PROGNOSTIC SIGNIFICANCE OF CONCENTRATIONS OF FOUR CLASSES OF PROTEIN-BOUND CARBOHYDRATES IN THE SERUM OF DOGS
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PROGNOSTIC SIGNIFICANCE OF CONCENTRATIONS OF FOUR CLASSES
OF PROTEIN-BOUND CARBOHYDRATES IN THE SERUM OF DOGS

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## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword (Nontechnical summary)</td>
<td>iii</td>
</tr>
<tr>
<td>Abstract</td>
<td>vii</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Materials and Methods</td>
<td>1</td>
</tr>
<tr>
<td>III. Results</td>
<td>3</td>
</tr>
<tr>
<td>IV. Discussion</td>
<td>8</td>
</tr>
<tr>
<td>References</td>
<td>11</td>
</tr>
</tbody>
</table>

## LIST OF FIGURES

Figure 1. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 400-rad dose of mixed gamma-neutron radiation. 3

Figure 2. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 230-rad dose of mixed gamma-neutron radiation. 5

Figure 3. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 225-rad dose of mixed gamma-neutron radiation. 6

Figure 4. Daily percent change from overall mean of protein concentration of each of various carbohydrates in individual dogs which survived 225 rads of mixed gamma-neutron irradiation with varying degrees of overt clinical symptomatology. 7
FOREWORD
(Nontechnical summary)

To establish criteria for assessment of continuing prognosis of an individual, tests should be selected which measure changes directly proportionate with the gravity of his condition. As a corollary, it may be stated that, with good prognosis, these changes should be minimal or absent.

To make the testing procedure objective, numerical boundaries should be established so that crossing from one range to another is indicative of changes in the probable course of the patient.

In the first two reports of this series (AFRRI SR68-4 and AFRRI SR69-24) it was suggested that the plasma concentration of protein-bound carbohydrates as neutral hexoses (uncharged 6-carbon sugars) is of significant prognostic value in following the course of radiation injury in mice and dogs. Thus, in both species, the animals that died showed a marked increase in plasma concentration of these bound carbohydrates, while the survivors of identical doses deviated only slightly from their preirradiation base-line values.

The presently reported experiments were designed to supplement the previous work on protein-bound neutral hexoses and to obtain a clearer picture of the prognostic value of serum carbohydrate constituents in radiation injury. Therefore, alterations in additional classes of carbohydrates bound to protein (sialic acid, hexosamines, and fucose) were followed as a function of time relative to irradiation.

Three groups of eight dogs each were given doses of 225, 230, and 400 rads of mixed gamma-neutron radiation, respectively. The doses were delivered at a rate of
approximately 20 rads/minute from the AFRRI-TRIGA reactor. Blood specimens were taken before irradiation and at intervals thereafter for 15 or 20 days or until death intervened.

The proteins were separated from the serum by precipitating them with alcohol. The various sugars were selectively removed from the proteins, and their concentrations were determined by chemical methods.

The actual amount of carbohydrate being incorporated into the proteins was estimated by calculating the amount (milligrams) of the various sugars bound per 100 mg total protein. In this way, the influence of changes in the blood volume of the animal was eliminated. This derived number is referred to as the protein concentration of the carbohydrate.

Moderate to marked fluctuations were seen during the testing period in the protein concentration of neutral hexoses, sialic acid, and hexosamines, especially in the two lower dose groups. The protein concentration of the neutral hexoses gave the clearest warning of unfavorable prognosis in the animals which died and exhibited the greatest stability in those which survived. No significant changes in the concentration of protein-bound fucose were seen in any of the 24 dogs.

These data, when analyzed in terms of observed clinical condition of the animals and their ultimate fate (survived or died), suggested that the following boundaries could be set:

1. Protein concentration of neutral hexoses below 1.60 was evidence of good prognosis. Thus, all the animals which maintained less than 1.60 mg neutral hexoses per 100 mg protein survived without treatment.
2. The interval 1.60 to 1.80 was assigned as the "guarded" prognosis range. That is, entry into this range within the first 15 days postirradiation presaged impending deterioration, and, if treatment is to be instituted, it probably should be started immediately. Such a warning occurred in a number of the dogs as early as the 1st day postirradiation, and always appeared several days before the sharp terminal rise. More importantly, entry into the "guarded" prognosis range preceded appearance of overt symptoms.

3. Protein concentrations of neutral hexoses greater than 1.80 mg per 100 mg protein signaled the beginning of the terminal rise and probably indicated that irreversible damage had occurred.

