

The effect of serum protein concentrations on the specificity of the radioimmunoassay of carcinoembryonic antigen in malignant neoplasia and non-neoplastic disease

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SYNOPSIS Carcinoembryonic antigen (CEA) has been measured in parallel with seven serum proteins and seromucoids in the sera of patients with malignant neoplasia and non-neoplastic disease. In the total group significant correlations were found between CEA and seromucoids and between CEA and several serum proteins. However, with two exceptions, when the individual disease groups were examined no correlation was seen. It is concluded that abnormal concentrations of the specific proteins measured do not consistently interfere in the CEA radioimmunoassay and do not explain the high CEA levels in patients with non-neoplastic diseases.

A major defect in the application of carcinoembryonic antigen (CEA) assay to diagnostic clinical practice lies in the lack of specificity for cancer as against inflammatory disease. This finding has been widely confirmed (Moore, Kupchik, Marcon, and Zamcheck, 1971; Laurence, Stevens, Bettelheim, Darcy, Leese, Turberville, Alexander, Johns, and Neville, 1972; Booth, King, Leonard, and Dykes, 1973), and although marginal differences exist between laboratories, elevated levels have been consistently found in a considerable proportion of patients with inflammatory disease of the bowel, cirrhosis, and chronic lung disease.

In neoplastic and inflammatory processes marked elevation of serum glycoproteins may occur (Macbeth and Bekesi, 1962; Bacchus, Kennedy, and Blackwell, 1967; Snyder and Ashwell, 1971), and the possibility exists that such elevation, of one or more of these proteins, might in some way be responsible for false positive CEA results. It may be difficult using radioimmunoassay as the method of measurement to distinguish between a highly active antigen such as CEA, itself a glycoprotein present in nanogram amounts, and other weakly cross-reacting proteins present in concentrations a thousand times greater. Interference with radioimmunoassay might also be due to entirely non-specific effects associated

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with marked changes in the concentration of certain serum proteins. Possible mechanisms include the masking of antibody binding sites for CEA and the effect on the coprecipitation of labelled antigen. An example of non-specific effects in radioimmunoassay has been reported by Girard and Greenwood (1969) who showed that salt and urea in urine simulated the presence of growth hormone. In a study comparing methods for the radioimmunoassay of plasma insulin (Cotes, Mussett, Berryman, Ekins, Glover, Hales, Hunter, Lowy, Neville, Samols, and Woodward, 1969) there was evidence that methods which used albumin solution instead of treated plasma to dilute insulin standards could not distinguish some non-insulin components of plasma from insulin.

Elevations of CEA concentration have been found in patients with ulcerative colitis and Crohn's disease (Lo Gerfo, Krupay, and Hansen, 1971; Booth *et al*, 1973). In these conditions there is marked elevation of the seromucoid fraction (Cooke, Fowler, Cox, Gaddie, and Meynell, 1958). This fraction is a heterogeneous mixture of glycoproteins characterized by their solubility in perchloric acid and is composed mainly of α_1 acid glycoprotein together with some α_1 antitrypsin, haptoglobin, and traces of many other serum proteins (Schultze and Heremans, 1966). It may be significant that the original method of CEA radioimmunoassay (Thom-

son, Kruey, Freedman, and Gold, 1969) employed a perchloric acid extract of serum, CEA itself being a glycoprotein. Although perchloric acid extracts of normal serum were added to the diluted CEA standards to minimize the effect of protein interference in the assay, it is possible that this may not compensate completely for abnormal protein concentrations in the perchloric acid extract of patients' sera. It has been stated by a member of that group (Thomson, personal communication, 1971) that simply doubling the quantity of serum extracted for the assay leads to a marked increase in the number of positive results, and halving the quantity of serum reduces the number of positive results after making allowance for the volume extracted. Such findings, therefore, raise doubts about the specificity of CEA radioimmunoassay.

In more recent methods whole serum or plasma is used instead of perchloric acid extracts, and it is possible that serum proteins, other than glycoproteins, might interfere when present in abnormal concentrations.

The purpose of this study was to investigate the possibility of non-specific interference in the radioimmunoassay of CEA by abnormal concentrations of serum proteins in patients with a variety of clinical conditions. In addition to seromucoids, seven serum proteins, of which the concentrations were known to range widely in disease, were chosen for measurement, selection being partly dependent upon the availability of satisfactory commercial antisera. Three of these proteins, with high carbohydrate content, are perchloric acid soluble (alpha₁ acid glycoprotein, alpha₁ antitrypsin, and haptoglobin) and four, with lower carbohydrate content, are perchloric acid insoluble (ceruloplasmin, transferrin, IgG, and IgA).

Materials and Methods

Blood was obtained from patients attending the

General Hospital, Birmingham, who were suffering from a variety of neoplastic and non-neoplastic conditions. The serum was separated within two hours of collection and stored in separate 1 millilitre aliquots at -20° until the measurements were made. The CEA measurements are reported elsewhere as part of a larger series (Booth *et al*, 1973). Carcinoembryonic antigen was measured by radioimmunoassay using the double antibody technique (Egan, Lautenschleger, Coligan, and Todd, 1972) as modified by Laurence *et al* (1972).

