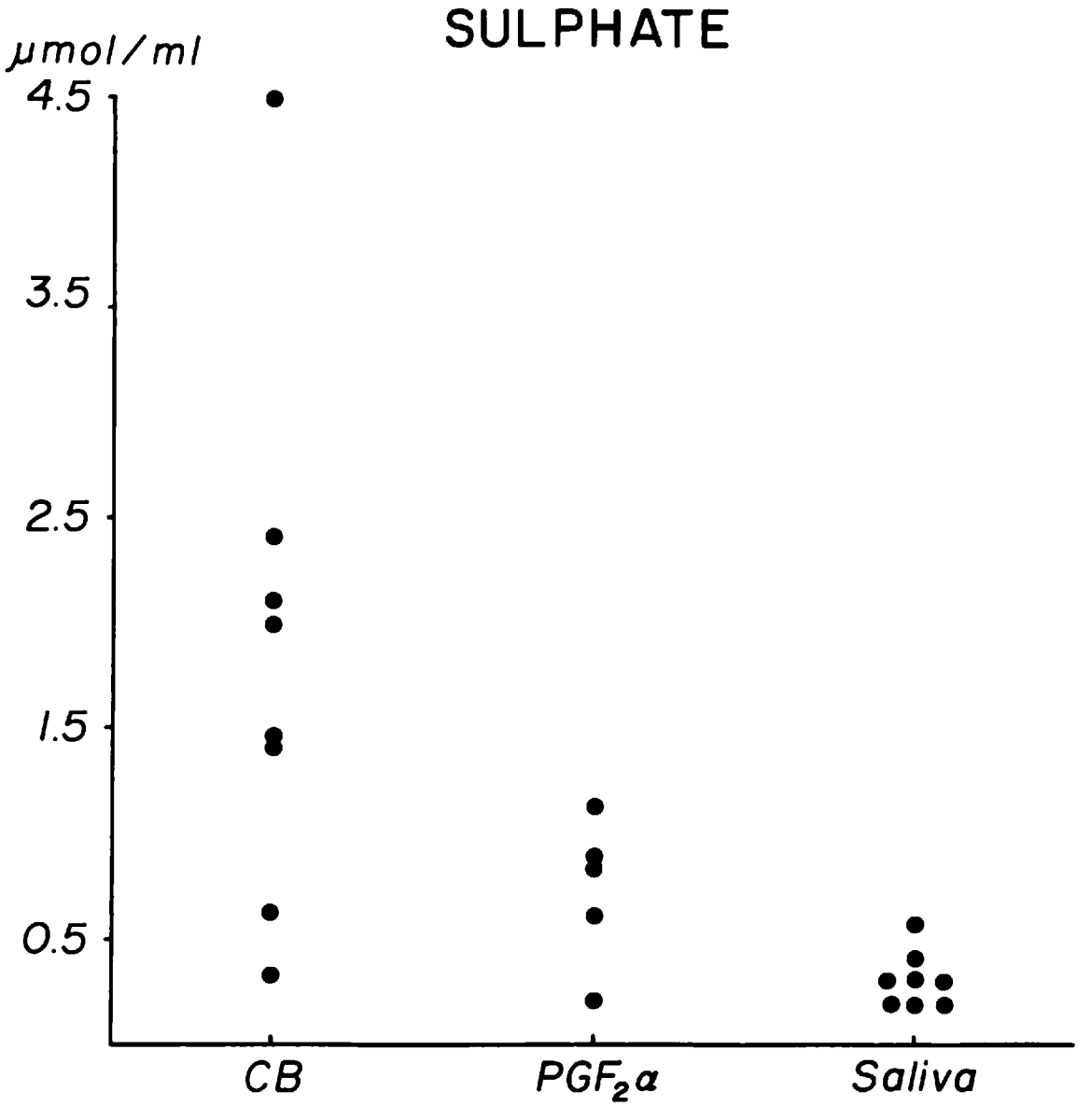


TABLE V,7

Mannose concentration in saliva and in sputum after inhalation of prostaglandin F2α.

Subjects	MANNOSE (µmol/ml)	
	Saliva	Sputum
1	0.41	0.29
2	0.06	0.10
3	0.06	0.28
4	0.15	0.21
5	—	0.41
6	0.33	0.21
7(a)	0.16	0.24
(b)	0.12	0.08
8(a)	0.26	0.23
(b)	0.11	0.09
9(a)	0.08	0.22
(b)	0.19	0.34
10(a)	0.12	0.48*
(b)	0.31	0.26
11	0.21	
12	0.11	0.11*
Mean	0.18	0.23
s.e.	0.02	0.02

t test 1.2561 not significant

(a) first experiment (b) second experiment

* excluded

Fig. V ,6 Absolute levels of mannose (µmol/ml) of mucoid chronic bronchitis sputum (CB), sputum produced after inhalation of PGF2α (PGF2α) and of saliva.

Fig. V,6.

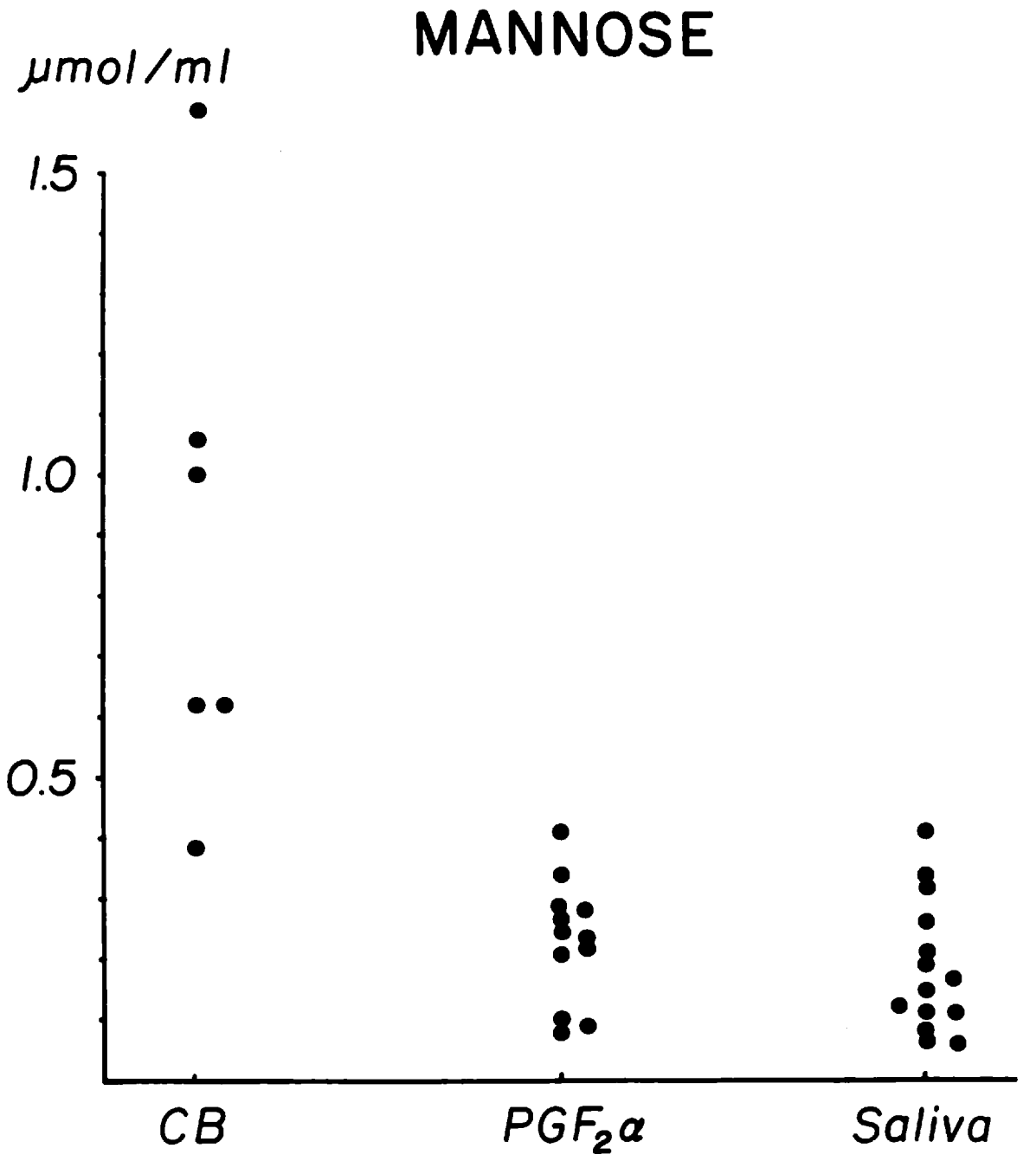


TABLE V,8

NANA/Fucose ratio in saliva and in sputum after inhalation of prostaglandin F2α.

Subjects	NANA/FUCOSE RATIO	
	Saliva	Sputum
1	0.25	0.27
2	0.12	0.15
3	0.52	0.18
4	0.20	0.29
5	0.14	0.22
6	0.33	0.29
7(a)	0.17	0.17
(b)	0.25	0.16
8(a)	0.53	0.36
(b)	0.50	0.33
9(a)	0.24	0.20
(b)	0.14	0.12
10(a)	0.31	0.80*
(b)	0.28	0.39
11	0.16	0.11*
12	0.32	0.25*
Mean	0.28	0.24
s.e.	0.04	0.02

Student's t test 0.7603 not significant

(a) first experiment (b) second experiment

* excluded

Fig. V,7 NANA/Fucose ratio of mucoid chronic bronchitis sputum, sputum produced after inhalation of prostaglandin F2α (PGF2α) and of saliva.

Fig. V,7.

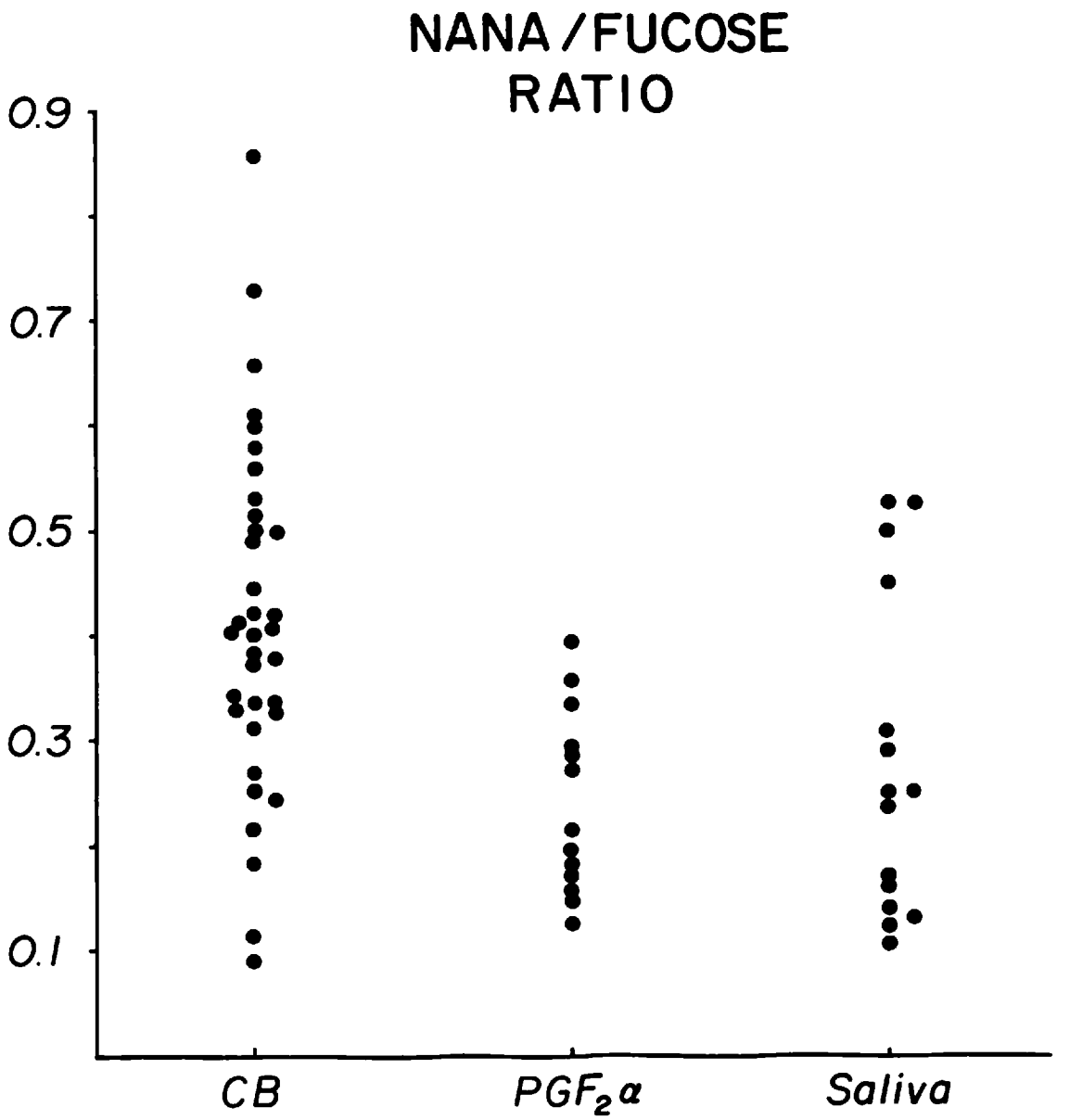


TABLE V,9

Albumin, IgA, IgG and transferrin concentrations (mg/100 ml) in saliva and in sputum produced after inhalation of prostaglandin F2α.

Subjects	Albumin		IgG		IgA		Transferrin	
	Saliva	Sputum	Saliva	Sputum	Saliva	Sputum	Saliva	Sputum
3	2.1	15.6	1.3	6.1	5.5	8.5	0.5	1.0
4	4.5	39.2	1.8	1.8	5.0	4.0	0.5	3.6
5	3.5	4.0	2.4	4.4	11.3	7.0	0.2	0.3
7(b)	8.2	23.4	2.8	1.3	11.4	7.8	0.5	2.2
8(b)	1.5	4.5	1.3	1.9	3.2	7.1	0.0	2.2
9(b)	8.2	19.0	4.4	5.4	2.4	6.0	0.6	0.3
10(b)	3.5	5.5	3.3	1.8	4.5	1.8	0.3	0.0
12	3.1	2.1*	1.8	1.2*	3.1	3.2*	0.0	0.0*
Mean	4.9	15.9	3.0	3.2	6.4	6.0	0.3	1.2
s.e.	0.9	4.4	1.6	0.7	1.1	0.8	0.1	0.4
Student's t test	2.3811		0.2233		0.2764		1.8543	
	$P < 0.05$		N.S.		N.S.		N.S.	

(b) second experiment * excluded

CHAPTER VI

NATURAL VARIATION OF CHEMICAL CONSTITUENTS OF SPUTUM

Rhythmic variations in biological functions such as body temperature, urine flow, plasma corticosteroid levels, plasma iron concentration etc. have been described in man (Aschoff, 1963; Mills, 1966). Bronchial secretion may also demonstrate a rhythmic variation; Ashcroft (1965) found, in a group of chronic bronchitic patients, that the morning sputum volume, that is the sputum expectorated in the first hour after waking, averaged 25% of the 24 hour sputum volume. A seasonal variation in dry macromolecular weight and neuraminic acid content of sputum from chronic bronchitic patients was reported by Keal (1970). The dry weight yield and neuraminic acid increased during the winter months and these changes seemed to be related to an increase in atmospheric pollution.

In order to study the natural cyclical variation of bronchial secretion, the chemical constituents of sputum from patients with chronic bronchitis, bronchiectasis, cystic fibrosis and asthma were examined. The studies included:

Diurnal variation

Daily variation

Monthly variation

Yearly variation.

DIURNAL VARIATION

Diurnal variation was examined in four groups of patients admitted to the wards of the Brompton Hospital with exacerbation of their disease. The studies were carried out when the disease had reached a stable phase. The sputum was studied over a period of 12 hours from 06.00 to 18.00 hrs.; sputum specimens were collected at three hours intervals and represented three hours production: 06.00-0.900 hrs.; 09.00-12.00 hrs.; 12.00-15.00 hrs. and 15.00-18.00 hrs.

Some of the patients were studied over 2 to 7 days; diagnosis, number of patients and number of samples collected at each time interval are given in Table VI,1.

Sputum volume was recorded and specimens were macroscopically classified as mucoid, mucopurulent or purulent. Chemical analysis of sputum included: dry macromolecular material, N-acetyl neuraminic acid (NANA) and fucose, the NANA/Fucose molar ratio was calculated from absolute values of NANA and fucose.

Each disease group was studied in a similar way.

Diurnal variation of chemical constituents of sputum in chronic bronchitis:

Eight patients suffering from chronic bronchitis (MRC, 1965) were included in the study: five patients were studied during a single day (Nos. 1,2,3,5 and 6) and three on several consecutive days (Nos. 4,7 and 8) (Table VI,2). Number of patients, number of samples collected at each time interval and macroscopic type of samples are given in Table VI,2. The macroscopic type of sputum from each patient remained unchanged throughout the study.

Sputum volume: Mean values and standard error of the mean for the group for each time of collection are given in Table VI,3. It was found that the sputum volume from 09.00 hrs. to 12.00 hrs. was higher than the 06.00 hrs. to 09.00 hrs. but the difference did not reach significant levels (Table VI,4). The sputum volume then decreased from 12.00 hrs. to 18.00 hrs. and the difference was significant when compared with the 06.00 to 09.00 hrs. and 09.00 to 12.00 hrs. values ($P < 0.02$, $P < 0.01$) (Table VI,4). When the 12.00 to 15.00 hrs. and the 15.00 to 18.00 hrs. sputum volumes were compared, no significant difference emerged (Table VI,4).

Dry macromolecular weight: The dry macromolecular weight yield expressed as mg/ml of sputum for the

group of patients showed no significant variation throughout the 12 hours study period although it was lower during the 12.00 to 15.00 hrs. and 15.00 to 18.00 hrs. periods. (Tables VI,3 and VI,4). This pattern of variation of high early morning values followed by a gradual decrease during the day was observed in four patients (Nos. 4, 5, 7 and 8), in the other four patients the 06.00 to 09.00 hrs. sputum samples had the lowest dry weight which then increased over the 0.900 to 12.00 hrs. and 12.00 to 15.00 hrs. periods and decreased during the 15.00 to 18.00 hrs. period.

N-acetyl neuraminic acid (NANA): Mean values and standard error of the mean for the group are given in Table VI,3. The NANA content of sputum produced during the 06.00 to 09.00 hrs. period was higher than that of the other three sputum collections and the difference reached significant levels when compared with the 12.00 to 15.00 hrs. ($P < 0.05$) and 15.00 to 18.00 hrs. ($P < 0.01$) values (Table VI,4). This pattern of variation was observed in four patients (Nos. 3,4,7 and 8) Fig. VI,1.

Fucose: Mean values and standard error of the mean are given in Table VI,3. Fucose content of sputum was found to be higher in the 06.00 to 09.00 hrs. period, then decreased slightly during the day

reaching the lowest levels during 15.00 to 18.00 hrs. When the 06.00 to 09.00 hrs. fucose levels were compared with the 15.00 to 18.00 hrs. levels, the difference was significant ($P < 0.02$), but no significant difference was seen when compared with the 09.00 to 12.00 hrs. and 12.00 to 15.00 hrs. levels (Table VI,4). This pattern of variation was observed in four patients (Nos. 3,4,7 and 8) (Fig. VI,1).

NANA/Fucose molar ratio: Mean values and standard error of the mean for the group are given in Table VI,3. The NANA/Fucose ratio varied little over the 12 hours study period, the lowest values were found in the 15.00 to 18.00 hrs. sputum samples, the ratio during the 06.00 to 09.00 hrs., 09.00 to 12.00 hrs. and 12.00 to 15.00 hrs. were very similar. No significant difference was seen between any of the values (Table VI,4). In six patients (Nos. 2,3,4,6,7 and 8) the pattern of variation was similar to that found for the group.

Comment: The dry macromolecular material varies little and seems to have no relation with the sputum volume produced, that is the concentration of macromolecular material of the bronchial fluid produced during the 12 hours study period is fairly constant. (Fig. VI,1)

Chemical constituents of sputum show a diurnal variation pattern of high early morning values followed by a gradual fall during the day, reaching the lowest levels during the 15.00 to 18.00 hrs. period. (Fig. VI,2).

Fucose content of sputum gradually decreased throughout the day, fucose being a marker substance of bronchial glycoprotein suggests that the bronchial secretion is subject to a diurnal variation being significantly lower in the evening.

The pattern of variation shown by NANA of high morning values followed by a marked decrease during the day is similar to that found for fucose although the trend is more marked. Since NANA is present both in bronchial and serum glycoproteins it would seem that in addition to a decrease in bronchial secretion there is also a decrease in serum transudate.

TABLE VI,1

DIURNAL VARIATION OF CHEMICAL CONSTITUENTS OF SPUTUM.

Diagnosis	No. of Patients	Time of collection and No. of samples			
		06-09 hrs	09-12 hrs	12-15 hrs	15-18 hrs
Chronic bronchitis	8	20	19	16	18
Bronchiectasis	6	10	10	10	10
Cystic fibrosis	3	5	5	5	5
Asthma	6	9	3	9	1

TABLE VI,2

Diurnal variation of chemical constituents of sputum in chronic bronchitis.

Patient	Time of sputum collection and number of samples				Macroscopic type
	6-9 hrs	9-12 hrs	12-15 hrs	15-18 hrs	
No. 1	1	1	1	-	MP
No. 2	1	1	1	1	P
No. 3	1	1	1	1	MP
No. 4	3	2	3	3	M
No. 5	1	1	1	1	MP
No. 6	1	1	-	1	M
No. 7	5	5	4	5	MP
No. 8	7	7	6	6	MP
Total No. samples	20	19	17	18	

TABLE VI,3

Diurnal variation of volume, dry weight and chemical constituents of sputum in chronic bronchitis.

Mean values and standard error of the mean ( )*

	Time of sputum collection			
	6-9 hrs	9-12 hrs	12-15 hrs	15-18 hrs
Volume (ml)	13.9 (1.5)*	15.1 (2.1)*	8.3 (1.1)*	6.8 (1.3)*
Dry macromolecular weight (mg/ml)	21.8 (2.5)	21.5 (3.1)	18.5 (2.4)	17.3 (2.2)
NANA µmol/ml	3.5 (0.2)	2.8 (0.2)	2.7 (0.2)	2.2 (0.2)
Fucose µmol/ml	4.9 (0.3)	4.0 (0.4)	4.1 (0.4)	3.6 (0.3)
NANA/Fucose molar ratio	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.7 (0.1)

TABLE VI,4

Diurnal variation of volume, dry weight and chemical constituents of sputum in chronic bronchitis. Comparison between times of collection (Student's t test).

	Volume (ml)	Dry Weight (mg/ml)	NANA µmol/ml	Fucose µmol/ml	NANA/ Fucose molar ratio
6- 9/ 9-12 hrs.	-0.44032	0.07309	1.78843	1.77034	-0.30308
6- 9/ 12-15 hrs.	2.58340**	0.94893	2.16260*	1.63062	-0.09971
6-9/ 15-18 hrs.	3.09998***	1.33773	3.34035***	2.63702**	0.36301
9-12/ 12-15 hrs.	2.54036**	0.75314	0.37402	-0.05402	0.18863
9-12/ 15-18 hrs.	2.97466***	1.08183	1.70426	0.78238	0.59588
12-15/ 15-18 hrs.	0.73592	0.36024	1.39401	0.80182	0.41999

* $P < 0.05$

** $P < 0.02$

*** $P < 0.01$

Fig. VI,1 Diurnal variation of N-acetyl neuraminic acid (NANA) and fucose in sputum from four patients with chronic bronchitis.

Fig. VI,1.

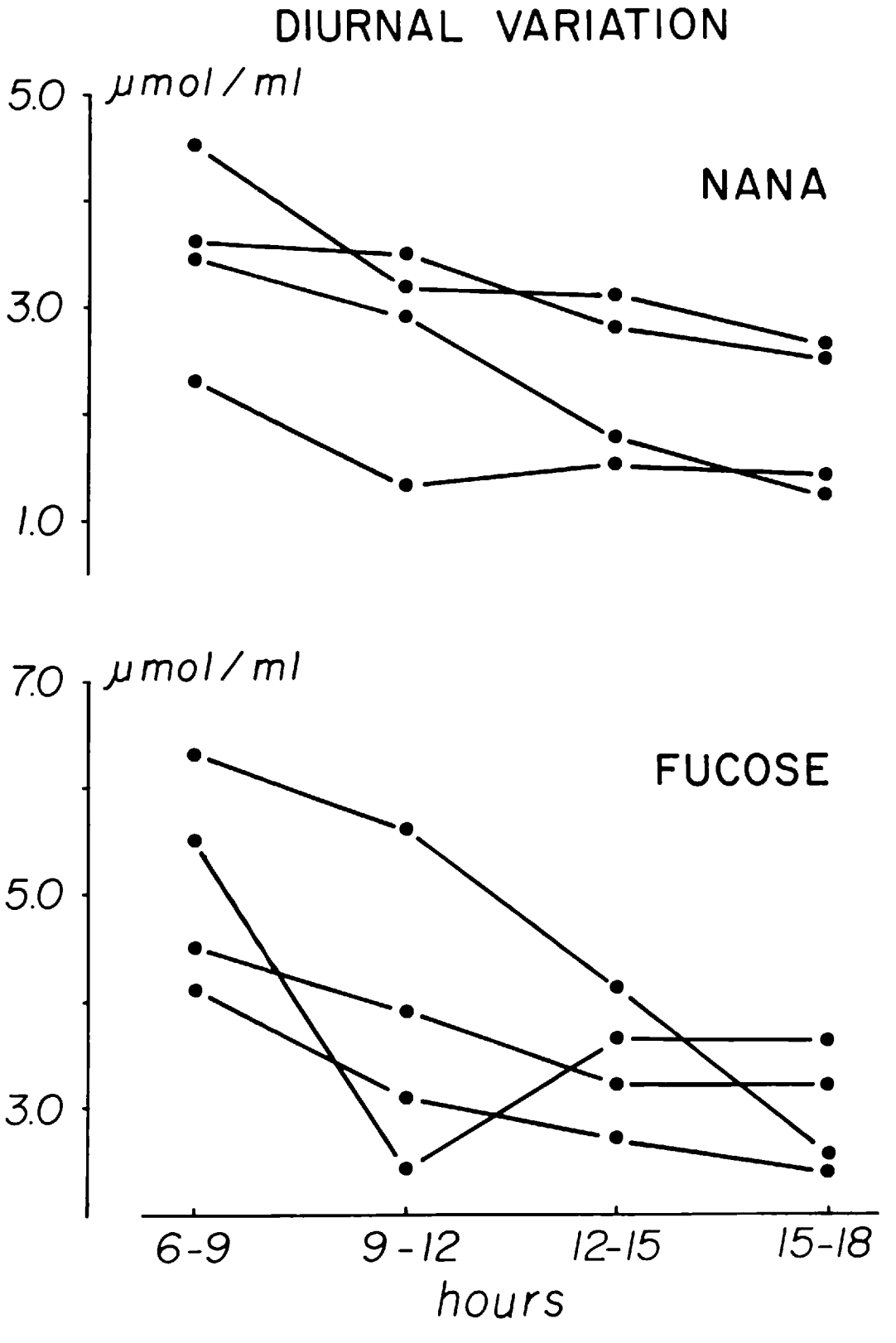
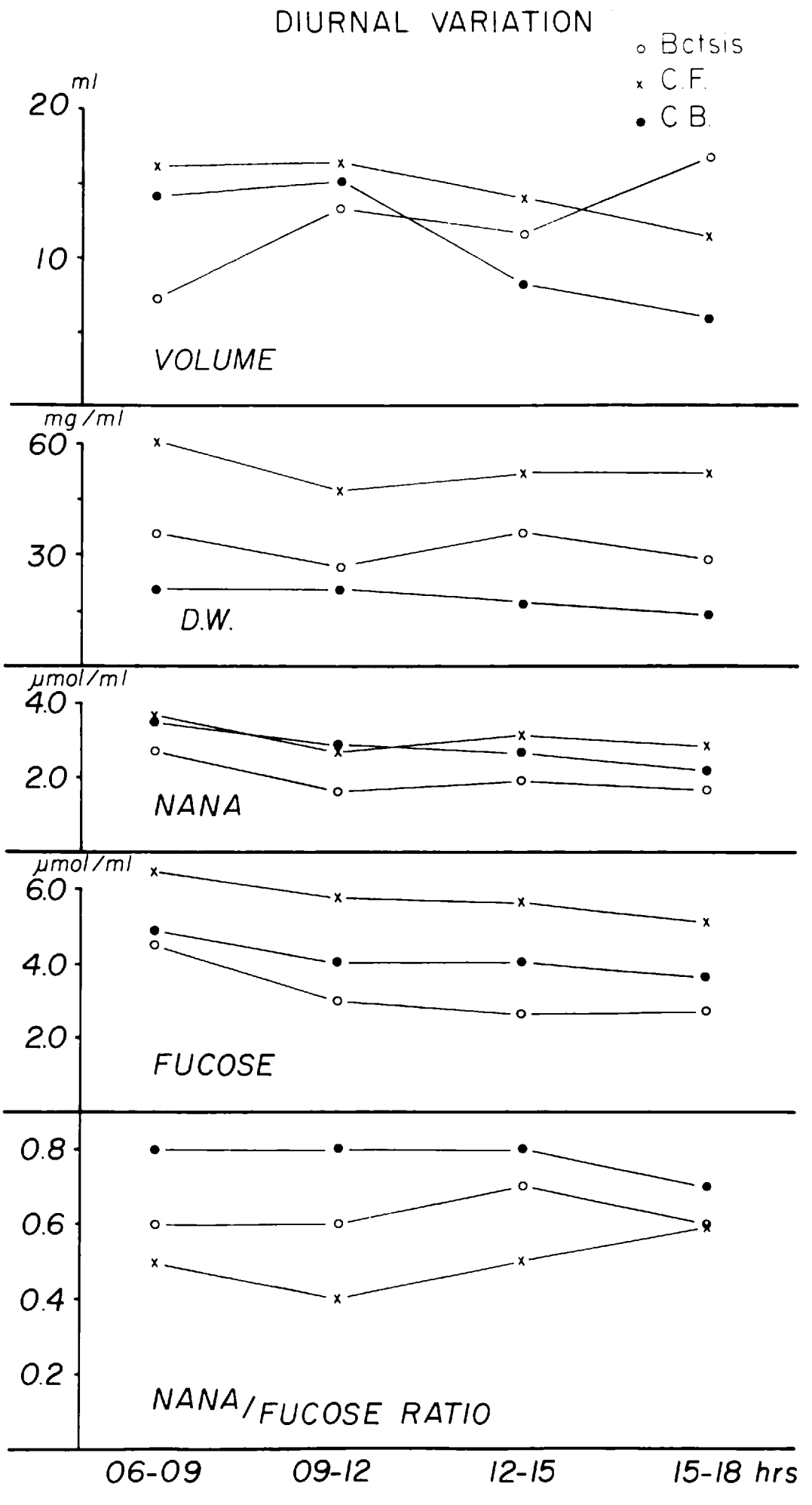

DIURNAL VARIATION
5.0
μmol/ml
NANA
3.0
1.0
7.0
μmol/ml
FUCOSE
5.0
3.0
6-9
9-12
12-15
15-18
hours

Fig. VI,2 Diurnal variation of sputum volume, dry weight (DW), N-acetyl neuraminic acid (NANA), fucose and NANA/Fucose ratio in sputum from patients with chronic bronchitis (CB), cystic fibrosis (CF), or bronchiectasis (Bctsis).