These tentative ranges were tested in nine additional dogs and were found to be effective for prediction.

These boundaries are obviously applicable only to dogs. Differences among various animals in their "normal" levels of protein concentration of neutral hexoses will require that appropriate ranges be established for each.
ABSTRACT

Three groups of eight dogs each were given doses of 225, 230, and 400 rads of mixed gamma-neutron radiation, respectively. The doses were delivered at a rate of approximately 20 rads/minute from the AFRRI-TRIGA reactor. In animals with poor prognosis, the serum protein concentrations (milligrams carbohydrate per 100 mg biuret protein) of neutral hexoses, hexosamines, and sialic acid started to rise at varying times postirradiation, continued upward, and remained high until the death of the animal. Moderate to marked fluctuations in the protein concentrations were seen in the time course of all these carbohydrates, especially in the two lower dose groups. The protein concentration of the neutral hexoses gave the clearest warning of unfavorable prognosis in the animals which died and exhibited the greatest stability in those which survived. No significant changes in the concentration of protein-bound fucose were seen in any of the 24 dogs. Serum protein-bound neutral hexose concentrations offer promise for development of a relatively simple, objective prognostic test to supplement clinical observation in cases of radiation injury. Thus, numerical ranges of protein concentrations of neutral hexoses are proposed to indicate good, guarded, and poor prognoses for this species. These tentative ranges were tested in nine additional dogs and were found to be effective for prediction.
I. INTRODUCTION

Previous reports from this laboratory\(^3,4\) have indicated that the plasma concentration of protein-bound carbohydrates as neutral hexoses is of significant prognostic value in following the course of radiation injury in mice and dogs. Thus, in both species, the animals which died showed a marked increase in plasma concentration of these bound carbohydrates, while the survivors of identical doses deviated only slightly from their preirradiation base-line values.

Electrophoretic and chromatographic analyses\(^3\) revealed that a large portion of the increase in protein-bound neutral hexoses in moribund animals could be accounted for operationally in glycoproteins concerned with iron conservation, transferrin and haptoglobin. Other affected components were the \(\beta_2\)-glycoproteins and \(\alpha_2\)-macroglobulin.

With the object of supplementing previous work on protein-bound neutral hexoses and to obtain a clearer picture of the prognostic value of serum carbohydrate constituents in radiation injury, alterations in additional classes of carbohydrates bound to protein were followed as a function of time relative to irradiation.

II. MATERIALS AND METHODS

Healthy, AKC registrable beagles of both sexes, 2-3 years of age, were the experimental animals.

Three groups of eight dogs each were given midline tissue doses of 225, 230, and 400 rads of mixed gamma-neutron radiation, respectively. The doses were delivered at a rate of approximately 20 rads/minute from the AFRRI-TRIGA reactor. The characteristics of the exposure field have been described previously.\(^5\)
During irradiation, the dogs were restrained in plastic boxes and positioned on an isokerma (free-in-air) curve with the center line of the animals approximately 400 cm from the vertical axis of the core. At the midpoint in time for each exposure the restraining boxes were rotated through 180° to achieve bilateral irradiation.

Blood samples (1–2 ml) were taken from the dogs prior to irradiation and at intervals thereafter for 15 or 20 days or until death intervened. The blood was allowed to clot; the serum was recovered by centrifugation and was stored in an ultra-low temperature freezer (−85°C) until analyzed.

Total protein was estimated by the biuret method using a commercial, stabilized reagent.* Commercially prepared, crystallized human albumin† was used as the standard.

To quantify the protein-bound neutral hexoses (as galactose and mannose) the sulfuric acid–orcinol technique of Weimer and Moshin⁷ was used.

Protein-bound hexosamines (as glucosamine) were quantified by Rimington’s modification of the Elson-Morgan method.⁶

The concentration of protein-bound sialic acid (as N-acetylneuraminic acid) was estimated by Winzler’s modification⁸ of the procedure of Ayala et al.¹

Protein-bound fucose concentration (as 6-deoxy-L-galactose) was determined by the method of Dische and Shettles.²

All of the determinations were made in duplicate on each sample. To eliminate any influence of hemodilution or hemoconcentration, the concentration of each class

* Hyceł No. 201A, Hyceł, Inc., Houston, Texas
† Dade Division, American Hospital Supply Corporation, Miami, Florida
of carbohydrate was converted to milligrams of carbohydrate per 100 mg biuret protein. For brevity, this value will be referred to as the protein concentration of the carbohydrate.

