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(54) Benævnelse: **ANVENDELSE AF OLIGOURONATER TIL BEHANDLING AF SLIMHYPERVISKOSITET**

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DESCRIPTION

[0001] This invention relates to treatment of human cystic fibrosis patients to combat elevated mucosal viscosity in the digestive tract, e.g. to facilitate faecal clearance, e.g. to combat constipation.

[0002] Cystic fibrosis is the most common lethal genetic disease in European populations. The disease is caused by a mutation in the gene that codes for the cystic fibrosis transmembrane regulator (CFTR), a chloride channel that is present in secretory and other cells in the body. The disease is characterized by the presence of thick, intractable mucus secretions in the body that can lead to lung disease, sinusitis, digestive problems and infertility. The normal pattern of mucociliary clearance in the lungs fails to clear the unduly viscous mucus which becomes colonized by microorganisms, which in turn increases mucus viscosity and may lead to chronic lung inflammation and obstruction. Lung disease is thus the biggest health problem for the majority of CF patients and is the major cause of death.

[0003] Mucus is a normal secretion of the entire respiratory tract, including the lungs. Its primary function is as part of the mucociliary clearance system that keeps the lungs clean and protects against infection. The mucociliary clearance system has three main components: mucus; cilia; and the airway surface liquid. The epithelial surface thus comprises mucus secreting goblet cells and ciliated epithelial cells with an overlying layer of airway surface liquid and above that a layer of mucus, into which the tips of the cilia protrude. The mucus is a sticky gel material composed primarily of water (about 95 % wt.) and mucins, gel forming molecules responsible for the physical properties of the mucus. The cilia are small hair-like projections from the surface of the epithelial cells, which beat rhythmically in the watery, non-viscous airway surface liquid with their tips immersed in the mucus layer. The mucus layer forms a sticky blanket on the lung surface that traps bacteria, viruses, inhaled particles, environmental pollutants and cell debris. The beating of the cilia serves to propel this mucus blanket and anything trapped in it towards the mouth and out of the lungs. Under normal conditions, the mucociliary clearance system functions effectively and the lungs are kept clean and free of infection. If the system is overwhelmed, there is a second line of defence - cough. Thus when increased levels of mucus are secreted in response to irritation or inflammation, e.g. due to inhaled particles or infection, the mucus is projected out of the lungs by the cough reflex.

[0004] In CF patients the mucus in the lung is thicker and more viscous than normal, and this thicker mucus is not so easily transported by the cilia. As a result the mucociliary clearance system is compromised and the lungs are more vulnerable to infection. In addition, the lungs of CF patients appear to be in a hyper-inflammatory state with a continual low level of inflammation and a heightened response to agents that normally cause inflammation. This is problematic as part of the response to inflammation is increased production of mucus. The increased mucus builds up if it is too thick to be cleared by the mucociliary clearance system or coughing, lung capacity is reduced and the exchange of oxygen across the mucosa is decreased. This provides an ideal environment for bacterial colonisation, a serious problem for

CF patients as it also causes inflammation and activates the immune response. This leads not only to increased mucus secretion but also an increased presence of immune response cells and agents such as macrophages and lysozymes. As bacteria and macrophages die, their cell contents are released into the mucus and these include viscous molecules such as DNA. Furthermore, some of the bacteria, e.g. *Burkholderia sp.* and *Pseudomonas aeruginosa,* also secrete highly viscous polysaccharides, in the latter case alginates. These molecules further increase the viscosity of the mucus, in the case of the alginates apparently by interaction with the mucin matrix of the mucus but in the case of DNA apparently by increasing the viscosity of the sol phase within the mucin matrix.

[0005] Maintaining the mucus in a form capable of being transported by the cilia is thus a key goal of treatment of CF. Agents which simply break down its gel-like structure would result in fluid which was as untransportable as the hyperviscous mucus of the CF patient. It is important therefore that any treatment agent should not break down the gel matrix formed by the glycoprotein mucins.

[0006] CF patients, especially infants, and the elderly in general, suffer particularly from constipation. By reducing mucosal hyperviscosity in the gut this problem also may be addressed.

[0007] US 2003/0013678 suggests the use of polygalacturonides (obtainable from pectin) as a food additive, and teaches that such molecules have a "roughage effect" which is beneficial in prevention or treatment of a number of colonic conditions including constipation, diverticulosis and colon carcinoma.

