ERYTHROPOIESIS AND FERROKINETICS IN CHRONIC RESPIRATORY DISEASE

by

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INTRODUCTION

Chronic exposure to high altitude leads to the development of secondary polycythemia which is roughly proportional to the degree of hypoxia (1,2). However, it has been suggested that many patients with chronic respiratory disease, particularly pulmonary emphysema do not respond normally to the hypoxic stimulus (3,4). The hematopoietic response to altitude hypoxia has been further characterized by measurement of the rate of movement of Fe^{59} out of the plasma and its subsequent incorporation in the red blood cells (5). Thus it was of interest to study the ferrokinetic and erythropoietic responses of patients hypoxic because of chronic respiratory disease, and it is the purpose of this paper to report these findings.

METHOD

1. Subjects

Studies were performed on 11 normal subjects and 25 patients with chronic hypoxia whose diagnosis, physical characteristics and measurements of pulmonary function are shown on Table I. The normal subjects had no clinical or laboratory evidence of respiratory, cardiac, hematologic, or neoplastic disease. In addition there was no evidence of chronic infection or blood loss.

The 12 patients with chronic bronchitis and emphysema had had daily cough and sputum for 10 - 40 years followed by the subsequent development of progressive dyspnea. All had physiologic evidence of increased resistance to air flow and impaired distribution of inspired gas.

The two patients with "pulmonary emphysema" had progressive dyspnea for five to 10 years with no antecedent history of cough and sputum.

The seven obese subjects weighed at least 25 per cent more than their ideal weight*. All complained of dyspnea on exertion.

Two patients with respiratory insufficiency and cor pulmonale due to pulmonary fibrosis were studied. In one this was due to extensive healed tuberculosis, complicated by a thoracoplasty. In the other, fibrosis was related to diffuse honeycomb lung, the cause of which was undetermined even at post mortem. The latter patient had chronic cough and mucoid sputum for the final five years of life.

There were two patients with congenital heart disease who were hypoxic due to a right to left shunt.

RESPIRATORY FUNCTION STUDIES

Vital capacity, maximum mid-expiratory flow rate and maximum breathing capacity were measured with a 9 liter Collins spirometer from which the CO_2 absorber and valves had been removed, and a high speed rotating drum incorporated. The maximum of at least three trials was recorded. Predicted values were obtained from Baldwin et al (6) and Leuallen and Fowler (7). The intrapulmonary distribution of inspired gas was assessed by determining the breath to breath washout of nitrogen from the lungs while breathing 100 per cent oxygen, and by measurement of the concentration of nitrogen in alveolar gas after seven minutes of oxygen breathing using an instantaneously recording nitrogen meter (8).

Arterial blood and expired gas were collected simultaneously over a two to three minute period after the patient had been in the semi-recumbent position for at least 20 to 30 minutes in order to assure a "steady state".

*Tables of Metropolitan Life Insurance Company.

Arterial oxygen and carbon dioxide tensions were measured by a modification (9) of the microtonometric technique of Riley, Proemmel and Franke (10). Carbon dioxide and oxygen contents were measured by the technique of Van Slyke and Neill (11). Expired air was collected in a Tissot spirometer and analyzed for oxygen and carbon dioxide with the Scholander apparatus (12). From this data was calculated the minute volume, tidal volume, oxygen consumption, respiratory quotient, physiologic dead space, dead space tidal volume ratio, and effective alveolar oxygen tension.

HEMATOLOGIC AND BLOOD VOLUME STUDIES

All patients had five or more estimations of hematocrit, on different days, by the technique of Wintrobe (13) or the microtechnique (14). There was no significant difference between values obtained by either technique. The mean of the determinations was recorded and used for each patient. The hemoglobin, leukocyte count with differential, platelet and reticulocyte counts were also measured.