The dogs were observed for overt clinical signs of acute illness (alertness, response to handling, respiration rate, mobility, etc.) several times each day.

III. RESULTS

All of the dogs which had received 400 rads of mixed gamma-neutron radiation died on or before the 11th day postirradiation. At this overwhelming dose, the protein concentrations of neutral hexoses, sialic acid, and hexosamines as a function of time were similar (Figure 1). Thus, in the seven animals which survived to the 10th or 11th day, these values started to increase on the 6th postirradiation day, continued upward at almost identical rates, and remained high until the death of the animal. These three classes of carbohydrates also followed a similar pattern in the one animal which died on the 4th day postirradiation, but significant increases over preirradiation values did not occur until the day of death.

Figure 1. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 400-rad dose of mixed gamma-neutron radiation. Open symbols: died on 4th day postirradiation. Closed symbols: died on day 10 or 11. o, o = neutral hexoses; △, △ = hexosamines; ◻, ◻ = sialic acid; ●, ● = fucose. The number of animals represented by each curve is in parentheses.
Seven of the eight dogs which had received 230 rads died. In the animals which
died, the protein concentrations of neutral hexoses, sialic acid, and hexosamines as
a function of time were similar to those seen in the 400-rad experiment (Figure 2A-C).
The time courses of these protein-bound carbohydrate concentrations, however, did
not parallel one another as closely as in the higher dose group (Figure 1). The one
surviving animal of the 230-rad group (Figure 2D) was acutely ill during the 2nd week postirradiation. When the neutral hexoses went above 1.60 mg carbohydrate per
100 mg protein and continued to creep upward, this animal was given a high protein,
soft diet, and special hygienic measures were taken to control routes of sepsis in its
environment. While it remained high, the protein concentration of neutral hexoses
stabilized after the 11th day, and clinical improvement continued for the remainder of
the 40-day observation period. At the end of this time, the concentration of protein-
bound neutral hexoses had returned to preirradiation levels.

At the lowest dose, 225 rads, three of the eight dogs died during the 40-day
observation period. In the animals which died, moderate to marked fluctuations were
seen in the protein concentrations of neutral hexoses, sialic acid, and hexosamines
during the course of the radiation sickness and terminated in a sharp rise shortly
preceding death (Figure 3A-C). The five survivors, taken as a group, exhibited no
remarkable changes in any of the protein-bound carbohydrate concentrations (Fig-
ure 3D). Clinically, however, there was considerable variation in the severity of the
radiation sickness among these survivors, and these differences were reflected in the
time course of protein-bound neutral hexose concentrations in the individual dogs (Fig-
ure 4). In Figure 4, the daily percent change in the protein concentration of each
Figure 2. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 230-rad dose of mixed gamma-neutron radiation. A, died on 9th day postirradiation; B, died on day 12 or 13; C, died on day 14 or 15; D, survived (with treatment).

• = neutral hexoses; △ = hexosamines; □ = sialic acid; o = fucose.

The number of animals represented by each curve is in parentheses.
class of carbohydrate from its overall mean for the 15 days was plotted. Thus, while variations follow the same course as a function of time as the raw data, the shift of coordinates permitted direct comparability in that all the carbohydrate concentrations are referenced to the same horizontal line, \( y = 0 \).

No definable pattern was seen in the moderate to marked fluctuations in the sialic acid and hexosamine concentrations of the survivors. The neutral hexose concentrations, however, were relatively stable as compared with the other carbohydrates,

![Graphs showing trends in carbohydrate concentrations](image)

Figure 3. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 225-rad dose of mixed gamma-neutron radiation. A, died on 16th day postirradiation; B, died on day 19; C, died on day 36; D, survived. ● = neutral hexoses; △ = hexosamines; □ = sialic acid; ○ = fucose. The number of animals represented by each curve is in parentheses.
and when ordered according to increasing variability (Figure 4A-D) the animals were at the same time ranked according to increasing observed clinical difficulty. The fifth survivor of this dose group (not shown) fell in the midrange between 4B and 4C when ordered by the same criteria.

No remarkable changes were seen in the protein-bound fucose concentration in any of the 24 animals of the three dose groups.

Figure 4. Daily percent change from overall mean of protein concentration of each of various carbohydrates in individual dogs which survived 225 rads of mixed gamma-neutron irradiation with varying degrees of overt clinical symptomatology. The broken line represents the level at which 1.60 mg neutral hexoses per 100 mg protein would fall for each individual.

