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Saturation of Serum with CO_2. A Simple Test for Hyperglobulinemia.*

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Ray[1] described the development of cloudiness or flocculation on dilution of the blood with distilled water in cases of kala-azar. Normal blood gives a clear solution on such dilution. An improvement of this test was introduced by Sia,[2] who used measured amounts of blood and distilled water and distinguished different degrees of reaction. The application of the water test in Bilharziasis was described by Salah.[3] The general applicability of this test in conditions of hyperproteinemia was pointed out by Bing.[4] A positive water test in Stiil's disease was described recently by Taussig.[5]

The test is based on the flocculation of euglobulin, the least soluble globulin fraction. Generally it is positive within an hour in diseases associated with high globulin concentrations such as kala-azar, lymphogranuloma-inguinale, multiple myeloma, etc. However, if the concentration of globulin ranges between 3 and 4%, a positive test is obtained only after standing for a considerable time. In these cases the test can be rendered more sensitive and the reaction accelerated by saturating the diluted serum with carbon dioxide of low tension or alveolar air. The flocculating tendency is increased by the lowering of the pH toward the isoelectric point of globulin, pH 5.2. The actual acidity produced under various conditions of dilution and carbon dioxide saturation, as measured with the quinhydrone electrode is given in Table I. The figures of 6.12 and 5.95 are approximately in agreement with the pH of 5.8 reported by Doladihle and Morel[6] in 1:17 diluted serum after saturation with carbon dioxide gas.

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1 Ray, S., *Ind. Med. Gaz.*, 1921, **56**, 9.

2 Sia, R. H. P., *Chin. Med. J.*, 1924, **38**, 35.

3 Salah, M., *J. Egypt. Med. Assn.*, 1937, **20**.

4 Bing, J., *Act. Med. Scand.*, 1937, **91**, 336.

5 Taussig, A. E., *J. Lab. Clin. Med.*, 1938, **23**, 833.

6 Doladihle, M., and Morel, C., *Compt. Rend. Soc. Biol.*, 1936, **122**, 1210.

TABLE I.
pH of Serum Diluted with Distilled Water and Saturated with Carbon Dioxide of Room Temperature.

	Serum dilutions 1:10	1:100
Without CO_2	7.60	7.75
Saturated with CO_2 of 40 mm Hg (alveolar air)	6.89	6.43
Saturated with CO_2 gas	6.12	5.95

Saturation of diluted serum with carbon dioxide precipitates the euglobulin more or less completely. Myttenaere and Bessemans[7] determined the total globulin in the serum by saturating the 1:10 diluted serum with carbon dioxide. However, they give no figures to demonstrate the accuracy of this method. In 4 cases of multiple myeloma Jacobson[8] found that the precipitate obtained by carbon dioxide saturation contained from 9-24% of total globulin. The present results as shown in Table II corroborate those of Jacobson. It would appear, therefore, that this method yields figures that are too low for the total globulin, so it can not be used as a quantitative test.

TABLE II.
Determination of Protein Precipitated by Carbon Dioxide Gas Saturation. (Micro-Kjeldahl).

Total protein	7.0	7.0	7.2	8.5	8.8
Globulin (21.5% Na_2SO_4)	2.8	2.0	3.8	3.6	4.7
Serum diluted 1:10 saturated with carbon dioxide gas	0.2	0.5	1.1	0.4	0.8
Percentage precipitated by carbon dioxide	7	25	29	11	17

It is of value, however, as a qualitative test for the detection of hyperglobulinemia. For diagnostic purposes saturation with carbon dioxide of 40 mm tension or with alveolar air is preferable to saturation with pure carbon dioxide gas for the latter gives a marked precipitation in normal serum. This 40 mm CO_2 test is performed as follows: 0.1 cc of clear non-hemolyzed serum is placed in a 10:100 mm test tube, 1 cc of distilled water added and the fluid saturated with alveolar air.

Evaluation of the test:

— Slight cloudiness, reflections on test tube observed against source of light sharp, no flocculation in standing.
± Cloudiness, reflections visible, slight deposit on bottom of test tube after 24 hours.
+ Definite cloudiness, reflections unsharp, distinct flocculation after 10-15 minutes.
++ Opaqueness, reflections not visible, coarse flocculation after 5-10 min.

[7] Myttenaere, F., and Bessemans, *Compt. Rend. Soc. Biol.*, 1922, **87**, 800.

[8] Jacobson, B. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1257.

The test was compared with the formol-gel, Takata-Ara and Bauer tests in a series of cases in which the albumin and globulin concentration of the serum was quantitatively determined using Minot and Keller's[9] modification of Greenberg's colorimetric technic. The results are given in Table III in which the cases are arranged according to decreasing globulin concentrations.

TABLE III.
Results of Serum Reactions in 25 Cases.