Blood volume determinations were carried out after the subject had been fasting for at least eight hours. The red cell mass was measured by the chromium^{51} tagged red blood cell technique (15) in 19 subjects. The plasma volume was measured by the T 1824 dye dilution technique of Gibson and Evans (16) in 30 subjects and in four patients it was determined from intravenously injected radioactive iodinated serum albumen (RISA) (17). In 17 patients the red cell mass was derived from the T 1824 plasma volume correcting the mean venous hematocrit for trapped plasma (18) and the body to venous hematocrit ratio (19).

FERROKINETIC STUDIES

Serum iron concentration was determined by the technique of Hamilton et al (20) and total iron binding capacity (TIBC) by the technique of Ressler and Zak (21). The rate of disappearance of Fe^{59} from the plasma was assessed by the technique of Huff et al (22). Between 1.0 and 1.5 g. of iron as ferrous citrate containing 12 to 18 microcuries of Fe^{59} was added to the patients plasma, incubated for 30 minutes at 37°C, and then injected intravenously. Plasma samples were obtained from the contralateral brachial artery after 4, 8, 12, 30, 60 and 90 minutes and counted in a well-type scintillation counter using a thallium activated sodium iodide crystal. The radioactivity in the samples was graphed semi-logarithmically and in most instances a straight line was obtained and extrapolated to zero time. Where the relationship was noted to have two components, the second slower component was extrapolated to zero time as suggested by Jensen et al (23). The time required for 50 per cent reduction in activity was calculated from the extrapolated line and was called the Fe^{59} T/2. All ferrokinetic studies were done in the morning with the patient fasting.

The plasma iron turnover rate (PIT) was calculated from measurements of plasma Fe^{59} T/2 and the serum iron concentration according to the method of Bothwell et al (24) using the formula:

$$\text{Plasma iron turnover mg/100 ml plasma/24 hours} = \frac{\text{Plasma Fe ugm/100 ml}}{Fe^{59}\ T/2\ \text{(minutes)}}$$

The red blood cell Fe^{59} incorporation was assessed by a modification of the technique of Huff et al (22). The theoretical maximum incorporation of iron if all of the injected Fe^{59} were utilized for red blood cell formation was calculated from the fraction:

$$\frac{\text{Total number of counts per minute injected}}{\text{Total red cell mas ml}}$$

Following the injection of Fe^{59}, a blood sample was taken every second day for a period of eight to 20 days. The radioactivity present in the blood at this time is due to reappearance of Fe^{59} in the newly formed red blood cells. The activity present in each sample was compared with the figure representing the theoretical maximum incorporation and the percentage of iron incorporated was graphed against the time in days.

The incorporation pattern did not differ significantly when the red cell mass was measured directly by Cr^{51} or derived indirectly from the T 1824 plasma volume in the eight control patients in whom it was compared.

RESULTS

Erythropoietic Studies

Blood gases are shown in Table I; hematocrits and red cell masses are depicted in Table II. The mean hematocrit in the normal male patients was 47.5 (SD = 4.7) and in the normal female patients was 39. It was found to be elevated more than two standard deviations in five patients with chronic respiratory disease, who were among the most hypoxic in the group. Figure 1 depicts the relationship of hematocrit to arterial oxygen tension in all subjects and indicates a significant inverse relationship between hematocrit and arterial oxygen tension ($r = -.564$) ($p < .001$). Also shown in Figure 1 are the ranges of normal hematocrit and oxygen tension in Hurtado's series of subjects at sea level and at 15,000 feet (25). The wide range of hematocrit in each of Hurtado's groups is apparent, and indicates the marked individ-

ual variation in the normal response to hypoxia. The range of hematocrits of the patients in the present study do not seem to differ from this group of normals although they are certainly not comparable in all respects.

The mean red cell mass in the male control patients was 25.3 ml/kg (SD = 1.83) and in females 23.1 ml/kg. It was found to be elevated significantly (more than two standard deviations above the mean for male controls) in 15 patients. Figure 2 demonstrates the relationship between the red cell mass and the arterial oxygen tension. It can be seen that the red cell mass increases with decreasing oxygen tension ($r = -.775$) ($p < .001$). Comparison of the correlation coefficients in Figures 1 and 2 reveals that the red cell mass is more closely related to the level of hypoxia than is the hematocrit.

