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Quantitative immunoelectrophoretic analysis in patients with tuberculosis and sarcoidosis

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A study of 12 immunologically distinct serum proteins has been made in two conditions, tuberculosis and sarcoidosis, which have in common the formation of giant-cell granulomata. Significantly ($P<0.001$) higher concentrations of five proteins [α_2 group component (GC), caeruloplasmin (caer), haptoglobin (hpt), haemopexin (hpx), and α_1 easily precipitable glycoprotein (α_1 PGp)] were found in tuberculosis. No significant differences from normal were found in patients with sarcoidosis. The results obtained compared to the available figures for conventional electrophoresis and the so-called 'acute phase reaction' are discussed.

Since tuberculosis and sarcoidosis both have the capacity to induce chronic giant-cell granulomata in man, it might be reasonable to look for the other shared properties as well as differences in the body's immunological and inflammatory responses. The serum proteins have been studied extensively already in both conditions, but the methods used have been superseded by others which are more accurate or more informative. This paper reports findings in the sera of patients with these two diseases, and was initiated as an attempt to find one parameter on which the differential diagnosis could be made with confidence.

MATERIALS AND METHODS

PATIENT SELECTION

General All patients were aged between 18 and 65 years, were of European stock, and were without current or past evidence of jaundice or major systemic disease apart from the disease under study. All had been at work up to the time of their hospital admission or were still at work. All blood samples were taken within 36 hours of admission to hospital. No steroid therapy had been given at any time. No patient was significantly below his or her mean normal weight, and none was anaemic, *i.e.*, haemoglobin below 70%.

Sarcoidosis The diagnosis was made independently by another physician on the usual clinical features of the case (such as erythema nodosum, uveitis, parotitis, hepatic or respiratory involvement, lymphadenopathy, and neurological signs, etc.), and was confirmed in all cases by a positive skin biopsy or Kveim test within the preceding year. All were judged to be in an active phase of their disease but were seen as out-patients.

Tuberculosis All patients were seen within the first 36 hours of their admission for a newly diagnosed tuberculous chest infection, or for a reactivation of a lesion treated over two years before. The diagnosis was based on clinical (weight loss, sweats, pyrexia, cough, and sputum, etc.), bacteriological, immunological (Mantoux conversion), and radiological information.

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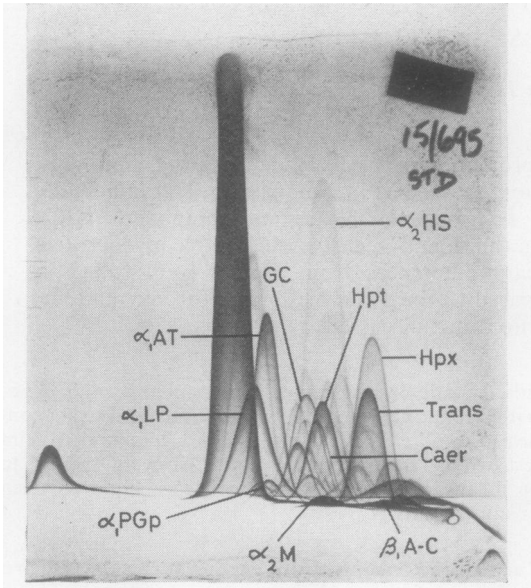
PROTEIN ANALYSIS

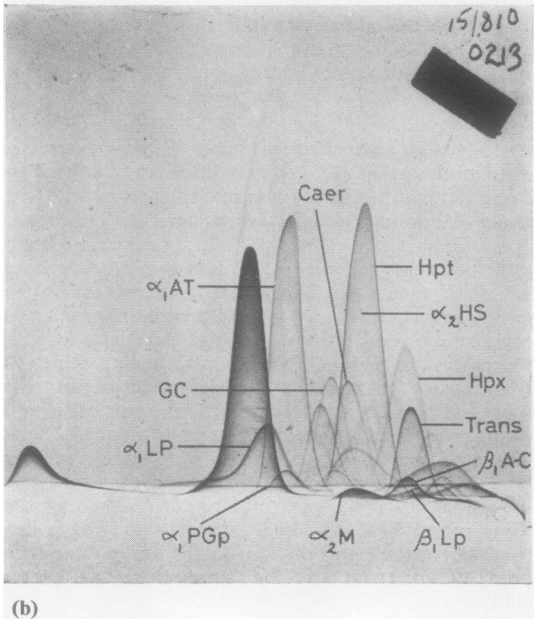
Protein analysis was performed by a modification (Clarke and Freeman, 1968) of the Laurell quantitative immunoelectrophoretic technique. A photograph of a normal serum run using this technique is shown (Fig. 1a). When performed in this way the areas under each protein curve are proportional to the concentration of that protein in the serum under study, and inversely proportional to the concentration of antibody to that particular protein. Thus, for the technique to be used quantitatively, it is necessary to compare the unknown serum with some standard. In this instance a pool of normal serum was used throughout as a standard. This is the same serum as was used as a reference preparation in a study of the normal concentration of individual plasma proteins previously reported (Clarke and Freeman, 1968). These values have been used as normals for this particular study. The same pool of antiserum was used throughout the present study.