[0008] We have found that mucosal hyperviscosity can be reduced using an oligouronate, in particular one containing two to thirty monomer residues.

[0009] Viewed from one aspect the invention provides a physiologically-tolerable oligoguluronate having a molecular weight in the range 350 Da to 6000 Da for use in a method of treatment of a human subject suffering from cystic fibrosis, wherein said treatment is to reduce mucosal hyperviscosity in the digestive tract, and said method comprises application of said oligoguluronate to a mucosal surface in the digestive tract of said subject. Viewed from a further aspect the invention provides the use of a physiologically tolerable oligoguluronate having a molecular weight in the range 350 Da to 6000 Da in the manufacture of a medicament for use in the treatment of mucosal hyperviscosity in the digestive tract of a human subject suffering from cystic fibrosis, wherein said treatment comprises application of said medicament to a mucosal surface in the digestive tract of said subject.

[0010] The application of the oligoguluronate may be by any means which delivers it to a mucus secreting or mucus carrying surface in the gastrointestinal tract of the patient. Introduction of the oligoguluronate may be oral or rectal, for example in the form of solutions, suspensions, dispersions, syrups, powders, tablets, coated tablets, capsules or suppositories. Where constipation is to be combatted, the oligoguluronate may be used as a food additive or supplement or may be present in a vitamin or mineral pill. The oligoguluronate for use according to the invention may thus be provided in the context of a foodstuff or food additive comprising a nutrient material, e.g. a material containing protein and/or carbohydrate, and the physiologically tolerable oligoguluronate.

[0011] The counterions for the oligoguluronate may be any of the physiologically tolerable ions commonly used for charged drug substances, e.g. sodium, potassium, ammonium, chloride, mesylate, meglumine, etc. Ions which promote alginate gelation, e.g. group 2 metals, however will preferably not be used.

[0012] While the oligoguluronate may be a synthetic material, it is preferably a derivative of a naturally occurring polysaccharide. It is preferably a 3- to 28-mer, e.g. a 4-to 25-mer, a 6- to 22-mer, an 8- to 15-mer or a 10-mer, e.g. having a molecular weight in the range 750 to 4500 Da. It may be a single compound or it may be a mixture of oligoguluronates, e.g. of a range of degrees of polymerization. Moreover, the monomeric residues in the oligoguluronate, i.e. the monosaccharide groups, may be the same or different.

[0013] Oligoguluronates are readily accessible from natural sources since many natural polysaccharides contain guluronic acid residues.

[0014] Polysaccharide to oligosaccharide cleavage to produce oligoguluronates useable according to the present invention may be performed using conventional polysaccharide lysis techniques such as enzymatic digestion and acid hydrolysis. Oligoguluronates may then be separated from the polysaccharide breakdown products chromatographically using an ion exchange resin or by fractionated precipitation or solubilization.

[0015] Examples of polysaccharides containing guluronates include naturally occurring polysaccharides (such as alginates) and chemically modified polysaccharides, including but not limited to polysaccharides modified to add charged groups (such as carboxylated or carboxymethylated glycans), and polysaccharides modified to alter flexibility (e.g. by periodate oxidation). Suitable polysaccharides are discussed for example in "Handbook of Hydrocolloids", Ed. Phillips and Williams, CRC, Boca Raton, Florida, USA, 2000. The use of alginates however is especially preferred as these naturally occur as block copolymers of manuronic (M) and guluronic (G) acids and G-block oligomers can readily be produced from alginate source materials.

[0016] Where alginates are used as the starting material for preparation of the oligoguluronate, the guluronic acid content may if desired be increased by epimerization with mannouronan C-5 epimerases from *A. vinelandii.*

[0017] Oligoguluronic acids suitable for use according to the invention may conveniently be produced by acid hydrolysis of alginic acid from *Laminaria hyperborea,* dissolution at neutral pH, addition of mineral acid to reduce the pH to 3.4 to precipitate the oligoguluronic acid, washing with weak acid, resuspension at neutral pH and freeze drying.