It is noted that in the patients with chronic bronchitis and emphysema the relationship of the hematocrit and the red cell mass to hypoxia was not different from that of the other chronically hypoxic patients.

FERROKINETICS

The plasma Fe^{59} disappearance rate (Fe^{59} T/2) is presented in Table II. It can be seen that the Fe^{59} T/2 varied over a wide range in the hypoxic patients. As is illustrated in Figure 3 the T/2 was related to the arterial oxygen tension ($r = .556$) ($p < .001$), being shorter in patients with lower oxygen tensions. No overall relationship of the plasma iron turnover to hypoxia was present except in the patients with oxygen tensions below 50 mm.Hg., in whom it appeared to be elevated.

The incorporation of Fe^{59} into the red blood cells in the normal subjects and patients is presented in Figure 4 and Table II.

In the control subjects 70 per cent or more of the injected dose of Fe^{59} was incorporated into the red blood cells by the seventh day after administration and between 80 per cent and 100 per cent by the eighth to tenth days. The obese subjects, those with the other cardiorespiratory and congenital heart diseases, behave similarly except for one (No.26). In contrast, none of the patients with chronic bronchitis and emphysema had incorporated 70 per cent of the dose by the seventh day. Of these; five patients were followed to a plateau of maximum incorporation (for a period of 15 - 20 days) during which only one had incorporated 80 per cent by the 11th day and four still had incorporated less than 70 per cent.

DISCUSSION

The data indicates that in chronic respiratory disorders of all types, secondary polycythemia develops proportionately to the degree of hypoxia. This differs from the opinions of Ratto et al (4), Wilson et al (3), and Hammarsten et al (26) who suggest that there is an abnormal erythropoietic response to hypoxia in these disorders. The data also indicate that there is a variation in the degree of hematopoietic response when elevation of the hematocrit is used as the index of polycythemia; but this spread does not appear to differ from that of the wide range of hematocrits in normal natives at high altitude (25,5). Because the red cell mass is more closely related to hypoxia than is the hematocrit, secondary polycythemia will be more frequently recognized by measurement of the red cell mass, i.e. significant polycythemia may well be present with a normal hematocrit, as is demonstrated by the presence of an elevated hematocrit in only five of the 15 subjects who had an elevated red cell mass. This is consistent with the findings of Berlin et al (27) and Abbatt et al (28) in patients with polycythemia vera.

From his early studies of residents at high altitude, Hurtado (2) suggested that the polycythemic response was depressed when hypoxia was severe (oxygen saturation below 60 per cent or an oxygen tension below 30 mm.Hg.). This was not confirmed in the present study, where the most hypoxic patients had the highest red cell masses. The difference between our data and Hurtado's may be explained by the inadequacy of the hematocrit as discussed above.

Ratto et al (4) and Wilson et al (3) have suggested that increased carbon dioxide tension (pCO_2) may limit the erythropoietic response in chronic bronchitis and emphysema. Inspection of our data indicates that the pCO_2 was generally elevated in the most hypoxic patients who also had the highest red cell masses. The mean pCO_2 of the polycythemic patients was 62 mm.Hg. and of the non-polycythemic was 53 mm.Hg. This difference is significant ($p < .02$) suggesting that, if anything, an elevated pCO_2 may stimulate erythropoiesis. However, it is only possible to infer the influence of the pCO_2 on the erythropoietic response by noting patients with comparable oxygen tensions who have differing carbon dioxide tensions. Reference to such patients in Tables I and II indicates that there is no apparent influence of carbon dioxide tension on the erythropoietic response.