Fig. VI,2


DIURNAL VARIATION
o Bctsis
x C.F.
• C.B.
20 ml
10
VOLUME
mg/ml
60
30
D.W.
µmol/ml
4.0
2.0
NANA
µmol/ml
6.0
4.0
2.0
FUCOSE
0.8
0.6
0.4
0.2
NANA/FUCOSE RATIO
06-09
09-12
12-15
15-18 hrs

Diurnal variation of chemical constituents of sputum in bronchiectasis:-

Number of patients, times of sputum collection, number of samples examined and macroscopic types are given in Table VI, 1 and V1,5. Patients Nos. 2 and 6 failed to produce sputum during the 15.00 to 18.00 hrs. period. The patients were divided into two groups: Group I included only those patients who produced sputum over the 12 hours study period and Group II included all the patients and represented those producing sputum over a period of 9 hours (excluding the 15.00 to 18.00 hrs. sputum collections). Mean values and standard error of the mean of sputum volume and chemical constituents for the two groups are given in Tables V1,6 and VI,7. No significant difference was found between the two groups for the sputum volume and chemical constituents and the pattern of variation was similar over the 9 hours study period, therefore only Group I is here reported.

Sputum volume: the first three hours collection showed the lowest sputum volume, the volume then increased during the day reaching the highest value between 15.00 and 18.00 hrs., the difference being significant when compared with the 06.00 to 09.00 hrs. sputum volume ($P < 0.05$) (Table VI,8). Dry

macromolecular weight, NANA and fucose levels decreased from 06.00 to 09.00 hrs. to 09.00 to 12.00 hrs., dry weight increased during the 12.00 to 15.00 hrs. period while the NANA and fucose levels continued to decrease. The 15.00 to 18.00 hrs. levels for all chemical constituents were lower when compared with the 06.00 to 09.00 hrs. levels, but the difference was only significant for fucose ($P < 0.05$) (Table VI,8).

The NANA/Fucose ratio varied little during the 12 hours study period.

These patterns of variation were observed in five patients (Nos. 1,2,3,4 and 5).

Comment: NANA and fucose values showed a similar pattern of variation as the chronic bronchitis group, that is of a high morning value and gradual fall during the day (Fig. VI,2). In contrast with the chronic bronchitis group the variation of NANA content was less marked suggesting that the serum transudate component in bronchiectasis is less subjected to variation, this is supported by the fact that the NANA/Fucose ratio was fairly constant.

TABLE VI,5

Diurnal variation of chemical constituents of sputum in bronchiectasis.

Patient	Time of collection and number of samples				
	6-9 hrs	9-12 hrs	12-15 hrs	15-18 hrs	Macroscopic type
No. 1	1	1	1	1	P
	1	1	1	1	MP
	1	1	1	1	MP
	1	1	1	1	MP
	1	1	1	1	MP
No. 2	1	1	1	-	MP
No. 3	1	1	1	1	MP
	1	1	1	1	MP
	1	1	1	-	P
No. 4	1	1	1	1	P
	1	1	1	1	P
	1	1	1	-	P
No. 5	1	1	1	-	MP
	1	1	1	1	MP
No. 6	1	1	1	-	MP

TABLE VI,6

Diurnal variation of chemical constituents of sputum in bronchiectasis. Group I.

Mean values and standard error of the mean of sputum volume, dry weight and chemical constituents of sputum.

	Time of sputum collection and number of samples			
	06-09	09-12	12-15	15-18
	n=10	n=10	n=10	n=10
Volume	7.3 (1.9)	13.6 (2.6)	11.6 (2.2)	16.8 (4.4)
Dry weight mg/ml	36.0 (3.7)	27.7 (3.5)	36.1 (2.9)	29.2 (1.9)
NANA (μmol/ml)	2.7 (0.5)	1.6 (0.4)	1.9 (0.3)	1.7 (0.3)
Fucose (μmol/ml)	4.5 (0.7)	3.0 (0.8)	2.6 (0.5)	2.7 (0.4)
NANA/Fucose molar ratio	0.6 (0.1)	0.6 (0.1)	0.7 (0.1)	0.6 (0.1)

TABLE VI,7

Diurnal variation of chemical constituents of sputum in bronchiectasis. Group II.

Mean values and standard error of the mean of sputum volume, dry weight and chemical constituents of sputum.

	Time of sputum collection and number of samples.		
	06-09	09-12	12-15
	n = 15	n = 15	n = 15
Volume ml	8.6 (2.1)	15.1 (2.8)	12.3 (2.6)
Dry weight mg/ml	34.8 (3.1)	28.5 (3.3)	33.3 (3.4)
NANA µmol/ml	3.0 (0.5)	2.1 (0.4)	1.9 (0.3)
Fucose µmol/ml	4.9 (0.6)	3.3 (0.6)	2.8 (0.3)
NANA/Fucose molar ratio	0.6 (0.05)	0.6 (0.06)	0.7 (0.05)

TABLE VI,8

Diurnal variation of chemical constituents of sputum in bronchiectasis.

Sputum volume, dry weight and chemical constituents of sputum: comparison between times of collection (Student's t test).

Time of collection	Volume (ml)	Dry Weight (mg/ml)	NANA (umol/ml)	Fucose (µmol/ml)	NANA/ Fucose Molar ratio
06-09/09-12	-1.94904	1.62173	1.59444	1.37770	-0.09745
06-09/12-15	-1.43430	-0.03156	1.19556	2.30420*	-1.37204
06-09/15-18	-2.19700*	1.62120	1.62872	2.35216*	-0.37058
09-12/12-15	0.60473	-1.83799	-0.52455	0.46525	-1.01699
09-12/15-18	-0.66201	-0.45324	-0.07518	0.40907	-0.23942
12-15/15/18	-1.17852	1.96575	0.49422	-0.11830	0.70656

* $P < 0.05$

Sputum volume and dry weight showed an inverse correlation (Fig. VI,2), that is the highest dry weight levels corresponded with the lowest sputum volume. The fact that these patients had postural drainage twice a day, between 09.00 to 12.00 hrs. and 15.00 to 18.00 hrs. might explain the increase in sputum volume and the decrease in dry weight.

Diurnal variation of chemical constituents of sputum in cystic fibrosis:-

Number of patients, times of collection, number of samples and macroscopic types are given in Tables VI,1 and VI,9. Mean values and standard error of the mean of sputum volume and chemical constituents of sputum for the group are given in Table VI,10.

Sputum volume: sputum produced during the 06.00 to 09.00 hrs. and 09.00 to 12.00 hrs. showed the highest values, the volume then gradually decreased reaching the lowest levels at 15.00 to 18.00 hrs.; the difference did not reach significant levels (Table VI,11).

Dry macromolecular weight and NANA content of sputum showed a similar pattern of variation of high early morning levels followed by a decrease during the 09.00 to 12.00 hrs. period and an increase during the 12.00 to 15.00 hrs. and then remained unchanged until the end of the study period. No statistically significant difference emerged between any of the periods (Table VI,11).

Fucose levels showed a gradual fall, similar to that seen in chronic bronchitis and bronchiectasis groups but no statistically significant difference emerged (Table VI,11). The NANA/Fucose ratio varied little throughout the day. This pattern of variation was seen in two patients (Nos. 1 and 3).

Comment: The pattern of variation observed in the cystic fibrosis group had characteristics of both the chronic bronchitis and bronchiectasis groups. The pattern of variation for the dry weight of high early morning levels followed by a decrease and then elevation towards the end of the day, was similar to that seen in the bronchiectasis group. (Fig.VI,2). The fact that in these two groups the macroscopic types of sputum were mucopurulent and purulent

TABLE VI,9

Diurnal variation of chemical constitutents of sputum in cystic fibrosis

Number of patients, times of sputum collection, number of samples and macroscopic types.

Patient	No. of samples and time of collection				
	06-09	09-12	12-15	15-18	Macroscopic type
No. 1	1	1	1	1	P
	1	1	1	1	P
No. 2	1	1	1	1	MP
No. 3	1	1	1	1	P
	1	1	1	1	P

TABLE VI,10

Diurnal variation of chemical constituents of sputum in cystic fibrosis

Volume and chemical constituents of sputum-variation with time. Mean values and standard error of the mean.

	Time of sputum collection			
	06-09	09-12	12-15	15-18
Volume	16.3 (2.9)	16.6 (3.3)	14.1 (2.8)	11.5 (3.9)
Dry weight mg/ml	60.6 (16.4)	48.5 (15.9)	52.5 (15.3)	52.3 (13.2)
NANA µmol/ml	3.6 (0.4)	2.7 (0.2)	3.1 (0.4)	2.9 (0.3)
Fucose µmol/ml	6.5 (0.8)	5.8 (0.4)	5.7 (0.9)	5.1 (0.6)
NANA/Fucose molar ratio	0.5 (0.03)	0.4 (0.06)	0.5 (0.04)	0.6 (0.06)

TABLE VI,11

Diurnal variation of chemical constituents of sputum in cystic fibrosis

Volume and chemical constituents of sputum. Comparison between times of collection (Student's t test).

Time of collection	Volume ml	Dry weight mg/ml	NANA µmol/ml	Fucose µmol/ml	NANA/ Fucose molar ratio
06-09/09-12	-0.06194	0.53156	1.71325	0.76583	-2.71304
06-09/12-15	0.50656	0.36108	0.89492	0.58412	0.22452
06-09/15-18	0.95024	0.39606	1.25548	1.30077	-0.49009
09-12/12-15	0.52998	-0.18302	-0.79029	0.02028	-1.58761
09-12/15-18	0.90636	-0.18524	-0.57995	0.82466	-1.76616
12-15/15-18	0.51853	0.01065	0.29957	0.54370	-0.59718

might explain the similarity. The diurnal variation of NANA and NANA/Fucose ratio were similar to that seen in bronchiectasis (Fig. VI,2), suggesting that in the presence of infection the serum transudate component varies little throughout the day. The pattern of variation for the fucose content of sputum was similar to that seen in chronic bronchitis, the bronchial glycoprotein component decreases during the day. Since the patients included in this group were in advanced stages of the disease, bronchial gland hypertrophy is expected to have taken place and therefore this could explain the similarity to the chronic bronchitis group.

Diurnal variation of chemical constituents of sputum in asthma.

Number of patients, times of sputum collection, number of samples and macroscopic types are given in Tables VI,1 and VI,12. Only one patient (No. 4) produced sputum during the 12 hours study period. Four patients failed to produce sputum between 09.00 to 12.00 hrs. and 15.00 to 18.00 hrs.

Mean values and standard error for sputum volume and chemical constituents of sputum are given in Table VI,13.

Sputum volume, dry weight, NANA and fucose levels of the 12.00 to 15.00 hrs. samples were lower than those produced during the 06.00 to 09.00 period, but the difference did not reach significant levels (Table VI,14). The NANA/Fucose ratio varied little. All the patients showed the same pattern of variation for the chemical constituents analysed.

The values of dry weight and chemical constituents of sputum from the single patient who produced sputum over the 12 hours study period are plotted in Fig. VI,3.

Dry weight, NANA, and fucose values followed the same pattern of variation observed in the patients who produced sputum only in two occasions over the 12 hours period. The 15.00 to 18.00 hrs. sputum samples showed a marked increase compared with the 12.00 to 15.00 hrs. sample, particularly for dry weight and NANA, the levels being higher than those of the early morning sample.

Comment:- Since asthmatic patients, even during attacks, produced little sputum over the 12 hours study period, full comparison with other diseases was not possible.

TABLE VI,12

Diurnal variation of chemical constituents of sputum in asthma.

Number of patients, time of sputum collection, number of samples and macroscopic type.

Patients	Time of sputum collection and number of samples				Macroscopic type
	06-09 hrs	09-12 hrs	12-15 hrs	15-18 hrs	
No. 1	1	-	1	-	M
	1	-	1	-	M
No. 2	1	1	1	-	M
	1	1	1	-	M
No. 3	1	-	1	-	M
No. 4	1	1	1	1	M
No. 5	1	-	1	-	M
No. 6	1	-	1	-	P
	1	-	1	-	P

TABLE VI,13

Diurnal variation of chemical constituents of sputum in asthma. Mean values and standard error of the mean ( ) of sputum volume dry weight and chemical constituents of sputum.

	Time of collection	
	06.00-09.00 hrs.	12.00-15.00 hrs.
Volume ml	8 (2.2)	6 (4.6)
Dry weight mg/ml	22.4 (6.2)	19.3 (6.2)
NANA µmol/ml	3.6 (0.9)	2.9 (0.9)
Fucose µmol/ml	8.4 (2.2)	6.6 (1.9)
NANA/Fucose molar ratio	0.56 (0.11)	0.54 (0.09)

TABLE VI,14

Diurnal variation of chemical constituents of sputum in asthma.

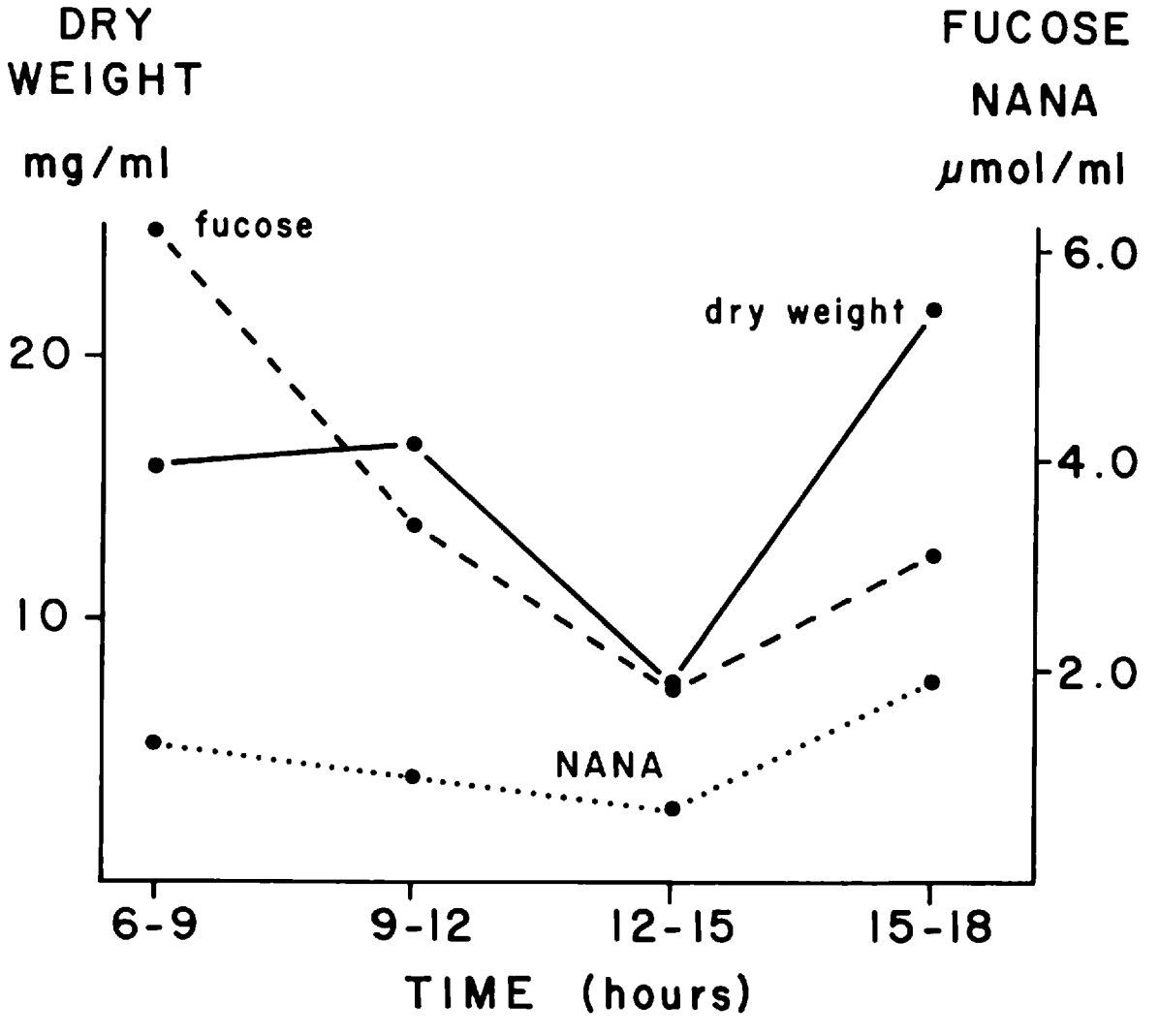
Sputum volume, dry weight and chemical constituents of sputum: comparison between times of collection (Student's t test).

06-09/12-15 hrs.

Volume (ml)	Dry weight (mg/ml)	NANA (μmol/ml)	Fucose (μmol/ml)	NANA/Fucose
0.06472	0.35895	0.49281	0.61039	0.14842

Fig. VI,3 Diurnal variation of dry weight (DW), N-acetyl neuraminic acid (NANA) and fucose in sputum from a patient with asthma.

Fig. VI,3


DRY
WEIGHT
mg/ml
FUCOSE
NANA
μmol/ml
fucose
dry weight
NANA
20
10
6.0
4.0
2.0
6-9
9-12
12-15
15-18
TIME (hours)

In those patients who produced sputum between 06.00 to 09.00 hrs. and 12.00 to 15.00 hrs., the pattern of variation for dry weight, NANA and fucose was similar to that seen in chronic bronchitis - high early morning levels followed by a decrease in the afternoon.

It is of interest that in the only one patient who produced sputum over the 12 hours study period, fucose levels showed a gradual decrease from 09.00 to 15.00 hrs. while NANA levels varied very little, suggesting that the serum transudate component remains constant while the bronchial gland secretion is subjected to a diurnal variation similar to that seen in chronic bronchitis.

DAILY VARIATION

Sputum samples were collected from 06.00 hrs. to 09.00 hrs. during 2 to 7 consecutive days. Diagnosis, number of patients and number of samples are given in Table VI,15.

Chemical estimations included: dry macromolecular weight, NANA and fucose. The average percentage coefficient of variation was calculated

from the formula:

$$\frac{\text{Standard deviation}}{\text{Mean value}} \times 100$$

Daily variation of chemical constituents of sputum in chronic bronchitis:-

Mean values, standard deviation, average percentage coefficient of variation for the chemical constituents of sputum for each individual patient and for the group are given in Table VI,16.

For individual patients the highest coefficient of variation was observed in patient No. 2, particularly for dry weight and NANA. All sputum samples from this patient were mucopurulent but one sample had very high dry weight and NANA levels falling within values commonly found for purulent sputum. When this sample was excluded the coefficient of variation fell within the range found for the other patients (26% and 4% respectively).

Taking all the samples the average coefficient of variation for the group showed that dry weight and NANA had the highest coefficient of variation (33% and 22% respectively). When the sputum sample with high levels of dry weight and NANA

TABLE VI,15

Daily variation of chemical constituents of sputum.

Diagnosis, number of patients and number of samples.

Diagnosis	No. of patients	No. of samples
Chronic bronchitis	3	15
Asthma	5	18
Bronchiectasis	4	13
Cystic fibrosis	5	17

TABLE VI,16

Daily variation of chemical constituents of sputum in chronic bronchitis.

Macroscopic type, number of samples, mean value, standard deviation and average percentage coefficient of variation of dry weight and chemical constituents of sputum for each individual patient and for the group.

	Patient	No. Samples	Macro-scopic type	Dry Weight mg/ml	NANA µmol/ml	Fucose µmol/ml	NANA/Fucose ratio molar ratio
Mean	No. 1	3	M	11.9	1.9	4.6	0.4
S.D.				2.5	0.4	0.8	0.03
Coef. Var.%.				21%	21%	17%	8%
Mean	No. 2	5	MP	23.9	3.7	7.0	0.5
S.D.				15.9	1.15	1.5	0.04
Coef. var.%.				66%	31%	21%	8%
Mean	No. 3	7	MP	22.8	4.4	3.7	1.2
S.D.				2.8	0.5	0.5	0.2
Coef. Var.%.				12%	13%	14%	18%
Coef. Var.%.	Group	15		33%	21%	17%	11%

from patient No. 2 was excluded the coefficient of variation decreased considerably (19% and 12% respectively) while the fucose and NANA/Fucose ratio varied little, and the coefficient of variation for all chemical constituents fell below 20%. (Fig. VI, 4 and 5).

Daily variation of chemical constituents of sputum in asthma.

Five patients were studied, three patients produced mucoid sputum during the study, one patient mucopurulent and one purulent. Number of patients and number of samples are given in Table VI,15. Chemical estimations included dry macromolecular weight, NANA and fucose.

Mean values, standard deviation and average percentage coefficient of variation for each individual patient and for the group for all chemical constituents of sputum and macroscopic types are given in Table VI,17.

Patients producing mucoid sputum showed the highest coefficient of variation of the asthma group for all chemical constituents tested, particularly for dry weight and fucose. In patient No. 4 whose sputum was mucopurulent during four

TABLE VI,17

Daily variation of chemical constituents of sputum in asthma.

Macroscopic type, number of samples, mean value, standard deviation and average percentage coefficient of variation of dry weight and chemical constituents of sputum for each individual patient and group.

	Patient	No. Samples	Macro-scopic type	Dry Weight mg/ml	NANA µmol/ml	Fucose µmol/ml	NANA/Fucose ratio Molar ratio
Mean	No. 1	5	M	12.4	1.7	4.9	0.4
S.D.				3.1	0.6	2.3	0.6
Coef. var.%.				25%	38%	46%	166%
Mean	No. 2	4	M	6.1	0.7	1.6	0.4
S.D.				3.0	0.3	0.7	0.05
Coef. var.%.				50%	46%	42%	11%
Mean	No. 3	3	M	15.3	2.6	3.0	1.3
S.D.				7.8	0.9	2.0	1.1
Coef. var.%.				51%	35%	66%	88%
Mean	No. 4	4	MP	46.4	8.4	9.8	0.8
S.D.				12.1	2.7	2.9	0.2
Coef. var.%.				26%	32%	30%	20%
Mean	No. 5	2	P	55.1	8.4	19.4	0.4
S.D.				0.8	0.3	0.9	0
Coef. var.%.				1%	4%	5%	
Coef. var.%.	Group	18		31%	31%	38%	57%

Fig. VI,4 Daily variation: percentage coefficient of variation for dry macromolecular weight, NANA, fucose and NANA/Fucose ratio in mucoid sputum from patients with chronic bronchitis or asthma

Fig. VI,4

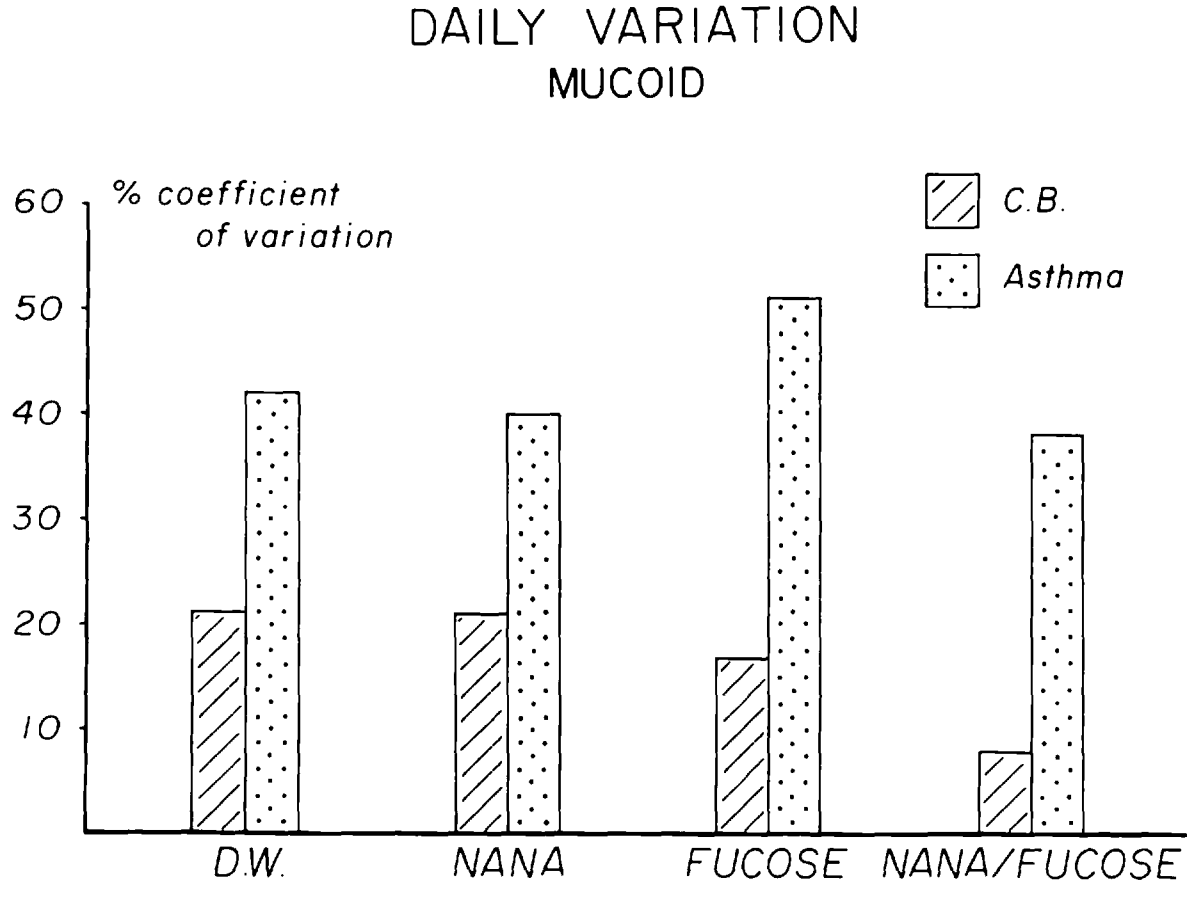
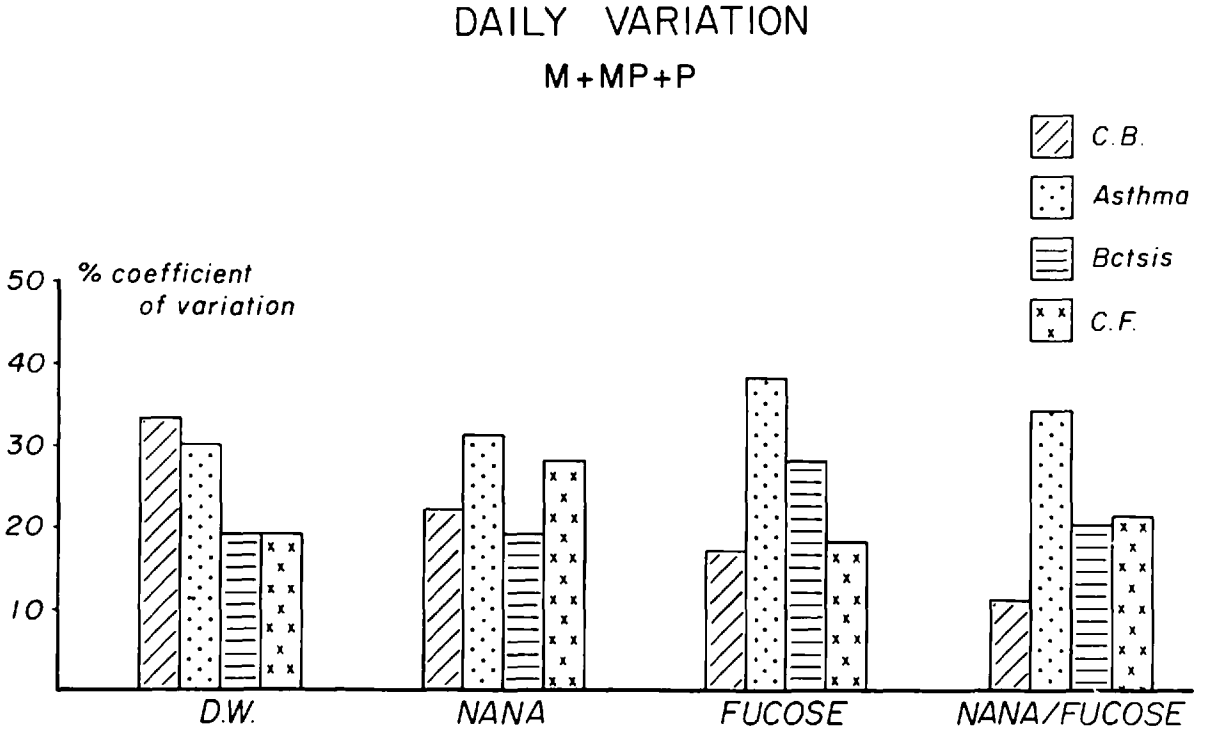

DAILY VARIATION
MUCOID
% coefficient of variation
60
50
40
30
20
10
C.B.
Asthma
D.W.
NANA
FUCOSE
NANA/FUCOSE

Fig. VI,5 Daily variation: percentage coefficient of variation for dry macromolecular weight, N-acetyl neuraminic acid (NANA), fucose and NANA/Fucose ratio in sputum (all macroscopic types included) from patients with chronic bronchitis (CB), asthma (A), cystic fibrosis (CF) or bronchiectasis (Bctsis).

Fig. VI,5


DAILY VARIATION
M+MP+P
C.B.
Asthma
Bctsis
C.F.
% coefficient of variation
50
40
30
20
10
D.W.
NANA
FUCOSE
NANA/FUCOSE

consecutive days the coefficient of variation for all chemical constituents fell within the range found for chronic bronchitis sputum. Patient No. 5 showed the lowest coefficient of variation for all chemical constituents.

The average coefficient of variation for the group for the chemical constituents of sputum was found to be above 30%, and fucose showed the highest coefficient of variation. (Table VI,17 and Fig. VI,4 and 5).

Daily variation of chemical constituents of sputum in bronchiectasis.

Number of patients included in the study and number of samples examined are given in Table VI,15. Chemical analysis included: dry macromolecular weight, NANA and fucose.

All the patients produced mucopurulent sputa; mean values and standard deviation and average coefficient of variation of chemical constituents for each individual patient and for the group are given in Table VI,18.

All patients, except No. 2, showed a coefficient of variation for dry weight, NANA, and fucose below 30%, the coefficient of

TABLE VI,18

Daily variation of chemical constituents of sputum in bronchiectasis. Macroscopic type, number of samples, mean value, standard deviation and average percentage coefficient of variation of chemical constituents of sputum for each individual patient and group.

	Patient	No. Samples	Macro-scopic type	Dry Weight mg/ml	NANA µmol/ml	Fucose µmol/ml	NANA/Fucose molar ratio
Mean	No.1	5	MP	33.1	1.3	3.1	0.4
S.D.				4.5	0.3	1.1	0.1
Coef. var.%.				14%	26%	37%	22%
Mean	No. 2	3	MP	29.8	4.0	6.2	0.7
S.D.				10.7	1.2	3.1	0.1
Coef. var.%.				36%	31%	50%	18%
Mean	No. 3	3	MP	44.1	5.4	7.9	0.7
S.D.				6.0	0.6	0.6	0.1
Coef. var.%.				14%	11%	8%	18%
Mean	No. 4	5	MP	29.3	2.8	3.3	0.8
S.D.				3.4	0.2	0.5	0.2
Coef. var.%.				12%	7%	16%	21%
Coef. var.%.	Group	16		19%	18.7	28%	20%

variation for the NANA/Fucose ratio was below 30% in all patients.

The coefficient of variation for the group for all chemical constituents and NANA/Fucose ratio fell within the range found for chronic bronchitis, but fucose showed the highest coefficient of variation. (Fig. VI,5).

Daily variation of chemical constituents of sputum in cystic fibrosis.

Number of patients included in the study and number of samples examined are given in Table VI,15. Chemical analysis included: dry macromolecular weight, NANA and fucose.

Macroscopic type, mean values and standard deviation of chemical constituents and average percentage coefficient of variation for each individual patient and for the group are given in Table VI,13.

All patients produced mucupurulent sputum during the study period. The individual coefficient of variation for the chemical constituents was below 40% in all patients. NANA content of sputum and the NANA/Fucose ratio showed the highest

TABLE VI,19

Daily variation of chemical constituents of sputum in cystic fibrosis.

Macroscopic type, number of samples, mean values, standard deviation and average percentage coefficient of variation of dry weight and chemical constituents of sputum for each individual patient and for the group.

