IMMUNOCHEMICAL DETERMINATION OF TRANSFERRIN IN THE BLOOD SERUM OF LEPROSY PATIENTS

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Investigations of iron metabolism during the past few years have given results which have improved our knowledge of iron transport in the body. It has been shown that the acid-soluble iron in plasma, which is usually called serum iron, is almost quantitatively bound to a special plasma protein ([2, 4]). This protein appears in the literature under the names of iron-binding component (protein), beta 1-metal-combining globulin ([10]), siderophilin ([8]), and transferrin ([5]). The last of these names is used in this report.

The function of transferrin is to establish an equilibrium between iron ion activities in the different organs of the body. Transferrin is not itself metabolized. The quantity of circulating transferrin is normally kept constant at 240 to 280 mgm. per 100 cc. serum, although it may be altered in disease. The iron-binding capacity of this concentration of transferrin (based on an established molecular weight of 90,000) is 300 to 360 micrograms of iron per 100 cc. serum ([10]).

In the study of the variations in the concentrations of transferrin under physiologic and pathologic conditions, indirect methods of investigation have usually been used. The fundamental principle of these methods is that serum can bind iron added *in vitro* or *in vivo* up to a certain limit (saturation limit) in a characteristic chemical bond. The quantity of iron which is bound in this specific way by 100 cc. of serum is usually used as an expression for the iron-binding capacity of serum ([6]).

Goodman *et al.* ([3]) determined the concentration of transferrin in human serum by an immunochemical method. A mean value of 270 mgm. per 100 cc. transferrin was obtained. To determine the iron-binding capacity the values were multiplied by 1.25, since each milligram of protein binds 1.25 micrograms of iron ([10]).

The status of the iron-binding capacity of the blood serum in leprosy has not been reported. It is desired to add to basic information this report of a study of serum transferrin occurring in the blood of patients with leprosy.

MATERIALS AND METHODS

Sera were obtained from 100 leprosy patients, of which 8 were of the tuberculoid and 92 of the lepromatous type. The controls consisted of sera from 20 nonpatient employees.

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Of the 92 lepromatous cases, 67 were bacteriologically positive and 25 were negative. The 8 tuberculoid cases were all bacteriologically negative. All patients were on sulfone therapy.

The procedure developed by Goodman *et al.* ([3]) was used for the immunochemical study. Dr. Goodman supplied us with the antichick transferrin antisera. A quantity of standardized pooled serum from cancer patients was also included.

The serologic reactions were routinely carried out in 13 per cent NaCl. The antigen solutions were also made up to this same concentration. A 1:15 dilution of the patient's serum was made up in 13 per cent NaCl, and 0.3 cc. of antichick transferrin antigen was then added. The reagent blank consisted of 0.3 cc. of 13 per cent NaCl and 3.0 cc. of transferrin antigen. The tubes were incubated in a water bath at 38°C for one hour. After incubation the precipitate was spun down in a centrifuge at 3,000 r.p.m. for 20 minutes. The supernatant was decanted and the tube drained. Then to each tube was added 1.1 cc. of 13 per cent NaCl. The precipitate was resuspended and the turbidity determined by spectrophotometry at 45 mμ against the reagent blank.

Hemoglobin determinations were performed on all patients by a cyanmethemoglobin method.

RESULTS

The distribution of transferrin levels in the individual tested is shown in Table 1. The last three groups—20° mgm. or higher—may be regarded as normal, being within the range of the normal controls.

TABLE 1.—*Distribution of serum transferrin levels in 100 leprosy cases, and 20 controls.*

Amount mgm./% (normal range 208-301)	Lepromatous		Tuberculoid	Controls (20 cases)
	Bact. pos. (67 cases)	Bact. neg. (25 cases)	Bact. neg. (8 cases)	
100–149	14	3	0	0
150–199	20	6	1	0
200–249	22	7	5	10
250–299	9	8	2	9
300–349	2	1	0	1
	67	25	8	20

Of the total 100 leprosy cases, 56 patients (and a like percentage) had normal serum transferrin levels. Low concentrations were found in 34 of the 67 lepromatous cases which were bacteriologically positive (i.e., 51%) and in 10 cases, 9 lepromatous and 1 tuberculoid of the 33, which were bacteriologically negative (30%).

The sulfone drugs are currently being used in the treatment of leprosy. Anemia due to sulfone drugs is not uncommon. Of the 100 cases in this series the hemoglobin levels fell below 10 gm. in 12 of the cases, and below 13 gm. in 66 of the cases; 22 of the cases had normal values, from 14 to 15 gm. The last group also showed normal serum transferrin levels.