The Fe^{59} T/2 was found to correlate with oxygen tension, hypoxia being associated with more rapid disappearance of Fe^{59}. The T/2 varied inversely with the venous hematocrit and the red cell mass, as would be expected from their mutual relationship to hypoxia (Figures 1 and 3). There was no significant relationship in the present study between the T/2 and the plasma volume. However, it is possible that the apparent increased rate of removal of Fe^{59} is a manifestation of the relationship between the plasma volume and an increased red cell mass as might occur

if the Fe^{59} moved from a relatively smaller plasma pool into a large red cell pool. The mean plasma iron turnover in all patients did not differ from that of the control subjects. This is similar to the data of Hammersten et al in patients with emphysema (26) and normal natives at high altitude (5). The most hypoxic patients, however, did have elevations of plasma iron turnover, particularly those in whom the oxygen tension was below 50 mm.Hg.

It was of great interest that the incorporation of Fe^{59} into the red blood cells was delayed and incomplete in the patients with chronic bronchitis and emphysema. This defect in red cell incorporation of Fe^{59} could not be related to any other function measured, and was associated only with the clinical physiological syndrome of chronic bronchitis and emphysema. The only exception was No.26, whose basic disease was honeycomb lung, but it is of interest that he had developed cough and sputum in the last five years of his life. Defective incorporation of Fe^{59} has been reported by Huff et al in four Peruvian miners with silicosis living at 15,000 feet (5). All were markedly polycythemic and had increased plasma iron turnovers. It has also been demonstrated in one patient with chronic respiratory failure and secondary polycythemia related to the sequelae of poliomyelitis (30). No explanation has been given for this observation.

The impaired incorporation noted in the present study could not be attributed to hypoxia or hypercapnia as the non-bronchitic patients with equivalent gas tensions had normal incorporation patterns. In addition, the defect was present in bronchitis whether they had minimal or marked hypoxia and hypercapnia. Because this defect was restricted to the bronchitics the role of chronic infection must be considered.

However, the ferrokinetics of hematologically significant infection as reported by Bush et al (29) include a low serum iron and total iron binding capacity, a consistently elevated plasma iron turnover rate and normal or accelerated incorporation of Fe^{59} into the red cells. The normal serum iron and iron binding capacity, and slow incomplete Fe^{59} red cell incorporation suggest that infection does not account for the findings in the patients with chronic bronchitis.

The plasma iron turnover rate was equal to or greater than that observed in normals in the patients with chronic bronchitis and emphysema, but the amount and rate of iron incorporated into the red blood cells was reduced. Despite this demonstrated defect, the patients were able to maintain a normal or increased red cell mass. As the present study has been only of the utilization of transferrin bound iron, it disregards other sources of iron for erythropoiesis (i.e. effete red cells). It is possible that these patients have impaired utilization of transferrin bound iron but are able to maintain a significant elevation in red cell mass by increased utilization of iron from senescent red cells. That the utilization of these two forms of iron may differ has been demonstrated in dogs by Freireich, Miller, Emerson and Ross (31). The impaired incorporation cannot be explained on the basis of the polycythemia as the non-bronchitic patients with equivalent polycythemia did not demonstrate the abnormality.

It is possible that the reduced incorporation of iron indicates the movement of iron into a pool other than that of red cell precursors in the marrow. Surface counting over the liver, spleen and marrow in patients with defective red cell incorporation has indicated increased pooling of Fe^{59} in the liver (32). This may offer at least a partial

explanation for the incomplete red cell incorporation in patients with chronic bronchitis and emphysema.

SUMMARY AND CONCLUSIONS

1. Patients with hypoxia due to chronic bronchitis and emphysema, as well as other respiratory diseases, develop secondary polycythemia roughly proportional to the hypoxic stimulus.

2. Secondary polycythemia may be present in chronic cardio-respiratory disease with a normal hematocrit; and is best demonstrated by measurement of the total red cell mass.

3. There is delayed and incomplete incorporation of Fe^{59} into the red blood cells of patients with chronic bronchitis and emphysema.

ACKNOWLEDGEMENTS

The author wishes to thank Drs. L.G. Israels and R.M. Cherniack for their guidance, Dr. Marion Ferguson and Mr. J.P. Mamacos for valuable advice, and Misses Elaine Macdonald, Kathleen Rasmussen, Joan Skanderbeg, Elizabeth Wintrup and Jean Zajac for technical assistance.