	Patient	No. Samples	Macro-scopic type	Dry Weight	NANA µmol/ml	Fucose µmol/ml	NANA/Fucose molar ratio
Mean	No. 1	5	MP	92.1	3.9	6.3	0.6
S.D.				23.2	1.2	1.7	0.1
Coef. var.%.				25%	30%	27%	21%
Mean	No. 2	3	MP	23.8	3.2	6.3	0.5
S.D.				9.0	0.9	1.6	0.05
Coef. var.%.				38%	30%	26%	10%
Mean	No. 3	3	MP	50.4	3.7	7.0	0.5
S.D.				3.4	0.8	1.1	0.06
Coef. var.%.				7%	23%	16%	11%
Mean	No. 4	3	MP	17.1	1.5	3.2	0.5
S.D.				4.4	0.3	0.4	0.1
Coef. var.%.				26%	24%	13%	27%
Mean	No. 5	3	MP	20.1	2.1	4.0	0.5
S.D.				2.7	0.7	0.3	0.2
Coef. var.%.				13%	34%	9%	37%
Coef. var.%.	Group	17		20%	28%	18%	21%

coefficient of variation.

The coefficient of variation for all chemical constituents for the group fell below 30%; NANA content of sputum and NANA/Fucose ratio showed the highest coefficient of variation (Table VI,19 : Fig. VI,5).

Comment:- Mucoid asthma samples showed the highest coefficient of variation for the chemical constituents tested and NANA/Fucose molar ratio, particularly for fucose, (Fig. VI,4) suggesting that the amount of bronchial mucus secreted by these patients varies considerably from day to day. The serum transudate component is also subjected to a variation but to a lesser extent since the coefficient of variation of NANA was lower than that of fucose.

When mucopurulent samples from the four disease groups were compared, the average percentage coefficient of variation for each disease fell below 30% for all chemical constituents tested, the values being very similar. It would seem that when pus is present in the sputum, the serum transudate/exudate component varies little from one disease to another since the NANA/Fucose ratio fell within the same levels for all diseases.

When all macroscopic types were included, the asthma group showed the highest coefficient of variation for NANA, fucose and NANA/Fucose ratio (Fig.VI,5).

MONTHLY VARIATION

Monthly variation of chemical constituents of sputum in chronic bronchitis

Sputum samples from fourteen chronic bronchitic patients were studied over a period of six months, from December to May. Seven patients were studied over a period of six consecutive months, six patients over five months, and four patients over four consecutive months (Table VI,20). Chemical estimations included: dry macromolecular weight, NANA and fucose. Sputum samples were examined macroscopically and classified as mucoid, mucopurulent or purulent; 34 samples were mucoid; 38 mucopurulent and 4 purulent (Table VI,20).

Mean values, standard deviation and average percentage coefficient of variation of dry weight. and chemical constituents for each individual patient in Table VI,21.

TABLE VI,20

Monthly variation of chemical constituents of sputum in chronic bronchitis.

Number of patients, number of samples, and macroscopic type of sputum studied.

Patient	No. Samples	Macroscopic type		
		M	MP	P
No. 1	6	3	2	1
No. 2	5	2	3	0
No. 3	6	5	1	0
No. 4	6	2	4	0
No. 5	6	5	1	0
No. 6	6	0	4	2
No. 7	5	0	5	0
No. 8	6	2	4	0
No. 9	6	3	3	0
No. 10	5	4	1	0
No. 11	4	2	2	0
No. 12	5	1	4	0
No. 13	5	1	3	1
No. 14	5	4	1	0
TOTAL				
14	76	34	38	4

All chemical constituents studied showed a wide range of values for the average coefficient of variation. The highest coefficient of variation for each individual and for the group was for NANA, in only two patients (Nos. 7 and 14) the percentage coefficient of variation was below 30%. In patient No. 7 the macroscopic type of sputum remained mucopurulent during the five months of the study and in patient No. 14 four out of five samples were mucoid.

When values of dry weight, NANA and fucose for each individual patient for the six months study period were compared, nine out of fourteen patients (Nos. 1,2,3,4,5,7,9,10 and 14) showed a similar pattern of variation consisting of high levels in December, a fall in January followed by an increase in February and March which was then followed by a decrease in April and then raised again in May. (Fig. VI,6, VI,7).

Mean values and standard error of the mean of dry weight, NANA, fucose and fucose ration for each month are given in Table VI,22; the pattern of variation for the group was similar to that described for individual patients, particularly for NANA and fucose levels (Fig. VI,8).

TABLE VI,21

Monthly variation of chemical constituents of sputum in chronic bronchitis.

Mean value, standard deviation and average percentage coefficient of variation for each individual patient and group.

Patient	Dry weight mg/ml			NANA (μmol/ml)			Fucose (μmol/ml)			NANA/Fucose molar ratio		
	Mean	s.d.	% c.v.	Mean	s.d.	% c.v.	Mean	s.d.	% c.v.	Mean	s.d.	% c.v.
No. 1	23.5	7.4	32	2.7	1.8	68	6.1	4.5	75	0.4	0.1	33
No. 2	25.8	5.1	20	3.9	2.4	63	8.1	3.2	40	0.5	0.1	31
No. 3	23.3	2.8	12	3.8	1.5	40	10.4	2.8	27	0.4	0.02	4
No. 4	34.5	19	55	4.1	2.1	52	14.3	7.8	55	0.3	0.1	28
No. 5	27.6	10.6	38	3.9	2.1	53	7.5	4.0	54	0.5	0.2	32
No. 6	29.8	5.6	19	3.3	1.5	46	7.6	1.2	16	0.4	0.1	40
No. 7	16.3	1.6	10	1.9	0.4	23	5.6	1.0	18	0.3	0.1	30
No. 8	10.0	2.6	27	1.2	0.5	41	3.1	0.9	30	0.4	0.1	35
No. 9	8.8	3.1	35	0.6	0.3	46	2.0	0.9	46	0.3	0.03	9
No. 10	14.7	5.4	37	2.8	1.3	47	3.9	1.5	39	0.7	0.1	18
No. 11	21.6	9.2	43	1.4	0.7	52	5.6	1.0	18	0.2	0.1	59
No. 12	24.8	8.2	33	3.2	1.3	42	7.1	2.4	35	0.5	0.2	36
No. 13	20.1	3.4	17	2.7	1.2	46	7.1	1.8	25	0.4	0.1	37
No. 14	17.2	2.2	13	2.6	0.7	28	4.5	1.2	26	0.5	0.1	20

TABLE VI,22

Monthly variation of chemical constituents of sputum in chronic bronchitis.

Mean values and standard error of the mean of dry weight and chemical constituents of sputum for each month.

Month	Dry weight mg/ml	NANA µmol/ml	Fucose µmol/ml	NANA/Fucose molar ratio
DECEMBER	22.67	2.73	7.9	0.47
	2.95	0.30	2.40	0.06
JANUARY	14.20	1.36	3.75	0.42
	3.40	0.20	0.80	0.05
FEBRUARY	21.06	3.98	7.08	0.54
	2.00	0.70	0.80	0.07
MARCH	26.98	2.60	7.33	0.37
	3.50	0.40	1.50	0.02
APRIL	27.02	2.16	4.78	0.34
	5.4	0.40	0.70	0.04
MAY	25.25	3.78	9.58	0.38
	4.00	0.80	2.00	0.04

Fig. VI,6 Monthly variation in dry macromolecular weight, (D.W.), N-acetyl neuraminic acid (NANA) and fucose in the sputum from a patient with chronic bronchitis.

Fig. VI,6

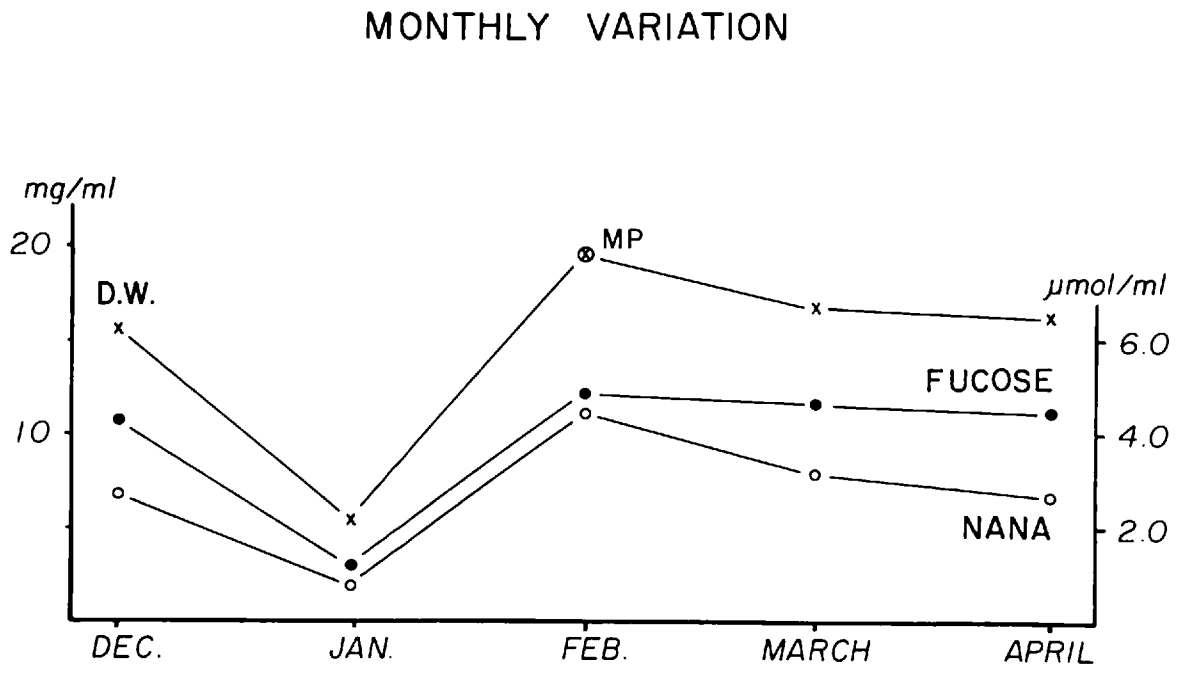

MONTHLY VARIATION
mg/ml
20
10
D.W.
MP
µmol/ml
6.0
4.0
2.0
FUCOSE
NANA
DEC.
JAN.
FEB.
MARCH
APRIL

Fig. VI,7 Monthly variation in dry macromolecular weight, (D.W.), N-acetyl neuraminic acid and fucose in the sputum from a patient with chronic bronchitis.

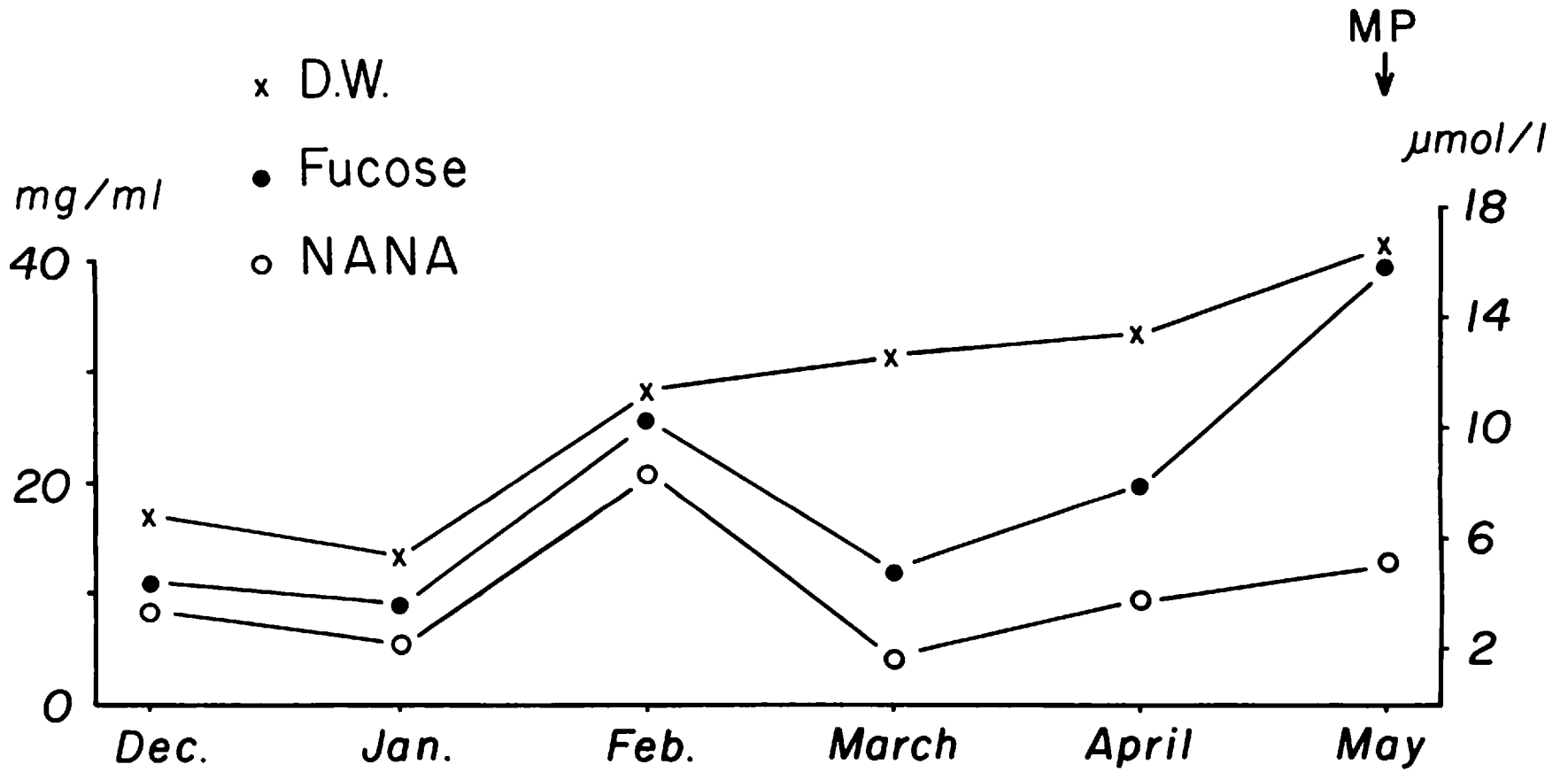
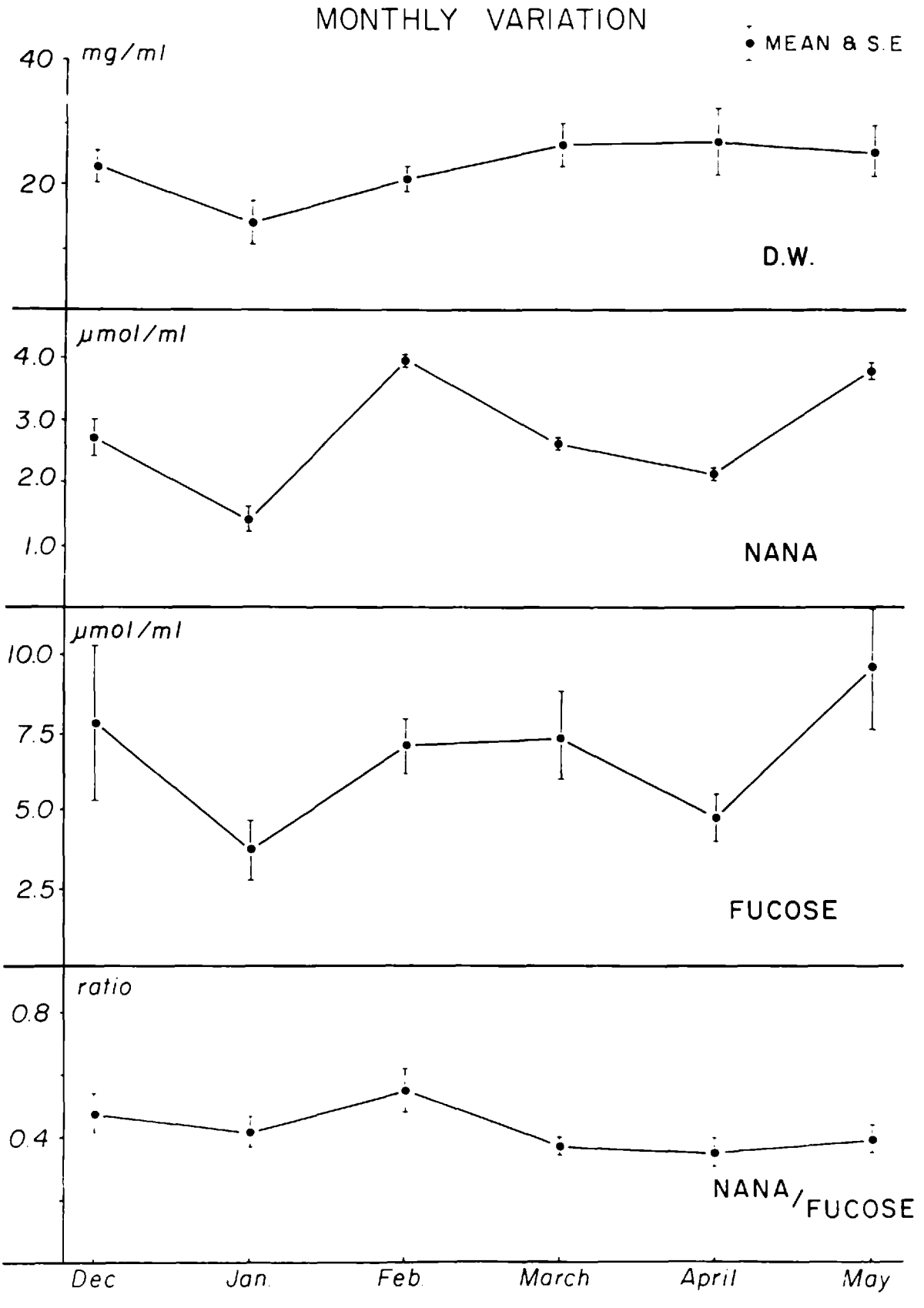

MONTHLY VARIATION
MP
x D.W.
• Fucose
o NANA
mg/ml
µmol/l
40
20
0
18
14
10
6
2
Dec.
Jan.
Feb.
March
April
May

Fig. VI,7

Fig. VI,8 Monthly variation in dry macromolecular weight (D.W.), N-acetyl neuraminic acid (NANA) fucose and NANA/Fucose ratio from sputum in a group of 14 patients with chronic bronchitis

Fig. VI,8


MONTHLY VARIATION
MEAN & S.E
mg/ml
40
20
D.W.
μmol/ml
4.0
3.0
2.0
1.0
NANA
μmol/ml
10.0
7.5
5.0
2.5
FUCOSE
ratio
0.8
0.4
NANA/FUCOSE
Dec
Jan
Feb
March
April
May

Comment: The high average percentage coefficient of variation found in this group of chronic bronchitis patients seems to be due to the variation in macroscopic type of sputum over the study period, since levels of NANA, a marker substance of both bronchial and serum glycoproteins, showed a higher variation than fucose. When the macroscopic type of sputum remained unchanged the coefficients of variation of dry weight, NANA, fucose and NANA/Fucose ratio were found to be below 30% whether the sputum was mucopurulent or purulent. It is also interesting to note that fucose levels for each individual patient showed less variation than NANA.

The seasonal variation pattern seen in this group of patients of high levels in December with a fall in January followed by an increase in February and March, a fall in April and then an increase in May could be due to the fact that the number of mucopurulent samples in January was the lowest while the number of mucopurulent samples in May was higher than in December, February and April. When individual patients were considered it was apparent that this pattern of seasonal variation was also seen in those patients whose sputum remained macroscopically unchanged (No. 7) or showed very

little variation (Nos. 3,5,10 and 14) and therefore other causes apart from macroscopic type could be responsible for this variation. Keal (1970) studied a group of early chronic bronchitis patients, with mucoid sputum, over three consecutive years and found a seasonal variation in the NANA content of sputum which was related to changes in atmospheric pollution.

YEARLY VARIATION

Yearly variation of chemical constituents of sputum in chronic bronchitis:-

Seven chronic bronchitic patients who were attending an out-patients clinic were studied over a period of six months from December to May during two consecutive years. Four patients were studied over six months, two during five months and one during four months (Table VI,23).

Number of patients, number of sputum samples and macroscopic type are given in Table VI,23.

In the first year 42% of the samples were mucoid, 53% were found to be mucopurulent and 5% purulent while in the second year 16% were mucoid, 76% mucopurulent and 8% purulent.

Chemical estimations included: dry weight, NANA and fucose. Mean values, standard error of the mean and average percentage coefficient of variation of sputum volume, dry weight and chemical constituents for each patient for the two years are given in Table VI,24.

It is interesting to note that the sputum volume for each individual patient varied little and those patients producing large amounts of sputum the first year produced similar volume the second year (Fig. VI,9).

Although the percentage of mucopurulent samples was higher during the second year, the dry macromolecular weight varied little and only in two patients (Nos. 4 and 6) did the difference reach significant levels (Table VI,25). The levels of NANA were higher during the second year and in two patients (Nos. 6 and 7) the difference was statistically significant (Table VI,25). Fucose levels varied little except in patient No. 1 in whom fucose levels were higher during the first year although the number of mucoid and mucopurulent samples was the same during the two years. No statistically significant difference was found for fucose levels between the two years for any of the

patients. (Table VI, 25, Fig. VI,10). In all patients the NANA/Fucose ratio was found to be higher during the second year and in four patients (Nos. 1, 2, 5 and 7, the difference was statistically significant (Table VI, 25). Patients Nos. 2, 5 and 7 produced more mucopurulent samples during the second year and the increase in inflammatory exudate may be responsible for the changes observed in dry macromolecular weight, NANA and NANA/Fucose ration.

When sputum samples from the same months for the two years study were compared, the pattern of variation over the six months was similar except for the dry weight which showed a different pattern during the second year consisting of an increase in January followed by a decrease during February and March and increase in April. Although the pattern of variation for NANA, fucose and NANA/Fucose ratio was similar during the two years the trend was more marked during the first year; NANA, fucose and NANA/Fucose ratio decreased in January then increased during February and March and then it was followed by a decrease in April (Fig. VI, 11; VI,12 and VI,13.).

The number of mucopurulent samples was higher during the second year, particularly in January and February and this could explain the higher levels of dry weight, NANA and NANA/Fucose ratio found during these months when compared with the first year. No significant difference was found for the chemical constituents for the same months except for dry weight in January and the NANA/Fucose ratio in April which were significantly higher in the second year (Table VI,26).

Comment:-

All the patients included in this study were suffering from long standing chronic bronchitis and all had marked and fixed airways obstruction, that is they represented a homogeneous group and the pathological changes of bronchial epithelium - gland hypertrophy and increase in number of goblet cells - should be expected to be irreversible.

From the results here presented it was found that the variations of sputum volume, dry weight, NANA and fucose concentrations were greater between patients (interpatients variation) than within a patient over a long period of time (intra-patient variation). In some patients the fucose concentration of sputum remained high over the study

period (Nos. 1, 2 and 3) while in one (No. 5) was very low. Similar inter-patient variation was observed for the sputum volume. For each individual patient fucose levels showed less variation than NANA or dry weight, this could be because NANA and dry weight are more influenced by changes in degree of purulence than fucose.

These findings suggest that the sputum produced by an individual patient has a characteristic biochemical profile.

TABLE VI,23

Yearly variation of chemical constituents of sputum in chronic bronchitis.

Number of patients, age, number of samples and macroscopic type for the first and second years.

		FIRST YEAR				SECOND YEAR			
Patients	Age	No samples	M	MP	P	No samples	M	MP	P
No. 1	69	6	2	4	0	6	2	4	0
No. 2	66	6	5	1	0	6	0	6	0
No. 3	66	6	0	4	2	6	0	6	0
No. 4	64	5	0	5	0	5	0	2	3
No. 5	68	6	3	3	0	6	1	5	0
No. 6	71	5	4	1	0	5	3	2	0
No. 7	66	4	2	2	0	4	0	4	0

TABLE VI,24

Yearly variation of chemical constituents of sputum in chronic bronchitis.
Mean value and standard error of the mean of dry weight, chemical constituents of sputum and sputum value for each individual patient for the two years study period.

	Dry weight mg/ml		NANA µmol/ml		Fucose µmol/ml		NANA/Fucose ratio		Volume ml	
Patients	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
No. 1	34.5	29.4	4.1	4.3	14.3	9.2	0.3	0.5	3.6	5.5
	7.8	1.8	0.9	0.4	3.2	0.6	0.04	0.05	0.6	0.3
No. 2	27.6	33.3	3.9	6.4	7.5	8.1	0.5	0.8	3.6	4.3
	4.3	3.6	0.8	0.8	1.6	1.2	0.07	0.02	0.6	0.5
No. 3	29.8	24.7	3.3	3.6	7.6	7.0	0.4	0.5	7.0	9.8
	2.3	4.2	0.6	0.5	0.5	0.8	0.07	0.03	0.9	2.7
No. 4	16.3	20.1	1.9	2.1	5.6	6.5	0.3	0.37	19.0	22.5
	0.7	1.5	0.2	0.2	0.4	1.6	0.05	0.06	1.0	1.3
No. 5	8.8	11.9	0.6	1.0	2.0	2.7	0.3	0.4	not	13.0
	1.2	1.2	0.1	0.2	0.4	0.4	0.01	0.02	recorded	1.1
No. 6	14.7	22.9	2.8	4.2	3.9	5.3	0.7	0.8	5.6	9.0
	2.4	2.4	0.6	0.4	0.7	0.7	0.06	0.05	0.6	2.8
No. 7	21.6	22.9	1.4	3.2	5.6	5.5	0.3	0.6	2.2	3.0
	4.6	2.4	0.4	0.5	0.5	1.7	0.07	0.08	0.2	0.7

TABLE VI,25

Yearly variation of chemical constituents of sputum in chronic bronchitis.

Comparison between two consecutive years for dry weight, chemical constituents and sputum volume (Student's t test).

Patient	Dry weight mg/ml	NANA µmol/ml	Fucose µmol/ml	NANA/Fucose molar ratio	Volume ml
No. 1	0.63947	-0.13210	1.56990	-2.43650*	-2.70713*
No. 2	-1.01068	-2.15770	-0.29332	-3.54490***	-0.81654
No. 3	1.06523	0.35426	0.28364	-1.04730	-0.98585
No. 4	-2.24050*	-0.64787	-0.51561	-0.30741	-2.15474
No. 5	-1.79206	-1.66936	-1.13835	-3.21158***	
No. 6	-2.36146*	-2.82013**	-1.44017	-1.42055	-1.18186
No. 7	0.12768	-2.58974*	0.07900	-3.26505**	4.13918***

* $P < 0.05$
** $P < 0.02$
*** $P < 0.01$

TABLE VI,26

Yearly variation of chemical constituents of sputum in chronic bronchitis.

Comparison between same months over two consecutive years for dry weight and chemical constituents of sputum.

Month	Dry weight	NANA	Fucose	NANA/Fucose
December	-0.17662	-0.08379	-0.06003	-0.05789
January	-1.89520	-2.69324*	-1.33666	-1.41828
February	-0.65646	-0.01635	0.81012	-0.90177
March	0.62759	-0.78659	0.07871	-0.50174
April	0.34653	-1.57604	-1.01083	-2.34395*
May	0.46926	0.00000	0.99997	-2.19627

* $P < 0.05$

Fig.VI,9 Yearly variation in sputum volume in seven patients studied over two consecutive years.

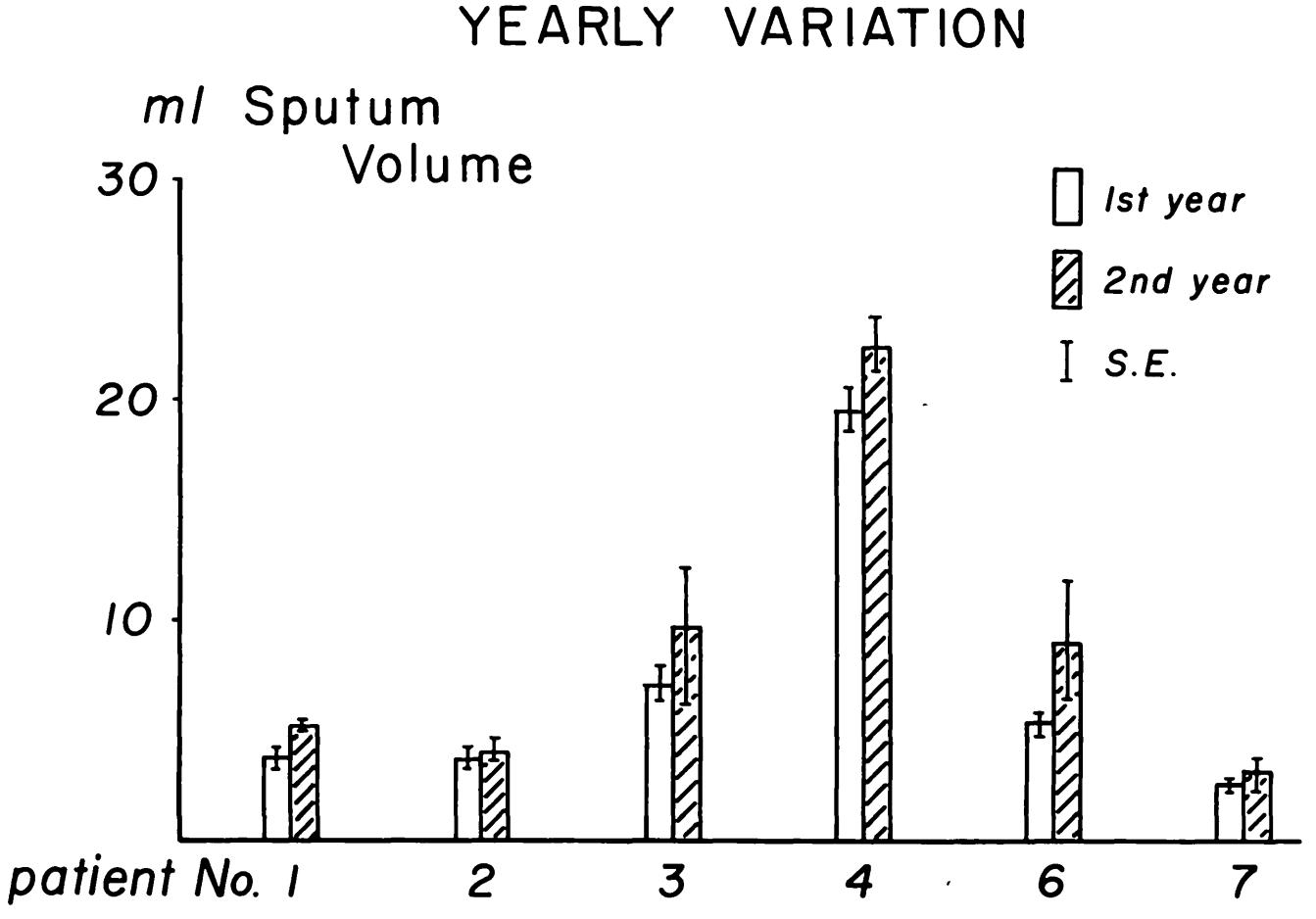

YEARLY VARIATION
ml Sputum Volume
30
20
10
1st year
2nd year
S.E.
patient No. 1
2
3
4
6
7

Fig. VI,9

Fig. VI,10 Yearly variation in fucose content of sputum in seven patients studied over two consecutive years.