[0018] The physiologically tolerable oligoguluronate for use according to the invention may be provided in the form of a pharmaceutical composition comprising the oligoguluronate together with a physiologically tolerable carrier or excipient, and preferably also a further physiologically tolerable mucosal viscosity reducing agent, e.g. a nucleic acid cleaving enzyme (e.g. a DNAse such as DNase I), gelsolin, a thiol reducing agent, an acetylcysteine, sodium chloride, an uncharged low molecular weight polysaccharide (e.g. dextran), arginine (or other nitric oxide precursors or synthesis stimulators), or an anionic polyamino acid (e.g. poly ASP or poly GLU). The use of a DNase is especially preferred. The oligoguluronate may be administered typically in doses of 1 to 10000 mg, especially 10 to 1000 mg for an adult human. The optimum dose may readily be determined by routine dosage ranging experiments, optionally following initial investigation of an animal model, e.g. a dog model.

[0019] Such compositions may be produced using conventional pharmaceutical carriers and excipients, e.g. solvents (such as water), osmolality modifiers, flavours, pH regulators, etc. They may contain additional active components, for example agents which serve to break down biopolymers not involved in the mucin matrix of the mucus (e.g. DNase, particularly rhDNase), antibacterial agents, and antiinflammatories. Combination therapy using a such further agent and the oligoguluronate, administered separately or together, is a particularly preferred aspect of the invention. Such further agents may be used in their normal doses or even at lower doses, e.g. 50 % of normal dose.

[0020] The invention will now be described further with reference to the following non-limiting Examples and the accompanying drawings, in which:

Figures 1a to If are plots of complex modulus over time for a mucin-alginate gel and for the same gel dosed with dextran, polyethylene glycol, DNA, sodium galacturonate oligomer and sodium guluronate oligomer respectively;

Figures 2 and 3 are bar charts showing the complex modulus (G*) and the phase angle of the same gels at the end of gelation as a percentage of the control;

Figure 4 is a bar chart showing the flow stress of the same gels, again as a percentage of the control; and

Figures 5a to 5c are photomicrographs of mucin untreated and treated with alginate and/or sodium guluronate oligomers.

EXAMPLE 1

[0021] The effect of five oligomeric materials on a model mucin-alginate gel were tested:-

1. a) Low molecular weight dextran (6000 Da)

2. b) Low molecular weight PEG (3350 Da)
3. c) Low molecular weight DNA
4. d) Sodium galacturonate oligomers
5. e) Sodium guluronate oligomers (G blocks)

[0022] The sodium guluronate oligomer was prepared as described above and had the following characteristics:
F_G 0.87, F_M 0.13, F_{GG} 0.83, F_{GM}=F_{MG} 0.05, F_{MM} 0.08, F_{GGM}=F_{MMG} 0.04, F_{MGM} 0.02, F_{GGG} 0.79, DP 19.

Materials

[0023] Control gel consisted of 18 mg/ml mucin + 0.6 mg/ml alginate in 0.05 M NaCl.

[0024] Test gels consisted of 18 mg/ml mucin + 0.6 mg/ml alginate + 4 mg/ml test material in 0.05 M NaCl

Rheological tests

[0025] Gelation - immediately after mixing the sample was loaded into the rheometer at 37°C. The temperature was cooled to 10°C (0.5 degree/min) and then held at 10°C for 18 hours. The behaviour of the sample and gel development was measured continuously.

[0026] Frequency sweep - after gelation the temperature of the sample was raised to 37°C and the rheological behaviour monitored as a function of frequency.

[0027] Stress sweep - at 37°C, the behaviour of the gel was monitored as a function of increasing applied stress.

Gelation

[0028] The studies demonstrated that sodium guluronate oligomers (G blocks) are able to displace alginate in mucin-alginate interactions as seen by the decrease in the complex modulus of the gel (G*) over time after the initial gelation. For the five test gels and the control gel this is shown in Figures 1a to If respectively.

[0029] Of the other oligomeric materials tested only sodium galacturonate oligomers had a similar effect, showing a decrease in the moduli values over time. The other materials (DNA, PEG, dextran) caused a delay in gelation but the gel continued to develop over time as shown by the increase in complex modulus.

[0030] The complex modulus and phase angle of the gels at the end of the gelling period are plotted in Figures 2 and 3. The complex modulus is a measure of the total response or strength of the system and the phase angle concerns the balance between liquid-like and solid-like behaviour with a higher phase angle indicating greater liquid-like behaviour. Sodium guluronate oligomers (G blocks) caused the greatest drop in G* and the greatest increase in the phase angle.

Gel behaviour from the frequency sweep

[0031] All the gels showed frequency sweep behaviour typical of weak viscoelastic gels.

