ANNUAL RESEARCH REPORT
1 JULY 1972 — 30 JUNE 1973

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE

DEFENSE NUCLEAR AGENCY
BETHESDA, MARYLAND 20014

PROTEIN-BOUND CARBOHYDRATES AS BIOCHEMICAL CRITERIA IN DIAGNOSIS AND PROGNOSIS OF MALIGNANT NEOPLASIA

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

The present study was designed to investigate the glycoprotein profile of patien with, or suspected of having, malignant neoplasia. These profiles were compared progressive stages of diagnostic work-ups and treatment regimens and related to th individual's response to therapy to define the utility of such a test battery (1) as an objective diagnostic aid for presurgical estimation of tumor activity, and (2) as a pr nostic tool to aid the physician in in-hospital management and outpatient follow-up.

During this period, 727 serum specimens were examined. Three hundred and seventy-one of these samples were from patients in (or outpatients of) the U. S. Na· Hospital, Bethesda, Maryland; 178 from the cancer therapy group, National Institu of Health (NIH) Clinical Center, Bethesda, Maryland; 177 from AFRRI personnel; 1 from the Frederick Memorial Hospital, Frederick, Maryland. In addition to the above human patients, sera from 30 cats, some with ^{89}Sr-induced osteosarcomas, were received from the Environmental Protection Agency.

The test battery consisted of total protein, total serum globulins, protein-boun neutral hexoses, hexosamines, sialic acid, and fucose. From these analytical data number of additional parameters were derived: ratios of the various carbohydrate the total protein (mg CHO/dg protein), carbohydrate to globulin ratios (mg CHO/dg globulin), and mole-fraction ratios of the various carbohydrates. The glycoprotein profile consisted of the graphical representation of these analytical and derived dat

Results from the glycoprotein profiles indicate excellent correlation for presu. cal differentiation of localized from metastatic tumors and for detection of seeding occult metastases 2-4 months before the lesions were demonstrable by presently a cepted clinical or laboratory procedures.

To illustrate, 178 samples were obtained on 82 individuals being followed by th cancer therapy group, NIH. These patients were 33 Ewing's sarcomas, 15 bronch genic carcinomas, 14 squamous cell carcinomas, head and neck, 17 breast tumors, 3 with no clinical disease. Interpretation of the profiles derived from the first sar ples obtained on each of the 65 patients on whom histories have been received agre with clinical assessment in only 87.7 percent (57 of 65) of the cases. However, cl cal follow-up 1 and 2 months later of the eight patients on whom there was disagre ment indicated that they had or were seeding metastatic lesions. In other patients including several cases who have shown no clinical evidence of recurrent disease f up to 9 years, follow-up profiles indicate that therapeutic control is deteriorating or that recurrent lesions are forming. These patients are being followed intensive by the NIH group.

