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ENCLOSURE 17

## PROTEIN-BOUND CARBOHYDRATES AS BIOCHEMICAL CRITERIA IN DIFFERENTIAL DIAGNOSIS OF VARIOUS DISEASE STATES

Principal Investigator: A. S. Evans  
Collaborator: M. F. Dolan, U. S. Naval Hospital  
Technical Assistance: F. A. Quinn, G. E. Routzahn and M. H. Gobbett

The present study was designed to investigate the glycoprotein profile of patients with various diseases at progressive stages of their treatment regimens and to relate those profiles with the individual's response to therapy to define the feasibility of such a test battery (a) as an objective diagnostic aid to estimate disease activity, and (b) as a prognostic tool to aid the physician in in-hospital management and outpatient follow-up.

To date, 507 serum specimens from 390 individuals have been examined. One hundred and twenty of these samples were from 111 individuals with no clinical disease (controls) and 39 were from patients with normal, uncomplicated pregnancies, approximately evenly divided amongst the trimesters. The remaining 348 specimens from 240 individuals were from patients in (or outpatients of) the Cincinnati General Hospital, Cincinnati, Ohio (66 specimens, 29 individuals) or the U. S. Naval Hospital, Bethesda, Maryland (282 specimens, 211 individuals).

The patients were categorized in accordance with the diagnostic nomenclature of the International Classification of Diseases, Eighth Revision, Adapted for Use in the United States (Public Health Service Publication No. 1693). Practically all the disease classes, as set forth therein, were represented in the sampling, with a considerable preponderance of class II, Neoplasms.

The test battery consisted of total protein, total serum globulins, protein-bound neutral hexoses, hexosamines, sialic acid, and fucose. From these analytical data, a number of additional parameters which are still in the process of interpretation were derived.

A major portion of the effort during this preliminary phase of the work has been directed toward critical evaluation and standardization of analytical methods, with especial reference to elimination of or correction for influences of extraneous substances including other carbohydrates on specificity of the various procedures. Consistency in results of protein-bound neutral hexoses was considerably enhanced by automation of the procedure, while interferences in testing for protein-bound hexosamines and sialic acid were apparently minimized by the simple expedient of utilizing a narrow band-pass spectrophotometer.

The most important technical modification, however, was derived from the work of Sobocinski et al.<sup>1</sup> Analyses of a matrix varying neutral hexoses (as galactose and mannose = G-M) and fucose concentrations over each of their ranges enabled derivation

of a correction factor to eliminate the influence of the neutral hexoses on the optical density readings and to obtain values for "true" fucose. Derivation of such a correction factor was made possible by the fact that each of the family of parallel lines generated by increasing amounts of neutral hexoses in the reaction mixture (Figure 31) was displaced by a distance proportionate to the added concentration of galactose-mannose throughout the ranges tested. To our knowledge, this is the first laboratory to be able to correct for nonfucose interference in analysis and to measure such true fucose values

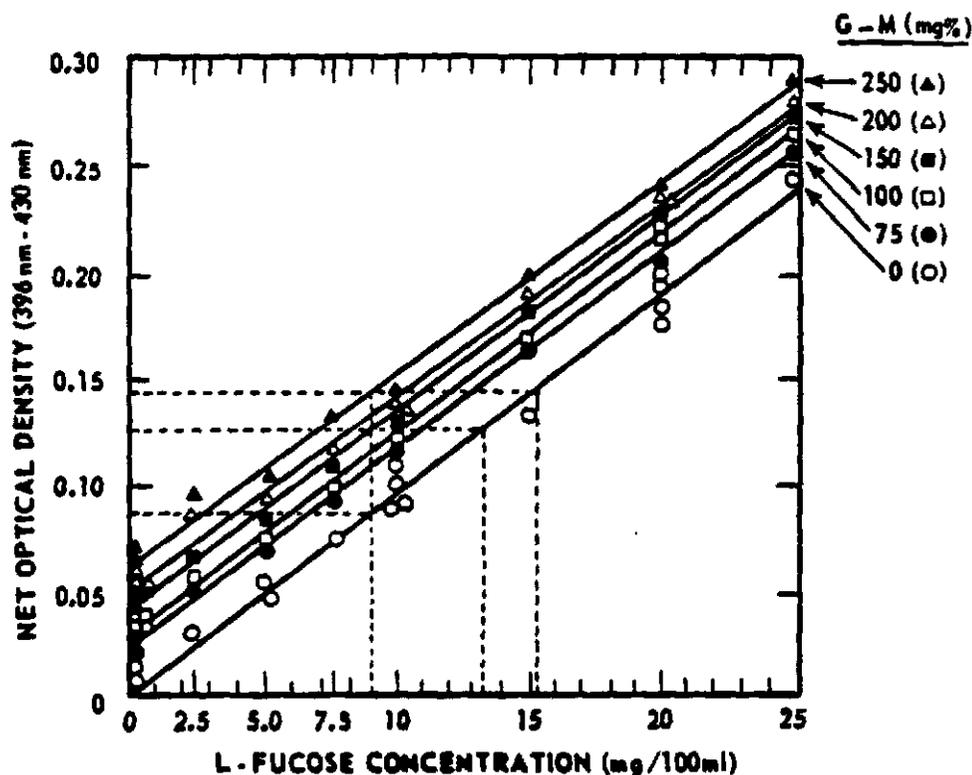


Figure 31. Influence of various concentrations of galactose and mannose (G-M) on the apparent concentration of L-fucose as estimated by the Dische-Shettles CyRS reaction. Each data point represents the mean of duplicate determinations. Regression lines were fitted by the method of least squares.