Seromucoids were measured by a semi-automated modification of the method of Mandel, Gorsuch, and Cooper (1955).

The specific proteins were measured by quantitative immunoelectrophoresis using the method of Laurell (1966). The specific antisera were supplied by Hoechst Pharmaceuticals (alpha₁ antitrypsin and alpha₁ acid glycoprotein), Hyland Laboratories (haptoglobin, transferrin, and ceruloplasmin), and the Department of Experimental Pathology, University of Birmingham (IgG and IgA).

Results

Protein and CEA measurements have been made on 115 sera, except for seromucoids studied in 94 only. For the purpose of analysis the diseases have been grouped into four diagnostic categories: gastrointestinal malignant disease (43 patients), non-gastrointestinal malignant disease (19 patients), inflammatory bowel disease (19 patients), and liver disease (16 patients).

The correlation coefficients for each protein and the seromucoids with CEA in the total and the four disease groups are given in the table. For the total group, a significant positive correlation (p < 0.01) was shown between CEA and alpha₁ acid glycoprotein and between CEA and alpha₁ antitrypsin; lesser correlations (p < 0.05) were shown between

	Total	Gastrointestinal Malignancy	Non-gastrointestinal Malignancy	Inflammatory Bowel Disease	Liver Disease
a Seromucoids	0.2508 ¹	0.1768	0.5220 ¹	0.3046	0.4298
b Alpha ₁ acid glycoprotein	0.2409 ²	0.3136 ¹	0.2126	0.1057	0.4590
c Alpha ₁ antitrypsin	0.2469 ²	0.2019	0.3459	-0.1580	0.2189
d Haptoglobin	0.1891 ¹	0.1009	0.3150	0.3457	0.3148
e Ceruloplasmin	0.2150 ¹	0.1417	0.1070	0.2052	0.0768
f Transferrin	-0.2317 ¹	-0.2673	-0.1481	-0.1581	-0.1219
g IgG	-0.944	-0.0504	-0.1522	0.1255	0.0888
h IgA	0.1004	0.1278	-0.1066	0.2296	0.1162
Degrees of freedom					
Seromucoids	93	36	18	12	11
Other proteins	114	42	18	15	15

Table Correlation of serum protein concentrations with the concentration of serum carcinoembryonic antigen

¹p = < 0.05

²p = < 0.01 For all other correlation coefficients p = > 0.05

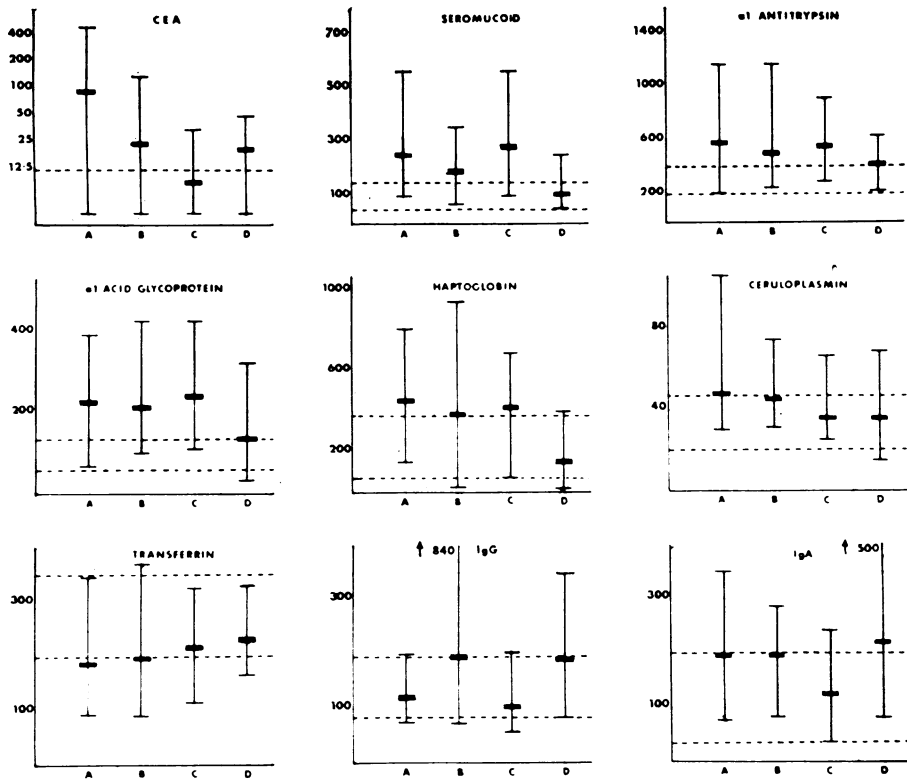


Fig 1 The observed range of results and their mean values for CEA, seromucoid, and individual protein measurements. The normal range for each measurement is shown by the dotted lines.

A = gastrointestinal cancer, B = non-gastrointestinal cancer, C = inflammatory bowel disease, and D = hepatic disease.