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Subj. No.	Sex	Age (yr)	Ht. (cm)	Wt. (kg)	Vital Capacity (% pred.)	Maximum Mid-Expiratory Flow (l/sec.)	Maximum Breathing Capacity (% pred.)	Alveolar N_2 After 7 min. O_2 (%)	Arterial pO_2 (mmHg)	Arterial pCO_2 (mmHg)	Dead Space (ml)	Dead Space (% tidal vol.)
NORMAL CONTROLS												
									82	45	209	49

TABLE

Subj. No.	Sex	Hematocrit (%)	Red Cell Mass (ml/kg)	Serum Iron (ug/100 ml)	Iro C (ug
NORMAL CONTROLS					
1	M	45	25.4	144	
2	F	33	22.1	74	
3	M	53	24.4	94	
4	M	50	25.7	107	
5	M	53	25.6*		
6	M	44		148	

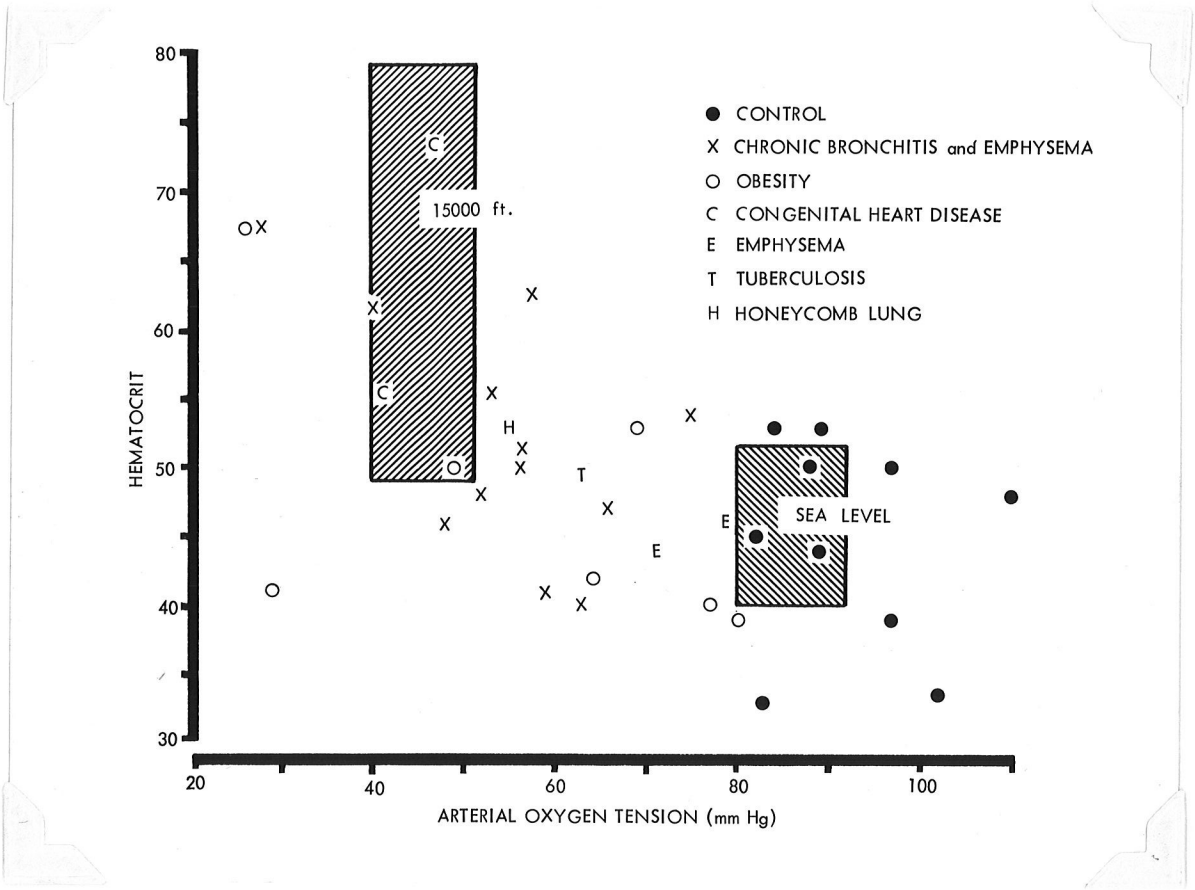