Interpretation of the data thus far has been empirical and dependent on two basic considerations. Thus, effects of changes in blood volume on the analytical concentration of the various carbohydrate classes were eliminated by calculating the amount of carbohydrate bound per 100 mg protein. More importantly, since albumin is not a glycoprotein, derivation of the carbohydrate to globulin ratio enabled differentiation of absolute from relative changes in the glycoprotein profile in disease states where hypoproteinemia results from loss of circulating albumin.

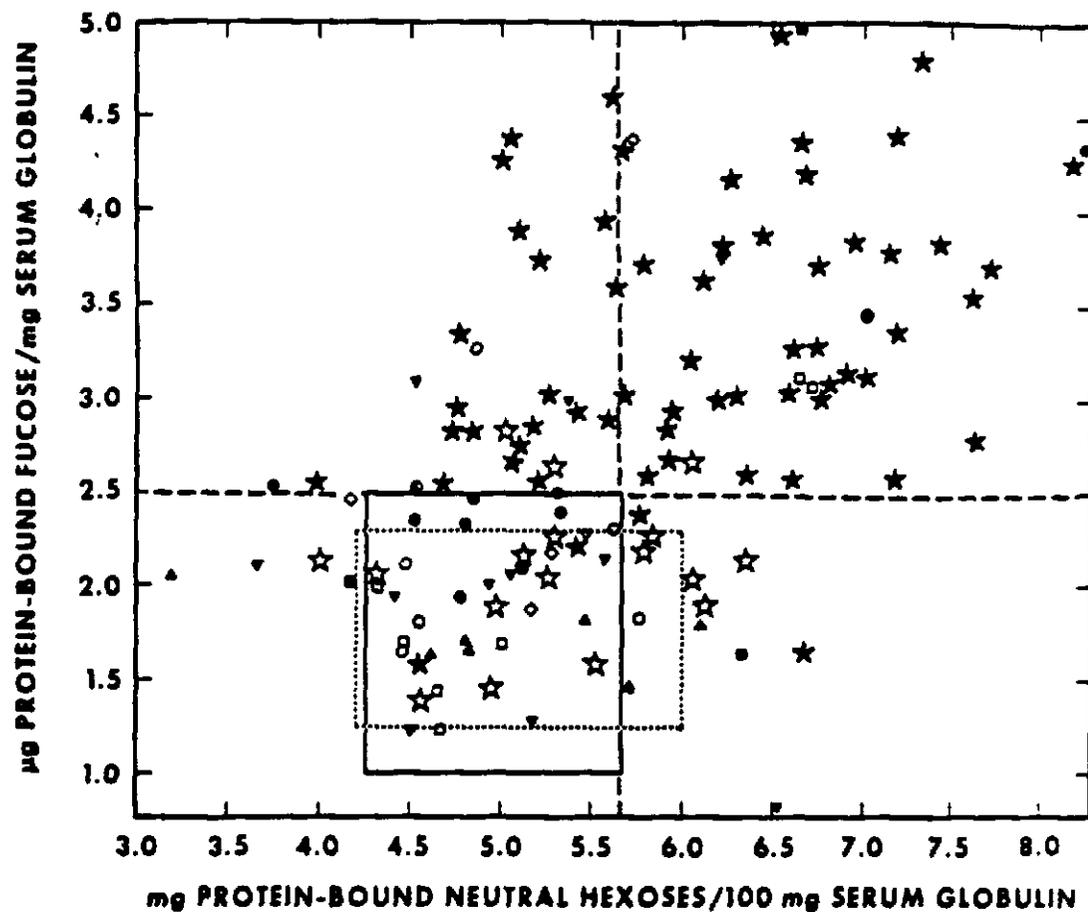
Figure 32 illustrates two of the 27 analytical and derived parameters under study in patients afflicted with a number of diseases who were not receiving treatment at the time of sampling. The most obvious and striking feature of this plot is the distribution of the values of patients with malignant neoplasms, with respect to the fucose content of their serum globulins. Thus, while only 61 of the 79 cases (77.2 percent) had elevated values, of those who were later found to have had some degree of dissemination of their disease, 58 of 62 (93.5 percent) had abnormal fucose to globulin ratios. By contrast, only 3 of 17 cases (17.6 percent) who were clinically or surgically assessed to have localized, potentially resectable tumors were found to have increased values. Such a dichotomy indicates excellent potential for presurgical estimation of the extent of the tumor process. The symbols to the right of the upper limit of normal of the neutral hexose to globulin ratio are interpreted as indicating concurrent infections or inflammatory processes or a proportionately greater metastatic involvement. The fact that 12 of the 50 cases (24.0 percent) with diagnoses other than cancer were abnormal in this test system indicates that these two parameters are not, by themselves, sufficient for original differential diagnostic application. It should be noted, however, that most of these individuals returned rapidly to the normal range with treatment.