• = neutral hexoses; △ = hexosamines; □ = sialic acid.
IV. DISCUSSION

To establish criteria for assessment of continuing prognosis of an individual, tests should be selected which measure changes directly proportionate with the gravity of his condition. As a corollary, it may be stated that, with good prognosis, these changes should be minimal or absent.

To make the testing procedure objective, numerical boundaries should be established so that crossing from one range to another is indicative of changes in the probable course of the patient.

The lack of response of the protein-bound fucose concentrations, together with the wide day-to-day fluctuations encountered in the protein concentrations of hexosamines and sialic acid, eliminated their usefulness in delineating the required boundaries.

The comparative stability of the neutral hexose concentrations in the survivors and their response in the diers, however, have enabled establishment of tentative levels which meet the requirements set above.

These data, when analyzed in terms of observed clinical condition of the animals and their ultimate fate (survived or died), suggested that the following boundaries could be set:

1. Protein concentration of neutral hexoses below 1.60 was evidence of good prognosis. Thus, all the animals which maintained less than 1.60 mg neutral hexoses per 100 mg biuret protein survived without treatment.

2. The interval 1.60 to 1.80 was assigned as the "guarded" prognosis range. That is, entry into this range within the first 15 days postirradiation presaged impending deterioration, and, if treatment is to be instituted, it probably should be
started immediately. While only one animal of these groups was treated, and that minimal compared to more heroic measures (complete asepsis, bone marrow transplants, etc.) which could have been used, initiation of treatment before the dog's condition became irreversible was undoubtedly critical. Such a warning occurred in a number of the dogs as early as the 1st day postirradiation, and always appeared several days before the sharp terminal rise. More importantly, entry into the "guarded" prognosis range preceded appearance of overt symptoms.

3. Protein concentrations of neutral hexoses greater than 1.80 mg per 100 mg protein was evidence of poor prognosis, signaled the beginning of the terminal rise, and probably indicated that irreversible damage had occurred.

The above criteria were applied to nine dogs which were bled at intervals after receiving 240 rads of $^{60}$Co $\gamma$-irradiation for another experiment. One of the five survivors fluctuated between the good and "guarded" ranges (high 1.71, low 1.56 mg neutral hexoses per 100 mg protein). The remaining four survivors maintained protein concentrations of neutral hexoses well below the 1.60 level. The four diers all gave early warning by excursion above 1.60 and later exceeded 1.80 (to go as high as 3.74) in the terminal phase. Entry into the "guarded" prognosis range occurred from 8 days prior to death in two dogs which died on the 17th day postirradiation to 29 days prior to one animal's death on the 35th day. Entry into the poor prognosis range in these dogs was 3 and 19 days before death, respectively.

These boundaries are obviously applicable only to dogs. Differences among mammalian species in "normal" levels of protein concentration of neutral hexoses will require that appropriate ranges be established for each.
Establishment of criteria for objective assessment of radiation injury to the human presents a complicated problem in experimental design. Thus, practically all the cases available for study are patients receiving therapeutic irradiation, and its effects must be separated from the damage inflicted by underlying disease. Preliminary data suggest that such a separation can be made on the basis of preirradiation studies on different tumor types.

Combined with careful in-hospital clinical management, these tests may prove of value in determining the tolerance limit of the individual to radiotherapy.
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Three groups of eight dogs each were given doses of 225, 230, and 400 rads of mixed gamma-neutron radiation, respectively. The doses were delivered at a rate of approximately 20 rads/minute from the AFRRI-TRIGA reactor. In animals with poor prognosis, the serum protein concentrations (milligrams carbohydrate per 100 mg biuret protein) of neutral hexoses, hexosamines, and sialic acid started to rise at varying times postirradiation, continued upward, and remained high until the death of the animal. Moderate to marked fluctuations in the protein concentrations were seen in the time course of all these carbohydrates, especially in the two lower dose groups. The protein concentration of the neutral hexoses gave the clearest warning of unfavorable prognosis in the animals which died and exhibited the greatest stability in those which survived. No significant changes in the concentration of protein-bound fucose were seen in any of the 24 dogs. Serum protein-bound neutral hexose concentrations offer promise for development of a relatively simple, objective prognostic test to supplement clinical observation in cases of radiation injury. Thus, numerical ranges of protein concentrations of neutral hexoses are proposed to indicate good, guarded, and poor prognoses for this species. These tentative ranges were tested in nine additional dogs and were found to be effective for prediction.