Figure 1. The relationship of hematocrit to arterial oxygen tension in all subjects studied. Shaded areas are the ranges of hematocrit and oxygen tension in the normal subjects at sea level and at 15,000 feet reported by Hurtado (25). The relationship of hematocrit to oxygen tension in patients with chronic respiratory disease does not appear to differ from the normal response depicted.

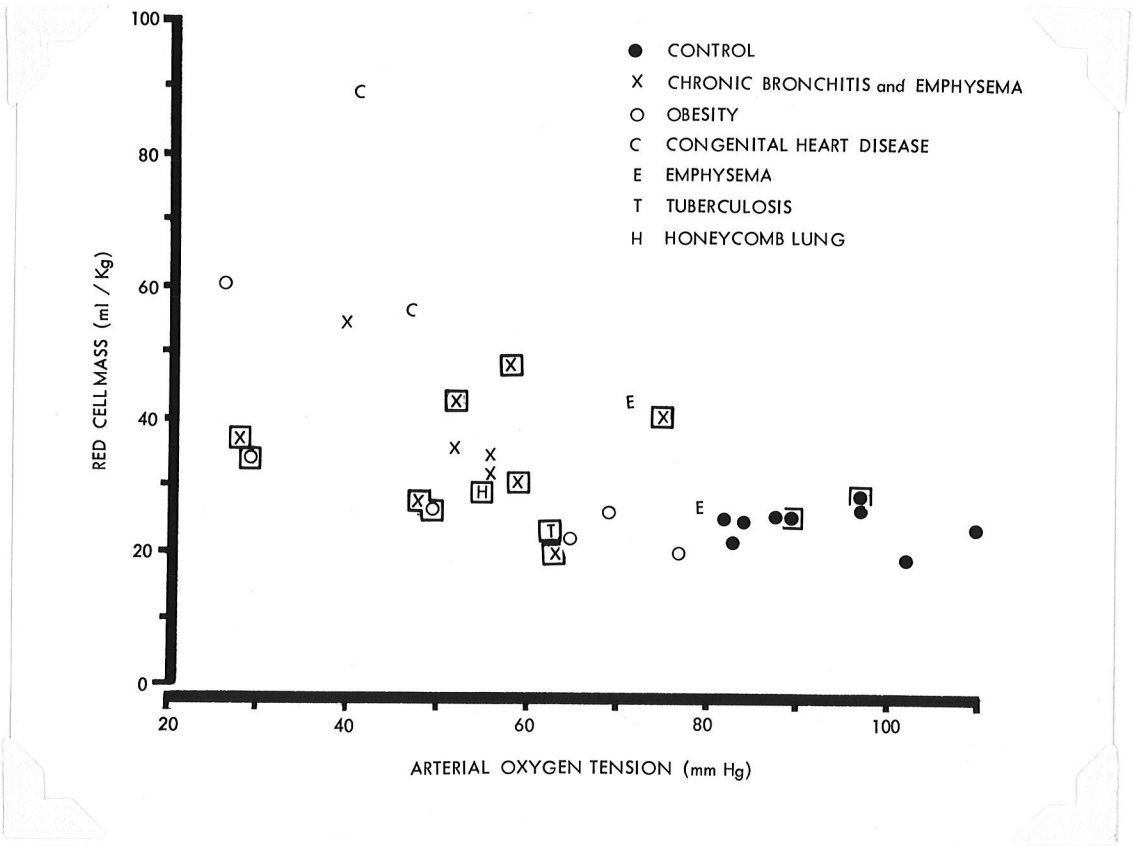

Figure 2. The relationship between red cell mass and arterial oxygen tension. Symbols which have been enclosed in a square indicate red cell mass calculated indirectly from the plasma volume. The remainder have been measured directly by Chromium^{51}.