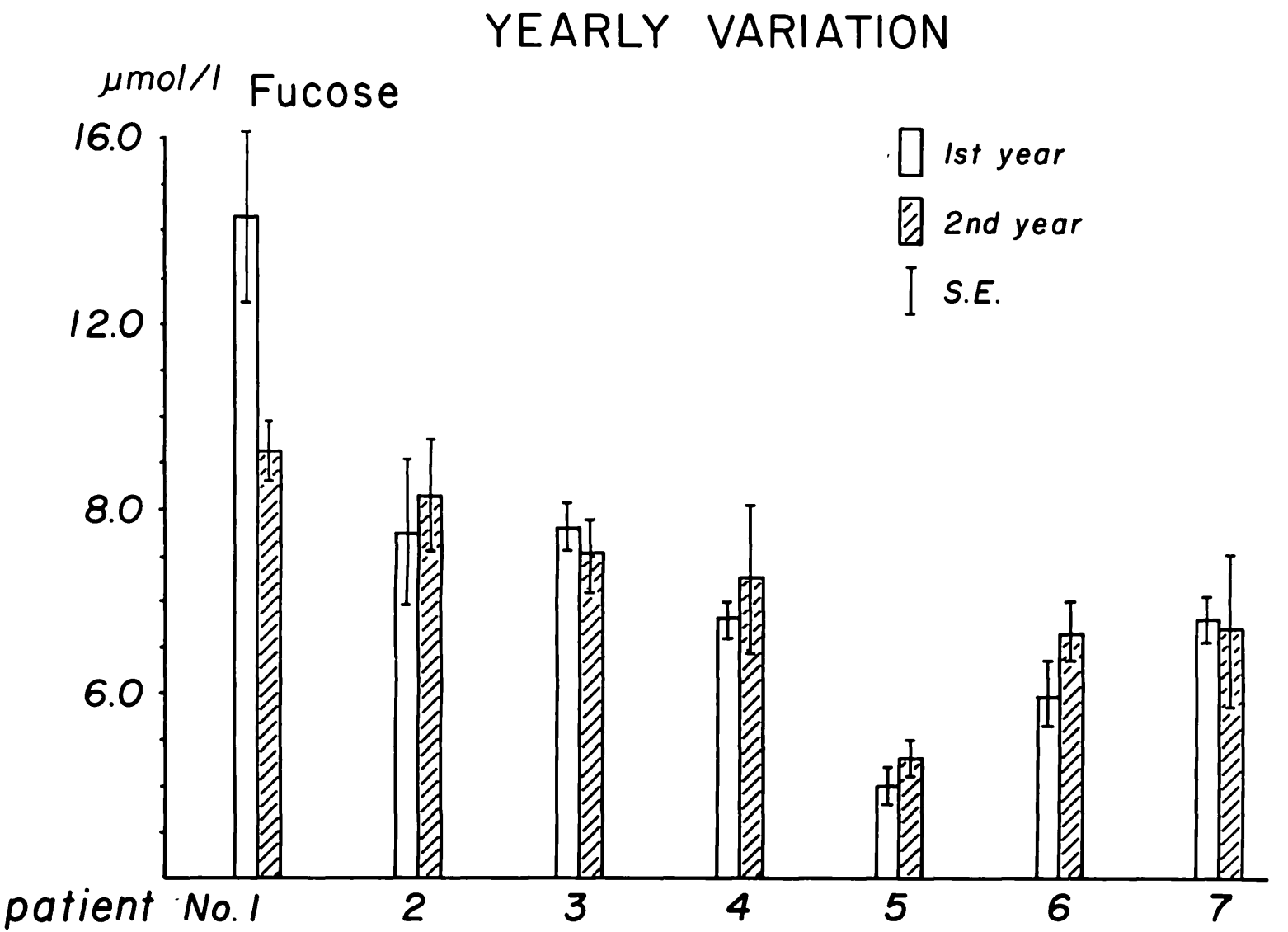

YEARLY VARIATION
μmol/l Fucose
16.0
12.0
8.0
6.0
1st year
2nd year
S.E.
patient No. 1
2
3
4
5
6
7

Fig. VI, 10

Fig. VI,11 Yearly variation in N-actyl neuraminic acid (NANA) content of sputum from a group of patients with chronic bronchitis studied over two consecutive years.

Fig. VI,11

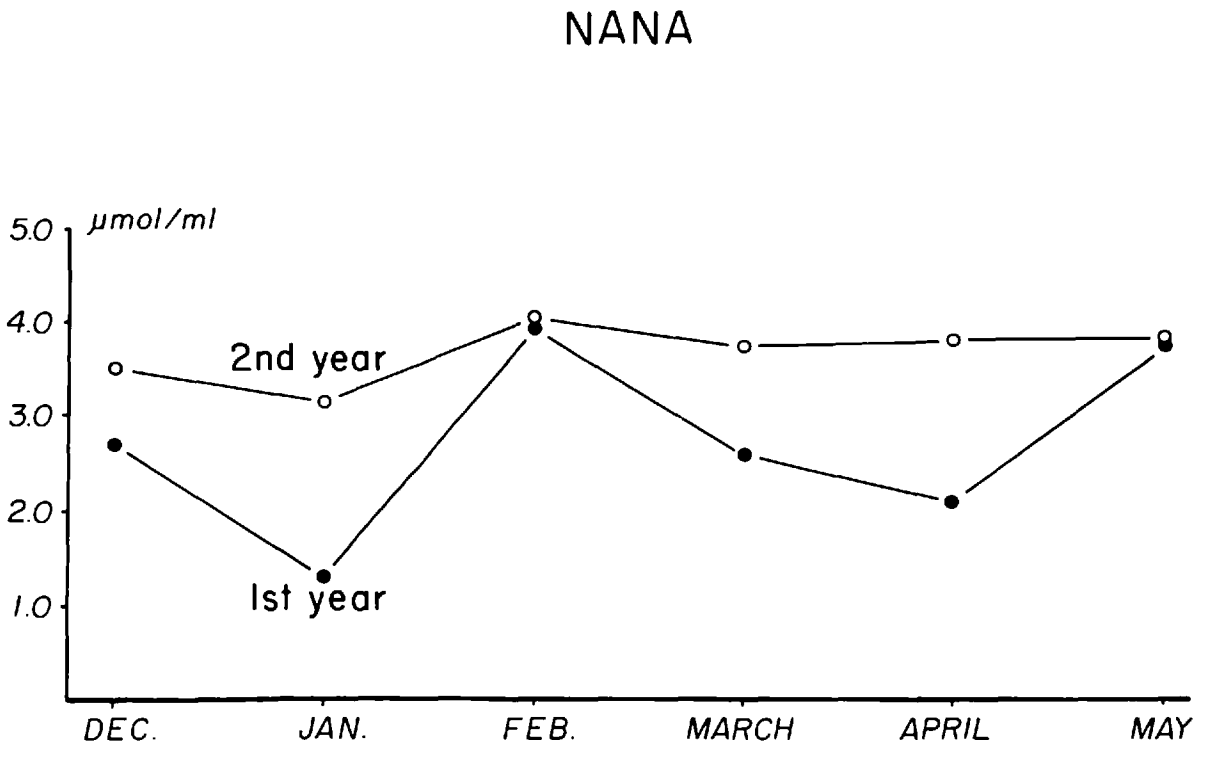

NANA
μmol/ml
5.0
4.0
3.0
2.0
1.0
2nd year
1st year
DEC.
JAN.
FEB.
MARCH
APRIL
MAY

Fig. VI,12 Yearly variation in fucose content of sputum from a group of patients with chronic bronchitis studied over two consecutive years.

Fig. VI,12

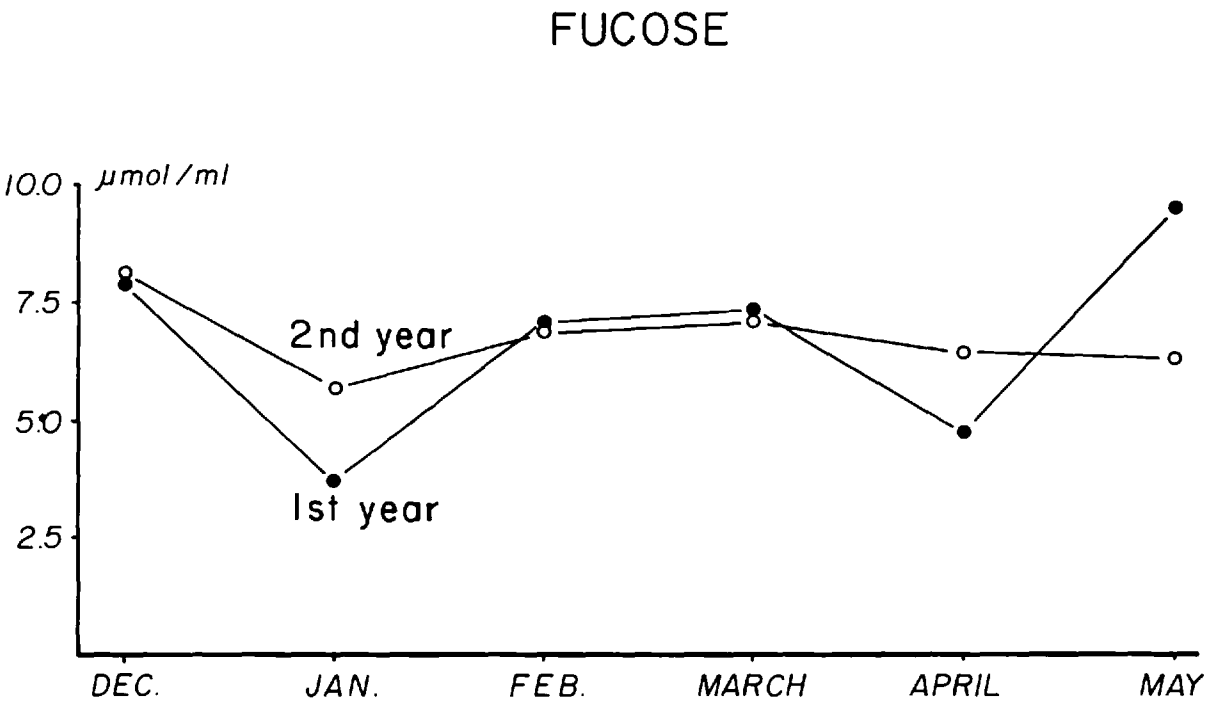
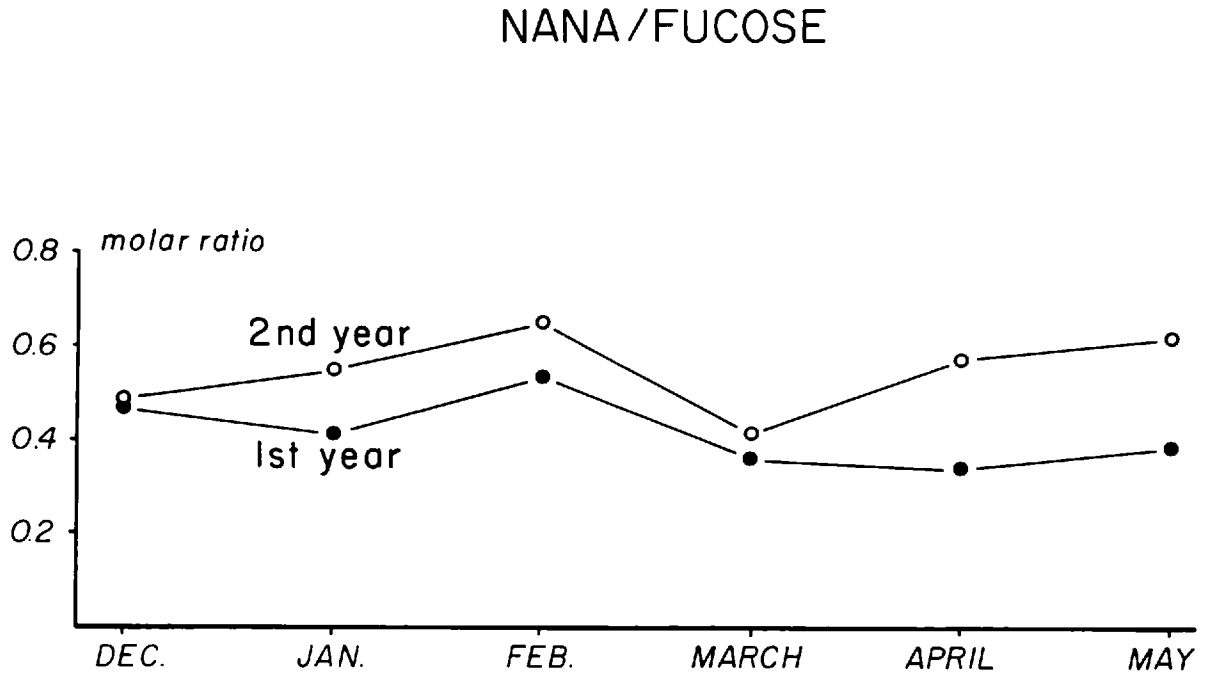

FUCOSE
10.0
μmol/ml
7.5
5.0
2.5
2nd year
1st year
DEC.
JAN.
FEB.
MARCH
APRIL
MAY

Fig. VI,13 Yearly variation in NANA/Fucose ratio of sputum from a group of patients with chronic bronchitis studied over two consecutive years

Fig. VI,13


NANA/FUCOSE
molar ratio
0.8
0.6
0.4
0.2
2nd year
1st year
DEC.
JAN.
FEB.
MARCH
APRIL
MAY

CHAPTER VII

EFFECT OF DRUGS ON SPUTUM PRODUCTION

THE EFFECT OF DRUGS ON SPUTUM PRODUCTION

When studying the effect of drugs on sputum production we have to bear in mind that sputum is a complex biological fluid and that the relative proportions of the various constituents particularly bronchial secretion and serum transudate may vary in different disease states. Any therapeutic attempt to reduce sputum volume should be concerned with the fundamental mechanisms that is mucus hypersecretion and/or increase in tissue fluid transudate.

Studies carried out in patients with chronic bronchitis (Medici et al 1973, Pham et al 1973; Lopez-Vidriero et al. 1973) and cystic fibrosis (Polgar and Denton, 1962; Feather and Russell 1970), have shown that the physical properties of sputum, particularly its viscosity, contribute to airways obstruction and impair gas exchange and mucociliary clearance. Mucolytic drugs are widely used to decrease sputum viscosity but contradictory results have been reported in the literature.

Selection of drugs:- The drugs in the study were:

- parasympatholytic: atropine
- corticosteroid hormone: ACTH or prednisone
- mucolytic agent: sodium 2-mercapto ethane sulphate.

Selection of patients:- The patients were divided into two groups: Group I included patients producing more than 100 ml. of sputum in 24 hours and Group II those producing less than 100 ml. of sputum in 24 hours.

EFFECT OF DRUGS ON SPUTUM PRODUCTION IN PATIENTS WITH BRONCHORRHOEA

Bronchorrhoea: definition:-

The patients included in this group had bronchorrhoea which has been defined as a condition in which more than 100 ml. of sputum is produced within 24 hours (Keal 1971), an amount in excess of that seen in chronic lung diseases (Ashcroft 1965; Miller et al 1965).

Bronchorrhoea may be idiopathic (Hartley and Davies 1923; Reid 1960) or associated with lung diseases such as chronic bronchitis (Kourilsky 1960, Calim 1972), asthma (Keal 1971) or alveolar cell carcinoma (Wood 1943; Kennamer 1951; Storey et al 1953; Schools and Ray 1961).

Macroscopic, rheological and chemical characteristics of bronchorrhoea sputum:

Bronchorrhoea sputum is transparent, resembles egg-white and is usually topped by a large layer of froth. Spontaneous separation into two layers may occur, a thicker more opaque gel-like material floating on a thin clear watery fluid (Lopez-Vidriero et al 1975). Some rheological properties are also characteristic, particularly the increase in viscosity with time and the absence of yield value. The apparent viscosity is always higher than saliva and falls within the lower end of mucoid sputum. Absolute levels of marker substances of bronchial glycoproteins, fucose and sulphate, are within the lower end of mucoid sputum but always above saliva values. This is important for the differential diagnosis between bronchorrhoea and hypersalivation (Keal 1971). Although NANA and mannose levels fall within mucoid sputum range the NANA/Fucose molar ratio is significantly higher suggesting that in bronchorrhoea there is an increase in serum transudate component.

Keal (1971) described two types of bronchorrhoea sputum one with low NANA levels which responded to steroids (reduction in sputum volume)

and another with high NANA levels which did not respond to steroid therapy.

Changes in response to corticosteroids.

Five patients were included in the present study, the 24 hours sputum volume was 100 ml. or more before corticosteroid treatment was commenced. The primary disease was intrinsic asthma (Nos. 1 and 2), extrinsic asthma in (No. 3), chronic bronchitis (No. 4) and scleroderma plus fibrosing alveolitis (No. 5). Sex, age, primary diagnosis and NANA content of sputum before steroid treatment are given in Table VII, 1.

The NANA content of the sputum was found to be low in three patients (Nos. 1, 3, 5) and high in two patients (Nos. 2 and 4).

Patient No. 1:-

A 27 year old student with intrinsic asthma which episodically caused severe wheeze and breathlessness accompanied by high eosinophilia. He was admitted to the Brompton Hospital complaining of copious sputum production. The 24 hours sputum volume varied from 250 ml. to 85 ml. He was treated with ACTH injections 80 units weekly and

TABLE VII.1

Effect of drugs on sputum production in patients with bronchorrhoea.

Number of patients, sex, age, primary diagnosis and NANA concentration in sputum before treatment.

Patient	Sex	Age	Primary diagnosis	NANA (μmol/ml)
No. 1	M	27	Intrinsic asthma	0.9
No. 2	M	53	Intrinsic asthma	2.6
No. 3	M	47	Extrinsic asthma	0.5
No. 4	M	52	Chronic bronchitis	2.6
No. 5	M	43	Scleroderma + Fibrosing alveolitis	0.6

prednisone 40 mg. daily for ten days. During the first four days of treatment the daily sputum volume changed very little and then sharply decreased to less than 25ml. and remained within these levels during the study. (Fig. VII,1). The reduction in sputum volume was accompanied by an increase in NANA and fucose content with a less marked increase in dry macromolecular weight. (Fig. VII,1).

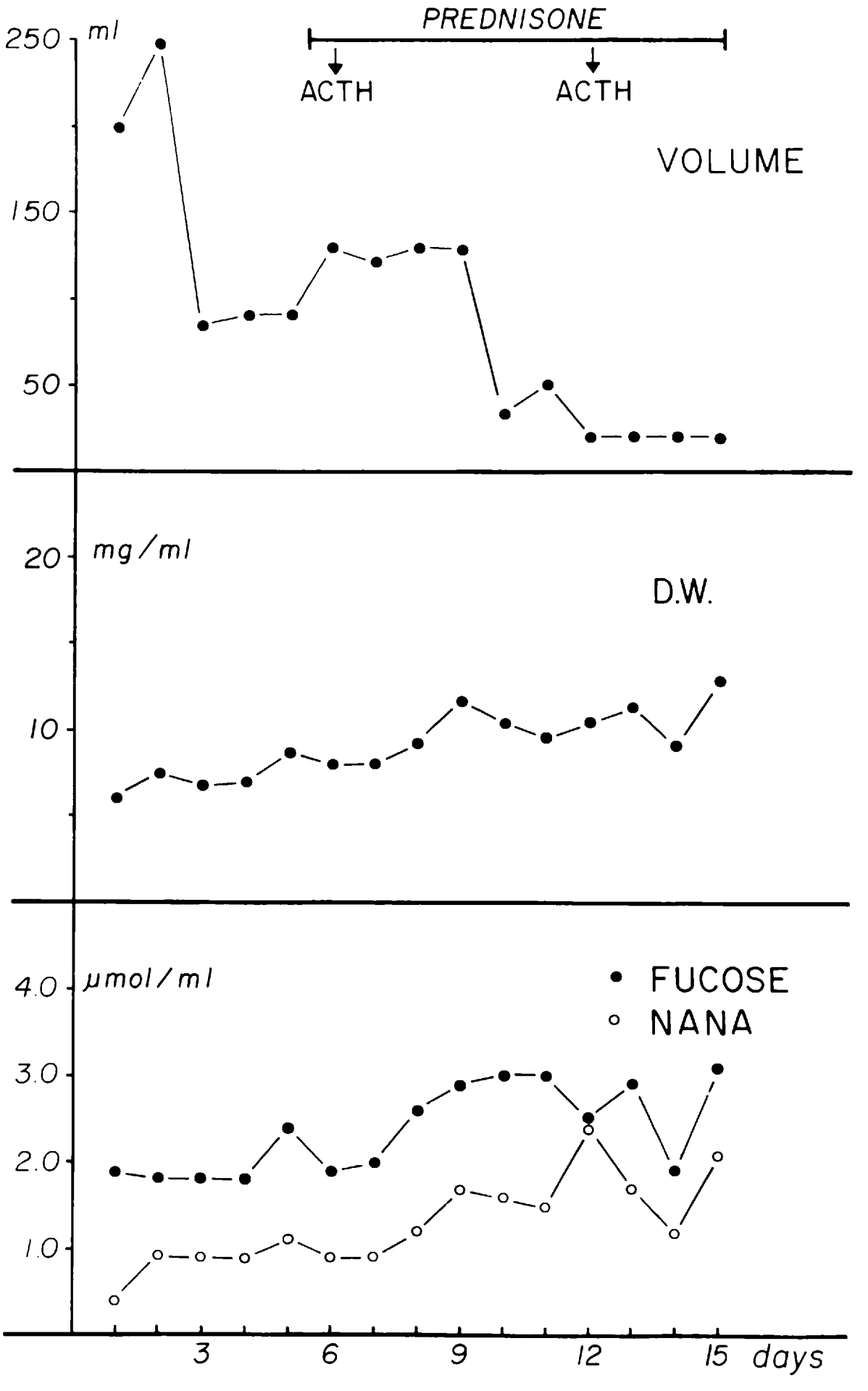
Comment:-

The low dry weight, NANA and fucose concentrations with high NANA/Fucose ratio suggest that in this patient fluid transudate was responsible for the main bulk of the sputum.

The good response to steroids, prednisone and ACTH, in this patient with intrinsic asthma and low levels of NANA seems to be due to a reduction in serum transudate since the fall in sputum volume was accompanied by an increase in dry weight, NANA and fucose concentrations. The fact that the marked reduction in sputum volume was accompanied by a moderate increase in dry weight and chemical constituents suggests that the effect of steroids in this case was to reduce not only the water content but also the

Fig. VII,1 The effect of steroids on sputum volume dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with intrinsic asthma associated with bronchorrhoea.

Fig. VII,1


PREDNISONE
ACTH
ACTH
250
ml
VOLUME
150
50
20
mg/ml
D.W.
10
4.0
µmol/ml
FUCOSE
NANA
3.0
2.0
1.0
3
6
9
12
15
days

concentration of large molecules such as serum glycoproteins.

Siltzbach (personal communication) reported that patients with bronchorrhoea associated with alveolar cell carcinoma respond better to ACTH than oral steroids. It could be that in this patient the effect of prednisone was enhanced by ACTH.

Patient No. 2:-

A 53 year old industrial chemist with a 4 years history of intermittent breathlessness and wheeze was admitted to the Brompton Hospital complaining of increasing shortness of breath and copious mucoid sputum up to 100 ml. per day. Examination of sputum showed scanty leucocytes, 30% of which were eosinophils and a diagnosis of intrinsic asthma was made.

The 24 hours sputum volume before treatment ranged from 100 ml. to 95 ml. and the NANA concentration was high (2.6 μmol/ml). Prednisone 30 mg. daily was commenced on the third day of the study. The daily sputum volume as well as the early morning (0600-0900 hrs) volume gradually decreased to 30 ml. and 10 ml. respectively (Fig. VII,2). The dry

macromolecular weight increased while the NANA and fucose content (μmol/ml) changed very little, this being due to a decrease in NANA and fucose as a percentage of the dry weight, particularly for NANA which was reduced from 6% to 3% (Fig. VII,2).

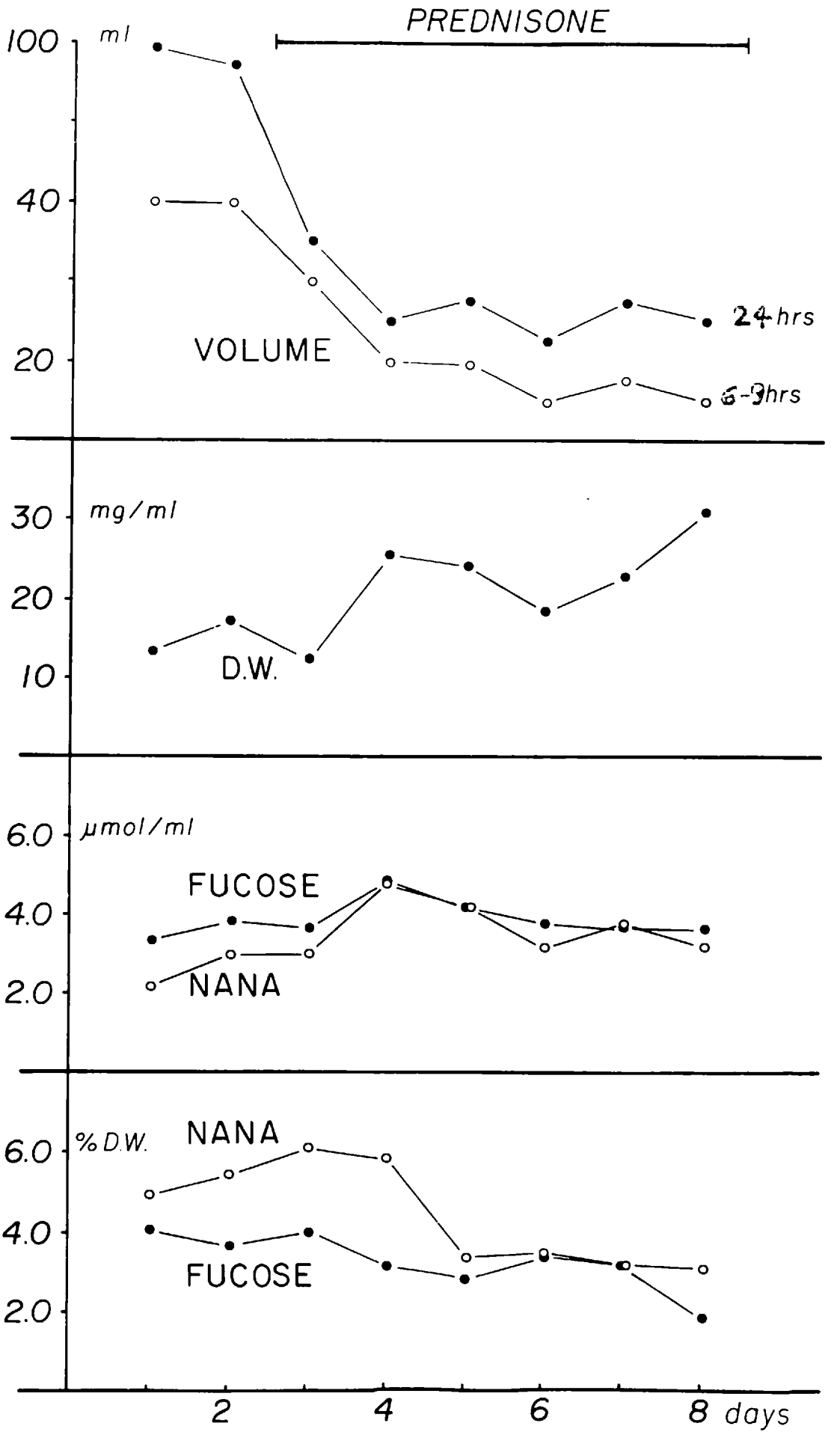
Comment:-

In this patient the yield of macromolecular material, NANA and fucose concentrations of the sputum fell within the lower range found for intrinsic asthma not associated with bronchorrhoea, while the NANA/Fucose ratio fell within the upper range suggesting that both bronchial secretion and tissue fluid transudate, particularly the latter, were contributing to the main bulk of the sputum.

The reduction in sputum volume accompanied by a marked increase in dry weight and a decrease in NANA and fucose as a percentage of dry weight, suggest that in this patient prednisone reduced both serum transudate and bronchial secretion. The decrease in NANA as a percentage of dry weight was not due to the presence of pus (Keal 1970), since the macroscopic type of the sputum remained mucoid throughout the study.

Fig. VII,2 The effect of steroids on sputum volume, dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum (concentration as a percentage of dry weight) in a patient with intrinsic asthma associated with bronchorrhoea.

Fig. VII,2


PREDNISONE
100
ml
40
20
VOLUME
24 hrs
6-9hrs
30
mg/ml
20
10
D.W.
6.0
µmol/ml
FUCOSE
4.0
NANA
2.0
6.0
%D.W.
NANA
4.0
FUCOSE
2.0
2
4
6
8
days

Patient No. 3

A 47 year old patient with extrinsic asthma was admitted to the Brompton Hospital complaining of profuse mucoid sputum. The daily sputum volume before treatment varied between 175 ml. and 50 ml. and the NANA content of the sputum was low (0.5 μmol/ml).

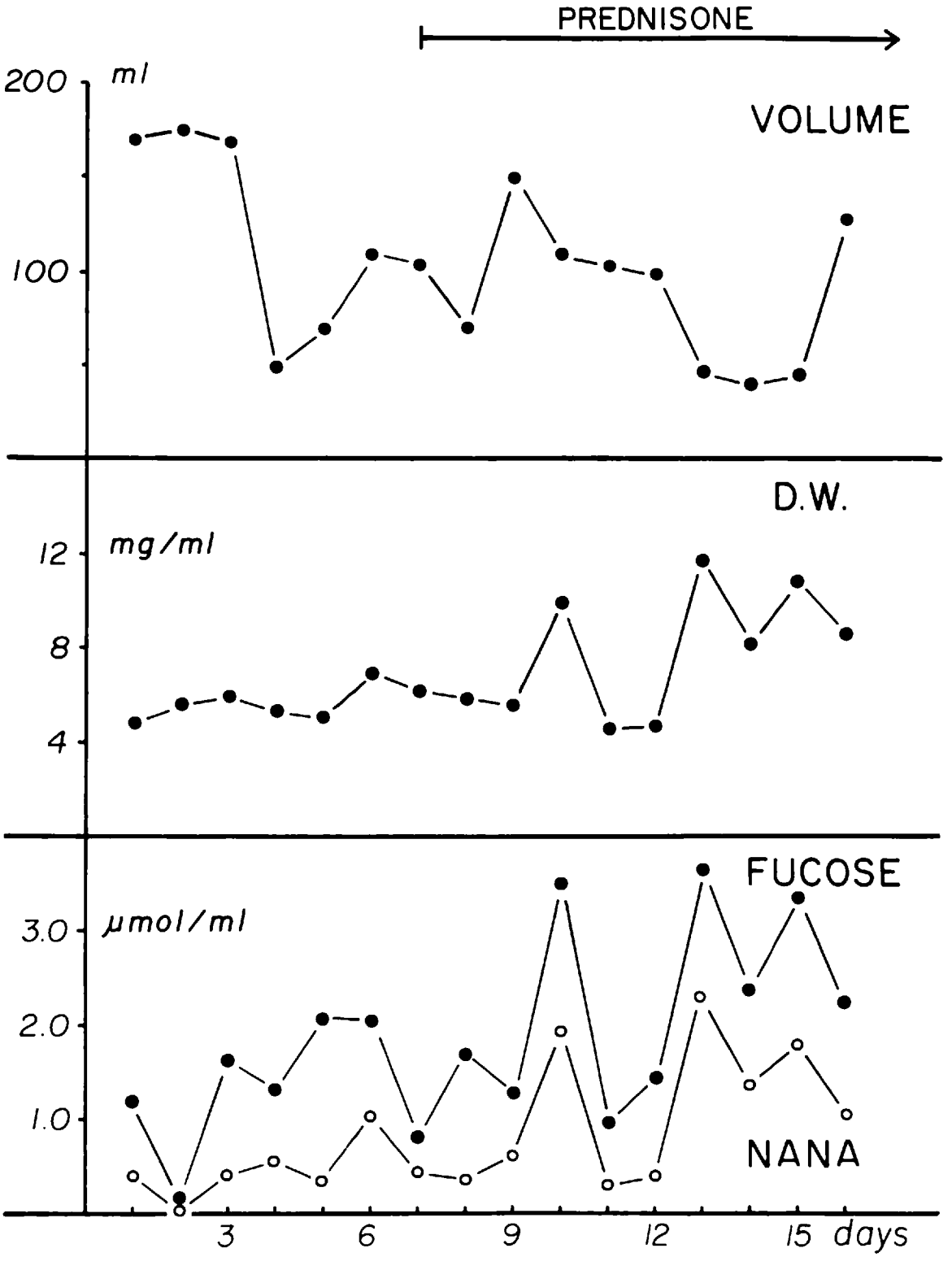
Prednisone 30 mg. daily were given for 10 days, the daily sputum volume changed very little except on the 7th, 8th and 9th treatment days when sputum volume fell below 50 ml. (47-49 ml.). Although a similar reduction in sputum volume was seen before prednisone treatment, dry weight, NANA and fucose levels showed a different pattern of variation. In spite of a marked decrease in sputum volume from 170 ml to 50 ml. before prednisone treatment, dry weight, NANA and fucose concentrations remained unchanged. In contrast the variation in sputum volume produced by prednisone, was accompanied by an increase in dry weight, NANA and fucose concentrations (Fig. VII,3).

