Simplification of the method of determining total iron-binding capacity of the serum (TIBC) for a routine laboratory test kit

N. Nakazawa, K. Maki, K. Nitta and H. Ogawa
Daiichi Radioisotope Laboratories, Ltd., Tokyo
H. Saito
Radioisotope Laboratory and Department of Radiology, Nagoya University School of Medicine, Nagoya

Purpose

Attempt was made to simplify the method of Saito for the determination of TIBC. Since ascorbic acid solution used in Saito's method for the separation of serum iron from transferrin was unstable at room temperature, we investigated various acids to replace ascorbic acid.

Material and Method

Hydrochloric acid, acetic acid and citric acid were tested. Resins such as Amberlite CG-120 and CG-400 were also tested in combination with these acids. The sera of normal subjects and patients with various diseases were used for the fundamental investigation of the method, and also for the determination of TIBC. Conditions of elimination of serum iron, those of incubation of iron-free serum with radioactive iron solution, and those of elimination of unbound iron ion after incubation were investigated.

Results

Although hydrochloric acid and acetic acid were not suitable, citric acid was available for the determination of TIBC instead of ascorbic acid.

Addition of 1 ml of 0.6% citric acid solution was sufficient to separate serum iron from 1 ml of serum.

Amberlite CG-400 resin powder removed more than 97% of serum iron in 15 minutes rotation with citric acid.

By the precedent addition of sodium bicarbonate solution to radioactive iron solution, the procedure of neutralization of citric acid serum mixture solution was omitted.

Free iron ion in the serum solution unbound to transferrin was removed by a resin strip by rotating it for 90 minutes just like the case of determining unsaturated iron-binding capacity of the serum (UIBC), and centrifugation was not needed.

We have obtained the satisfactory results in determining TIBC of normals and patients by the improved method as compared with the TIBC of the same sera by colorimetry and immunodiffusion (micro Ouchterlony) method. Serum iron values as obtained by subtracting UIBC from TIBC radioiron method each coincided with the values by colorimetry.

In vitro measurement of globulin synthesizing capacity of lymphocytes using ^{75}Se-selenomethionine

M. Hasegawa, H. Yoshioka and I. Iwasaki
Second Department of Internal Medicine, Okayama University Medical School, Okayama

Stimulation of lymphocytes has been observed as increases in synthesis of DNA, RNA and globulin in the presence of phytohemagglutinin (PHA). Globulin synthesizing capacity of human peripheral lymphocytes has been measured in terms of the incorporation of ^{75}Se-eleno-

methionine (^{75}Se) into the globulin protein. Lymphocytes culture in medium contained ^{75}Se with and without PHA were incubated for 96 hr at 37°C. Globulin was separated by adding ammonium sulfate and radioactivity of ^{75}Se incorporated into globulin of 10^6 lymphocytes was measured in a gamma well scintillation counter. The ratio of radioactivities of stimulated to unstimulation cells represents an index of lymphocyte stimulation by PHA.

In lymphocytes of normal subject incorporation of ^{75}Se into globulin was demonstrated in unstimulated cells (basal synthesis) and was well stimulated by PHA. Index of stimulation of PHA was 4.4 ± 2.8.

In Hodgkin's disease basal synthesis was normal but PHA reactivity was suppressed in 2 of 3 cases. In chronic lymphatic leukemia (CLL) basal synthesis and PHA reactivity were remarkably suppressed in 2 cases whose serum gammaglobulin was high. But in a case of CLL whose serum gammaglobulin was low basal synthesis was high and lymphocytes were remarkably stimulated by PHA. In 2 cases of IgG myeloma basal synthesis was normal but synthesis was not stimulated by PHA. In 2 cases of Bence-Jones myeloma globulin synthesis was not stimulated by PHA. In 2 cases of Bence-Jones myeloma globulin synthesis was not stimulated by PHA.

In 5 cases of SLE, one case of dermatomyositis, one case of scleroderma and one case of autoimmune hemolitic anemia PHA reactivity was suppressed. But in one case of scleroderma basal synthesis was high and the synthesis was well stimulated by PHA. Decreased globulin synthesis was seen in uremia, infectious mononucleosis, hypoplastic anemia and hypoglobulinemia of unknown origin.

Index of lymphocytes stimulation by PHA was low in Hodgkin's disease, leukemia, autoimmune disease. Namely lymphocytes reactivity to PHA was suppressed in these diseases.