RESULTS

PATIENTS The clinical characteristics of the two groups of patients (20 with sarcoidosis and 16 with pulmonary tuberculosis) are summarized in Table I, from which it may be seen that the mean age of the sarcoid patients was 10 years above



(a)



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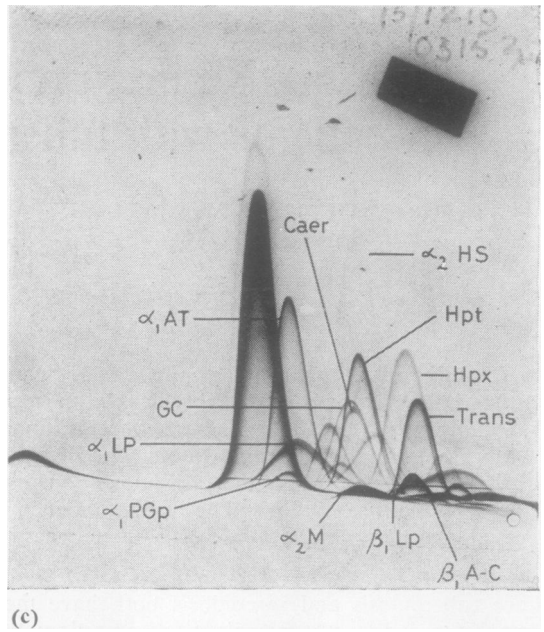

(c)

FIG. 1. *Shows a typical separation of (a) a normal serum, (b) serum from a patient with tuberculosis, and (c) serum from a patient with sarcoidosis.*

TABLE I

CLINICAL CHARACTERISTICS OF PATIENTS STUDIED

	Tubercu-losis	Sarcoidosis
No. of patients	16	19
Male	12	9
Female	4	10
Mean age on admission (yr)	36·5 (range 18–62)	46·4 (range 22–63)
Estimated duration of illness		
< 3 mth	10	2
< 6 mth	5	1
< 36 mth	1	9
36+ mth	0	7
Full/part-time employment or capable of work	15	18
Past infection known	2	0
Mantoux positive	15	3
negative	0	8
not known	1	9
Mean weight loss in past year (lb.)	10	Nil
Chest *x*-ray		
Shadow and glands	1	Reticulation 5
Shadow	2	Hilar glands 7
Calcium	2	Both 3
Cavitation	7	Normal 4
Normal	4	
Positive sputum (AFB)	6	0
Mean ESR fall (mm/hr)	30 (range 10–66)	Less than 20
Positive biopsy (liver and other)	0	15
Positive Kveim test	0	11
Lymphadenopathy	—	7
Bone involvement	0	2
Renal involvement	0	0
Skin involvement	0	11
CNS involvement	0	4
Eye involvement	0	8

that of those with tuberculosis. Seven of the 20 patients with sarcoidosis had been so diagnosed for over three years, but this duration was never found among the tuberculosis patients. In each group (on admission to the series), only a single patient was not known to be at work or capable of it. Weight loss averaged 10 lb. (4·5 kg.) in the past year among patients with tuberculosis, but usually no loss occurred among the others. Other clinical parameters are self explanatory and are presented simply to give an idea of the usual degree of involvement of each disease.

PROTEINS The protein concentrations found in these subjects are presented in Table II. These are presented as means and standard deviations for each protein, together with the normal values from the series reported previously. The results obtained for each series have been compared with each other and with the normal series, and the significance of differences found is presented. Correlations were made, using a digital computer, between each of the protein concentrations and a variety of clinical criteria, including, for tuberculosis, duration of illness, evening sweats, *x*-ray findings, and ESR; and for sarcoidosis, duration of illness, birth area, *x*-ray findings, ESR, biopsy findings, and results of Kveim testing. Some of the correlations found are shown in Table III.