Comment:-

In this patient the dry weight, NANA and fucose concentrations during prednisone treatment, particularly on the 7th, 8th and 9th days, suggest

Fig. VII,3 Effect of steroids on sputum volume, dry macromolecular weight (D.W.), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with extrinsic asthma associated with bronchorrhoea.

Fig. VII,3


PREDNISONE
200
ml
VOLUME
100
D.W.
12
mg/ml
8
4
FUCOSE
3.0
µmol/ml
2.0
1.0
NANA
3
6
9
12
15
days

that this patient had an irregular response to steroids probably by an effect on the water absorption and/or reabsorption mechanisms.

Patient No. 4:-

A 51 year old chronic bronchitic was admitted to St. Olave's Hospital with acute pericarditis. Viral studies failed to identify a cause. He developed bronchopenumonia with purulent sputum and he responded satisfactorily to ampicillin although he continued to expectorate large amounts of mucoid sputum.

The 24 hours sputum volume ranged from 220 and 80 ml. before prednisone treatment and the NANA content of the sputum was high (2.6 μmol/ml).

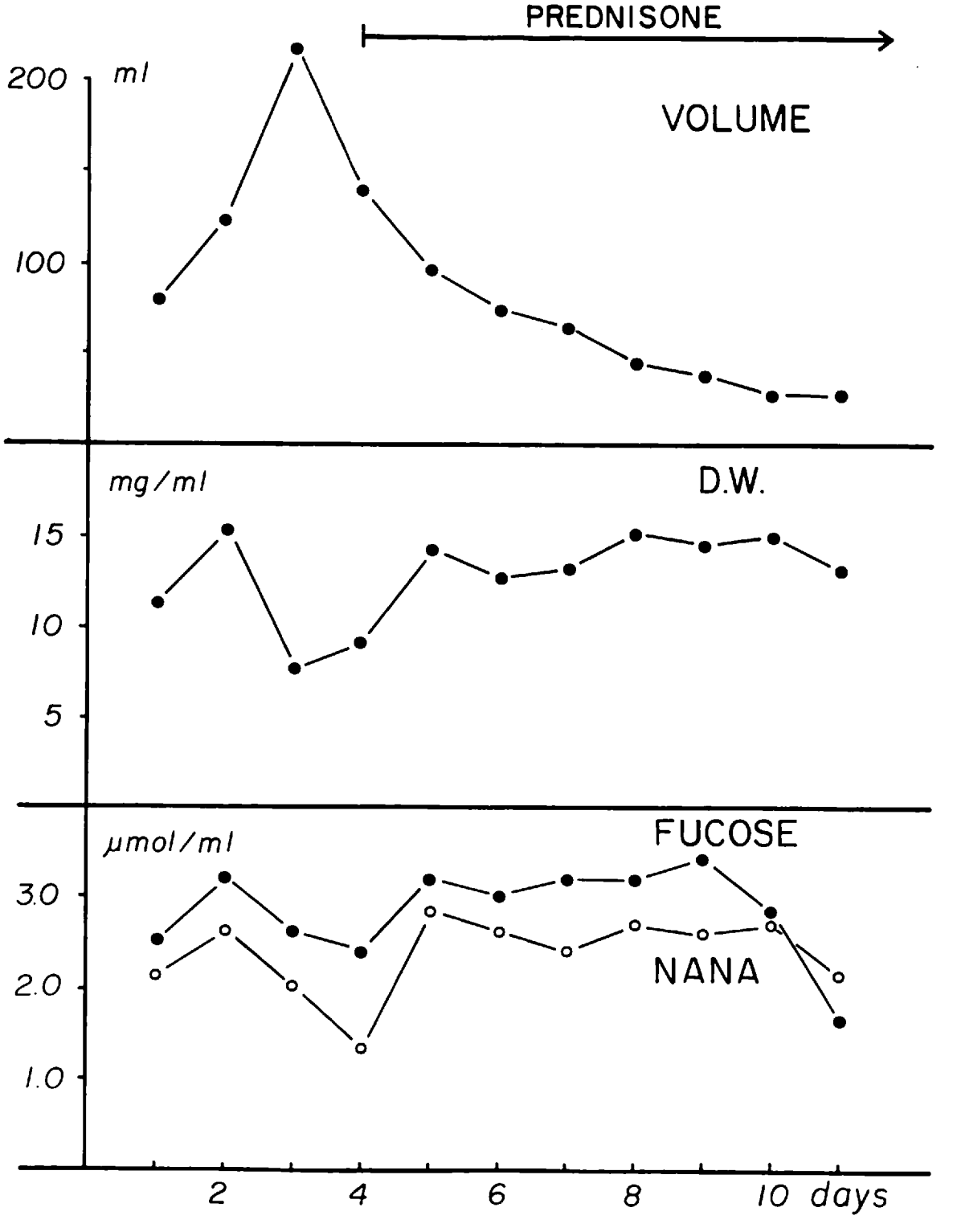
Prednisone 40 mg. daily were given for 8 days. The daily sputum volume gradually decreased to 25 ml. (Fig. VII,4). Macromolecular dry weight, NANA and fucose content of the sputum remained fairly constant during treatment.

Comment:-

In this patient levels of dry weight, NANA and fucose fell within the lower end of mucoid chronic bronchitis sputum suggesting that in this

Fig. VII,4 Effect of steroids on sputum volume, dry macromolecular weight (D.W.), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with chronic bronchitis associated with bronchorrhoea.

Fig. VII,4


PREDNISONE
200
ml
VOLUME
100
mg/ml
D.W.
15
10
5
µmol/ml
FUCOSE
3.0
2.0
NANA
1.0
2
4
6
8
10 days

case bronchial gland secretion was contributing to the main bulk of the sputum.

The response to steroids, reduction in sputum volume with no change in yield of dry weight, NANA or fucose concentrations, seems to be due to an inhibitory effect of prednisone on bronchial gland secretion.

Patient No. 5

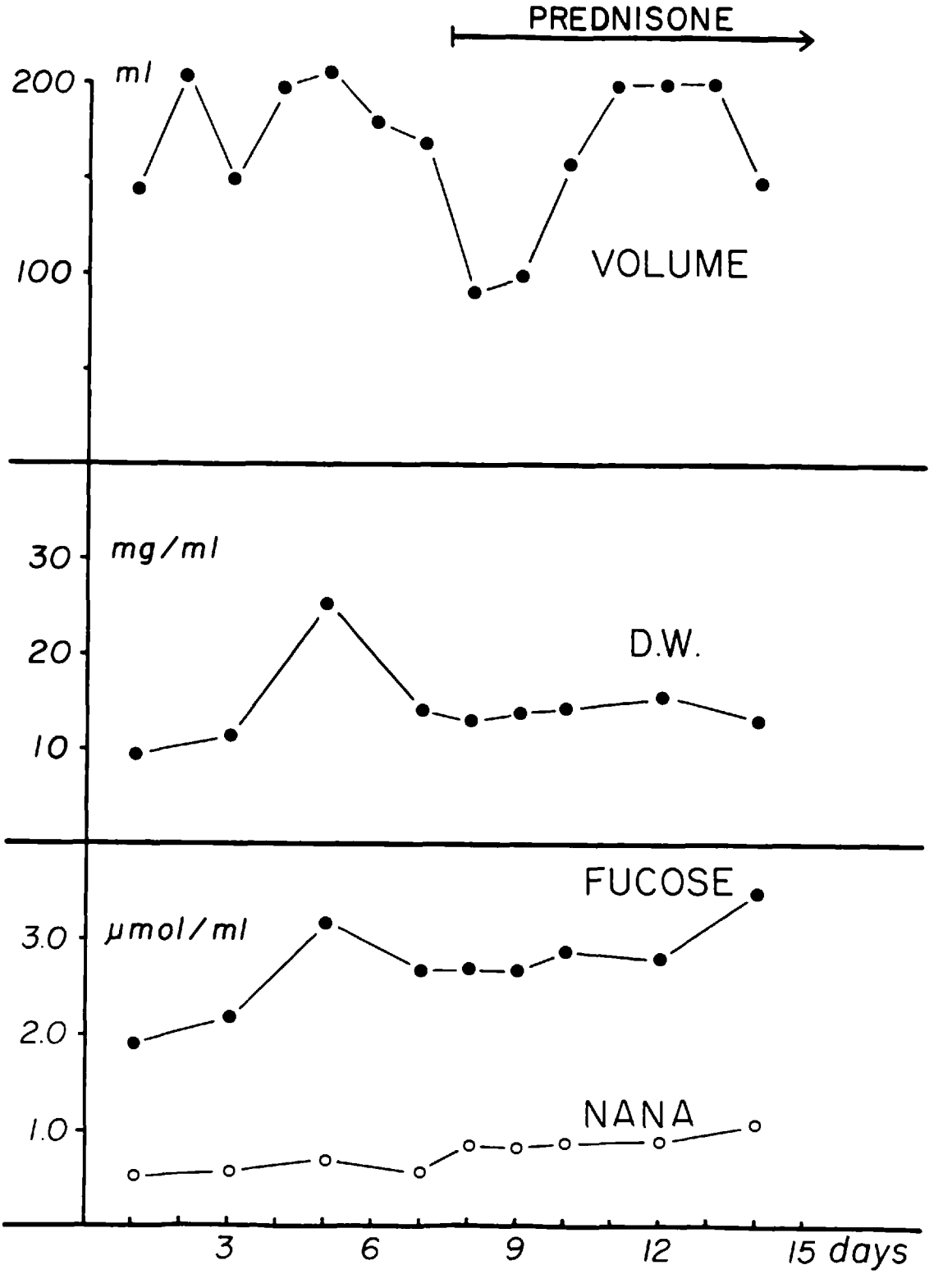
A 43 year old patient with scleroderma was admitted to the Brompton Hospital complaining of increasing breathlessness and productive cough. A diagnosis of fibrosing alveolitis was based on the histopathological findings in tissue from lung biopsy material.

The daily sputum volume ranged between 200 and 150 ml. Since the NANA content of the sputum was found to be low 0.6 μmol/ml it was decided to treat him with prednisone.

Prednisone 30 mg. daily were given for a week. The daily sputum volume decreased from 180 ml. to 90 ml. in the first two days of treatment and then increased reaching pretreatment levels (Fig. VII,5).

Fig. VII,5 Effect of steroids on sputum volume dry macromolecular weight (DW) N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with scleroderma and fibrosing alveolitis associated with bronchorrhoea.

Fig. VII,5


PREDNISONE
200
ml
100
VOLUME
30
mg/ml
20
D.W.
10
FUCOSE
3.0
µmol/ml
2.0
NANA
1.0
3
6
9
12
15 days

Macromolecular dry weight, NANA and fucose levels remained unchanged throughout the study (Fig VII,5).

Comment:-

This patient's sputum had some interesting chemical features: dry weight yield and fucose levels fell with the range found for mucoid chronic bronchitis sputum while NANA concentration and NANA/Fucose ratio were very low. It seems therefore very likely that this patient was producing large amounts of bronchial mucus with very little serum transudate component.

The reduction in sputum volume during the first two days of prednisone treatment was greater than before prednisone was commenced, and it was not accompanied by any change in dry weight or chemical constituents. These facts suggest that in this patient prednisone had a transient inhibitory effect on bronchial gland secretion.

Changes in response to atropine

This study was divided into two parts:

- changes in response to a single dose of intramuscular atropine

- changes in response to long term treatment with oral atropine.

Response to single dose of intramuscular atropine:-

The patients were admitted on the trial in a stable state and treatment other than atropine remained unchanged. On each day of the study the sputum was collected:

- daily volume was measured
- chemical studies were made in the three hours sputum collections from 06.00-09.00 hrs. and 09.00-12.00 hrs. The 3 hours sputum volume was also recorded. Chemical analysis included macromolecular dry weight, NANA and fucose.

Pulse rate was recorded at hourly intervals during control and atropine days, if the patient did not have a significant rise (more than 10%) in pulse rate or complained of dry mouth after his first dose of atropine, the dose was doubled next day. Atropine was given at 10.00 hrs.

Patient No. 1:-

A 53 year old male patient with intrinsic asthma, producing more than 100 ml. per day.

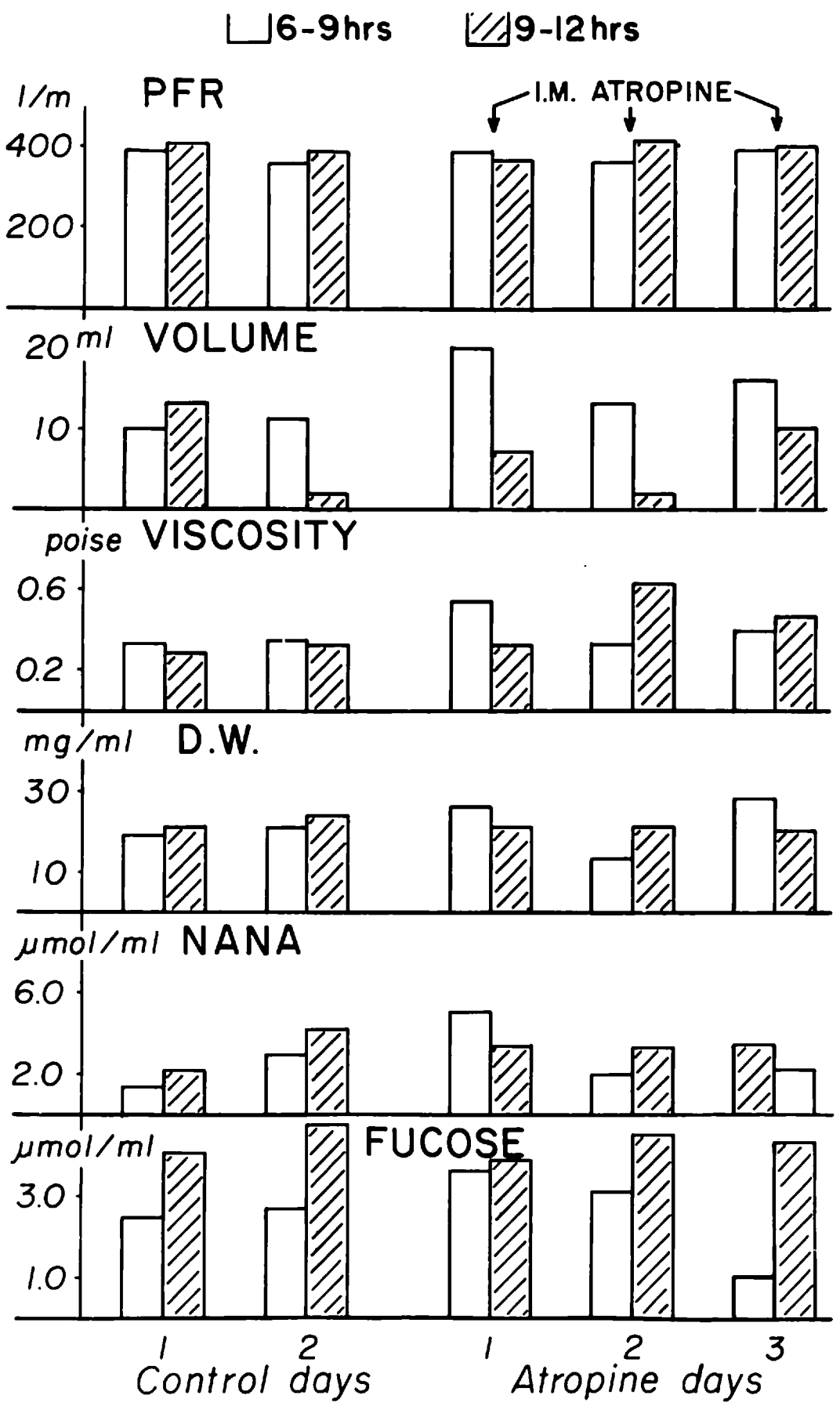
Atropine 0.6 mg. intramuscularly was administered on the third day of the study. The reduction in sputum volume was similar to that seen during the second control day. When atropine was increased to 1.2 mg. a similar reduction was observed on the fourth day while on the fifth day the sputum volume changed very little (Fig. VII,6). The 24 hours sputum volume did not change during atropine days.

The pattern of variation of fucose content of sputum in control and atropine days was similar, that is the 09.00-12.00 hrs. levels were higher than the 06.00-09.00 hrs (Fig. VII,6). The changes in NANA content and macromolecular dry weight during control and atropine days were inconsistant and they did not follow the variation seen in sputum volume.

Comment:

In this patient intramuscular atropine did not have any effect on bronchial secretion since the changes in sputum volume and fucose were similar to those observed during control days. It is probable that in this patient the excess sputum production was mainly due to an increase in serum transudate component, since the NANA/Fucose ratio

Fig. VII,6 Effect of atropine on peak expiratory flow rate, sputum volume, apparent viscosity (at 1350 sec^{-1}), dry macromolecular weight (DW), N-acetyl, neuraminic acid (NANA) and fucose levels of sputum in a patient with intrinsic asthma associated with bronchorrhoea.


6-9hrs
9-12hrs
l/m
PFR
I.M. ATROPINE
400
200
20 ml
VOLUME
10
poise
VISCOSITY
0.6
0.2
mg/ml
D.W.
30
10
μmol/ml
NANA
6.0
2.0
μmol/ml
FUCOSE
3.0
1.0
1
2
1
2
3
Control days
Atropine days

was higher (0.867) than in mucoid sputum not associated with bronchorrhoea.

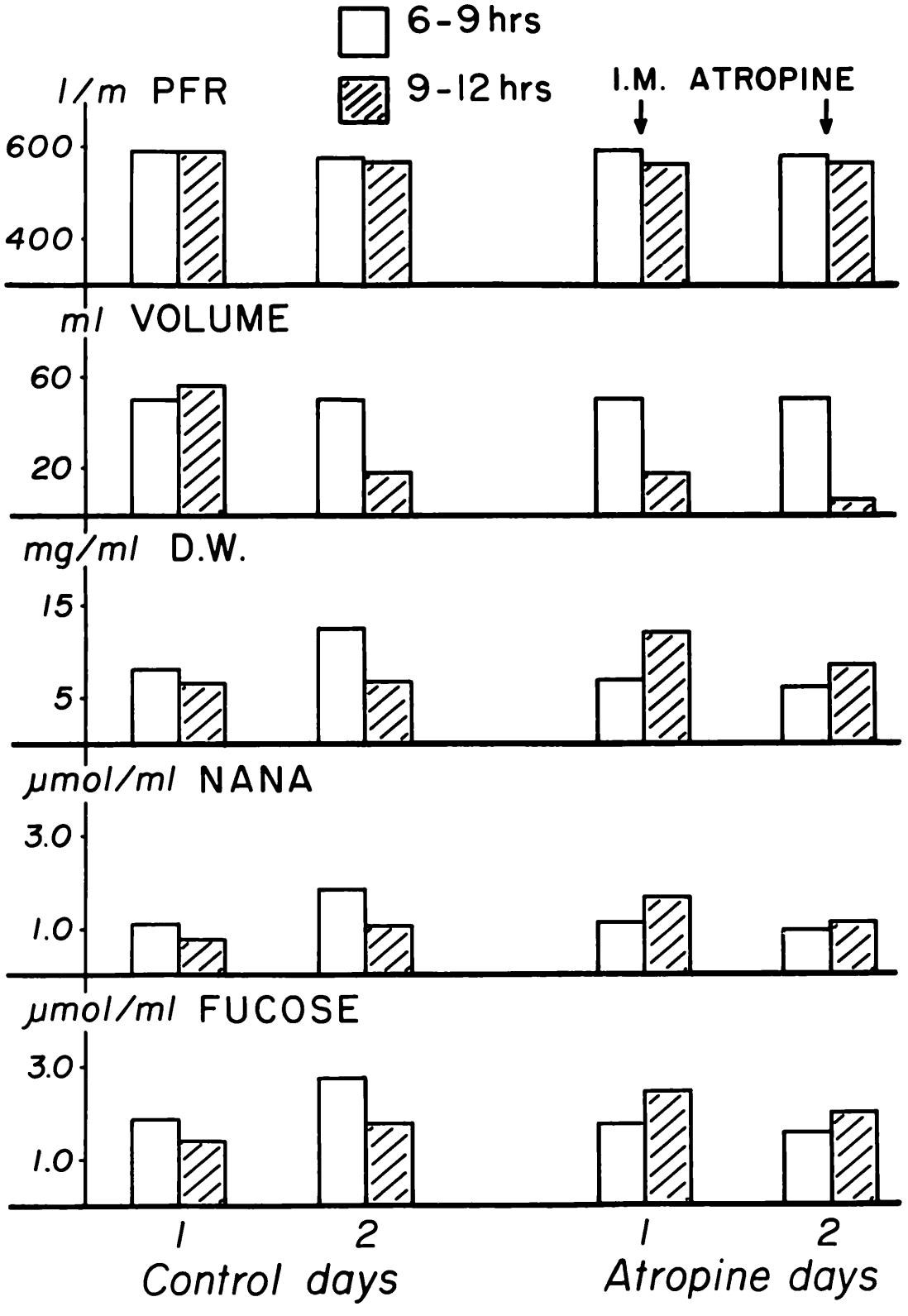
Patient No. 2:-

A 38 years old male patient with bronchiectasis confirmed on bronchography, he gave a history of increasing sputum production over the last 2-3 years. It appeared that most of the material he was expectorating was saliva and that this was probably related to his slightly obsessional nature in that the idea of purulent sputum in his chest was abhorrent to him.

Intramuscular atropine 0.6 mg. was given on the third, fourth and fifth days of the study. The 09.00-12.00 hrs. sputum volume was lower than the 06.00-0900 hrs. volume but a similar reduction was seen in the second control day (Fig. VII,7), and the 24 hrs. sputum volume remained unchanged throughout the study.

Macromolecular dry weight, NANA and fucose levels of the 09.00-12.00 hrs. sputa in control days showed a decrease compared with the 06.00-12.00 hrs. samples, while in atropine days they increased (Fig. VII,7).

Fig. VII,7 Effect of atropine on peak expiratory flow rate (PEFR), sputum volume, dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with bronchiectasis and hypersalivation.


6-9 hrs
9-12 hrs
I.M. ATROPINE
l/m PFR
600
400
ml VOLUME
60
20
mg/ml D.W.
15
5
μmol/ml NANA
3.0
1.0
μmol/ml FUCOSE
3.0
1.0
1
2
1
2
Control days
Atropine days

Comment:-

The reduction in sputum volume after intramuscular atropine was similar to that observed during control days; but whereas the fall in sputum volume, during control days, was accompanied by a decrease in dry weight, NANA and fucose concentrations, the reduction in sputum volume after atropine was accompanied by an increase in dry weight and NANA and fucose concentrations - that is the sputum produced was more concentrated. These changes in sputum volume, dry weight and chemical constituents suggest that atropine had an inhibitory effect on salivary secretion which in this case was contributing to the main bulk of the sputum.

Changes in response to long-term oral atropine:-

In this study atropine was given orally, the duration of the treatment varied from one week to five weeks.

The 24 hrs. sputum volume was measured and chemical analysis including macromolecular dry weight, NANA and fucose were made on the 06.00-09.00 hrs. sputum samples.

Patient No. 1:-

A 54 year old postman with chronic bronchitis was admitted with an acute respiratory illness with cough and producing large amounts of mucoid sputum. Virology studies confirmed mycoplasma pneumonia infection. The acute symptoms subsided except for the excess sputum production. The 24 hrs. sputum volume varied between 213 ml. and 60 ml.

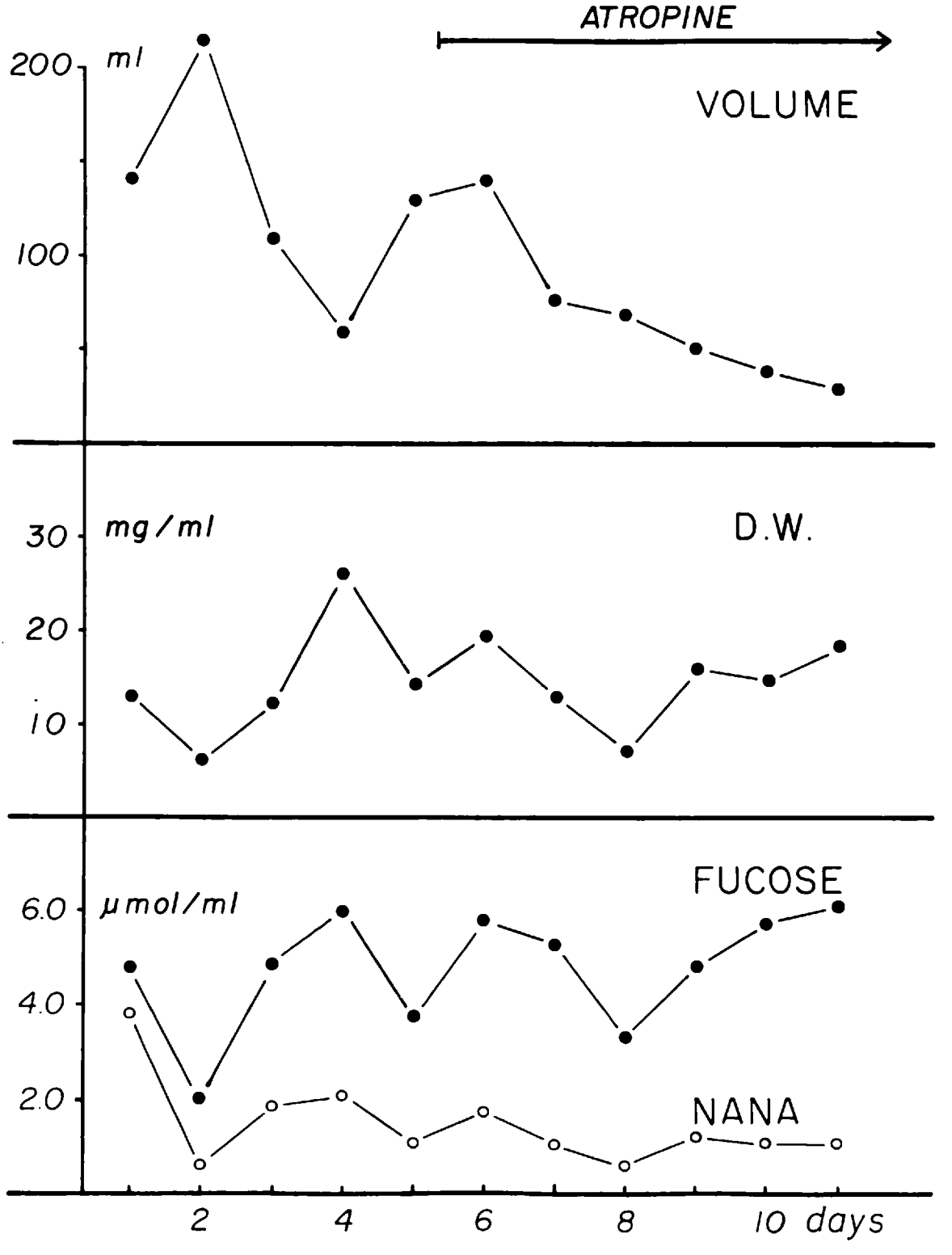
Atropine 0.6 mg. three times a day were given orally for a week. The daily sputum volume gradually decreased and on the seventh day of treatment the volume was within the patient's usual daily sputum volume (Fig. VII,8). The macromolecular dry weight, NANA and fucose levels varied very little during atropine treatment (Fig. VII,8).

Comment:-

Dry weight, NANA and fucose levels in the sputum produced before atropine treatment, fell within the range found for mucoid chronic bronchitis not associated with bronchorrhoea suggesting that in this patient increased volume of gland secretion was responsible for the excess sputum production. This is supported by the fact that NANA/Fucose ratio was very low (0.3-0.2).

Fig. VII,8 Effect of long-term treatment with atropine on sputum volume, dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with chronic bronchitis associated with bronchorrhoea.

Fig. VII,8


ATROPINE
200
ml
VOLUME
100
30
mg/ml
D.W.
20
10
6.0
μmol/ml
FUCOSE
4.0
2.0
NANA
2
4
6
8
10 days

In this patient the reduction in sputum volume did not affect the yield of macromolecular material or NANA and fucose concentrations this being in favour of an inhibitory effect on bronchial gland secretion.

Patient No. 2:-

A 44 year old male patient with intrinsic asthma with severe airways obstruction was admitted to the Brompton Hospital complaining of increasing sputum production over the last two years. He had received steroid treatment, prednisone up to 60 mg. daily and in some occasions ACTH 80 units twice a week to relieve his airways obstruction. Although the sputum volume was not recorded at that time, the patient did not notice any significant change on sputum production.

Oral atropine was commenced at a dose of 0.3 mg. twice a day and it was gradually increased up to 0.6mg. four times a day over a period of five weeks. Prednisone was reduced from 60 mg. daily to 10 mg. daily over the same period.

During the first three weeks the sputum volume showed little variation but when atropine was increased to 0.6 mg. three times a day, the

sputum volume gradually decreased and this effect was even more marked when atropine was increased to 0.6 mg. four times a day (Fig. VII,9).

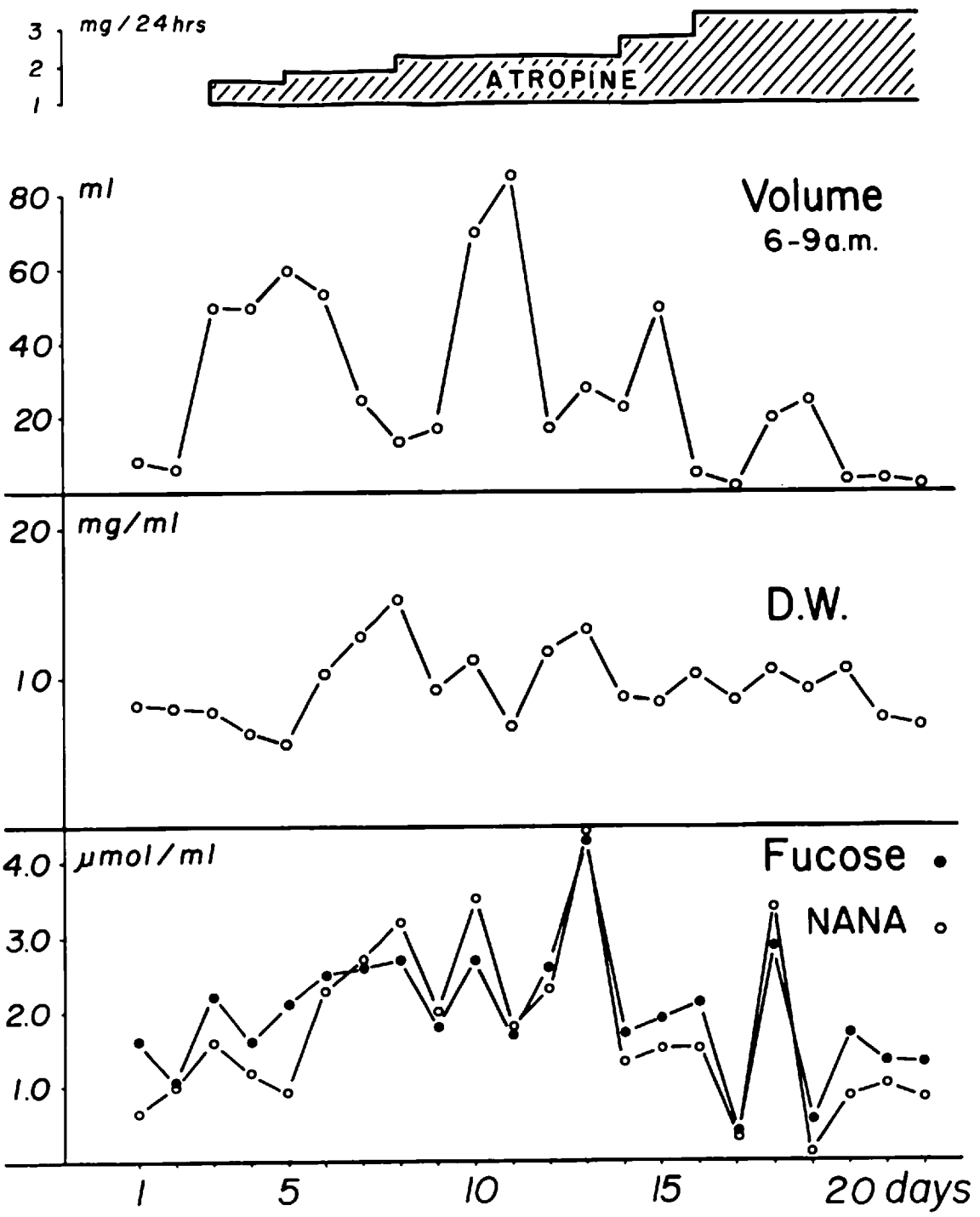
The NANA and fucose content of the sputum, as well as the macromolecular material, showed a great day to day variation but no significant change was seen (Fig. VII,9).