DISCUSSION

Moore *et al.* ([7]) state in substance that the plasma iron is influenced by and may be regarded as an index of (*a*) the quantity of iron absorbed

from the intestines, (*b*) the adequacy of the tissue iron reserves, (*c*) the capacity of the bone marrow to utilize iron from hemoglobin synthesis, and (*d*) the activity of the hemolytic processes.

Disturbances of iron metabolism are evidenced by (*a*) decreased formation of hemoglobin, (*b*) decrease in circulating hemoglobin, (*c*) abnormalities in the serum iron concentration, or (*d*) abnormal deposition of iron-containing pigment in the tissues. Certain forms of hypochromic and microcytic anemias are dependent primarily upon inadequate supply or absorption of iron, the latter occurring particularly in the presence of gastric acidity ([7]).

The iron in food is neither completely absorbed nor wholly available. When it is combined in complex chemical linkages, the iron may not be useful because, to be absorbed, it must be convertible to an ionizable form. The iron in foods is almost always in the trivalent or ferric state and is organically bound. Reduction to the ferrous state must take place before absorption can be accomplished.

Brennan *et al.* ([1]), by performing serial determinations of serum transferrin in patients suffering from cancer, have demonstrated a distinct fall in transferrin as disseminated cancer progresses. These changes are reversed with the induction of clinical remissions. In all infections of the liver, except viral hepatitis, serum iron falls below normal levels ([9]).

In the cases tested in the present study the serum transferrin levels were lower among the active (i.e., bacteriologically positive) group. Since lepromatous leprosy is a systematic disease in its dissemination throughout the body, the lowering of the serum transferrin levels could also be associated with low hemoglobin, which is not studied in direct relations in this article.

SUMMARY

Serum transferrin has been studied in 20 normal individuals and 100 leprosy patients, of whom 92 were of the lepromatous type and 8 were tuberculoid.

In the 100 cases, 56 had serum transferrin levels within the normal range of the control group. Low concentrations were found in 34 of the 67 lepromatous cases (51%) which were bacteriologically positive, and in 10 cases, 9 lepromatous and 1 tuberculoid, of the 33 (30%) which were bacteriologically negative.

The iron-binding capacity as evidenced by the serum transferrin is to establish an equilibrium between iron ion activities in the different organs of the body, this equilibrium seems to be altered to a greater extent in the bacteriologically positive lepromatous cases.

RESUMEN

Se ha estudiado la transferencia de suero en 20 individuos normales y 100 leprosos, de los cuales 92 eran de la forma lepromatosa y 8 tuberculoideos.

En los 100 casos, 56 mostraron valores de transferencia de suero que quedahan dentro de los límites normales del grupo testigo. Observáronse concentraciones bajas en 34 casos lepromatosos (51%) que eran bacteriológicamente positivos, y en 10 casos, 9 lepromatosos y 1 tuberculoideo, de los 33 (30%) que eran bacteriológicamente negativos.

La capacidad ferro-fijadora, según la expresan las cifras de transferencia del suero, quedó alterada en 44 (44 por ciento) del groupo completo. Como la función de la transferencia es establecer un equilibrio entre las actividades de los yones de hierro en los distintos órganos del cuerpo, este equilibrio parece alterarse en mayor grado en los casos lepromatosos positivos bacteriológicamente.

RESUMÉ

La transferrine du sérum a été étudiée chez 20 individus normaux et chez 100 malades de la lèpre, parmi lesquels 92 étaient lépromateux et 8 tuberculoïdes.

Chez les 100 cas de lèpre, 56 ont présenté des taux de transferrine qui ne s' écartaient pas des limites de la normale trouvées dans le groupe témoin. Des taux diminués ont été trouvés chez 34 des 67 cas lépromateux (51%) qui étaient bacteriologiquement positifs et chez 10 malades, dont 9 lépromateux et 1 tuberculoïde, des 33 (30%) qui étaient bactériologiquement négatifs.

La capacité de fixer le fer, telle qu'elle est mise en évidence par les de transferrine ferrique, était modifiée chez 44 des cas considérés (44%).

Vu que le rôle de la transferrine est d'établir un équilibre entre les activités de l'ion fer dans les différents organes du corps, cet équilibre parît devoir être compromis dans une grande mesure chez les malades lépromateux bactériologiquement positifs.

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