CEA is measured in ng/ml, seromucoid, alpha₁ antitrypsin, alpha₁ acid glycoprotein, haptoglobin, ceruloplasmin, and transferrin in mg/100 ml, and IgG and IgA in International units/litre.

CEA on the one hand and seromucoids, ceruloplasmin, and haptoglobin on the other. A significant negative correlation ($p < 0.05$) was found for CEA and transferrin but there was no significant correlation with either of the immunoglobulins measured. For the individual disease groups the only significant correlations ($p < 0.05$) were between CEA and alpha₁ acid glycoprotein in gastrointestinal malignant disease and between CEA and seromucoids in non-gastrointestinal neoplasia. No other relationship reached a level of statistical significance.

The observed range of results and the mean values for the serum proteins for each disease category, together with the normal ranges, are shown in figure 1. Figure 2 illustrates the detailed relationship between CEA and alpha₁ antitrypsin and between CEA and alpha₁ acid glycoprotein indicating the separate diagnostic subgroups in-

involved. Elevated glycoprotein concentrations in the presence of normal CEA values appear in each of these groups.

Discussion

The double antibody radioimmunoassay estimates the CEA concentration of whole serum. It has been shown to give results comparable with those obtained using other radioimmunoassay techniques where CEA concentration is measured on perchloric acid extracts of serum (Laurence *et al*, 1972). Thus if non-specific protein interference accounts for some falsely high CEA levels, it is likely that the interfering protein is extracted by perchloric acid.

Correlation between CEA and other serum protein concentrations appears poor, and, although levels of significance are reached in a number of instances,

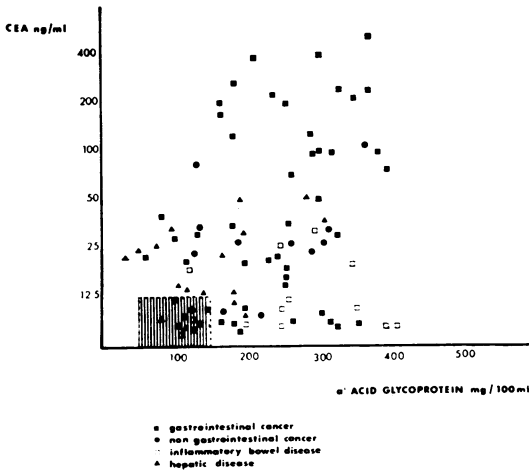


Fig 2a

Fig 2a Relationship between CEA and α_1 acid glycoprotein

Fig 2b Relationship between CEA and α_1 antitrypsin.

The normal range for each measurement is denoted by the hatched area.

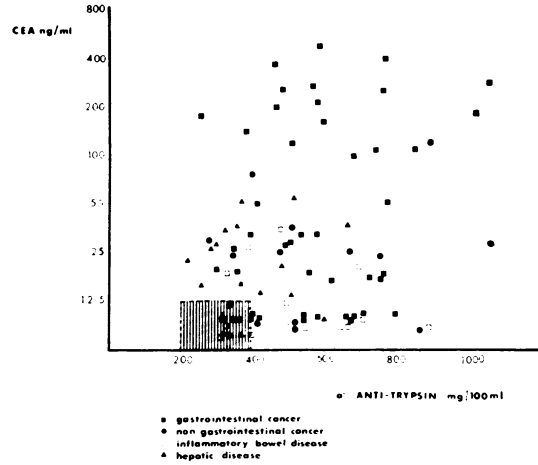


Fig 2b

such correlation may well be misleading. The relationships were demonstrated mainly when all patients were grouped together and reached their highest levels of significance for two perchloric-acid-soluble proteins, α_1 acid glycoprotein and α_1 antitrypsin. Elevation of the serum levels of these two proteins has been shown in a wide variety of conditions including neoplasia, cirrhosis, and inflammatory disorders (Simmons, Penny, and Goller, 1969). Thus in such a heterogeneous group of patients some degree of correlation might be expected. This does not necessarily imply a causal relationship and the absence of significant correlation, α_1 acid glycoprotein ($P < 0.05$) excepted, in the group of patients with gastrointestinal malignancy and high CEA concentrations strongly supports the specificity of the assay in this situation.

It might be expected that the non-specific interference would account for the high CEA levels in disease states where specific CEA elevation is not expected. The absence of a demonstrable correlation in patients with inflammatory bowel disease and liver disease is therefore a further argument against interference and again favours the specificity of the test.

Thus we have been unable to establish a direct relationship between the concentration of CEA and seven other serum proteins or seromuroids. That many sera from all four disease groups showed marked increases in one or more of the protein fractions and yet had CEA levels within the normal range (fig 2) is a strong argument against consistent

interference by these proteins. These findings do not, of course, exclude the possibility that other serum proteins, including individual seromuroids not measured in this investigation, either separately, or in combination with other factors, may in some cases be responsible for false positive CEA results, but this seems unlikely.

Recently attempts have been made to provide a diagnostic index for cancer by analysing serum protein profiles (Snyder and Ashwell, 1971). Although as yet the results only merit cautious optimism, the addition of CEA measurements might improve this to a clinically useful level. This hypothesis, however, will require further detailed investigation and analysis.

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