Mucopolysaccharide from Patients with Cystic Fibrosis of the Pancreas

Abstract. *Physical and immunologic methods were used to demonstrate the presence of an apparently unique glycoprotein in stool and tissue of patients having cystic fibrosis of the pancreas. The detection of a molecular structure specific for the disease is consistent with the apparent hereditary nature of the affection.*

Cystic fibrosis of the pancreas (CFP), a hereditary congenital disorder, is characterized by the production of a mucus of abnormal viscosity (*1*). Elaboration of an abnormal mucus is considered the pathophysiologic defect in the disease, but chemical studies have failed to reveal unambiguous differences between the organic constituents of mucus from patients with CFP and from normal individuals.

Dische *et al.* (*2*) isolated from the duodenal fluid of patients with this disease a mucoid that, in certain physiochemical aspects, differed from corresponding material present in normals; it was susceptible to denaturation and lost its solubility in water after exposure to a mixture of ethanol and benzene; analyses indicated an increase in fractions having limited solubility and low content of sialic acid. These results do not point directly to the presence of a unique glycoprotein; they could be adequately explained by a shift in the ratios of the constituent glycoproteins normally present as a heterogenous mixture in duodenal fluid.

If a unique glycoprotein were present in mucus secreted by patients with the disease, structural abnormality would probably be reflected in antigenic properties, and immunologic methods might be used to study this material (*3*). We now describe the demonstration and isolation of an apparently unique glycoprotein found by immunologic and physical techniques in stool of patients with CFP.

The preparative procedure includes the following steps: Stool (1 part) is mixed with water (2 parts) in a Potter-Elvejehm-type homogenizer and centrifuged for 20 minutes at 26,000*g*. To 1 volume of the clear supernatant is added 9 volumes of 1 : 1 (vol : vol) mixture of benzene and absolute ethanol; the mixture is allowed to stand for 2 hours at 4°C. The precipitate is compacted by centrifugation and washed three times with suitable volumes of

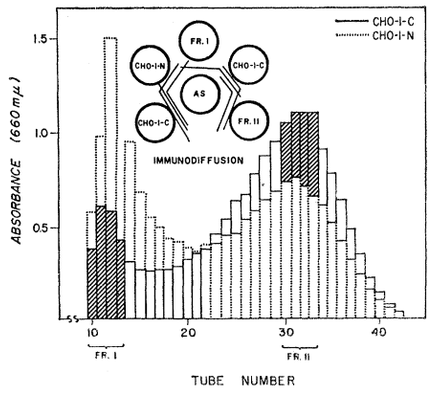

Fig. 1 (Bottom). Separation of CHO-I-C and CHO-I-N on Sephadex G-200; eluent, 1-percent acetic acid. (Top) A drawing of an agar-gel immunodiffusion plane shows fractions 1 and 2 (*FR. I, FR. II*) of CHO-I-C (as indicated on the curve) in comparative analysis with CHO-I-C and CHO-I-N tested against an antiserum to CHO-I-C (*AS*).

95-percent ethanol; when all traces of benzene disappear it is extracted by vigorous stirring with 3 portions each of 10 ml of H_2O per 1 ml of packed sediment. Extraction yields two fractions: one is soluble in water; the other, insoluble.

The remaining water-insoluble precipitate is dried by washing with successive portions of ethanol and finally ether. To each 200 mg of dry powder, 10 ml of 1-percent (by volume) acetic acid is added, and the mixture is placed in a boiling-water bath for 4 hours before the sediment is removed by centrifugation. The sediment is discarded and the acetic acid solution is dialyzed

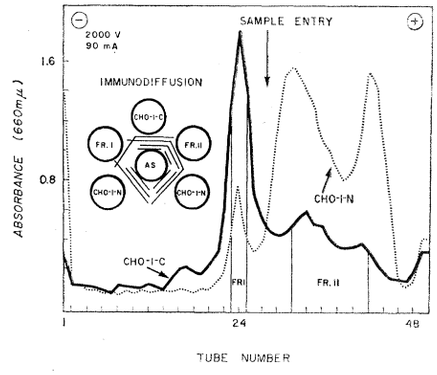

Fig. 2. Separation of CHO-I-C (solid line) and CHO-I-N (broken line) with continuous-flow electrophoresis in 1.0*N* acetic acid, *p*H 2.3. Optical density at 600 mμ was measured after reaction with Bial's reagent. Fractions 1 and 2 (*FR. I, FR. II*) of CHO-I-C (as indicated on the curve) are drawn in comparative analysis with CHO-I-C and CHO-I-N tested against an antiserum to CHO-I-C (*AS*) on agar-gel immunodiffusion.

against distilled water for 78 hours.