Comment:-

The features of the response to long term atropine in this patient are different from the pattern of response to prednisone described by Keal (1971) in patients with bronchorrhoea associated with asthma, in whom a fall in sputum volume was accompanied by an increase in concentrations of macromolecular weight and NANA. Since the patient here described had intrinsic asthma any gland hypertrophy might be expected to be minimal and therefore more susceptible to inhibition by atropine. The decrease in sputum volume without any change in chemical constituents suggests an inhibitory effect of atropine on bronchial gland secretion.

Fig. VII,9 Effect of long-term treatment with atropine on sputum volume, dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with intrinsic asthma associated with bronchorrhoea.

Fig. VII,9


3
2
1
mg / 24hrs
ATROPINE
80
60
40
20
ml
Volume
6-9a.m.
20
10
mg/ml
D.W.
4.0
3.0
2.0
1.0
µmol/ml
Fucose •
NANA ○
1
5
10
15
20 days

EFFECT OF DRUGS ON SPUTUM PRODUCTION IN A PATIENT WITH ALVEOLAR CELL CARCINOMA

A clinical feature of alveolar cell carcinoma is the production of large amounts of sputum. Storey, et al (1953) reviewing a series of 302 patients reported that 10 patients produced sputum in excess of 240 ml. and two of these more than 3 litres.

Case report:-

A 57 year old male solicitor's clerk was admitted to the Brompton Hospital with a five weeks history of wheezing and cough productive of a small amount of transparent frothy sputum. The chest radiograph showed patchy consolidation of the left lower lobe. On seven occasions the cytological examination of the sputum failed to reveal neoplastic cells. He was discharged from hospital and a week later he was producing large quantities of watery sputum which he expectorated simply by bending forward. He was readmitted and at that time the daily sputum volume increased up to 700 ml. The chest radiograph showed increased shadowing in the left lower lobe and two weeks later some abnormal shadows were seen for the first time in the right lower lobe. Alveolar carcinoma was diagnosed from positive cytological findings in four of six sputum specimens.

For three weeks all the sputum was collected and volumetric, rheological and chemical analyses were carried out. The daily sputum volume varied from 630 ml. to 1 litre with a mean value of 843 ml.

Mean levels for viscosity, dry weight, NANA, fucose, sulphate and mannose concentrations were found to be below the range of other bronchorrhoea sputum and significantly lower than mucoid chronic bronchitis sputum but higher than in saliva. The NANA/Fucose ratio was within bronchorrhoea range and significantly higher than in mucoid chronic bronchitis or saliva. (Table VII,2).

Sputum samples were sent to Prof. Pattle for surfactant studies. The stability tests and treatment with antifoam showed that the froth was of a gelatinous nature consisting of typical alveolar bubbles lined with lung surfactant.

Response to steroids:- Prednisone (40 mgs. daily) was given for six weeks, there was no significant change in either sputum volume or chemical constituents (Fig. VII,10 and VII,11).

Response to atropine:- Subcutaneous atropine, to

tolerance levels (0.6 mg) was given at 10.00 hrs. and 16.00 hrs. The sputum volume was measured from 09.00-12.00 hrs. and from 16.00-20.00 hrs. during preatropine, atropine and postatropine days.

Subcutaneous atropine did not have any effect on the sputum volume (Fig. VII,10).

Fluid restriction:- Fluid intake was restricted for four days to a daily volume of 1-1.5 litres. The 24 hours intake, urine output and sputum volume were measured. Sputum, plasma and urine osmolality measurements were carried out before and during fluid restriction. During fluid restriction neither sputum nor plasma osmolality changed but that of urine increased (Table VII,3). The urine output decreased from 2 litres to 730 ml., while the sputum volume remained unchanged (Fig. VII,11).

Comment:-

The rheological and chemical studies indicate that this patient's bronchial fluid was partly bronchial mucus secretion, partly serum transudate and that some of the fluid was of alveolar origin.

The failure to reduce the sputum volume with agents that might produce some reduction either

TABLE VII,2

Rheological and chemical constituents of saliva and sputum from chronic bronchitis, bronchorrhoea and alveolar cell carcinoma.

	SALIVA	CHRONIC BRONCHITIS	BRONCHORRHOEA	ALVEOLAR CELL CARCINOMA
Viscosity ($135 Osec^{-1}$) (poise)	0.23	0.34	0.24	0.10
Dry weight (mg/ml)	2.50	15.90	9.39	5.78
NANA (umol/ml)	0.06	2.50	1.27	0.42
Fucose (umol/ml)	0.24	5.30	2.15	0.73
Sulphate (umol/ml)	0.10	1,80	0.84	0.30
Mannose (umol/ml)	0.16	0.90	1.24	0.11
NANA/Fucose ratio	0.26	0.40	0.66	0.77

TABLE VII,3

Sputum, plasma and urine osmolalities before and during fluid restriction. (mosmol/l).

Material	Before fluid restriction	During fluid restriction
SPUTUM	288	304
PLASMA	300	292
URINE	477	993

Fig. VII,10 The effect of prednisone and atropine on the sputum volume, dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a patient with alveolar cell carcinoma.

Fig. VII,10

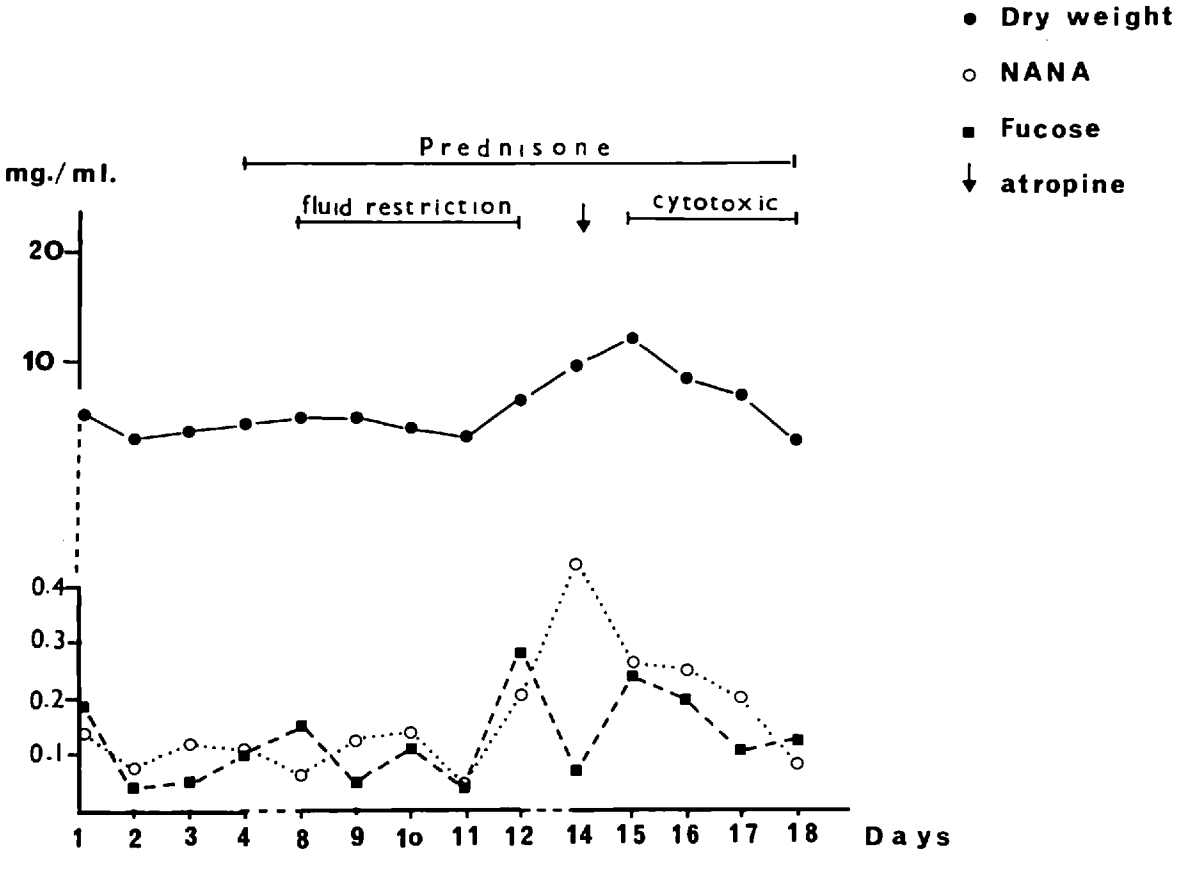
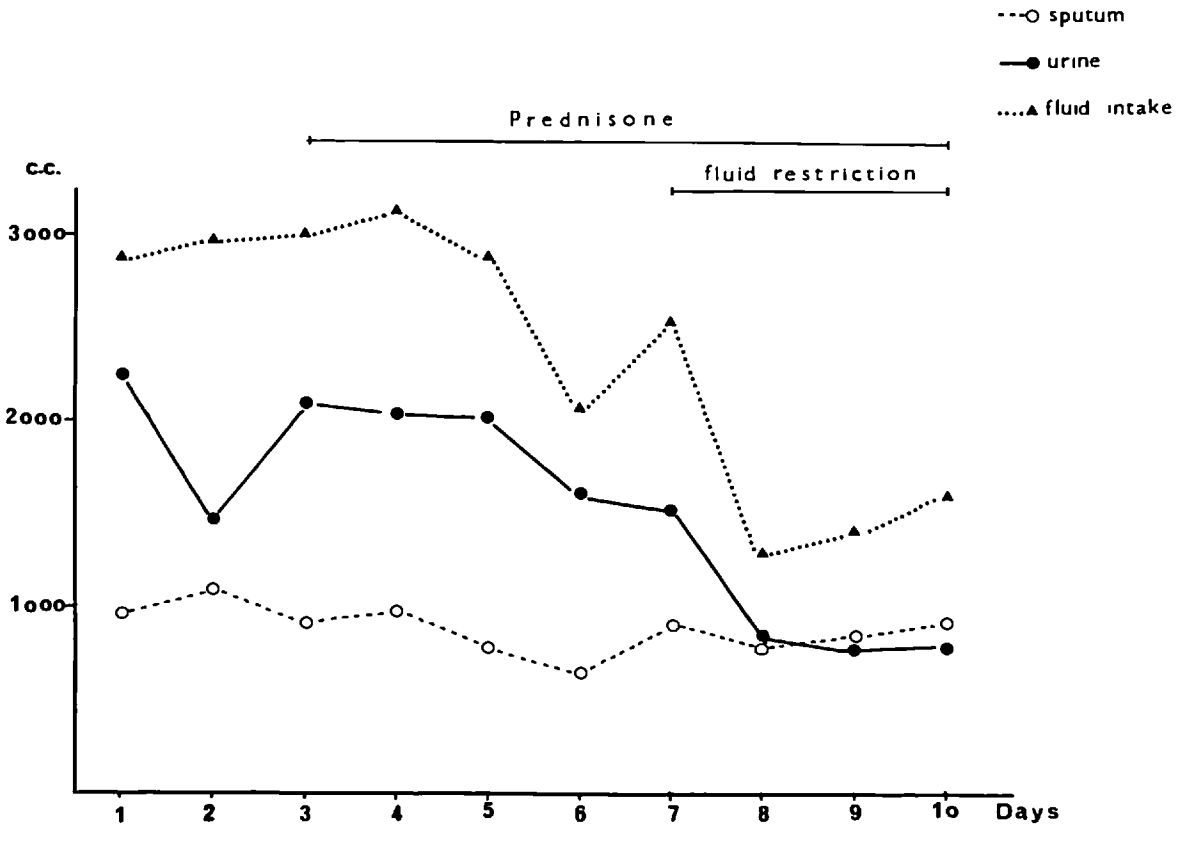

Dry weight
NANA
Fucose
atropine
Prednisone
mg./ml.
fluid restriction
cytotoxic
20
10
0.4
0.3
0.2
0.1
1
2
3
4
8
9
10
11
12
14
15
16
17
18
Days

Fig. VII,11 The effect of prednisone and fluid restriction on sputum and urine volumes in a patient with alveolar cell carcinoma.

Fig. VII,11


sputum
urine
fluid intake
Prednisone
fluid restriction
c.c.
3000
2000
1000
1
2
3
4
5
6
7
8
9
10
Days

on bronchial gland secretion or tissue fluid transudate or both, suggests that the cells of alveolar cell carcinoma have an autonomous secretion.

EFFECT OF DRUGS ON SPUTUM PRODUCTION IN PATIENTS WITH CHRONIC BRONCHITIS, ASTHMA, BRONCHIECTASIS OR CYSTIC FIBROSIS

Changes in response to atropine

The study was designed to determine whether atropine administration caused any change in sputum production in terms of volume produced, viscosity and chemical constituents.

The patients were selected because they produced sputum rather than because of the underlying disease. All the patients were in a stable phase of their disease and their usual treatment remained unchanged, throughout the period of study.

Clinical date of the six patients studied and the details of their atropine administration are summarized in Table VII,4.

Atropine methonitrate was used for inhalation and atropine sulphate for intramuscular injection. With the exception of patient No. 2 (who opted out of further study) each patient had two consecutive

TABLE VII,4

Clinical data. Macroscopic type of sputum. Route of atropine administration and dose.

Patient	Sex	Age	Diagnosis	Macroscopic type	Route	Dose	
						Day 1	Day 2
No. 1	M	48	Extrinsic asthma	M	Inhalation	1.2mg.	2.4mg
No. 2	M	80	Chronic bronchitis	MP	I.M.	0.6mg	-
No. 3	M	53	Extrinsic asthma	M	Inhalation	2.0mg	2.0mg
No. 4	M	18	Bronchiectasis	P	I.M.	1.2mg	1.2mg
No, 5	M	46	Chronic bronchitis	MP	Inhalation	1.2mg	1.2mg
No. 6	M	42	Intrinsic asthma	M	I.M.	0.6mg	0.6mg

treatment days. If there were no significant atropine effects - rise in pulse rate or dry mouth - the dose was doubled on the second or third day (Table VII,4). Atropine was administered at 10.00 hrs.

Sputum collections were made at three hours intervals from 06.00-21.00 hrs. on control and atropine days. The volume of the three hours specimens and the overnight sputum volume were measured. Viscosity measurements and chemical analysis, including macromolecular dry weight, NANA and fucose were made on the first two specimens of the day (06.00-09.00 hrs. and 09.00-12.00 hrs.)

Volume:- The results of the sputum volume measurements are given in Table VII,5.

Two patients (Nos. 2 and 6) produced no sputum after atropine. Both these patients had produced only small amounts of sputum on the preatropine days and both complained of very marked dry mouth and thirst after their intramuscular injection.

In patient No. 5 the 09.00-12.00 hrs. sputum volume was significantly reduced (-67%) compared

TABLE VII,5

Effect of atropine on sputum volume.

	CONTROL DAY			ATROPINE DAY		
Patient	06.00-09.00hrs.	09.00-12.00hrs.	Daily	06.00-09.00hrs.	09.00-12.00hrs.	Daily
No. 1	7	5	84	11	14	104
No. 2	2	2	12	1	0	10
No. 3	9	2.5	36	8	3	30
No. 4	10	7	38	30	22	65
No. 5	2	2	12	3	1	10
No. 6	1	0.5	4	1	0	3
Mean	5.17	3.17	25.50	9	10	37
S.E.	1.62	0.97	12.8	4.51	4.92	16.26

with the variation seen during control days (Table VII,5).

The changes in sputum volume in patients 1, 3 and 4 was similar both during control and atropine days (Table VII,5).

The mean sputum volume for the group shows that there was no statistically significant difference either in the immediate post-treatment volume or in the total daily volume (Table VII,8).

Viscosity:- Absolute levels of apparent viscosity on control and treatment days are given in Table VII,6. The figures represent the maximum variation during the two control and two atropine days.

It is interesting to note that in all the patients with asthma or chronic bronchitis the 09.00-12.00 hrs. sputum viscosity was lower than that of the early morning specimen. This confirms the diurnal variation already described in Chapter VI.

The only one patient in whom the 09.00-12.00 hrs. sputum viscosity was higher than that of the early morning sample had bronchiectasis and this type of variation has also been described in Chapter VI.

TABLE VII,6

Effect of atropine on apparent viscosity of sputum at 1350 sec^{-1} (poise).

Patient	Control day		Atropine day	
	06-09hrs	09-12hrs	06-09hrs	09-12hrs
1	0.38	0.11	0.29	0.07
2	0.97	0.58	0.83	-
3	0.61	0.12	0.39	0.73
4	0.69	2.29	1.14	2.47
5	3.11	1.54	2.35	1.43
6	0.10	0.11	0.26	-
Mean value	0.98	0.79	0.88	1.18
S.e.	0.44	0.38	0.33	0.51

In only one patient (No. 3) the sputum viscosity was higher after atropine (+84%) while on the control day the 09.00-12.00 hrs. sputum viscosity was lower (-80%) than that of the 06.00-09.00 hrs. sputum samples.

There is a wide variation within a single patient and between patients but the mean values show that atropine had no significant effect on sputum viscosity (Table VII,8).

Chemical constituents:- Absolute values of macromolecular dry weight, NANA and fucose of control and atropine sputum samples are given in Table VII,7.

In patients with mucoid sputum (Nos. 1,3 and 6) absolute values of macromolecular dry weight, NANA and fucose were found to be lower on the 09.00-12.00 hrs. sputum than on the early morning samples both on control and atropine days, while in patients with mucopurulent or purulent sputum the dry weight of the 09.00-12.00 hrs. sputum samples was higher than that of the early morning samples. Fucose levels varied little or decreased during both control and atropine days; NANA increased in two patients (Nos. 2 and 4) and decreased in one (No. 5).

TABLE VII,7

Effect of atropine on dry weight and chemical constituents of sputum.

Patient	Dry weight (mg/ml)				NANA(umol/ml)				Fucose(umol/ml)			
	Control day		Atropine day		Control day		Atropine day		Control day		Atropine day	
	06-09 hrs	09-12 hrs	06-09 hrs	09-12 hrs	06-09 hrs	09-12 hrs	06-09 hrs	09-12 hrs	06-09 hrs	09-12 hrs	06-09 hrs	09-12 hrs
1	8.5	6.4	13.8	8.1	1.3	0.6	2.2	1.1	3.0	1.8	5.8	3.2
2	45.7	51.3	61.8	-	4.2	5.6	6.1	-	4.1	4.3	5.6	-
3	13.6	7.5	14.5	10.8	2.7	1.2	3.6	1.8	2.8	1.4	3.0	1.8
4	58.5	87.4	48.8	80.7	2.2	5.5	2.4	3.1	7.9	5.3	5.9	5.4
5	33.8	37.1	42.5	48.1	12.3	9.1	7.7	5.2	9.1	9.1	8.5	9.1
6	7.3	5.7	8.4	-	1.0	0.7	0.8	-	2.0	1.9	2.1	-
Mean value	27.9	32.6	31.6	36.9	3.9	3.8	3.8	2.8	4.8	3.9	5.1	4.9
S.e.	8.7	13.4	9.1	17.2	1.7	1.4	1.1	0.9	1.2	1.2	0.9	1.6

TABLE VII,8

Comparison between 06-09 hrs1 and 09-12 hrs sputum samples on control and treatment days. Comparison between control and treatment days. (Student's t test)

	Volume	Viscosity	Dry weight	NANA	Fucose
Control days 06-09 hrs/09-12 hrs	1.4500	0.3298	-0.2902	0.0759	0.4999
Atropine days 06-09hrs/09-12 hrs	-1.5000	-0.4988	-0.2498	0.7185	0.1465
Control/Atropine 06-09 hrs	-0.7996	0.1833	-0.2961	0.0738	-0.2165
09-12 hrs	-1.3369	-0.6196	-0.2011	0.5946	-0.4571

No statistically significant change in dry weight, NANA or fucose was seen on treatment days (Table VII,8).

Comment:

Atropine administration appears to have little effect on the quantity and quality of the sputum produced by these patients. The two patients who produced no sputum after atropine administration both had severe dry mouth and pharynx and complained of difficulty on coughing. As they both went on to produce a volume of sputum very close to their control day level it is probable that their difficulty in expectoration was due to the drying action of atropine on the salivary secretion and not any change in the physical or chemical characteristics of the sputum.

Changes in response to a mucolytic agent: Sodium 2 mercapto ethane sulphonate:-

The mucolytic agent selected in these studies was sodium 2 mercapto ethane sulphonate (Mistabron).

Formula: $HS - CH_2 - CH_2 - SO_3^- - Na+$

The sulphydryl group breaks the disulphide bonds present in the mucus and the sulphonate radical, due to its high hydrophilic function, solubilises the

fragments released.

Effect of sodium 2 mercapto ethane sulphonate on chronic bronchitic sputum:-

This trial was designed to study the short term effect of sodium 2 mercapto ethane sulphonate on sputum volume, rheological properties and chemical constituents of sputum. Mucociliary clearance studies were carried out at the London School of Hygiene by Drs. Thomson and Pavia.

A 20% solution of Mistabron was given by inhalation, the particle diameter being 5 μm. A placebo supplied by the pharmaceutical firm (containing 1% Mistabron) was administered in the same way as the drug. Drug and placebo were assigned randomly.

Number of patients, sex, age and tobacco consumption are given in Table VII,9. All patients fulfilled criteria of chronic bronchitis (MRC 1965).

TABLE VII,9

Number of patients, sex, age and tobacco consumption (x 10^3 pack-years).

Patient	Sex	Age	Tobacco consumption
No. 1	F	58	1.1
No. 2	M	59	4.7
No. 3	M	53	10.5
No. 4	M	46	6.3

Sputum samples were collected at the same time of the day during control, placebo and drug periods, the volume was measured. Viscosity measurements were carried out within 1-2 hours of production and the remaining of the sputum was stored at -20°C for chemical analysis, these included macromolecular dry weight, NANA and fucose.

Patient No. 1:-

The macroscopic type of the sputum changed from mucoid during control and drug periods to muco-purulent during placebo period.

Sputum volume increased both during placebo and drug periods compared with control value. Dry weight, NANA and fucose concentrations also

increased on placebo and drug days. The increase in dry weight and NANA was greater with placebo while the increase in fucose was similar on placebo and drug days. The sputum viscosity after drug treatment was higher than during placebo or control days (Table VII,10).

TABLE VII,10

Sputum volume, apparent viscosity and chemical constituents.

Patient No. 1.

	CONTROL	MISTABRON	PLACEBO
Volume (ml)	9	18	15
Viscosity (poise) at 1350 sec^{-1}	0.47	0.99	0.46
Dry weight (mg/ml)	11.91	18.6	29.33
NANA (µmol/ml)	1.48	2.6	3.1
Fucose (µmol/ml)	3.95	8.8	8.8
NANA/Fucose ratio	0.37	0.29	0.35

Comment:-

Since the macroscopic type of the sputum changed from mucoid to mucopurulent it is difficult to compare the effect of placebo and Mistabron. When control and Mistabron data are compared it seems that Mistabron increased the proportion of bronchial mucus secretion expectorated since the

macromolecular dry weight, NANA and fucose concentrations were higher and the viscosity was also higher than in control sputum.

Patient No. 2:-

The macroscopic type of the sputum on control and placebo days was mucopurulent and it became mucoid during drug period.

Sputum volume increased after placebo and drug treatment compared with control day.

After placebo the dry weight of the sputum was higher than on control day and the apparent viscosity was also higher; NANA and fucose concentrations were within the levels found in control sputum. After Mistabron dry weight yield and NANA concentrations were found to be lower than control sputum while fucose was higher and viscosity varied little. (Table VII,11).

TABLE VII,11

Patient No. 2:-- Sputum volume, apparent viscosity and chemical constituents

	CONTROL	PLACEBO	MISTABRON
Volume (ml)	13	19	20
Viscosity (poise) at 1350 sec-1	0.55	0.68	0.58
Dry weight (mg/ml)	17.31	22.32	13.48
NANA (µmol/ml)	3.75	3.88	2.78
Fucose (µmol/ml)	2.86	2.85	3.36
NANA/Fucose ratio	1.31	1.36	0.83

Comment:-

The decrease in dry weight and NANA concentration after Mistabron could be due to the fact that the sputum became mucoid and therefore the absence of pus and the reduction in serum transudate could be responsible for these changes.

Mistabron increased the sputum volume and fucose concentration suggesting that the amount of bronchial secretion was increased.

Patient No. 3:-

The macroscopic type of the sputum remained mucoid throughout the trial.

, The sputum volume and dry weight decreased during placebo and drug days, particularly the latter. Sputum viscosity, NANA and fucose concentrations showed a marked decrease after placebo while after Mistabron sputum viscosity and fucose varied little compared with control values and NANA concentration increased (Table VII,12).

TABLE VII,12

Patient No. 3:- Sputum volume, apparent viscosity and chemical constituents.

	CONTROL	PLACEBO	MISTABRON
Volume (ml)	18	15	10
Viscosity (poise) at 1350 sec -1	0.49	0.29	0.59
Dry weight (mg/ml)	13.14	4.33	9.41
NANA (μmol/ml)	1.73	0.44	3.00
Fucose (μmol/ml)	2.63	1.37	2.72
NANA/Fucose ratio	0.66	0.32	1.10

Comment:-

The decrease in viscosity and chemical constituents after placebo was probably due to a dilution effect.

The effect of Mistabron in this patient's

sputum seems to be an increase in serum transudate component since the dry weight was lower and the NANA concentration increased markedly, the NANA/Fucose ratio was much higher 1.103 after the drug than in control sputum (0.658).

Patient No. 4:-

The macroscopic type of the sputum remained mucopurulent throughout the trial.

The sputum volume decreased after placebo and Mistabron treatment while macromolecular dry weight, NANA and fucose, particularly fucose, increased. Sputum viscosity varied little after placebo and was found to be lower after Mistabron when compared with control values (Table VII,13).

TABLE VII,13

Patient No. 4:- Sputum volume, apparent viscosity and chemical constituents.

	CONTROL	MISTABRON	PLACEBO
Volume (ml)	5	2	1.5
Viscosity (poise) at 1350 sec -1	1.51	1.19	1.44
Dry weight (mg/ml)	35.40	45.10	32.60
NANA(μmol/ml)	4.23	6.17	5.42
Fucose (μmol/ml)	10.20	15.80	12.20
NANA/Fucose ratio	0.41	0.39	0.44

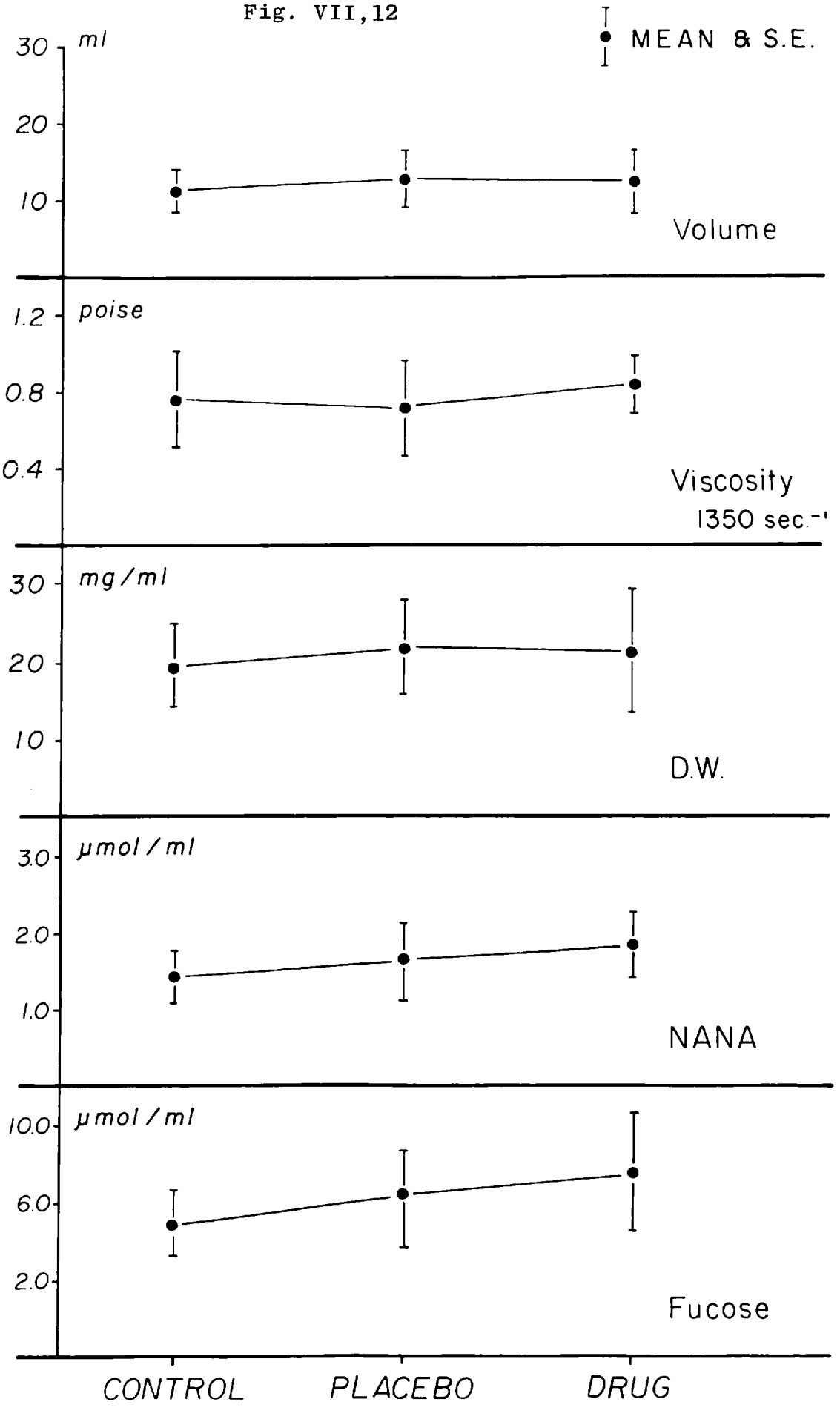
TABLE VII,14

Comparison between control, placebo and drug of sputum volume, apparent viscosity and chemical constituents. (Student's t test).

	Control vs Placebo	Control vs Mistabron	Placebo vs Mistabron
Volume	-0.29210	-0.25192	0.02317
Viscosity	0.32515	-0.27440	-0.41600
Dry weight	-0.32515	-0.21281	0.06252
NANA	-0.32778	-0.76505	-0.32014
Fucose	-0.45156	-0.78387	-0.34392

Fig. VII,12 Effect of sodium 2 mercapto ethane sulphonate on sputum volume, apparent viscosity (at 1350 sec^{-1}) dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a group of patients with chronic bronchitis.