Similar results were obtained on patients of the U. S. Naval Hospital. More of these cases, however, were sampled before any definitive treatment had been undertaken. A typical profile series is given in Figures 30-32. This patient, a 49-year-old caucasian male, was sampled 16 days presurgery and had a profile compatible with the (later) surgical diagnosis of a localized squamous cell carcinoma of the esophagus (Figure 30). Two hundred seventy-eight days after resection of the circumscribed, noninvasive tumor, the pattern (Figure 31) altered to one indicating a recurrent or metastatic lesion. At this time, no clinical or other evidence suggested the presence of tumor burden. Eighty-one days later (on the patient's next 3-month check-up), 359 days postoperative (Figure 32), this patient's profile indicated active, metastatic disease, but clinical examination still did not demonstrate a tumor. Twenty-seven days after this last profile (3 months and 27 days after the profile illustrated in Figure 31), the patient presented with malignant cervical lymph node involvement. Similar changes have been seen in intestinal and lung tumors, the latter, however, only given 2 to 2-1/2 months' warning.
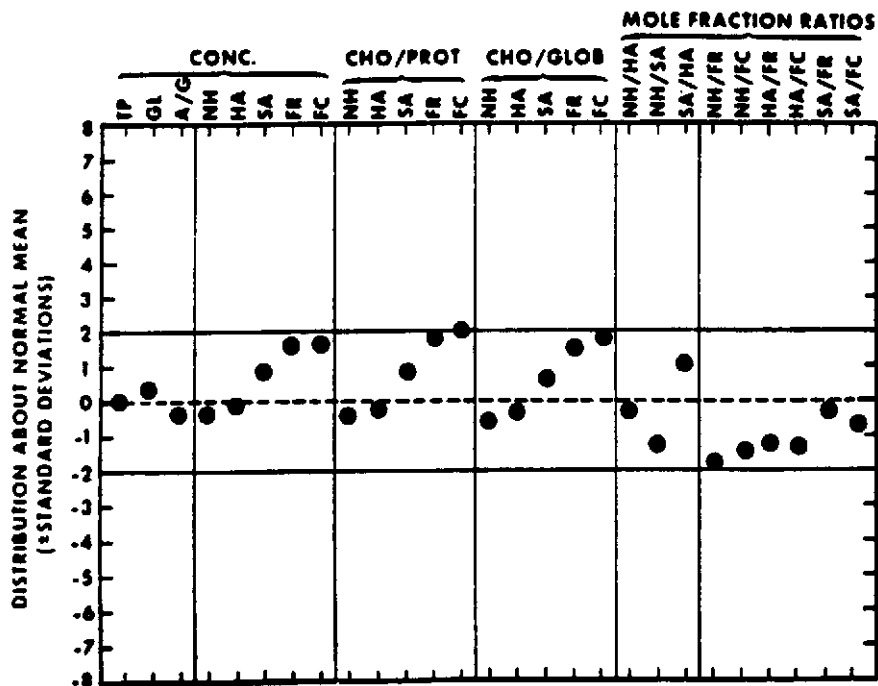

Figure 30. Localized squamous cell carcinoma of the esophagus 16 days preoperative
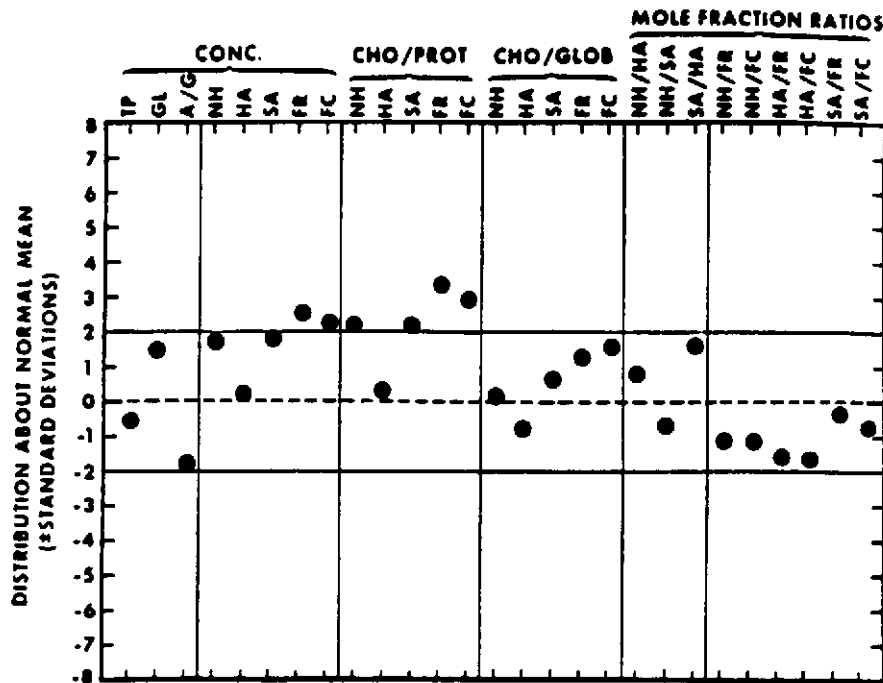
Figure 31. Follow-up on carcinoma esophagus 278 days postoperative. No evidence of disease.
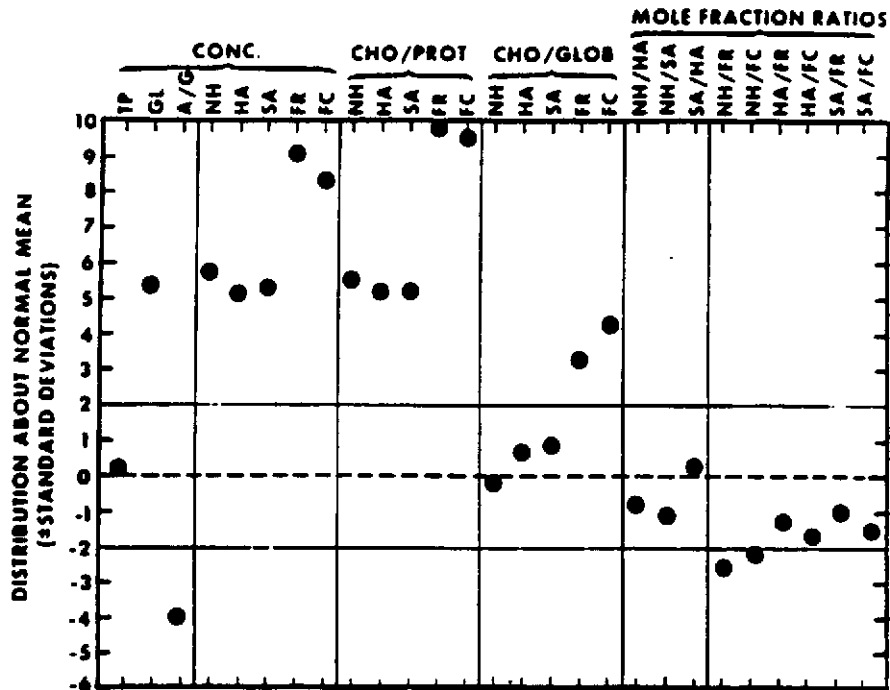

