Changes in serum α_2 globulins in reticuloses

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SYNOPSIS The total concentrations of α_2 globulin and two of its components, haptoglobin and caeruloplasmin, have been determined in the sera from 78 patients with primary malignant diseases of lympho-reticular tissue (reticuloses). The total α_2 globulin fraction was significantly raised only in Hodgkin's disease. The serum haptoglobin was also significantly increased in Hodgkin's disease and some other reticuloses, including acute leukaemia, lymphosarcoma, and reticulum cell sarcoma. The serum level of caeruloplasmin was raised in all reticuloses. There was possibly some correlation between the rise in haptoglobin and caeruloplasmin concentrations and the total α_2 globulin in Hodgkin's disease but this was not statistically significant. The significance of these results is discussed, and it is concluded that the increase in α_2 globulin which occurs in Hodgkin's disease is due to an increase in several of its components and not to a single component.

Elevation of the α_2 globulin fraction of the serum has been noted in a number of conditions, including infections, liver disease, the nephrotic syndrome, and malignant diseases, particularly primary malignant diseases of lympho-reticular tissue (reticuloses). The ease with which the glycoprotein haptoglobin may be measured colorimetrically by methods based on the work of Jayle (1951), and the colorimetric estimation of the copper-containing protein caeruloplasmin by the methods of Ravin (1956), have allowed the rise in these α_2 globulin fractions in reticuloses to be demonstrated as well.

We decided to study the concentration of α_2 globulin, haptoglobin, and caeruloplasmin in the serum of a large number of patients with reticuloses, to determine if there was any correlation between the increases in these fractions, and if there was any connexion between them and the duration of the disease particularly in Hodgkin's disease.

SELECTION OF CASES

The term 'reticulosis' has been used to include all primary malignant diseases of lympho-reticular tissue. Leukaemia was diagnosed by the usual criteria of infiltration of the bone marrow and the blood by 'blast' cells in acute leukaemia, lymphocytes in chronic lymphocytic leukaemia, and developing granulocytes in chronic myeloid leu-

kaemia. Hodgkin's disease, reticulum cell sarcoma, and lymphosarcoma were diagnosed by the characteristic histology of lymph node biopsies, and myelomatosis by infiltration of the bone marrow with abnormal plasma cells, as well as abnormalities in gamma globulin synthesis.

As far as possible, patients were not being treated when the blood samples were taken, and in particular were not receiving corticosteroids. Many patients had been treated previously, and blood was not collected for at least a week after blood transfusion, as this lowers the serum haptoglobin concentration in some patients (Langley, Owen, and Padanyi, 1962). For the same reason patients with proven haemolysis were not included. Normal controls were chosen from hospital staff, medical students, and patients with no evidence of organic disease.

METHODS

TOTAL SERUM PROTEIN CONCENTRATION This was determined by the biuret method (Gornall, Bardawill, and David, 1949).

SERUM α_2 GLOBULIN CONCENTRATION This was determined by electrophoresis using the method of Franglen and Martin (1961). The serum was applied to Whatman no. 3 MM paper on a hanging strip apparatus. Electrophoresis was carried out using a barbiturate buffer pH 8.6 with a current of 0.4mA/cm. width of paper for 18 hours. The paper was dried at 115°C. for 30 minutes, stained with bromocresol green, and the various fractions measured after elution on an absorptiometer at 617 m μ . The absolute values were then calculated from the total serum protein concentration.

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SERUM HAPTOGLOBIN CONCENTRATION This was measured colorimetrically by the colour change in guaiacol resulting from the peroxidase activity of the haemoglobin-haptoglobin complex (Owen, de Gruchy, and Smith, 1960). A standard curve was prepared each week from pooled normal serum.

SERUM CAERULOPLASMIN CONCENTRATION This was measured by the method of Ravin (1956, 1961), which employs the colour change induced in paraphenyldiamine by the oxidase activity of caeruloplasmin. The optical density readings were converted to milligrams per cent of caeruloplasmin using the factor 87.5 (Ravin, 1956).

RESULTS

Table I shows the total protein concentrations in 106 patients and controls, and as expected the only significant change was found in multiple myeloma where the concentration was increased due to the abnormal myeloma protein.

Table II shows the serum α_2 globulin concentrations in 105 patients and controls, and here the

only significant increase was in cases of Hodgkin's disease, where the mean concentration was 0.94 g./100 ml. compared with the controls in whom it was 0.65, although in the other reticuloses some of the values fell outside the normal range.