Flow stress from the stress sweep

[0032] A stress sweep was used to determine the stress needed to induce flow in the system. This is plotted in Figure 4 as a % of the stress needed to induce flow in the control gel. This gives an indication of how much the added material has weakened the gel matrix. G blocks (sodium guluronate oligomers) had the most pronounced effect on the flow stress followed by sodium galacturonate oligomers.

<u>Example 2</u>

<u>Ferning</u>

[0033] Mucin compositions were prepared as follows:

1. a) 18 mg mucin per mL 0.05 M aqueous NaCl;
2. b) 18 mg mucin and 1 mg alginate per mL 0.05 M aqueous NaCl; and
3. c) 18 mg mucin, 0.4 mg alginate, and 0.6 mg sodium guluronate oligomer (as in Example 1) per mL 0.05 M aqueous NaCl.

[0034] The compositions were air dried and photomicrographs were recorded. These, at the same magnifications, are shown in Figures 5a to 5c for compositions (a) to (c) respectively. It can clearly be seen that the guluronate oligomer promotes ferning.

<u>EXAMPLE 3</u>

Constipation Medicament

[0035] A 5-50 ml oral dose of an aqueous liquid consists of 5-30 % of the sodium salt of a guluronic acid block with a purity of at least 75 % guluronate and a degree of polymerisation between 5 and 20, and if necessary, a preservative agent. The liquid may also be combined with other compounds which have additional health benefits, e.g. vitamins.

REFERENCES CITED IN THE DESCRIPTION

Cited references

Patent documents cited in the description

- US20030013678A [0007]

Non-patent literature cited in the description

- Handbook of HydrocolloidsCRC20000000 [0015]

Patentkrav

1. Fysiologisk acceptabel oligoguluronat med en molekylevægt i området 350 Da til 6000 Da til anvendelse i en fremgangsmåde til behandling af et menneskeligt individ, som lider af cystisk fibrose, hvor nævnte behandling skal reducere slimhindehyperviskositet i fordøjelseskanalen, og nævnte fremgangsmåde omfatter anvendelse af nævnte oligoguluronat på slimhindeoverfladen i fordøjelseskanalen hos nævnte individ.

2. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge krav 1, hvor nævnte oligoguluronat er en 3- til 28-mer.

3. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge krav 2, hvor nævnte oligoguluronat er en 4- til 25-mer.

4. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge et hvilket som helst af kravene 1 til 3, hvor nævnte oligoguluronat har en molekylevægt i området fra 750 Da til 4500 Da.

5. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge krav 4, i form af en oral dosis af en vandig væske omfattende 5-30% af natriumsaltet af en oligoguluronat omfattende mindst 75% guluronat og med en polymeriseringsgrad på mellem 5 og 20.

6. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor nævnte behandling er mod forstoppelse.

7. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor nævnte oligoguluronat i nævnte fremgangsmåde indføres i tarmen ved oral eller rektal indgivelse.

8. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor nævnte oligoguluronat er indeholdt i en farmaceutisk sammensætning omfattende nævnte oligoguluronat og en fysiologisk acceptabel bærer eller excipiens.

9. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge krav 8, hvor nævnte farmaceutiske sammensætning yderligere omfatter et yderligere fysiologisk acceptabelt slimhindeviskositetsreducerende middel valgt fra et nukleinsyrespaltende enzym, gelsolin, et thiolreducerende middel, acetylcystein, natriumchlorid, et uladet polysaccharid med lav molekylevægt, et nitrogenoxid-udgangsstof eller en syntesestimulator eller en anionisk polyaminosyre.

10. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge krav 8 eller 9, hvor nævnte farmaceutiske sammensætning yderligere omfatter et antibakterielt eller antiinflammatorisk middel.

11. Den fysiologisk acceptable oligoguluronat til anvendelse ifølge krav 6, hvor nævnte oligoguluronat indgives i form af et fødevareadditiv eller -supplement eller i en vitamin- eller mineralpille.

12. Anvendelse af en fysiologisk acceptabel oligoguluronat som defineret i krav 1, til fremstilling af et medikament til behandlingen af slimhindehyperviskositet i fordøjelseskanalen hos et menneskeligt individ, som lider af cystisk fibrose, hvor nævnte behandling omfatter anvendelse af nævnte medikament på en slimhindeoverflade i fordøjelseskanalen hos nævnte individ.

13. Anvendelsen ifølge krav 12, hvor nævnte oligoguluronat, tilstand, indgivelse eller sammensætning er som defineret i et hvilket som helst af kravene 2 til 11.