The product obtained by dialysis and lyophilization of the acid hydrolyzate was designated CHO-I; it was prepared individually from 50 CFP patients (CHO-I-C), from 25 healthy controls (children and adults; CHO-I-N), and from a depancreatized but otherwise normal adult. Antiserums against CHO-I-C and CHO-I-N were prepared in rabbits by six weekly injections of the antigen in Freund's adjuvant.

When the antigens were tested in agar diffusion against a rabbit antiserum induced with CHO-I-C, CHO-I-C regularly produced three or four precipitin lines, while CHO-I-N produced either two or three lines—which always gave reactions of identity with all the precipitates of similar positions in the preparations from CFP patients. However, CHO-I-C always produced one band that failed to give a reaction of identity with any of the precipitates formed by CHO-I-N, regardless of whether the CHO-I-N produced two or three precipitation lines. Absorption studies yielded further confirmation of the presence of an apparently unique antigen in CHO-I-C: when antiserums against CHO-I-C were absorbed with CHO-I-N, they still gave rise to a precipitin band when tested against CHO-I-C, while the reaction with CHO-I-N was completely abolished.

The polysaccharide was further purified by three physical methods: (i) molecular sieving, (ii) continuous-flow electrophoresis, and (iii) equilibrium-gradient centrifugation; each method showed quite clearly that CHO-I-C and CHO-I-N differed in composition. These differences can best be appreciated by comparing the shapes of the curves obtained by fractionation of each material with Sephadex G-200 (Fig. 1) and by continuous-flow electrophoresis (Fig. 2). Each method permitted isolation of the apparently unique mucopolysaccharide (fraction 1) from CHO-I-C. The fractions 1 obtained by the three methods gave reactions of identity with each other and with a single component of unfractionated CHO-I-C; fraction 1 was not present in CHO-I-N.

By immunofluorescence it was also possible to localize the unique glycoprotein in the cytoplasm of epithelial cells of colonic and pulmonary glands. When tissues from CFP patients were treated with the rabbit antiserum against CHO-I-C that was exhaustively absorbed with CHO-I-N, and were then exposed to fluorescein-labeled goat

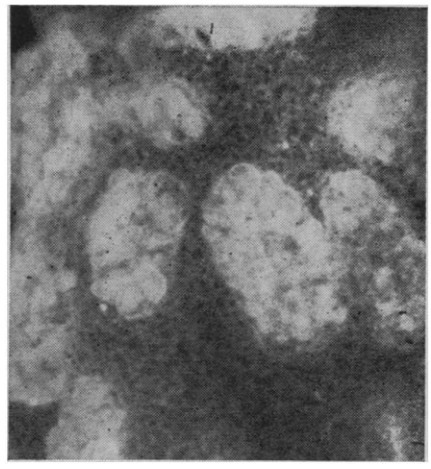

Fig. 3. Biopsy of rectal mucosa, from a patient with CFP, treated first with rabbit antiserum to CHO-I-C and then with fluorescein-labeled antiserum to rabbit γ-globulin; under the fluorescent microscope.

antiserum (*4*) against rabbit γ-globulin, antibodies were specifically absorbed onto these glandular structures (Fig. 3). Also, when the absorbed rabbit antiserum to CHO-I-C (which was specific for the abnormal unique glycoprotein) was conjugated with fluorescein (*5*) and used to stain the tissues, the results were the same. Appropriate control experiments confirmed the specificity of the reaction.