Case No.	Diagnosis	Albumin	Globulin	40 mm CO_2 test	Formol-gel	Takata	Bauer
1 L.D.	Lymphogran. inguin.	3.5	5.1	++	+++	+++	+++
2 A.B.	Hepatic cirrhosis	3.8	4.9	++	+++	+++	+++
3 J.D.	Secondary lues	4.9	4.6	++	++	++	++
4 L.S.	Icterus (unknown origin)	4.4	4.3	++	+++	+++	+++
5 H.M.	Carcinomatosis	3.3	4.0	—	—	±	±
6 W.R.	Pulmonary tuberculosis	3.4	3.7	+	++	++	—
7 R.H.	Pneumonia	4.3	3.5	++	++	±	+
8 L.D.	Secondary lues	4.2	3.4	++	++	+	++
9 G.W.	" "	4.3	3.0	+	±	±	—
10 W.J.	Pulmonary tuberculosis	3.8	2.9	++	+	+++	+++
11 J.C.	" "	4.3	2.8	±	±	+	—
12 J.G.	" "	3.4	2.7	+	—	++	+
13 M.K.	Hepatic cirrhosis	5.8	2.7	+	—	±	+++
14 J.M.	Secondary lues	4.0	2.4	—	±	±	±
15 F.A.	Pneumonia	3.9	2.4	—	—	—	—
16 K.N.	Secondary lues	4.8	2.4	—	±	+	—
17 J.H.	Pneumonia	3.7	2.2	±	—	±	—
18 W.R.	Paget's disease of the bone	4.1	2.1	++	—	+	—
19 J.L.	Secondary lues	5.3	2.1	±	—	—	+
20 A.R.	Pneumonia	4.4	2.0	±	—	—	+
21 A.S.	Pneumonia	4.2	1.9	±	—	±	—
22 E.M.	Paget's disease of the bone	4.4	1.9	—	—	±	—
23 C.D.	Pulmonary tuberculosis	3.9	1.8	—	—	—	±
24 I.G.	Cholecystitis	4.7	1.7	—	—	—	—
24 H.D.	Pneumonia	5.0	1.6	—	—	—	—

It will be seen from the table that in cases Nos. 1-13, with globulin concentrations ranging from 5.1 to 2.7% the 40 mm CO_2 test was ++ or +, in general agreement with the result of the formol-gel, Takata and Bauer tests. A noteworthy exception is case No. 5 with negative CO_2 and formol-gel tests in the presence of a globulin concentration of 4%. On the other hand, cases Nos. 12 and 13 with globulin concentrations of 2.7% and case No. 18 with a globulin concentration of 2.1% gave positive 40 mm CO_2 tests while negative results were obtained with the formol-gel tests. The Takata-Ara test was positive in 2 of these cases and the Bauer test positive in

[9] Minot, A. S., and Keller, M. A., *J. Lab. Clin. Med.*, 1936, **21**, 743.

the third. A negative test in the presence of hyperglobulinemia may be explained by the fact that high globulin concentration is due to an increase in the concentration of the less readily precipitated pseudoglobulin I and II rather than to an increase in the euglobulin fraction. On the other hand, a positive test in the presence of a normal total globulin concentration, may have been caused by a relative increase in the euglobulin fraction. A more detailed discussion of these problems is reserved for another paper.[10]

From the results as presented above it can be concluded that the 40 mm CO_2 test generally is positive, if the globulin concentration of the serum is increased beyond 3% and that the reaction is in accord with the Takata-Ara, Formol-gel, and Bauer tests. As a test for hyperglobulinemia the 40 mm CO_2 test has the advantage of simplicity and speed.

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Specificity of Kerateine Derivatives.

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In a previous communication,[1] it was shown that species specificity is an individual characteristic of the keratins. The suggestion was offered that the redox state and spatial arrangement of the amino acids cystine and cysteine may be intimately connected with the specificity of these proteins.

In this article it will be shown that the substitution of the hydrogen in the –SH groups of reduced keratin (kerateine) with organic halogen compounds gives the protein a new immunological character dependent on the introduced "determinant group."

Since keratins contain a very high percentage of di-sulfide sulfur (10 to 15% cystine) and are readily reduced to sulphydryl proteins by alkaline thioglycolate, a large number of substituent groups may be introduced into their molecules by reaction with simple organic halogen compounds according to the following formula:

$$A — SH + R — X \rightarrow A — S — R + HX$$

Such a reaction occurs readily in a mildly alkaline environment,[2]

[10] Greene, C. H., and Naumann, H. N., to be published shortly.

[1] Pillemer, L., and Ecker, E. E., *Science*, 1938, **88**, 16.

[2] Goddard, D. R., and Michaelis, L., *J. Biol. Chem.*, 1935, **112**, 361.