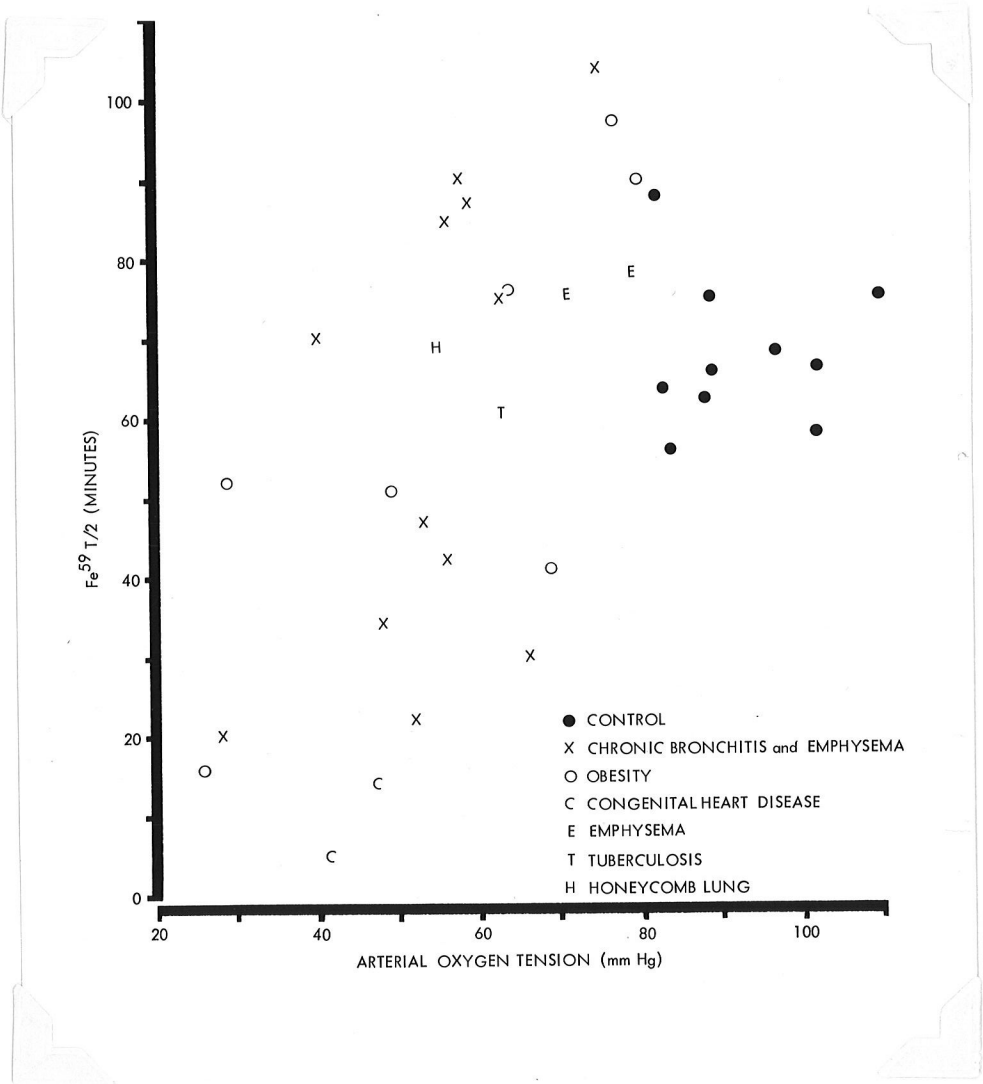

Figure 3. The relationship of rate of removal of Fe^{59} to arterial oxygen tension in all subjects.