Figure 32. Follow-up on carcinoma esophagus 359 days postoperative. No evidence of disease.

Immunoassay of 16 protein species (albumin and 15 glycoproteins) has been completed on 16 serum samples from five individuals with different tumor types and varied responses to therapy.

Preliminary results from these assays indicate, as one would expect, that the acute phase reactants: α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin vary directly with the severity of the patient's general condition. However, the consistent depression of the Gc-globulin, transferrin, and hemopexin, together with a uniform elevation of β_1 E-globulin (C'4) suggests that these assays may be of value in adding specificity to the profile battery to aid differential diagnosis.

Calculation and summing of the various carbohydrates bound to the glycoproteins assayed indicate good correlation with the chemical analytic data except in the hexosamines. The presently used technique appears to be underestimating the actual concentrations. It appears also, as was suspected, that the raw fucose values as obtained by the Dische-Shettles CyR3 reaction are being overcorrected for the influence of the neutral hexoses. Both of these procedures are being reexamined.

Blood serum samples were obtained every 2 weeks for 3 months from 20 AFRRI personnel, 10 males and 10 females, to test for individual variation in the glycoprotein profile. The patterns maintained remarkable stability in both sexes for the entire period, indicating that changes seen in pathological specimens are not related to cyclic variation but are responses to the disease state.

The cat appears to be a less than ideal animal model for following the progress of osteogenic malignancies with our present procedures. Certain of the values obtained indicated that the methodology applicable to analysis of human sera is not valid when directly applied to the cat.

●●●●●●●●

CORRELATES OF MARROW INJURY AND RADIATION SENSITIVITY

Principal Investigator: *M. E. Ekstrom*
Collaborators: *A. S. Evans, J. E. West* and *J. F. Taylor*
Technical Assistance: *G. D. Lee*

The objective of this project was to further evaluate the possible relationship between preirradiation levels of plasma protein-bound carbohydrates (PBC), measured as neutral hexoses (mannose and galactose), and the relative sensitivity or resistance of animals (C_3H mice) to doses of ionizing radiation producing hematopoietic death.