DRAWINGS

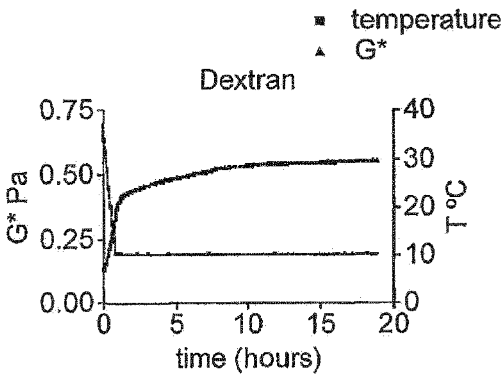


FIG. 1a

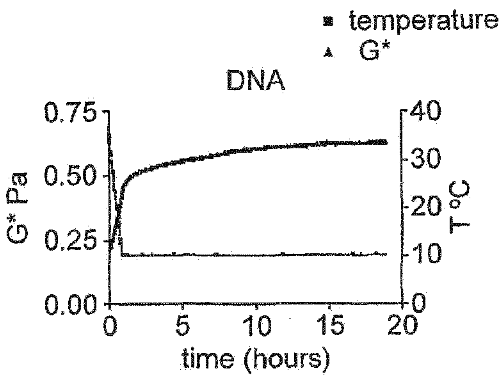


FIG. 1c

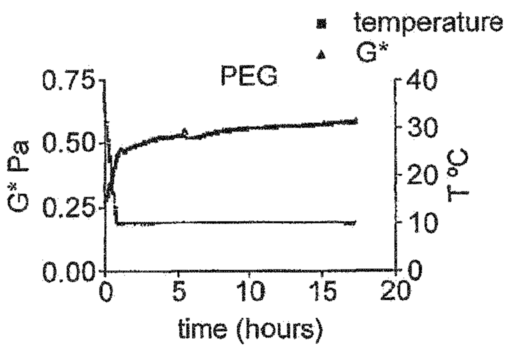


FIG. 1b

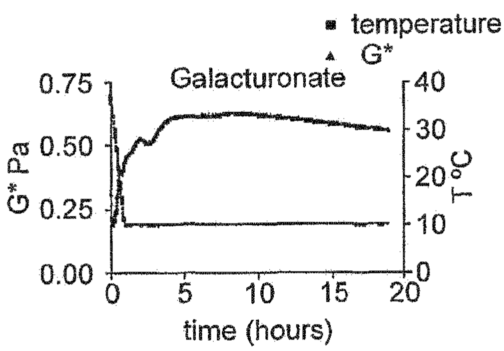


FIG. 1d

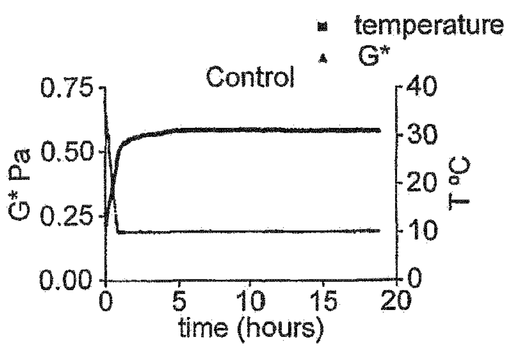


FIG. 1f

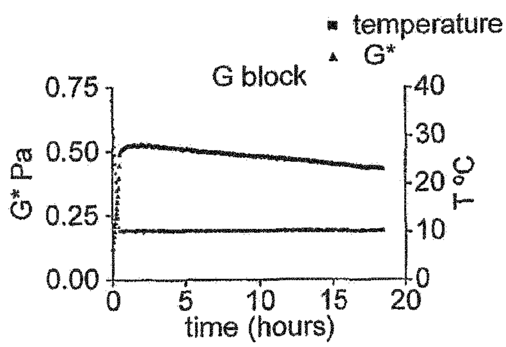