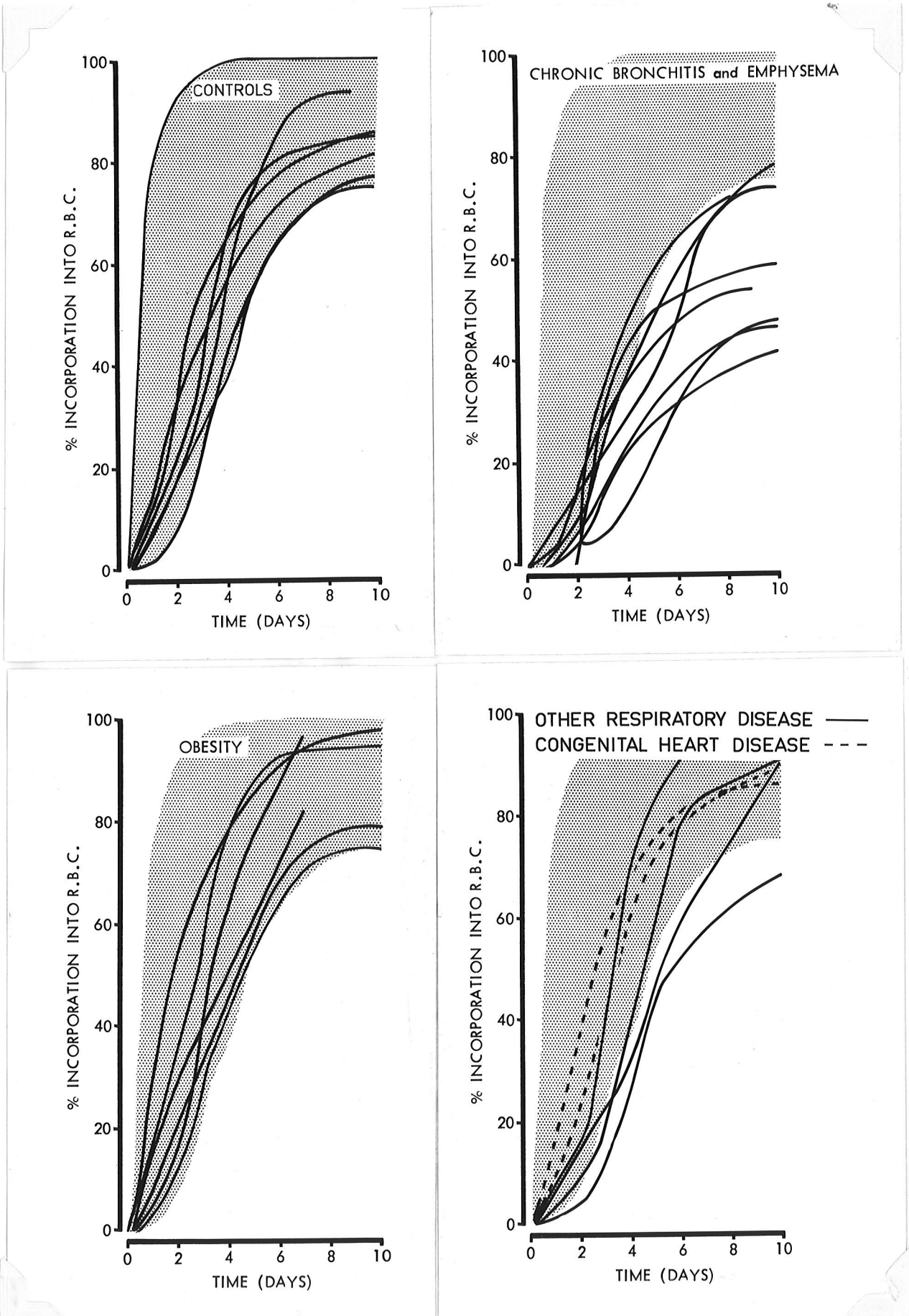

Figure 4. Incorporation of Fe^{59} into erythrocytes. The stippled areas represent the incorporation pattern of the normal control subjects. Each curve represents one patient. There is delayed and incomplete incorporation of Fe^{59} in the group with chronic bronchitis and emphysema.