kemic population during the next 20 h (Table 1). These changes were evidently due to the blocking action of QD on cells in the late phase of DNA synthesis in the second division cycle. An important result of secondary blocking by QD of transition of the cells from the phase of DNA synthesis into the postsynthetic period was a considerable decrease in the rise of the second wave of labeled mitoses and of the mitotic index. The mechanism of action of the inhibitor of cobalamine-dependent methionine synthetase which we have examined thus determines the increased effectiveness of cyclodependent antimetabolites, and above all of the S-phase-specific preparation cytosar during combined treatment of L-1210 leukemia.

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MODIFICATION OF AMINO-ACID RESIDUES OF BLOOD ALBUMIN FROM CANCER PATIENTS

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The writers previously have described changes in the blood albumin in various diseases. These changes were recorded by many different physicochemical methods, including peptide mapping [5]. When the structural changes observed in albumin were discussed, the possibility of post-translation modification of this protein under pathological conditions was considered. Later experiments showed that besides the native form of albumin, a conformationally changed (modified) form also circulates in the patients' blood, and the structural changes observed largely depend on the ratio between these forms [6]. This paper describes the study of this modified form of albumin with a view to the possible discovery of amino-acid residues modified as a result of disease.

EXPERIMENTAL METHOD

Preparations of blood albumin from patients and normal subjects were obtained by preparative electrophoresis in polyacrylamide gel [2]. The purity of the isolated preparations was tested by analytical electrophoresis in the same gel and by an immunochemical method. The modified form of albumin (A_m) was separated from the native form (A_n) from the electrophoretic fraction (A_e) by the method described in [3]. Free SH-groups, ionization of free hydroxyl groups, and N- and C-terminal amino acids were determined in the isolated albumin preparations [1]. The N-terminal sequence as far as the 6th residue in the albumin preparation was determined on a Model 890 "Sequenator" (Beckman, USA) [1]. Mass-spectrometric analysis also was used in the investigation [4].

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TABLE 1. Results of N-Terminal Analysis

Albumin preparation	Number of preparations	N-terminal aspartic acid	
		identified	not identified
		number of preparations	
A_e of healthy subjects	25	25	0
A_e of cancer patients	38	18	20
A_m of cancer patients	38	2	36

Altogether 25 albumin preparations isolated from normal subjects and 38 from cancer patients (carcinoma of the liver and other organs with metastases in the liver) were studied.

EXPERIMENTAL RESULTS

When the content of free SH-groups in healthy human albumin was determined, its values fluctuated from 0.8 to 1.2 mole SH/mole protein. Verification of the degree of polymerization of these two groups of albumin preparations showed that polymerization of albumins from cancer patients does not take place even if the preparations are kept for 30 days, whereas preparations of albumins from healthy blood donors polymerized after 7 days.

These results are evidence of modification of the single free SH-group of cysteine in the amino-acid sequence of serum albumin from cancer patients.

Ionization of free hydroxyl groups of tyrosine residues of the albumin preparations described above was studied by spectrophotometric titration in guanidine chloride. Virtually complete ionization of all tyrosine residues (about 17) in healthy human albumin was observed, whereas there were far fewer in albumin from cancer patients. For instance, for A_e about nine residues were calculated, but only five for A_m. Ionization of only 50% of tyrosine residues is thus observed in albumin from cancer patients, and this number was even smaller for the purified modified form (A_m); consequently, the rest of the tyrosine residues were modified.

High reactivity of phenolic groups of tyrosine residues has been reported in the literature, including *in vivo* [12]; this was evidently observed in the present experiments also. In one study, based on the primary structure of serum albumin, the writers showed that 50% of tyrosine residues may lie at the end of helical regions of the molecule, and the rest in the helices themselves [7]. Modified tyrosine residues are perhaps located on the ends of the helical regions and, for that reason, are more accessible to metabolites capable of modifying them.

N- and C-terminal analysis was undertaken on all isolated samples of albumin. In every case (for A_e, A_n, and A_m) the C-terminal amino acid was leucine.

It will be clear from Table 1 that the N-terminal aspartic acid in the electrophoretic fraction of albumin from cancer patients was covered in about 50% of cases, and in the modified form it was not identified in nearly every case. Considering data in the literature on glycosylation of proteins [10], similar glycosylation of the N-terminal amino-acid residue can be postulated, as with a Schiff base, more especially since carbohydrate components, including fucose [8], were found in albumin from cancer patients. This hypothesis was confirmed after treatment of albumin with a covered N-end with 10% formic acid. In every case, aspartic acid was discovered in A_m after this treatment.

Analysis of clinical data showed that the modified form of albumin appears in severe forms of cancer, and that the quantity of this form correlates with the severity of the patients' condition [9].

This information on modification of the SH-group of cysteine, of a definite number of tyrosine residues, and of the N-terminal amino acid, obtained by these experiments, served as the basis for a study of the N-terminal sequence in order to discover any other possible modifications.

The sequence known from the literature [11] was confirmed by analysis of the N-terminal sequence of six residues for healthy human albumin. A subsequent study of the sequence of six residues in the modified form of albumin isolated from patients with liver cancer (seven samples) revealed abnormal behavior of the phenylthiohydrantoin (PTH) of lysine in the 4th posi-

tion. To obtain more concrete information, mass spectrometric analysis was undertaken of the phenylthiohydrantoin of the amino acid in the 4th position. The results of these analyses showed the presence of normal lysine residues, with at the same time certain peaks with a molecular weight of about 300 daltons that were difficult to interpret. We give below the amino-acid sequence with N-terminal amino acids for six amino-acid residues.

For healthy human albumins: Asp-Ala-His-Lys-Ser-Glu-....

For albumins from cancer patients (modified form): Asp-Ala-His-Lys (modified)-Ser-Glu-....

The following modified amino acids were thus discovered in albumin from cancer patients: cysteine, about 50% of tyrosines, mainly those located in the amino-acid sequence under numbers 84, 140, 148, 150, 160, 333, 340, 410, 496, and the lysine residue in the 4th position from the N-end. It is here, in our opinion, that in all probability the post-translation modification of the albumin is observed, although genetic modification of individual amino acids likewise cannot be ruled out for certain other proteins.

It is also possible that other modifications may be found in the amino-acid sequence of albumin in pathology. It can be concluded from these data that such modifications are most probably not rare for functioning proteins *in vivo* and that, to some degree, they may be linked with pathogenesis.

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