It may be argued that the immunologic techniques that we used are insufficiently sensitive to detect minor amounts of the unique antigen that may be present in CHO-I-N. Even if the antigen were present in an amount too small to give a positive reaction with the appropriate antibody, it might be capable of inducing formation of specific antibody when used for immunization. This possibility was tested: the precipitin produced by the unique antigen did not appear when CHO-I-C was allowed to react with an antiserum to CHO-I-N. Applications of other immunologic methods, such as hemagglutination and latex-particle agglutination, for identification of the antigen in CHO-I-N have failed thus far.

We considered the possibility that the absence of pancreatic enzymes might be responsible for the appearance of the mucopolysaccharide in CHO-I-C, because the unique antigen could not be demonstrated in the CHO-I-C prepared from stool of patients with CFP and receiving pancreatic enzyme (Cotazyme) orally. However, the antigen peculiar to cystic fibrosis of the pancreas could be detected under these conditions in the water-soluble fraction of the ethanol-benzene precipitate; this same fraction from normal subjects did not react with the antiserums against CHO-I-C to produce the unique precipitate, nor did the CHO-I prepared from stool of a depancreatized adult react in this way.

Our findings justify two conclusions: (i) absence of pancreatic enzymes is not responsible for the appearance of the unique mucopolysaccharide in the patients, and (ii) pancreatic enzymes influence the susceptibility of the unique material to denaturization, but do not alter its antigenic structure. When bile was used as source material for preparation of CHO-I-C the unique antigen appeared in the water-soluble fraction.

We have but limited knowledge of the antigen's chemical structure. Velocity centrifugation indicates a molecular weight exceeding 1 million. Strong-acid hydrolysis released reducing sugars (Somogyi-Nelson) (*6*) and also a substance that gives a color reaction, with Bial's orcinol (*7*) reagent, characteristic of pentoses. Treatment with a peptidase, Pronase (*8*), did not alter the specific antigenic structure although a considerable amount of amino acid was split off—as revealed by titration and amino acid analysis (*9*). The fact that the digested product gave a reaction of complete identity with the undigested material when tested by immunodiffusion does not exclude the possibility that amino acids are at least partly responsible for the specific antigenic structure; hydrolysis in strong acid, after Pronase treatment and subsequent dialysis, revealed a number of amino acids. The intrinsic structure of the mucopolysaccharide thus probably includes amino acids in addition to carbohydrates.

Our data agree with a hypothesis that the histologic pathology in cystic fibrosis of the pancreas reflects the elaboration of a mucus that is abnormal in structure and physical characteristics.

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Nucleic Acid Guanine: Reaction with the Carcinogen N-Acetoxy-2-Acetylaminofluorene

Abstract. *Reaction of* N-*acetoxy-2-acetylaminofluorene with DNA or RNA at* p*H 7 causes marked increase in absorption at 280 to 320 millimicrons and marked decrease in guanine content. Reaction with guanosine-8-*^{14}*C yields a radioactive fluorescent derivative. The data suggest that metabolic esters of* N-*hydroxy-2-acetylaminofluorene may be intermediates in the binding of this proximate carcinogen to nucleic acids in vivo.*

Studies in our laboratory have shown that the carcinogen 2-acetylaminofluorene (AAF) is metabolized by the rat and other species to *N*-hydroxy-AAF, which is more carcinogenic than the parent amide (*1*). Administration of 9-^{14}C-labeled AAF to rats results in low levels of DNA- and RNA-bound radioactivity in the liver; 2- to 3-times-higher levels are obtained when the *N*-hydroxy derivative is administered (*2, 3*). Identification of the metabolite(s), implicated in this reaction, with tissue nucleic acids is of particular importance in view of the possible causal relation between the reaction of carcinogens with DNA and RNA and the induction of neoplasia (reviewed by Miller and Miller, *4*).

Kriek (*5*) has shown that *N*-hydroxy-2-aminofluorene (*N*-hydroxy-AF) reacts with the guanine bases of RNA or DNA in vitro at *p*H lower than 6. This report shows that the guanine bases of the nucleic acids react readily at neutral *p*H with *N*-acetoxy-AAF,