Of interest, also, was the finding that, contrary to other biochemical criteria for assessment of tumor activity, normal, uncomplicated pregnancies did not give false positives.

When patients were treated and they experienced varying degrees of amelioration of their disease, their values tended to return toward the norm. The full significance of this finding is yet to be determined, but it appears at this stage to promise objective criteria for at least short-term prognosis.

A unique effort is being made to generate total glycoprotein profiles on these patients, utilizing the entire gamut of the 27 parameters mentioned previously. While trends are evident visually which enable differentiation of some disease classes from others, computer analyses will be necessary to relate the profile of the individual with the medical history and current status of that person.

Comparison of these studies with results with carcinoembryonic antigen and phosphohexoisomerase determinations, which are to be added to the test battery, may give clues to the mechanism of the changes seen and therefore enable refinement of its ultimate (clinical) application.



**INTERPRETATION OF SYMBOLS**

(THE NUMBERS IN PARENTHESES ARE NUMBER OF PATIENTS IN EACH DISEASE CATEGORY.)

- THE SOLID RECTANGLE DELIMITS THE RANGE OF THE NORMAL CONTROLS (120)
- THE DASHED RECTANGLE REPRESENTS THE LIMITS OF THE PREGNANCIES (39)
- ★ = METASTATIC MALIGNANT NEOPLASIA (62)
- ☆ = LOCALIZED MALIGNANT NEOPLASIA (17)
- = ENDOCRINE, NUTRITIONAL, AND METABOLIC DISEASES (6)
- = DISEASES OF THE BLOOD AND BLOOD-FORMING ORGANS (2)
- ⊙ = DISEASES OF THE NERVOUS SYSTEM AND SENSE ORGANS (1)
- = DISEASES OF THE CIRCULATORY SYSTEM (7)
- ▲ = DISEASES OF THE RESPIRATORY SYSTEM (2)
- = DISEASES OF THE DIGESTIVE SYSTEM (10)
- △ = DISEASES OF THE GENITOURINARY SYSTEM (5)
- ▼ = DISEASES OF THE MUSCULOSKELETAL SYSTEM AND CONNECTIVE TISSUE (7)
- = CONGENITAL ANOMALIES (1)
- ▽ = INFECTIVE AND PARASITIC DISEASES (5)
- ◇ = ACCIDENTS, POISONINGS, AND VIOLENCE (4)

Figure 32. Distribution of pretreatment serum protein-bound fucose to globulin and neutral to globulin ratios in 79 patients with primary diagnoses of malignant neoplasia patients with a variety of nonneoplastic diseases.

## REFERENCE

1. Sobocinski, P. Z., Canterbury, W. J. and Hartley, K. M. Determination of L-fucose in glycoproteins. I. Effect of nonfucose moieties of serum glycoproteins. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR72-6, 1972.



## DEVELOPMENT OF A HYPOTHROMBOGENIC BLOOD OXYGENATOR MEMBRANE

Principal Investigator: *P. K. Weathersby*

Collaborator: *T. Kolobow, National Institutes of Health*

Despite its current sporadic performance, the membrane oxygenator holds the greatest promise for long-term pulmonary assistance.<sup>1</sup> Most of the remaining problems concern the membrane itself. Prominent among these is thrombogenicity. The development of nonthrombogenic or even hypothrombogenic membranes has been retarded by the use of poorly characterized industrial products rather than homogeneous, pure materials.

The objectives of this research are to produce pure materials, study their chemical and physical nature and evaluate their blood compatibility. The family of silicone rubbers has been chosen since they have a very high permeability to gases and a history of relative inertness toward blood.

Commercially obtained silicone polymers were analyzed for average (viscosity) molecular weight and chemical composition. A computer-controlled, pulse infrared spectrometer at the National Institutes of Health was used to detect contaminant substituents; in some cases the sensitivity reaches one group per average polymer chain.

The polymer is applied to suitable substrates and cured by ionizing radiation in a controlled gaseous environment. Mechanical tests and infrared spectra determine the type of cure and the occurrence of side products, such as oxidation residues. Radiation curing produces a purer product than traditional peroxide catalysts, but side groups can nevertheless be changed.

The cured silicone rubbers are tested for thrombogenicity. Fresh human blood requires over 45 minutes to clot in test tubes coated with these silicone rubbers, rather than the 15-25 minutes in commercial silicones (Lee and White test). Additional