Fig. VII,12


MEAN & S.E.
30
ml
20
10
Volume
1.2
poise
0.8
0.4
Viscosity
1350 sec.-1
30
mg/ml
20
10
D.W.
3.0
µmol/ml
2.0
1.0
NANA
10.0
µmol/ml
6.0
2.0
Fucose
CONTROL
PLACEBO
DRUG

Comment:-

In this patient both placebo and Mistabron seemed to have had the same effect and although the sputum produced after Mistabron was less viscous the percentage change (-20%) fell within the intra-specimen variation reported for chronic bronchitis sputum. (Charman 1973).

Conclusion:-

When mean values of control, placebo and drug periods were compared, no statistically significant difference of sputum volume, viscosity or chemical constituents were found between control and placebo, control and Mistabron or placebo and Mistabron. (Table VII,14; Fig. VII,12).

Although the number of patients was small it seemed that sodium 2 mercepto ethane sulphonate (Mistabron) did not have an immediate effect on sputum volume, viscosity or chemical constituents.

Effect of sodium 2 mercapto ethane sulphonate on cystic fibrosis sputum.

Nine patients with a diagnosis of cystic fibrosis confirmed by history, examination and a sweat sodium greater than 70 mEq/l were studied. Six were female and three were male and their ages ranged from 8 to 16.

The severity of the disease was assessed before entering the trial in terms of forced vital capacity as a percent of the predicted normal for height: four had a vital capacity greater than 80%, four between 60 and 80% and one less than 60%. All patients had been on routine therapy including physiotherapy, dietary control, pancreatic enzymes, vitamins and oral antibiotics as necessary. These treatments were continued during the trial. Two patients were on carbenicillin inhalation therapy and continued this throughout the trial.

The trial was designed as a double-blind cross-over, a one month baseline period was followed by two-two months treatment periods (Placebo or drug). Mistabron 20% solution was compared with a 1% solution of Mistabron in normal saline as placebo.

Three millilitres of the solution were inhaled twice daily following physiotherapy and were administered through a short, wide bore tube to the mouth, having been nebulized by a Wright nebulizer (particle size 0.5-5μm).

Viscosity measurements and chemical analysis were carried out in mid morning sputum at monthly intervals. Only those samples received within 3 hours of production were included in the study. Most of the samples being purulent it was very likely that enzymatic degradation had taken place.

Results:

In this series of patients levels of viscosity, dry weight, NANA and fucose fell within the range found for cystic fibrosis sputum.

Number of samples analyses and macroscopic type are given in Table VII,15.

TABLE VII,15

Macroscopic type of the sputum samples studied

Patient	CONTROL	PLACEBO	DRUG
No. 1	P	P	P
No. 2	M	MP	MP
No. 3	MP	M	MP
No. 4	MP	MP	MP
No. 5	MP	MP	MP
No. 6	MP	MP	MP
No. 7	MP	P	P
No. 8	MP	MP	P
No. 9	MP	MP	P

In four patients, Nos. 1,4,5 and 6 the macroscopic type remained unchanged throughout the trial. In patient No. 2 the sputum was mucoid during control and then became mucopurulent. In two patients, Nos. 8 and 9 the sputum was mucopurulent during control and placebo periods and became purulent during drug period. In patient No. 7 the sputum was mucopurulent during control and became purulent during control and drug periods.

Individual values and mean values for the group and standard error of the mean for control

placebo and drug periods are given in Table VII,16.

Patient No. 1:-

No significant change was seen either during placebo or drug periods for apparent viscosity and chemical constituents.

Patient No. 2:-

Viscosity decreased during placebo and drug periods, particularly in the latter. A similar pattern of variation was seen for NANA and fucose while the dry weight increased during placebo.

Patient No. 3:-

Sputum viscosity showed little change during placebo and drug periods although it tended to be lower than in control sputum.

Macromolecular dry weight, NANA and fucose concentrations were higher during placebo treatment and fell within control values with Mistabron.

Patient No. 4:-

Apparent viscosity of both placebo and drug sputum were found to be higher than in control sputum. Dry weight and fucose concentration were higher in placebo sputum and NANA lower. Dry

TABLE VII,16

Absolute values, mean and standard error of the mean of sputum viscosity (at 1350 sec^{-1}) dry macromolecular weight, NANA and fucose content of sputum during control, placebo and treatment days.

Patient	Viscosity(poise)			Dry weight (mg/ml)			NANA (umol/ml)			Fucose(umol/ml)		
	Control	Placebo	Drug	Control	Placebo	Drug	Control	Placebo	Drug	Control	Placebo	Drug
No. 1	0.5	0.8	0.4	34.5	31.4	38.8	0.8	1.4	1.0	4.4	3.4	2.3
No. 2	0.4	0.3	0.2	15.5	19.5	15.7	3.4	2.2	0.7	3.9	3.6	2.8
No. 3	0.3	0.2	0.2	13.3	23.1	10.1	0.1	3.2	0.1	2.9	7.9	1.8
No. 4	0.1	0.1	0.2	16.8	28.1	9.3	2.2	0.2	0.1	2.7	4.3	2.0
No. 5	0.1	0.3	0.2	25.3	10.8	14.1	0.4	0.6	0.5	2.9	3.8	1.6
No. 6	0.1	0.3	0.2	11.8	17.7	15.3	0.9	1.1	1.1	2.7	3.7	2.3
No. 7	0.2	0.3	0.3	23.6	28.9	26.3	0.7	0.5	1.5	4.1	0.1	5.1
No. 8	0.6	0.2	0.2	38.4	15.3	34.2	5.9	1.1	3.2	8.0	0.4	3.7
No. 9	0.4	0.4	0.4	30.5	27.3	28.2	1.3	1.7	2.7	6.3	4.9	4.9
Mean value	0.3	0.3	0.2	23.3	22.5	21.3	1.7	1.3	1.2	4.2	3.6	2.9
s.e.	0.1	0.1	0.03	3.2	2.3	3.6	0.6	0.3	0.4	0.6	0.8	0.4

weight, NANA and fucose concentrations in drug sputum were lower than the control values.

Patient No. 5:-

Apparent viscosity of sputum in placebo and drug periods was higher than in control. Macromolecular weight was found to be lower in placebo and drug sputa while the NANA concentration varied little and fucose levels were found to be lower during drug treatment.

Patient No. 6:-

Apparent viscosity and dry weight yield followed the same pattern of variation - they were both higher in placebo and drug sputa than in control. NANA concentration showed very little change and fucose increase in placebo sputum and was lower in drug sputum than in control.

Patient No. 7:-

Apparent viscosity, dry weight yield and NANA concentration varied little during placebo and drug periods. Fucose concentration was found to be lower during placebo and fell within control values during drug treatment.

Patient No. 8:-

Apparent viscosity, dry weight, NANA and fucose concentrations showed the same variation during placebo and drug periods, that is all of them were found to be lower than in control period particularly in placebo sputum.

Patient No. 9:-

Apparent viscosity and dry weight yield in placebo and drug sputa remained within control levels. NANA showed a slight increase during placebo and drug periods while fucose was found to be lower also in placebo and drug sputa than in control.

Comment:-

When mean values of viscosity, dry weight, NANA and fucose for control, placebo and drug periods were compared, no statistically significant difference emerged between control and placebo, control and drug or placebo and drug (Table VII,17).

Sputum viscosity, dry weight, NANA and fucose concentrations particularly fucose and NANA, were lower during drug treatment than in control and placebo periods and although chemical constituents also decreased during placebo, the percentage

reduction was greater during Mistabron treatment.

Fucose was the only chemical constituent which showed a more consistent change during drug treatment since it decreased in eight patients and increased only in one.

In a mucoid sputum a fall in fucose might be expected to be accompanied by a fall in dry weight; the dry weight in this group of cystic fibrosis varied little, suggesting that in these patients pus was relatively more important in determining the rheological properties of sputum than the bronchial glycoprotein.

TABLE VII,17

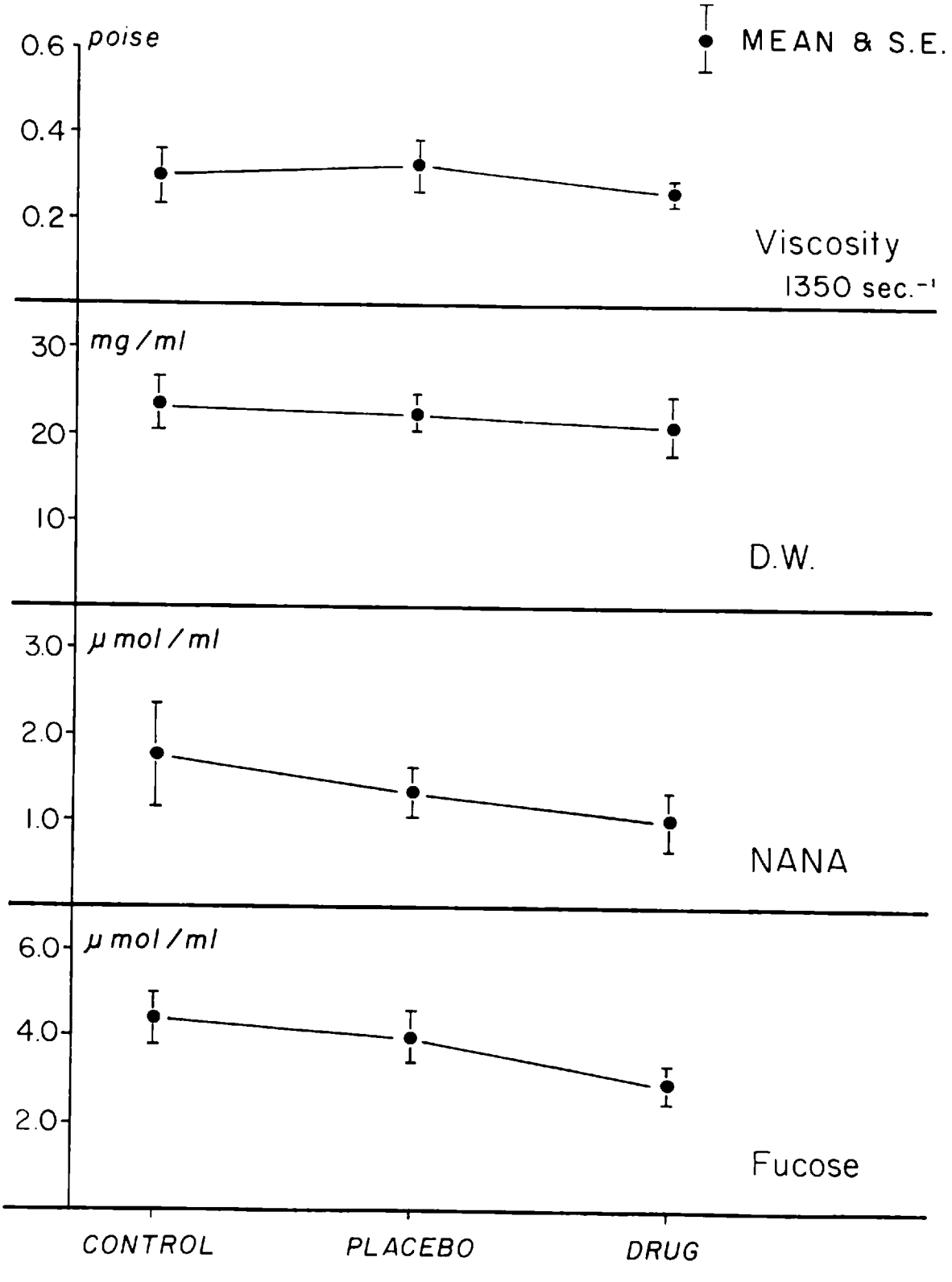
Comparison between control, placebo and drug for sputum viscosity, dry weight, NANA and fucose content (Student's t test).

	Control/Placebo	Control/Drug	Placebo/Drug
Viscosity	-0.2122	0.6711	0.9396
Dry weight	0.2088	0.4071	0.2633
NANA	0.6085	0.7666	0.2707
Fucose	0.6582	1.6885	0.6946

P not significant

Fig. VII,13 Effect of sodium 2 mercapto ethane sulphonate on apparent viscosity of sputum (at 1350 sec^{-1}), dry macromolecular weight (DW), N-acetyl neuraminic acid (NANA) and fucose levels of sputum in a group of patients with cystic fibrosis.

Fig. VII,13


0.6
poise
0.4
0.2
MEAN & S.E.
Viscosity
1350 sec.-1
30
mg/ml
20
10
D.W.
3.0
μ mol/ml
2.0
1.0
NANA
6.0
μ mol/ml
4.0
2.0
Fucose
CONTROL
PLACEBO
DRUG

CHAPTER VIII

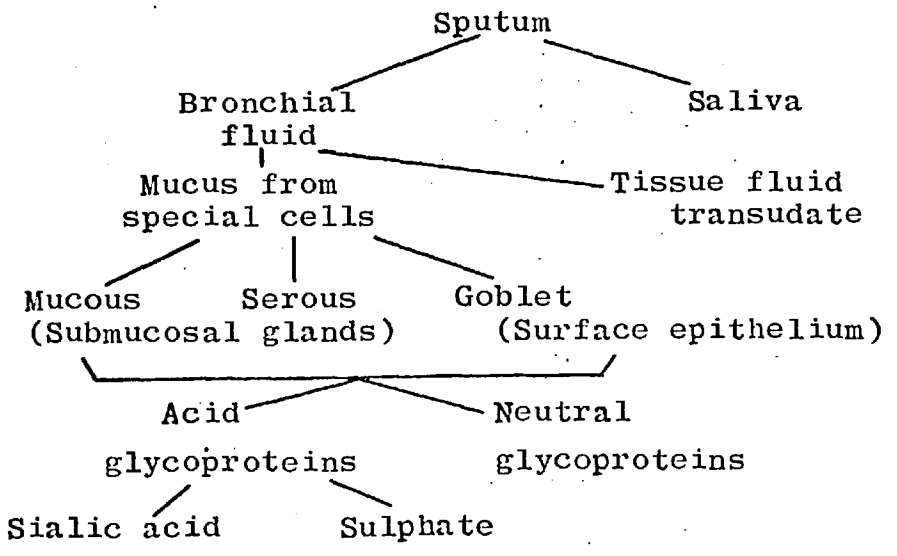
DISCUSSION

DISCUSSION

Marker substances of bronchial and serum glycoproteins: their use in tracing the origin of bronchial fluid.

Sputum was defined by Hooper in 1831 thus: "that which is cast out of the mouth merely by spitting or hawking, as the spittle, is properly sputum; but it also applies to expectorated matter, or that which comes from within the chest and it is spit out". In this century beginning with the studies of Muller (1901) interest in sputum has steadily increased particularly over the last twenty years when the multidisciplinary approach to its study has aimed to characterise the nature of its constituents and to trace their origin.

The constituents of sputum can be schematically represented as follows:



In normal conditions saliva seems to contribute very little to the chemical constituents of sputum (Masson et al 1965; Keal and Reid 1972; Brogan et al 1971); the other component, bronchial fluid, consists of two major constituents: bronchial secretion and tissue fluid transudate.

The contribution of tissue fluid transudate to bronchial fluid has been demonstrated by various methods:

- using fluorescein, a specific label for albumin, it has been shown that it appears on the surface of the bronchial mucosa within minutes of intravenous injection (Steinmann1956)
- in malformed fetal lambs with no communication between trachea and amniotic cavity the tracheal fluid was similar to that of normal lambs and it was an ultrafiltrate of plasma, (Adams et al 1963).
- in normal subjects 0.5% of a dose of radioiodinated albumin, injected intravenously, was present in protein bound form in sputum (Bonomo and D'Addabo 1964).
- immunoelectrophoretic studies in bronchial washings and sol phase of sputum have demonstrated the presence of albumin and serum glycoproteins, including immunoglobulins. (Keimowitz 1964; Masson et al 1965; Turgeon et al, 1971; Brogan et al 1971;

Masson and Heremans 1973; Havez et al 1973).

The serum glycoproteins most commonly found in sputum are: α1 acid glycoprotein, α 1 antitrypsin, α 1 antichymotrypsin, haptoglobin, transferrin, β 1c globulin, ceruloplasmin, hemopexin, IgA and IgG.

Serum glycoproteins differ from bronchial glycoprotein in the relative low levels of hydroxyaminoacids and proline, low carbohydrate content (3-35%) the presence of large amounts of mannose and low levels of fucose and galactosamine (Spiro 1973, Roberts 1974).

Characterisation of their sugar components have shown that they contain relatively large amounts of mannose and sialic acid and little or no fucose (Winzler 1958; Heimburger et al 1964; Dawson and Clamp 1968). Mannose, sialic acid and fucose content of some serum glycoproteins identified in bronchial fluid are as follows:

	Moles/mole of protein		
	Mannose	Sialic acid	Fucose
α 1 acid glycoprotein	12	15	3
Haptoglobin	31	30	2
Ceruloplasmin	22	9	2
Transferrin	8	4	-
α 2 macroglobulin	94	48	13
Hemopexin	16	19	-
α 1 antitrypsin		6	1
IgG	5	1	2
IgA	14	5	2

Studies carried out in purified bronchial glycoprotein (Roberts 1974) have shown that they are rich in fucose and sialic acid (NANA), they contain sulphate but mannose has not been identified:

	Fucose	NANA (g/100 g of dry material)	Sulphate
Patient A	17.9	5.9	3.8
Patient B	12.2	8.0	1.2

Lamb (1968) and Keal (1970) correlated the chemical analysis of sputum, including fucose, NANA and sulphate, with quantitative studies of acid glycoprotein in the mucosal glands of the same patients and reported that levels of fucose, NANA and sulphate in sputum were related to the percentage

of mucous cells. Based on all these facts, in the present study fucose and sulphate were used as marker substances of bronchial glycoprotein, mannose of serum glycoprotein and NANA of both bronchial and serum glycoproteins.

Levels of dry weight, fucose, sulphate, NANA and mannose in serum, saliva and sputum from normal subjects.

To support further the use of marker substances for assessing the relative contributions of bronchial secretion and serum transudate, saliva, serum and sputum produced after inhalation of prostaglandin F2α from two normal subjects were recently studied. The results are as follows:

	Subject 1			Subject 2		
	Serum	Saliva	Sputum	Serum	Saliva	Sputum
Dry weight mg/ml	82.5	3.5	7.2	73.3	2.1	5.4
Fucose µmol/ml	0.1	0.6	2.9	0.2	0.3	1.7
Sulphate µmol/ml	0.07	0.21	0.9	0.1	0.2	1.1
NANA µmol/ml	1.5	0.2	0.6	1.3	0.2	0.6
Mannose µmol/ml	1.7	0.08	0.22	1.1	0.2	0.2

It is apparent from these results, that absolute levels of fucose and sulphate will give us an indication of the amount of bronchial

mucus present in sputum and absolute levels of mannose that of serum transudate. Variations in NANA concentrations could be either due to an increase in serum transudate or to an increase in mucus. It has been shown that there is a high correlation between fucose and NANA in mucoid chronic bronchitis sputum (Lopez-Vidriero et al 1973) and by using the regression line with 95% confidence limits for the relationship between fucose and NANA concentrations, the NANA equivalent to fucose can be calculated.

Changes in levels of marker substances in response to infection: mannose as a marker of inflammatory exudate:-

Changes in all marker substances analysed will be given with more detail in page 418
It is the first time that mannose has been used as a marker substance of serum glycoproteins in sputum and from the results presented it is evident that mannose levels are very high in serum, absent in purified bronchial glycoprotein and low in bronchial fluid from normal subjects.

Mannose concentration in sputum produced after inhalation of PGF2α from normal subjects was below

levels found in mucoid chronic bronchitis or asthma sputum suggesting that the serum glycoprotein contribution to normal bronchial fluid is negligible. This is supported by Bonomo and D'Addabo's findings that in patients with chronic bronchitis the radio-iodinated albumin in sputum, in protein-bound form, was 16 times higher than in normal subjects.

When mucoid and purulent sputa were compared, mannose levels showed a marked increase (100%) while fucose and sulphate (marker substances of bronchial secretion) increased only slightly 23% and 37% respectively. An increase in albumin and serum glycoproteins with acute infection has been reported by several authors Matthews et al 1963; Dennis et al 1964; Schultze and Heremans 1966.).

Inhalation of Prostaglandin F2α: a method for obtaining sputum from normal subjects:-

To obtain bronchial secretion uncontaminated with tissue fluid transudate from normal subjects or from patients with mucus hypersecretion is impossible; even to obtain bronchial fluid presents many technical problems and it is not practical for studying large series of individuals. The material more easily obtainable and readily

available is sputum, but sputum is only produced in hypersecretory states or when there is an increase in tissue fluid transudate such as in pulmonary oedema.

Different methods are being used to obtain bronchial fluid from the normal bronchial tree, some of them are only applicable to laboratory animals (Perry and Boyd 1941; Wardell et al 1970; Proctor et al 1973; Gallagher et al 1975). Other methods used in man require tracheostomised (Toremalm 1960) or laryngectomised patients (Potter et al 1963), and in these situations the physiological conditions are abnormal and irritation might induce changes either in the serum transudate component or in the intracellular acid glycoprotein - e.g. shift to a sialo or sulphomucin.

Bronchial fluid can also be obtained at bronchoscopy; the main objections to this method are that the patient is usually premedicated with atropine or hyoscine and that it is necessary to irrigate the bronchial tree with saline since in a normal bronchial tree the amount of fluid is too small to be aspirated, and the volumetric baseline is lost (Masson and Heremans 1973).

Aerosols with hypertonic saline solutions - originally used to obtain sputum for cytological studies (Bickerman et al 1958; Leilop et al 1961) increase serum transudation and therefore the bronchial fluid is no longer normal (Lopata et al 1974).

In halation of prostaglandin F2α seems to be a reliable method for obtaining bronchial fluid from normal bronchial tree since all the subjects except one produced sputum. When the same subjects inhaled other drugs sputum production was less successful; after acetyl choline only 6 out of 11 produced sputum after histamine 4 out of 11 and after citric acid 4 out of 9. The experiments were conducted on two separate occasions, in the first,all subjects inhaled the drugs in the following order: PGF2α, acetyl choline and histamine; in the second experiment the subjects were divided into three groups, Group 1 histamine, acetylcholine, PGF2α and citric acid were inhaled in that order, in Group 2 PGF2α was inhaled first then histamine, acetylcholine and citric acid, in Group 3 acetylcholine was first followed by PGF2α, histamine and citric acid. Regardless of the order in which PGF2α was inhaled all subjects except one produced sputum.

The method does not involve complicated techniques and apart from the bronchial constriction and cough, which only lasts for 15-30 minutes, no other unpleasant effects were noticed.

The material expectorated after inhalation of PGF2α has the rheological properties and chemical characteristics of mucoid sputum:-

Absolute levels of viscosity, dry weight, fucose, NANA and sulphate fell within the lower range of mucoid sputum but some dissimilarities were apparent. Mannose levels in sputum produced after inhalation of PGF2α were below those found for mucoid sputum suggesting that the serum transudate component in normal bronchial fluid is negligible. Albumin, IgG and IgA concentrations were also lower than those reported for mucoid chronic bronchitis or asthma sputum (Brogan et al 1971; Brogan et al 1975)and fell within the range found for normal subjects (Salvaggio et al 1973). It is interesting to note that the two subjects with the highest albumin levels (Nos. 4 and 7) experienced a marked reduction in PEFR (21 and 24% respectively); (Mathé and Hedqvist 1975) have reported a considerable variability in the airway conductance in healthy subjects after inhalation of PGF2α and the response did not differ when inhalation was repeated 1 and

17 months later.

Sputum produced after inhalation of PGF2α also differed from mucoid chronic bronchitis sputum in that it contained relatively more fucose than NANA or sulphate. Although absolute levels of fucose were found to be in the lower range of mucoid chronic bronchitis sputum, the NANA/Fucose ratio and sulphate levels were lower than in mucoid sputum suggesting that the bronchial glycoprotein secreted by normal mucus secreting structures is less sialylated and/or less sulphated than in disease. Histochemical studies carried out in normal and chronic bronchitic patients (McCarthy and Reid 1964; Lamb 1968; Lamb and Reid 1969, 1970, 1972 a,c) have shown that with disease there is a shift to sialo or sulphomucin.

CHANGES IN CHEMICAL CONSTITUENTS OF SPUTUM WITH INFECTION AND IN DISEASE.

When studying a complex and intricate material as sputum to interpret one's own results is a difficult and sometimes disappointing task but to interpret and compare other workers' results can be a nightmare.

Some of the contradictory findings reported in the literature may arise for various simple reasons: the inhomogeneity of the sputum sample studied, analysis may have been carried out in different material (whole sputum from an individual patient, pooled sputum from several patients, bronchial washings or sol phase). Time of the day at which the specimen was collected may also account as well as methods of collection and storage of samples. This is important since enzymatic degradation takes place if sputum is left at room temperature or at 4°C (Leach 1963; Woodcraft et al), this could be avoided by quick freezing and storing at -20°C or boiling at 100°C (Woodcraft et al). Lack of information concerning macroscopic type of sputum, presence of pus and degree of purulence may be also responsible for some of the dissimilarities between reports.

Changes with infection in chemical constituents of sputum. Macroscopic appearance of sputum was found to be a reliable method of assessing degree of purulence. Estimation of DNA, which has been used as an objective criterion for assessing the degree of infection (Burgi et al 1968), showed that those samples classified as

mucoid by naked eye contained no DNA or only a trace. Some overlap between mucopurulent and purulent samples for levels of DNA was expected since the mucopurulent group included samples with various degrees of purulence (MMP, MP and MPP). These three subgroups correspond to types M2, P1 and P2 used by Miller and Jones (1963)

MMP (M2): predominantly mucoid with a suspicion of pus

MP (P1): pus amounting to less than one third of the specimen

MPP (P2): pus amounting to more than two thirds of the specimen.

These authors observed an overlap between mucopurulent and purulent samples in total cell count but their conclusion was that with experience macroscopic examination is a reliable method of assessing degree of purulence.

Mannose levels of purulent sputum were found to be significantly higher than those of mucoid sputum:- Changes in mannose concentration with infection have already been discussed (see page 412)

NANA concentration of sputum increases with degree of purulence. NANA was the only marker substance

which showed a statistically significant increase from mucoid to mucopurulent and from mucopurulent to purulent. Its dual origin, from bronchial and serum glycoproteins may account for this: if each component is increased it will reinforce the effect. The increase in NANA content of sputum could be due to a greater inflammatory exudate contribution, to an increase in the number of cells producing sialomucin, to a shift to acid glycoprotein or to a combination of these.

Although infection is a common complication or the cause of many chest diseases, little attention has been paid to the effect of infection per se on mucus production.

Bronchial gland hypertrophy and increase in number of goblet cells with a change in the histochemical nature of the glycoprotein secreted - a shift from the production of neutral to acid glycoprotein - have been produced experimentally in animals by exposure to various irritants but with no evidence of infection: sulphur dioxide (Lamb and Reid 1968; Mawdesley-Thomas et al 1971; Barker et al 1973), nitrous oxide (Freeman and Haydon 1964) and tobacco smoke (Leuchtenberger et al 1958; Lamb

and Reid 1969; Jones et al 1973).

Jones et al (1975) have described for the first time the changes in histochemical nature of glycoproteins in the hypertrophied glands of pigs after experimentally induced enzootic pneumonia (Mycoplasma hyorhinis). There was a shift from neutral to acid glycoprotein, the mucous cells containing more sulphate and sialic acid resistant to sialidase than in control animals.

Reid and de Haller (1967) reported that in cystic fibrosis children with pulmonary infection the gland size can increase within a few weeks from normal size to an enormous hypertrophy and the percentage of glandular acini entirely susceptible to sialidase was higher than in cystic fibrosis children who died from meconium ileus.

Keal (1970) found that in patients with cystic fibrosis there was a relationship between clinical radiological grading and the NANA content of sputum: increasing levels of NANA with increasing severity of the disease.

Sulphate content of sputum increases with degree of purulence:-

Since sulphate is virtually absent in serum glycoprotein, the increase in sulphate content of sputum with degree of purulence may be due to either an increase in the number of cells producing sulphomucin, to an increase in the amount of intracellular sulphate or to a combination of the two.

Inhalation of sulphur dioxide or tobacco smoke, in the absence of infection, produces an increase in number of goblet cells with extension to distal airways and there is a shift from sialo to sulphomucin (Lamb and Reid 1968, 1969). Histochemical changes in glycoprotein without increase in number of goblet cells has been produced after exposure to tobacco smaoke with phenylmethyloxodiazole (Jones et al 1973) Submucosal gland hypertrophy with an increase in intracellular acid glycoprotein has also been reported following exposure to tobacco smoke and after administration of isoprenaline (Jones et al 1973; Sturgess and Reid 1973).

Lamb (1968) and Lamb and Reid (1972c) reported that in patients with cystic fibrosis the number of mucus cells staining for sulphated mucin was

increased, particularly in the older patients with marked lung damage and infection, and suggested that these changes were due to lung damage rather than the disease itself since similar changes were found in patients with bronchiectasis.

Fucose content of sputum increases with degree of purulence:- The statistically significant increase in fucose content of sputum with degree of purulence was unexpected since serum glycoproteins contain hardly any fucose and this could represent increase in the amount of bronchial glycoprotein; a shift from acid to neutral glycoprotein has not been reported in histochemical studies.

Biochemical analysis of purified bronchial glycoprotein, after treatment of the original mucin with thiol compounds, have demonstrated three types of glycoproteins: fucomucin, sialomucin and sulphomucin. Although this separation into three types may be artificial (Roberts 1974), it shows that regardless of the acidic character of the mucin all three molecules contain similar amounts of fucose.

Lamb (1968) reported that fucose levels in sputum were directly correlated to the number of

mucous cells as a percentage of total gland cells. Gland hypertrophy results in mucus hypersecretion and therefore independently of the type of acid glycoprotein produced the total amount of fucose will increase. It is possible that gland hypertrophy will develop as a result of infection only in those situations where gland hypertrophy is absent or mild as in asthma or cystic fibrosis . When changes in fucose content of sputum with degree of purulence were analysed in each disease group it was apparent that in chronic bronchitis fucose content varied very little with degree of purulence while in asthma and cystic fibrosis there was a great increase in fucose levels.

Differences in chemical constituents of sputum between macroscopic types were found to be greater than between diseases when the same macroscopic type was compared. The results of the present study show that the changes in chemical constituents of sputum with degree of purulence are due to variations in both bronchial and serum components - an increase in tissue fluid transudate or inflammatory exudate, as well as in sialo and sulphomucin. Similar findings have been reported for sputum viscosity (Charman and Reid 1972; Charman 1973). When

studying differences between diseases it is of great importance to give detailed information about the macroscopic type of sputum since some dissimilarities may be due to the presence of infections rather than an abnormal glycoprotein being produced.

Chemical constituents of sputum: difference between diseases

In recent years international bodies have been concerned with the definition of respiratory diseases in particular chronic bronchitis and asthma but in spite of all the efforts there is still a great confusion. Terms like chronic obstructive pulmonary disease, wheezy bronchitis, reversible airways obstruction, are widely used in the literature and there is a relationship between the term used and the country of origin.

Asthma is probably the most vaguely defined and authors very seldom specify if the sputum samples analysed were from patients with extrinsic or intrinsic asthma and whether the patients also fulfilled criteria of chronic bronchitis. In cystic fibrosis, the pathological changes of bronchial epithelium vary according to the state

of the disease (Lamb 1968; Lamb and Reid 1972c).

Asthmatic sputum. The results presented in this study illustrate the importance of detailed clinical information when comparing sputum from various diseases.

Keal (1971) found that the variance of NANA content of sputum from a group of asthmatic patients was greater than that seen in chronic bronchitis. Similar findings have been reported for sputum viscosity (Keal 1971; Charman and Reid 1972; Charman 1973) and for levels of albumin (Brogan et al 1971; 1975). No distinction between extrinsic and intrinsic asthma was made by these authors; in Brogan's paper (1971) the average age for the group was 53 with a range of 34-72, it is therefore very likely that some of these patients had intrinsic asthma (with or without chronic bronchitis), since extrinsic asthma patients tend to be in a younger age group.