Table III shows the serum haptoglobin concentrations in 108 patients and controls, and here the levels were significantly increased in Hodgkin's disease and acute leukaemia ($p < 0.001$), as well as in lymphosarcoma and reticulum cell sarcoma ($p < 0.01$).

Table IV shows the serum caeruloplasmin concentrations in 106 patients and controls, and here the levels were significantly increased in all the reticuloses.

Only in cases of Hodgkin's disease were the serum levels of α_2 globulin, haptoglobin, and caeruloplasmin all significantly increased. Comparing the α_2 globulin concentrations with the haptoglobin levels the correlation coefficient was +0.29, and with the caeruloplasmin levels it was +0.44. Although these values are not statistically significant

TABLE I

TOTAL SERUM PROTEIN CONCENTRATIONS IN RETICULOSES

	No.	Mean (g./100ml.)	Standard Deviation	t	p
<i>Cases</i>					
Acute leukaemia	12	6.39	1.34	0.28	—
Chronic myeloid leukaemia	9	6.2	0.78	1.38	—
Chronic lymphatic leukaemia	12	6.3	0.81	0.61	—
Hodgkin's disease	14	6.4	1.05	0.357	—
Multiple myeloma	14	8.7	2.65	3.79	<0.01
Lymphosarcoma and reticulum cell sarcoma	15	6.67	0.75	0.46	—
<i>Controls</i>	30	6.52	± 1.13		
Total	106				

TABLE II

SERUM α_2 GLOBULIN CONCENTRATIONS IN RETICULOSES

	No.	Mean (g./100ml.)	Standard Deviation	t	p
<i>Cases</i>					
Acute leukaemia	11	0.68	0.235	1.08	—
Chronic myeloid leukaemia	9	0.58	0.165	1.59	—
Chronic lymphatic leukaemia	12	0.63	0.138	0.24	—
Hodgkin's disease	14	0.94	0.263	3.78	<0.1
Multiple myeloma	14	0.83	0.335	0.84	—
Lymphosarcoma and reticulum cell sarcoma	15	0.83	0.316	1.99	—
<i>Controls</i>	30	0.65	± 0.21		
Total	105				

TABLE III

SERUM HAPTOGLOBIN CONCENTRATION IN RETICULOSES

	No.	Mean (mg./100ml.)	Standard Deviation	t	p
<i>Cases</i>					
Acute leukaemia	12	215	19	5.67	<.001
Chronic myeloid leukaemia	9	119	33	1.66	—
Chronic lymphatic leukaemia	12	139	41	0.68	—
Hodgkin's disease	16	202	103	4.2	<.001
Multiple myeloma	14	156	133	1.89	—
Lymphosarcoma and reticulum cell sarcoma	15	180	125	2.92	<.01
<i>Controls</i>	30	100.6	± 54		
Total	108				

TABLE IV
SERUM CAERULOPLASMIN CONCENTRATIONS IN RETICULOSES

	No.	Mean (mg./100ml.)	Standard Deviation	t	p
<i>Cases</i>					
Acute leukaemia	11	21	13	3.59	<0.01
Chronic myeloid leukaemia	9	31	14	6.48	<0.001
Chronic lymphatic leukaemia	11	18	16	2.69	<0.05
Hodgkin's disease	16	24	11	5.4	<0.001
Multiple myeloma	14	20	14	4.2	<0.001
Lymphosarcoma and reticulum cell sarcoma	15	23	13	4.5	<0.001
<i>Controls</i>	30	10.3	± 5.7		
Total	106				

they do suggest that there may be some correlation. Certainly the rise in α_2 globulin cannot be due to an increase solely in one component of α_2 globulin.

DISCUSSION

Changes in the α_2 globulin levels in reticuloses have been recorded previously, *e.g.*, Rottino, Suchoff, and Stern (1948) studied a number of cases of Hodgkin's disease at all clinical stages and found that a rise in the α_2 globulin was particularly noticeable in the later stages of the disease. We have been unable to confirm this as there was no correlation in our patients between the duration of the disease and the α_2 globulin concentration, the haptoglobin levels, or the caeruloplasmin levels. Neely and Neill (1956) found an increase in the mean α_2 globulin values in all reticuloses, particularly Hodgkin's disease and lymphosarcoma; and Brackenridge and Csillag (1962) also noted that the highest levels of α_2 globulin were to be found in Hodgkin's disease and lymphosarcoma. Goulian and Fahey (1961) found an increase in α_2 globulin and protein-bound hexose in two patients with Hodgkin's disease. In the present study α_2 globulin was significantly raised only in Hodgkin's disease, but the number of cases of lymphosarcoma and reticulum cell sarcoma were possibly too small to give a significant result.