FIG. 1e

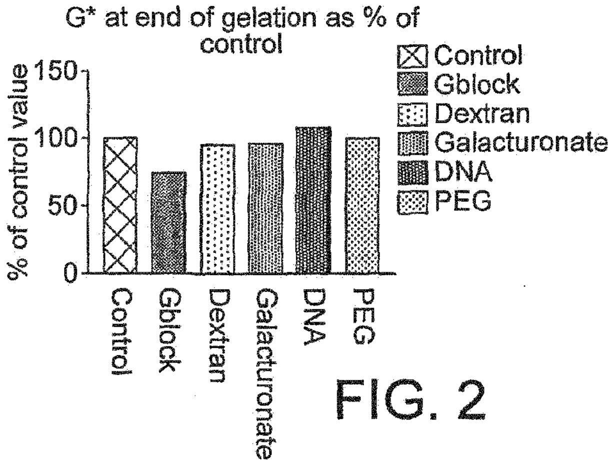


FIG. 2

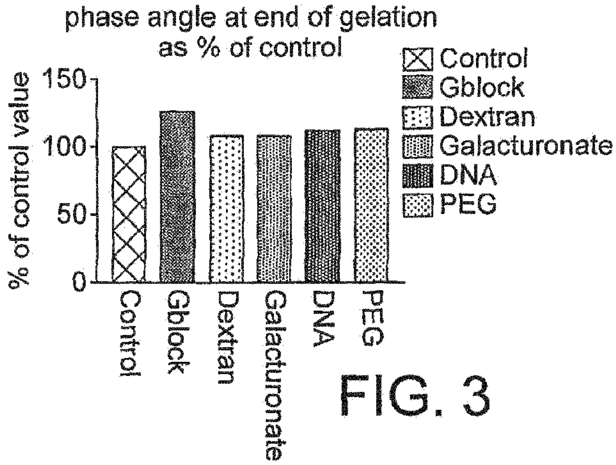


FIG. 3

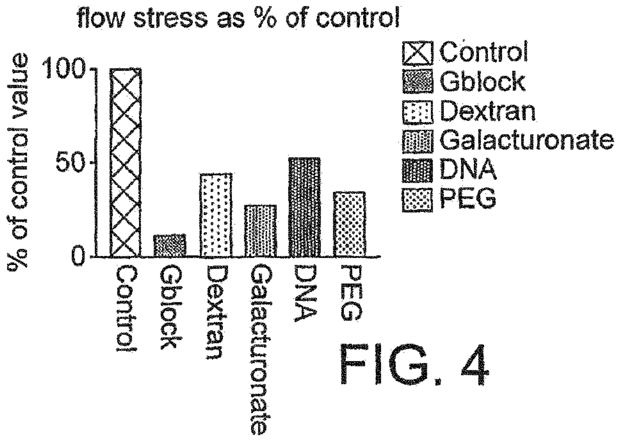
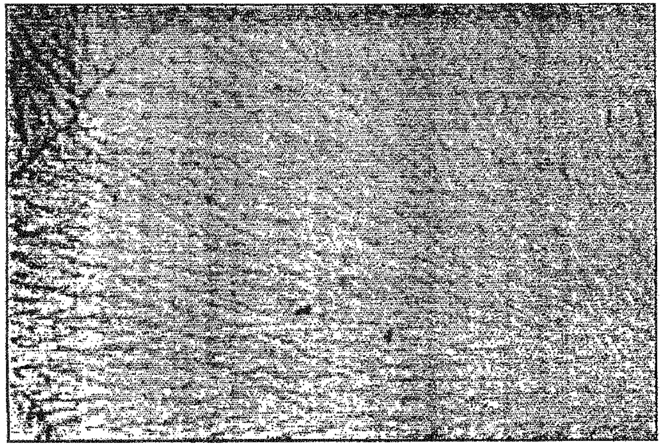


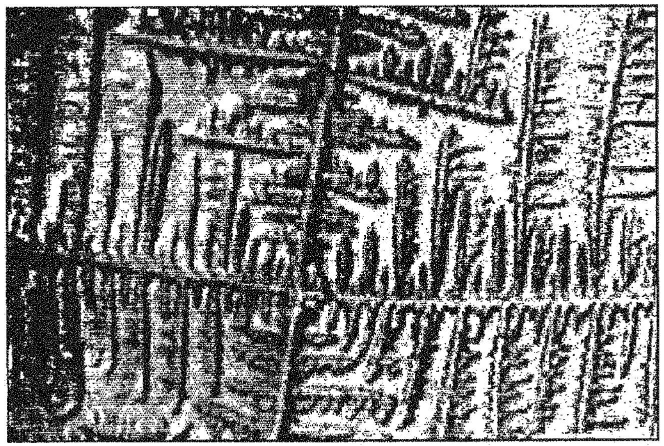
FIG. 4

FIG. 5

A



B



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