The high degree of variance found in the present study, particularly for dry weight, fucose and NANA contents, urged for a more detailed analysis of the clinical data. Luckily the patients were

studied at the Brompton Hospital which means that extensive information was available; clinical history (smoking habits, previous history of cough and sputum) and exhaustive laboratory investigations (skin tests, serum precipitins, eosinophils in sputum and blood eosinophilia, respiratory function tests, response to bronchodilators).

In asthma most of the pathological studies have been carried out on necropsy material from patients dying in status asthmaticus which represents an extreme degree of pulmonary change. Dunnill (1975) reported that in asthmatic patients who died as a result of road traffic accident, bronchial occlusion due to mucus plugs was absent, while occlusion of bronchial lumen down to the level of terminal bronchiole was one of the main pathological features in patients dying in status asthmaticus.

Changes in submucosal glands vary from absence of any quantitative and qualitative changes (Houston et al 1953) to marked gland hypertrophy similar to that found in chronic bronchitis (Dunnill 1975) with a wide spectrum of intermediate changes (Earle 1953; Turiaf et al 1958; Glynn and Michaels 1960; Gordon 1964; Vidal et al, 1968). Few studies

related the pathological changes to clinical features. Turiaf et al(1958) found that in the young adult group (probably extrinsic asthma) bronchial gland hypertrophy was less common than in the old adult group of late onset (intrinsic asthma). Glynn and Michaels (1960) studied bronchial biopsy material and divided their patients into different groups: with or without chronic bronchitis or whether chronic bronchitis preceded or followed asthma and found that mucus hypersecretion and increase in number of goblet cells was present particularly in those patients with chronic bronchitis. Histochemical studies from large series of patients are not available; Keal and Reid (1975) reported a case of an 11 year old boy who died in status asthmaticus, they found gland hypertrophy with a gland/wall ratio of 0.5 (normal 0.3) and an increase in the number of cells producing acid glycoprotein, particularly sulphomucin, but they suggested that these changes could have been produced by isoprotenerol (Sturgess and Reid 1973).

It is of interest that the biochemical features of intrinsic asthma sputum were closer to chronic bronchitis than to extrinsic asthma which seems to correlate with the pathological findings. When

chronic bronchitis was associated with intrinsic or extrinsic asthma, it seemed that gland hypertrophy was more important in determining the chemical features of sputum. Extrinsic asthma sputum showed the highest NANA/Fucose ratio compared with intrinsic asthma, intrinsic asthma + chronic bronchitis or even extrinsic asthma + chronic bronchitis suggesting an increase in serum transudate.

Recent studies on purified bronchial glycoprotein from patients with chronic bronchitis, extrinsic asthma or intrinsic asthma (Creeth et al 1976) have shown that bronchial glycoprotein from extrinsic asthma contained relatively less NANA than that from chronic bronchitis which supports the difference between extrinsic asthma (without gland atrophy) and those diseases where gland hypertrophy is present with shift to acid glycoprotein.

Cystic fibrosis sputum. Since Farber (1944) coined this name for the disease as mucoviscidosis, most of the research has been aimed to prove or disprove that cystic fibrosis mucus is more viscous than in other diseases and that the glycoprotein secreted is abnormal (Di Sant'Agnese et al 1957; Dische et

al 1959; Chernick and Barbero 1959; Bauer 1960; Johansen 1963; Potter et al 1963; Matthews et al 1963; Roussel et al 1968; Potter et al 1969; Charman and Reid 1972; Charman 1973).

From the results presented in this study it is apparent that when the same macroscopic type is compared, although cystic fibrosis sputum differed little from other diseases, some differences emerged.

Macroscopically mucoid cystic fibrosis sputum contained more DNA than mucoid chronic bronchitis sputum. Of the 11 mucoid chronic bronchitis sputum analysed only two contained traces of DNA while all the mucoid cystic fibrosis sputa contained considerable amounts, within mucopurulent range, suggesting that in cystic fibrosis there is a greater cellular contribution either from inflammatory exudate or desquamative process. Similar findings have been reported for purulent sputum, cystic fibrosis sputum contained more DNA than purulent bronchiectasis sputum (Chernick and Barbero 1959; Matthews et al 1963; Potter et al 1963).

Mucoid cystic fibrosis sputum contains relatively less bronchial secretion than mucoid chronic bronchitis sputum: This is supported by the following findings:

- dry macromolecular weight of mucoid cystic fibrosis sputum lower than that of mucoid chronic bronchitis in spite of the fact that mucoid cystic fibrosis sputum contained more DNA.
- fucose content of cystic fibrosis sputum was found to be significantly lower than that of mucoid chronic bronchitis sputum
- NANA/Fucose ratio was higher in cystic fibrosis sputum than in chronic bronchitis.

It was surprising to find that sulphate levels were lower in cystic fibrosis than in chronic bronchitis since histochemical studies have shown that in cystic fibrosis the number of mucous cells secreting sulphomucin is increased (Lamb 1968; Lamb and Reid 1972c). This could be due to the loss of sulphate during dialysis, Keal (1970) reported that 23% of sulphate is lost during dialysis possibly because sulphate may be attached to a small molecule.

This relatively low contribution from bronchial secretion could also explain, in terms

of an increase in serum transudate or more likely inflammatory exudate since DNA was present in mucoid cystic fibrosis samples and the NANA/Fucose ratio was higher than in mucoid chronic bronchitis sputum and fell within extrinsic asthma levels. Brogan et al (1975) have reported that in some cystic fibrosis patients, the albumin content of the sputum was closer to asthma than to chronic bronchitis sputum.

NATURAL VARIATION OF CHEMICAL CONSTITUENTS OF SPUTUM

Diurnal variation:-

From the results of the present study it seems that chronic bronchitis sputum is subjected to a diurnal variation. The findings supporting this being:

- sputum volume decreases during the day, the reduction being most marked between 12.00 hrs and 18.00 hrs.
- the yield of macromolecular material decreases during the day, particularly in the evening.
- fucose and NANA contents of sputum gradually decrease during the day
- NANA/Fucose ratio varies little although it tends to be lower towards the evening.

Blanshard (1955) reported, in two chronic bronchitic patients, that the first sputum produced after wakening was more viscous than that of sputum produced during the day and he claimed that the high viscosity was merely due to dehydration. In contrast, Charman and Reid (1972) found that when sputum collections were made over shorter periods of time, the early morning sputum was not always more viscous. More recently Keal (1974) reported a 25% reduction in sputum viscosity between specimens collected in the morning and afternoon. If dehydration was the only cause of variation of the rheological properties of sputum, a significant decrease in dry weight, fucose and NANA should be expected in the 09.00-12.00 sputum samples and this was not the case in the present study.

Since all the patients were studied in the same hospital and during the same period of the year, it seems very unlikely that atmospheric conditions or uncontrolled fluid intake may have been the cause of the variation. It seems likely therefore, that the variation of chemical constituents of sputum was due to a reduction in bronchial secretion. A similar pattern of variation of high morning levels at 18.00 hrs. has been reported in chronic bronchitic

patients for secretory IgA (Havez et al 1973; Michel et al 1974).

When studying the effect of drugs on the rheological and chemical properties of sputum, it is very important to bear in mind that they are subjected to a spontaneous or natural variation.

Seasonal variation:-

In the group of chronic bronchitis patients studied over six consecutive months, a pattern of seasonal variation with high levels of dry weight, fucose and NANA in December and a fall in January with a gradual increase from January to May was observed in nine out of the 14 patients.

Since the macroscopic type of sputum varied throughout the study virological studies were carried out and on no occasion had any of the patients had a positive titre for influenza A virus. Therefore the seasonal variation could not be caused by a viral infection.

Keal (1971) reported a seasonal variation in dry weight and NANA (high levels during winter months and low levels over spring and summer), in a group of chronic bronchitics with mucoid sputum

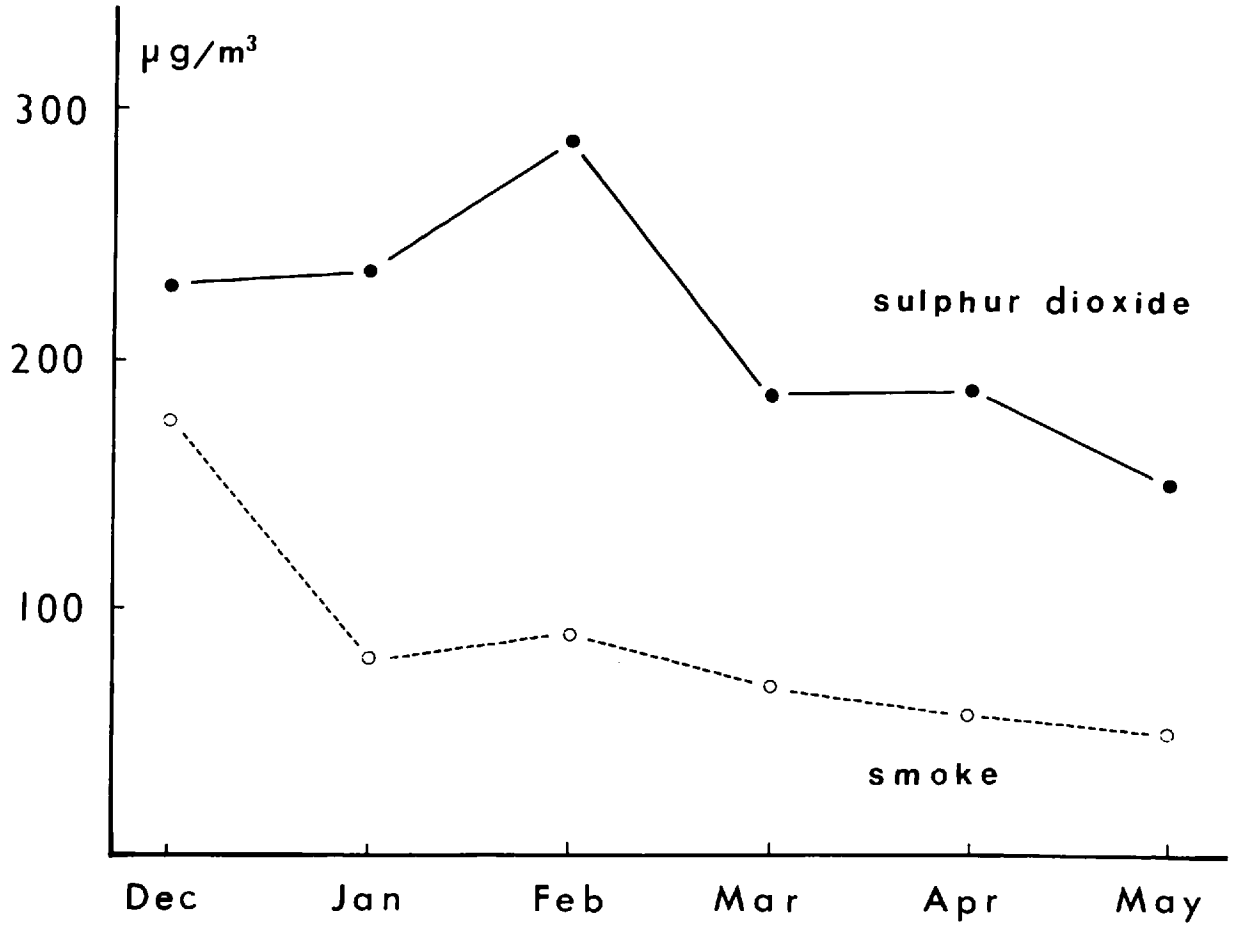
and suggested that changes in atmospheric pollutants were possibly responsible. Taking into account Keal's findings, the sulphur dioxide and smoke atmospheric concentrations ($\mu g/m^3$) recorded in the area were the patients lived, were plotted (Fig.VIII,1). It was apparent that the smoke concentration was at its highest in December and sulphur dioxide in February, these changes may be partly responsible for the seasonal variation seen in some of the patients with mucoid sputum throughout the study period.

The "virulence-enhancing" properties of mucus have been demonstrated (Olitzki et al, 1946; Olitzki 1948; Smith 1953) since changes in atmospheric pollutants result in mucus hypersecretion, it is very likely that the increase in mucus facilitates infection (Ogilvie 1967; Stuart-Harris 1971).

Studies carried out in laboratory animals (Boyd and Boyd 1971; Boyd 1973) have shown that there is a seasonal variation in the effect of drugs on bronchial fluid and it has been suggested that these are related to physiological variations which occur mainly in the autumn and winter months such as a

Fig. VIII,1 Average concentration of atmospheric sulphur dioxide and smoke in the Borough of Wandsworth from December to May 1971.

Fig. VIII,1


µg/m³
300
200
100
sulphur dioxide
smoke
Dec
Jan
Feb
Mar
Apr
May

decrease in vagal tone, endocrine activity, body temperature or to environmental weather conditions.

Yearly variation:-

In chronic bronchitis the levels of chemical constituents of sputum for an individual patient - biochemical profile - vary little over relatively long period:- It was interesting to note that in the group of chronic bronchitic patients studied during two consecutive years, levels of dry weight, fucose and NANA for each individual patient vary little from year to year. Even more striking was the fact that the sputum volume for each patient whether large or small, remained constant over the two year study period.

It was also apparent that there was a marked inter-patient variation for fucose and NANA levels, in some patients fucose content was very high while in others, was very low and a similar variation was seen for NANA.

These observations are in agreement with the results of histochemical studies carried out in normal human bronchi (Lamb and Reid 1972a; Jones and Reid 1973b), a wide variation in histochemical types of acid glycoprotein between

individuals while for each individual the pattern of distribution of the types of acid glycoprotein was similar throughout the bronchial tree. In some bronchi the percentage of mucous cells staining red after sialidase AB-PAS was small (2.5%) while the percentage of those staining blue was high (75%), in other bronchi from a different patient, the percentage of mucous cells staining red or blue was similar (21% and 20% respectively). Jones and Reid (1973b) reported similar findings, the proportion of mucous cells producing sulphomucin or sialomucin was 1:2 in some bronchi while in others was 1:4 and in one 3:1.

Havez et al (1973) reported that chronic bronchitic patients could be divided into three groups according to the levels of secretory IgA in sputum and it has been shown that there is a relationshop between concentrations of secretory immunoglobulins in sputum with various factors such as age, sex and race (Orfanakis et al 1973) and damage to bronchial epithelium (Medici and Burgi, 1971).

The reasons for the presence of a characteristic biochemical profile for each individual have not been yet established although there is some evidence that a genetic factor may be responsible. Studies carried out in purified bronchial glycoprotein, from

chronic bronchitis sputum, have shown that the sugar content of the blood group substances varies with the type of blood group activity, mucins with blood group activity H or B have a galactose/hexosamines ratio of 1 or more than 1 while those with blood group activity A, the ratio is less than 1. Variations of NANA, sulphate and fucose content have also been demonstrated (Degand et al 1973) and there is evidence that the action of fucosyl transferases are influenced by genetic factors (Lloyd and Kabat 1968).

CHANGES IN CHEMICAL CONSTITUENTS OF SPUTUM IN RESPONSE TO TREATMENT

The effect of three different types of drugs has been investigated: steroids, atropine and sodium 2 mercapto ethane sulphonate.

Patients with respiratory diseases particularly chronic bronchitis, asthma or cystic fibrosis, come to the doctor complaining of: production of large amounts of sputum; difficulty in coughing up sputum (tenacious sputum) or dyspnoea.

Steroids have been used with success in reducing the sputum volume in patients with bronchorrhoea by decreasing the serum transudate component. Their beneficial effect in improving airways obstruction

in asthmatic patients is well known.

The use of atropine as a bronchodilator has been hampered by its effect on mucus secretion and in particular by its effect on the viscosity of sputum.

Mucolytics are widely used but the results reported in the literature either lack objective criteria, are contradictory or their beneficial effect has been based on in-vitro studies.

Bronchorrhoea sputum: changes in response to steroid or atropine:- The patients included in this group were producing large amounts of sputum, more than 100 ml in 24 hours, their primary disease was extrinsic or intrinsic asthma, chronic bronchitis or scleroderma.

Previous studies (Keal 1970, 1971) suggested that the response to steroid treatment in these patients could be predicted from the NANA content of sputum and the primary disease. Those patients with extrinsic asthma and low levels of NANA responded better to steroids than those with chronic bronchitis and high levels of NANA in the sputum, the reduction of sputum volume being due

to a decrease in serum transudate component rather than an inhibitory effect on bronchial secretion.

From the results of the present study it seems that the action of steroids can be either a reduction in serum transudate, an inhibitory effect on bronchial secretion or a combination of the two. In two patients, one with intrinsic asthma and the other with chronic bronchitis, both with originally high levels of NANA in sputum, the reduction of sputum volume was due to an inhibitory effect on bronchial gland secretion since the yield of macromolecular weight, NANA and fucose concentrations varied very little. In one patient with low levels of NANA but with a low NANA/Fucose ratio, suggesting that the main constituent of the bronchial fluid was mucus, there was a transient response to steroids.

In contrast in a patient with intrinsic asthma and low levels of NANA the reduction of sputum volume was accompanied by an increase in dry weight, NANA and fucose concentrations; in this case the effect of steroid treatment was a reduction of the serum transudate component.

A combination of inhibitory effect on bronchial secretion and reduction of serum transudate component was observed in one patient with intrinsic asthma and high levels of NANA where the sputum volume decreased, the dry weight increased but the NANA and fucose levels remained unchanged.

Organ culture studies (Sturgess 1970, Sturgess and Reid 1972) have shown that steroids decrease the secretory index of human bronchial sub-mucosal glands, but that this response is not constant (about 1 in 3) and is dose related. The variation in response was attributed to differences in tissue sensitivity and this could explain the discrepancies between Keal's results - failure to respond to steroids in patients with chronic bronchitis and high levels of NANA - and those presented in this study.

In patients with bronchorrhoea, long term atropine reduced the sputum volume without any change in dry weight or chemical constituents. In one patient with intrinsic asthma, whose bronchorrhoea failed to respond to steroids, the sputum volume was reduced after long term atropine therapy suggesting that in this patient the main bulk of the

sputum was bronchial secretion. Sturgess and Reid (1972a,b) have shown, in organ culture studies, that atropine decreases the secretory index of both mucous and serous cells of the submucosal glands, that there is a wide variation in the degree of inhibition in bronchi from different subjects and that there is an inverse relationship between gland size and inhibitory effect - the larger the gland the less the inhibitory effect of a given dose of atropine. At all sizes of gland, there was a dose related response to atropine. Since the patient had intrinsic asthma, some degree of gland hypertrophy should be expected and this could explain why very high doses of atropine were required to decrease bronchial secretion.

A good response to long term atropine was also observed in a patient with chronic bronchitis who developed bronchorrhoea following a mycoplasma infection. Excessive bronchial secretion has been reported in pigs after experimentally induced enzootic pneumonia (Baskerville 1972) as a result of bronchial gland hypertrophy (Jones et al 1975). It has been suggested that bronchial gland hypertrophy may occur as a reflex response since mycoplasma are known to produce peroxide (Cherry and Taylor-Robinson 1970) which acts directly on

epithelial cells and may act as an irritant to receptors within the airways.

It seems likely that the response to atropine in this patient could have been due to a blocking effect of stimulus at receptors level since the patient continued producing his baseline chronic bronchitic sputum volume.

Alveolar cell carcinoma: the failure to reduce sputum volume either with steroids, atropine or fluid restriction suggest that the secretion is autonomous. A temporary reduction of sputum volume in patients with alveolar cell carcinoma has been achieved with infiltration of the stellate ganglion (Bourgeois et al 1950) subcutaneous atropine and oral belladona (Gernez-Rieux et al 1961) or ACTH (Siltzbach-personal communication) while in other cases treatment with atropine, steroids, intravenous procaine radiotherapy or antihistamine failed to reduce sputum volume (Bourgeois et al 1950; Rubinstein and Philheu 1954; Turiaf et al 1957; Gernez-Rieux et al 1961). The effect of surgical treatment or cytotoxic drugs on sputum production has not been reported in the literature. The patient reported in the present study received cytotoxic chemotherapy,

a combination of cyclophosphamide, 5-fluouracil, vincristine and procarbazine, but only for a week and they had no effect on sputum production.

In the absence of bronchorrhoea, atropine had no effect on bronchial gland secretion. When atropine was administered by aerosol or intramuscularly in a single dose over two or three consecutive days, the 24 hour sputum volume, sputum viscosity, dry weight, NANA or fucose content, remained unchanged. Two patients, a chronic bronchitic and an intrinsic asthma, failed to produce sputum immediately after intramuscular injection of atropine but this was due to the drying up effect of salivary secretion since the patients complained of dryness of the mouth and throat and difficulty on coughing up the phlegm which they could feel in their throats. The effect of atropine on salivary secretion was even more striking in a patient with bronchiectasis, who presented with a history of increasing sputum production. It was originally thought that he was suffering from bronchorrhoea, but the dramatic effect of atropine on sputum volume with an increase in dry weight yield, NANA and fucose concentrations without altering the 24 hour volume, suggested that the main bulk of the sputum was saliva.

One of the biological functions of epithelial secretions is lubrication, Hillis (1952) claimed that stimulation of salivary flow lubricates the irritable fauces of patients with chronic bronchitis thus easing cough and expectoration.

The presence of gland hypertrophy may be responsible for the lack of response to atropine (Sturgess and Reid 1972). The dose given may have been too small to have an inhibitory effect on bronchial gland secretion although it was sufficient to cause a dry mouth and a significant rise in pulse rate.

Sodium 2 mercapto sulphonate had no effect on the physico-chemical properties of sputum in patients with chronic bronchitis. Although the number of patients studied was small, sodium 2 mercapto ethane sulfonate seemed to have no effect on sputum volume, viscosity or chemical constituents. It could be claimed that the lack of response was due to changes in macroscopic type of sputum during the trial in two patients, but this is not so since no effect was seen when the macroscopic type remained unchanged. Similar changes were observed during placebo treatment.

Experimental studies in animals have shown that the physical properties of mucus can affect the mucociliary clearance mechanism (Kilburn 1968). Lung clearance studies were carried out in these patients by Dr. Thomson and Dr. Pavia at the London School of Hygiene. Lung clearance was assessed by the rate of removal of previously inhaled particles (5μ - tagged with radioisotope 99^{m} Tc) before and after inhalation of sodium 2 mercapto ethane sulphonate and placebo.

Mean values of rate of clearance for the group for control, placebo and drug are shown in Fig. VIII,2. A faster clearance was observed after the drug than during control but an identical effect was produced by placebo. Similar results have been reported with bromhexine (Thomson et al 1974).

Sodium 2 mercapto ethane sulphonate had no effect on the physicochemical properties of cystic fibrosis sputum. The failure to reduce sputum viscosity in this group of cystic fibrosis patients could be due to the fact that all samples studies, except two, were macroscopically mucopurulent or purulent. Burgi (1973) has shown that DNA fibres envelop the glycoprotein fibres. This is

important since the action of sodium 2 mercapto ethane sulphonate is to break the disulphide bonds present in the mucus and the DNA fibres can prevent the mucolytic agent reaching the glycoprotein fibres.

It is surprising to see the excellent results obtained in vitro studies with different mucolytic agents while the in vivo clinical trial results are so disappointing. During in vitro studies with mucoid sputum, van de Walle et al (1970) obtained an 85% liquefaction effect and Hirsch et al (1969) an 80% reduction in sputum consistency with a 10% solution of sodium 2 mercapto ethane sulphonate.

When studying the effect of mucolytics agents we have to take into consideration the complexity of the chemical basis of the visco-elastic properties of sputum. Although the bronchial glycoprotein content is mainly responsible, other factors such as hydration (free and bound water), levels of IgA, presence of DNA fibres, may also contribute to the rheological properties.

Fig. VIII, 2 The effect of sodium 2 mercapto ethane sulphonate on mucociliary clearance in a group of patients with chronic bronchitis.

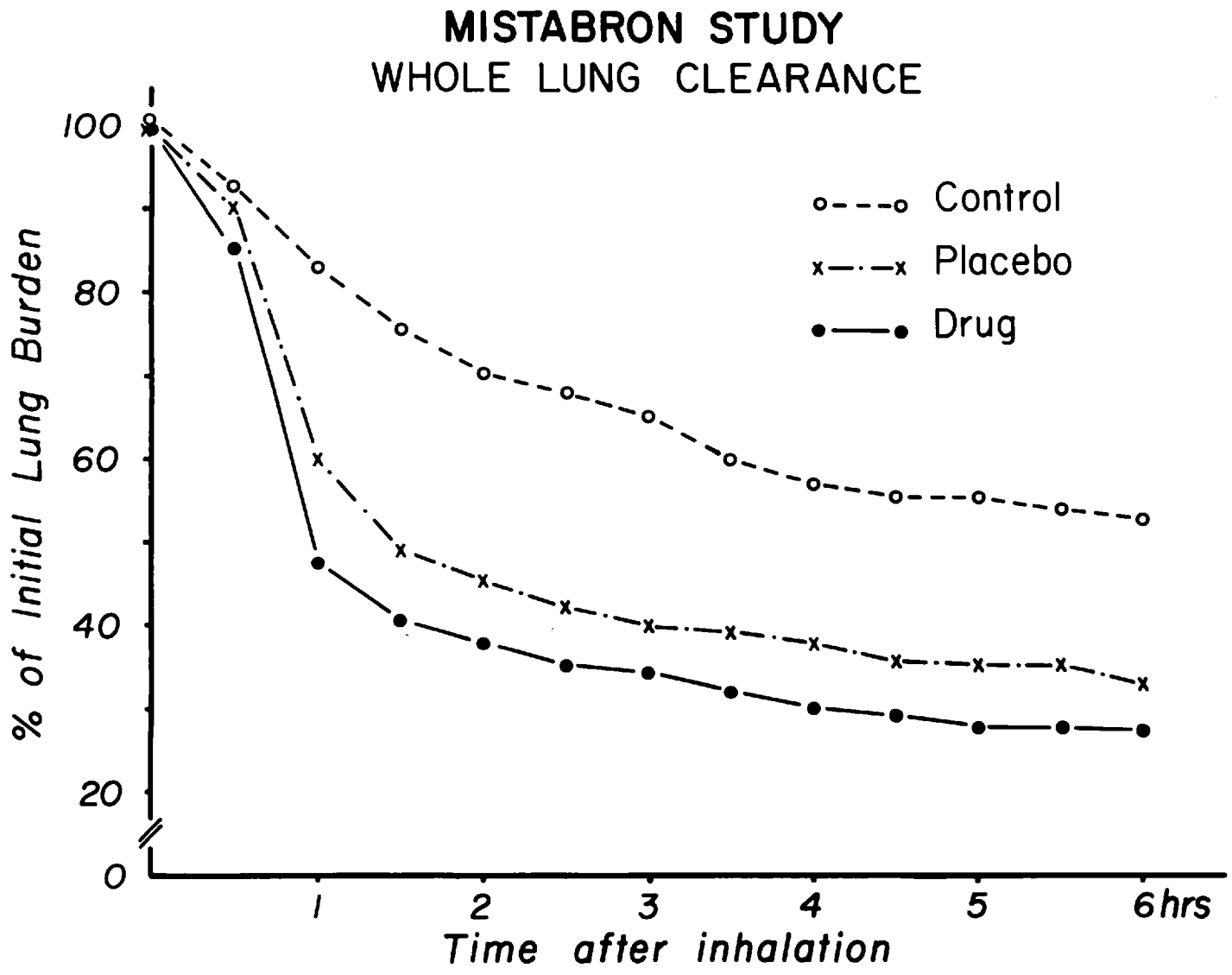

MISTABRON STUDY
WHOLE LUNG CLEARANCE
100
80
60
40
20
0
% of Initial Lung Burden
Control
Placebo
Drug
1
2
3
4
5
6hrs
Time after inhalation

Fig. VIII,2

SUMMARY OF FINDINGS

SUMMARY OF FINDINGS

1. Fucose, neuraminic acid, sulphate and mannose have been estimated in sputum of normal subjects and of patients with chronic bronchitis, asthma, cystic fibrosis, bronchiectasis and bronchorrhoea. Saliva and serum have also been examined.

2. Fucose and sulphate have been used as marker substances of bronchial glycoprotein, mannose as a marker of serum glycoproteins and neuraminic acid of both bronchial and serum glycoproteins.

3. Mannose has been used for the first time as a marker of serum glycoproteins and seems to be a satisfactory marker of inflammatory exudate.

4. Absolute levels of marker substances can be a useful tool for assessing the relative contributions of serum transudate and bronchial gland secretion to sputum.

5. Inhalation of prostaglandin F2α has been shown to be a simple and reliable method to obtain sputum from normal subjects.

6. The sputum produced after inhalation of prostaglandin F2α has the rheological and chemical features of mucoid chronic bronchitis sputum.

7. The serum transudate component in bronchial fluid from normal bronchial tree is negligible.

8. The bronchial glycoprotein secreted by normal mucus-secreting structures is less sialylated and less sulphated than in disease.

9. Macroscopic examination of sputum is, by an experienced observer, a reliable method for assessing degree of purulence.

10. Infection increases the serum transudate component as well as the concentration of bronchial mucus secretion.

11. Infection produces a shift to acid glycoprotein, increase in sialo and sulfomucins.

12. Differences in chemical constituents of sputum between macroscopic types were found to be greater than between diseases.

13. Detailed clinical information is essential when comparing sputum from various diseases, in particular in asthmatic patients.

14. Comparison between diseases should be always carried out in the same macroscopic type of sputum.

15. The chemical features of intrinsic asthma sputum are closer to chronic bronchitis sputum than to extrinsic asthma.

16. When chronic bronchitis is associated with the features of either intrinsic asthma or extrinsic asthma, the chemical features of sputum are closer to chronic bronchitis sputum than to that of extrinsic asthma sputum.

17. In cystic fibrosis, even macroscopically mucoid sputum samples contain considerable amounts of DNA. Estimations of DNA should be carried out in mucoid cystic fibrosis sputum since the presence of pus may contribute to the rheological and chemical features of sputum.

18. A diurnal variation in neuraminic acid, fucose and dry weight has been found in the sputum

of patients with chronic bronchitis. Evidence is presented that the variation is due to variation in the amount of bronchial secretion rather than tissue fluid.

19. A seasonal variation in neuraminic acid, fucose and dry weight has been found in sputum from patients with chronic bronchitis. It is suggested that changes in atmospheric pollution may be partly responsible for this seasonal variation.

20. Natural variation in chemical constituents of sputum should be taken into account when planning a drug trial and assessing the effect of drugs on the rheological properties and chemical constituents of sputum.

21. In chronic bronchitis the levels of chemical constituents of sputum for an individual patient vary little over the relatively long period of several years. It is suggested that the sputum produced by an individual has a characteristic chemical profile.

22. Steroids reduced sputum volume by inhibiting bronchial gland secretion in patients with

bronchorrhoea associated with chronic bronchitis or intrinsic asthma with high levels of NANA and/or low NANA/Fucose ratio.

23. In patients with bronchorrhoea associated with extrinsic or intrinsic asthma with low levels of NANA and high NANA/Fucose ratio steroids reduced sputum volume by decreasing the serum transudate component.

24. In a patient with alveolar cell carcinoma the failure to reduce sputum volume either with steroids, atropine or fluid restriction suggests that the secretion is autonomous.

25. Long term atropine reduced sputum volume by inhibiting bronchial gland secretion in patients with bronchorroea associated with chronic bronchitis or intrinsic asthma.

26. In patients with or without bronchorroea a single dose of atropine had no inhibitory effect on bronchial gland secretion. Evidence is presented that atropine did not increase sputum viscosity.

27. The difficulty in producing sputum after a single dose of atropine is due to its inhibitory effect on salivary secretion.

28. In patients with chronic bronchitis, sodium 2 mercapto ethane sulphonate had no immediate effect on either sputum viscosity, volume or chemical constituents of sputum or on lung clearance.

29. In patients with cystic fibrosis, producing mucopurulent or purulent sputum, long term treatment with sodium 2 mercapto ethane sulphonate had no effect on sputum viscosity or on chemical constituents. It is suggested that the failure to reduce sputum viscosity is due to the presence of deoxyribonucleoprotein fibres thus preventing the mucolytic reaching the glycoprotein fibres.

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