Owen *et al.* (1960) found a raised haptoglobin in some cases of acute leukaemia, chronic myeloid leukaemia, lymphosarcoma, and reticulum cell sarcoma. In a 10-year study of 40 patients with Hodgkin's disease Jayle, Marnay, Ennuyer, and Ridremont (1961) showed that haptoglobin levels were raised in untreated patients or in patients towards the termination of their disease. He considered it to be a better guide to activity of disease than any other protein change, including erythrocyte sedimentation rate, total α_2 globulin, β lipoprotein, and γ globulin. The patients with Hodgkin's disease and acute leukaemia in this series showed the most significantly increased levels in haptoglobin. Whether this was due to haptoglobin only, or another haemoglobin-binding protein reported by Herman-Ott (1963), is not known. It was also noted that in three patients with acute leukaemia very low values of

haptoglobin (40, 40, 55 mg./100 ml.) were obtained when there was no overt evidence of haemolysis. By contrast, several patients with Hodgkin's disease, who were not included in the series because they had a proven haemolytic anaemia, had normal haptoglobin levels, demonstrating that the haemolysis was not intravascular; possibly without haemolysis their haptoglobin levels would have been higher.

Brenner (1953), in a comprehensive review of the metabolism of copper, noted that a few patients with acute leukaemia and chronic forms of leukaemia had a rise in the serum copper levels; six patients with Hodgkin's disease also showed a rise. Underwood (1962), confirmed these findings on a large number of acute and chronic leukaemias and Hodgkin's disease; in his series the level of cellular copper was not raised. Adelstein and Vallee (1961) reported a rise in the caeruloplasmin levels in a wider variety of conditions but particularly reticuloses and metastatic neoplasia and that a favourable response to treatment is accompanied by a fall in caeruloplasmin. Our results show that the levels of caeruloplasmin were increased in all reticuloses; least change occurred in patients with chronic lymphatic leukaemia; one patient with acute leukaemia showed a value of 2 mg./100 ml. which was below the lower limit of normal of 5 mg. Wintrobe, Cartwright, and Gubler (1953) have also recorded occasional very low values in acute leukaemia.

Lahey, Gubler, Brown, Smith, Jager, Cartwright, and Wintrobe (1953) found a positive correlation between serum copper and α_2 globulin levels. The correlation coefficient between caeruloplasmin levels and α_2 globulin concentration in Hodgkin's disease in this series was +0.44 which is not statistically significant, but with a small number of patients it is not possible to exclude a correlation. Similarly the correlation between α_2 globulin and haptoglobin in our patients with Hodgkin's disease was +0.29 which is not statistically significant.

Possible explanations for the rise in the α_2 globulin levels have been that it replaces the osmotic activity of the plasma protein lost by the fall in albumin, or α_2 globulin may be produced directly from tissue destruction (Jayle and Boussier, 1955; Adelstein

and Vallee, 1961) or may result from involvement of the liver in these diseases (Owen, Mackay, and Got, 1959; Williams, Speyer, and Billing, 1961). Another possibility is that haptoglobin and possibly other components of the α_2 globulin fraction are manufactured by cells of the lymphoid series.

In patients with reticuloses we have found that increased α_2 globulin levels have preceded by months or years the fall in albumin which is frequently a terminal event. The origin of these proteins awaits radioisotope studies, for it is not clear whether synthesis is increased or degradation diminished. Haptoglobin levels are sometimes raised in carcinoma involving the liver and in obstructive jaundice (Owen *et al.*, 1959) but this may simply be due to obstruction and not to increased production, for Williams *et al.* (1961) have shown that in some cases of liver damage, for example, infective hepatitis, low values of haptoglobin may occur.

In the case of caeruloplasmin, Underwood (1962) suggests that there is an increased intestinal absorption of copper and the liver is induced to form caeruloplasmin more rapidly, but this would not explain the increase in the other components of the α_2 globulin fraction. Though marked changes may occur in the α_2 globulin levels the significance is, at the moment, not well understood, but the increase in α_2 globulin which occurs in Hodgkin's disease is due to an increase in several of its components and not to a single component.

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