TABLE II

SERUM PROTEIN CONCENTRATIONS IN TUBERCULOSIS AND SARCOIDOSIS, EXPRESSED AS A PERCENTAGE OF A REFERENCE SERUM, MEAN AND SD

Protein	Active Tuberculosis		Sarcoidosis		Normal	
α_1 Lp	104	34[1]	112	35	127	20
α_1 PGp	158	25[3]	122	32	115	19
α_1 AT	241	67[1]	126	31	114	18
α GC	155	22[3]	117	25	117	15
α_2 HS	105	29	101	23	—	—
α_2 M	156	25[3]	131	41	122	25
Caer	252	58[3]	153	41	137	25
Hpt	379	145[3]	115	37	103	44
Hpx	154	52[3]	114	19	107	13
Trf	100	30	110	28	110	15
β Lp	143	54	160	82	177	34
β_1A-c	137	55[1]	116	56	107	24
n	16		18		100	

Significance of difference between tuberculosis, sarcoidosis, and normal
[1]P = < 0·05. [2]P = < 0·01. [3]P = < 0·001.

TABLE III

CORRELATIONS FOUND BETWEEN PROTEINS AND BETWEEN PROTEINS AND CLINICAL DATA

Tuberculosis			
Caeruloplasmin: haptoglobin	r=0·79	n=14	P= < 0·001
Haptoglobin: α_1 antitrypsin	r=0·78	n=14	P= < 0·001
Sarcoidosis			
Birth area: Kveim	r=0·80	n=12	P= < 0·001
β-lipoprotein: ESR	r=0·79	n=12	P= < 0·001
α-lipoprotein: Kveim	r=0·71	n=12	P= < 0·01

n= degrees of freedom= number of pairs of observations minus two.

DISCUSSION

There seems to be general agreement that there is an increase of α_1 and β_2 globulins in tuberculosis (Baldwin and Iland, 1953; Levi-Valensi and Akoun, 1957; Johnson, Wakefield, and Turk, 1967). Some authors find similar changes in sarcoidosis (Ingestad and Tryding, 1966). Our results for tuberculosis are consistent with previous work in that all the α_1 and α_2 proteins, with the exception of α_1 lipoprotein and α_2 HS, were significantly increased. In contrast, none of the levels of the proteins studied in sarcoidosis differed significantly from those of normal subjects.

The normal or low values found in tuberculosis for α_2 HS, albumin, and transferrin indicate that the other increased values observed are unlikely to be due to haemoconcentration. The low α_1 lipoprotein value may be due to deterioration of this protein during storage.

The correlations shown in Table III are interesting, but in view of the small number of cases studied should not be accepted without reservation, unless confirmed by further work, despite the statistical probability values. The relationships previously reported in tuberculosis between α_1 and α_2 proteins and cavitation (Gilliland, Johnston, Stradling, and Abdel-Wahab, 1956), severity (Chievitz and Thiede, 1960), and fever (Johnson *et al.*, 1967) may be due either to the stage of disease at the time of sampling or to the effect of grouping a number of patients, in terms of their electrophoretic mobility, as α_1, α_2, β or γ.

The low β globulin found by Groulade, Guillermand, Duché, and Tizzani (1956) is probably due to a low transferrin level despite the increase of haemopexin, which is normally present in one-third its concentration of transferrin.

The changes reported for sarcoidosis include increases in α_1, α_2, β and α globulins, although any group may be normal in stationary cases: Norberg (1964) and Ingestad and Tryding (1966) reported similar findings. Their significance is not clear due to the presence of impaired liver function as indicated by bromsulphthalein excreted in a number of his cases. Böttiger and Norberg (1964) related the activity of the disease to the protein-bound carbohydrate and seromucoid levels. Our patients could be regarded as

being in a stationary phase (although clinically still active) in view of the normal ESR and lack of current need for steroid therapy. Thus our findings are in agreement with those of Norberg (1964).

No changes were observed in patients with clinically active but not progressive sarcoidosis, in contrast to the definite abnormalities observed among patients with newly-diagnosed tuberculosis who were not gravely ill. These changes are doubtless related to humoral defence, but we feel that the ill-defined term 'acute phase' proteins is misleading, because it is at least possible that each protein concentration in serum is controlled by an independent mechanism. More exact studies of individual concentrations may reveal differences within the so-called 'acute phase' proteins consequent upon different pathological stimuli.

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