TABLE I.

Instrument	Reading below which Beer's law was followed
Beckman B	1.300
Coleman	.700
Leitz	.800
Klett	500

spectively. When the level of added chloride was 100 meq/l, in a similar experiment, mean recovery for urine was 100% and for serum 97%.

Precision. Twenty-eight urine specimens were analyzed in duplicate. The standard deviation calculated from these duplicate determinations was 1.86 meq/l and the 95% confidence limits were ± 2.1%. A similar experiment using 28 serum specimens yielded a standard deviation of 2.32 meq/l and 95% confidence limits of ± 4.6%.

Comparison with other methods. The same urine specimens were analyzed by the open Carius method of Van Slyke and Sendroy(3) and the serum specimens by the method of Schales and Schales(4). Values for urine averaged 2.3 meq/l lower than the reference method and values for serum averaged 1.25 meq/l higher than the reference method. By the use of the t test, these differences were not significant at the 5% level (t = 1.51 for urine; t = 1.71 for serum).

Summary. A simple procedure for determination of chloride in biological fluids, based on the mercuric thiocyanate method, is described. It is shown that results obtained by this procedure do not differ significantly from those yielded by the Van Slyke and Sendroy method for urine chloride and the Schales and Schales method for serum chloride.

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Received March 15, 1963. P.S.E.B.M., 1963, v113.

Circulating Antibodies to Egg Albumin in Man.* (28316)

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This study presents an estimate of the incidence of circulating antibodies to egg albumin (EA) in a general hospital population. Egg albumin was chosen as the antigen since it a) is a ubiquitous dietary item, b) is available in reasonably pure form, c) is a small protein (MW 40,000) and perhaps more readily absorbed, d) is well adapted for use in the hemagglutination test system, and e) is an effective antigen in rabbits so that positive control sera can be obtained.

Methods and materials. Sera were obtained from 1086 unselected patients admitted to a 325 bed general hospital and from 38 second year medical students. The bloods were stored and centrifuged at 4°C and the sera frozen at −70°C. The usual elapsed time from collection of blood until freezing was about 4 hours although approximately 20% were held overnight at 4°C before freezing.

Egg Albumin, 1x crystallized and bovine-γ-globulin (BGG) were obtained from the Mann Research Laboratories and stored at −20°C.

Antibody assays employed both hemagglutination and microprecipitation technics. The hemagglutination method used was that of Ingraham(1) with slight modifications. Instead of storing the formalinized erythrocytes in concentrated form at 4°C, the cells were diluted to final concentration and kept frozen at −70°C until use. For titrations 12 x 75

* This investigation was supported in part by a research grant from Nat. Inst. of Allergy and Infect. Dis., USPHS.

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mm acid-cleaned tubes were used.

Screening was done using 0.5 ml of a 1:20 dilution of serum in PS (*i.e.* phosphate saline at pH 7.3 containing 0.5% normal rabbit serum) to which was added 0.1 ml of coupled cells. Higher concentrations of sera produced large numbers of positive reactions. Tubes were read after standing overnight at 22°C; readings were carried out on a 0 to 4+ basis using a stand over a mirror to avoid disturbing the patterns. Positive and negative controls were run with each set.

Those sera giving a 2+ or higher reading in this screening procedure were then serially titrated in 2-fold steps from a 1:10 dilution. Sera so titrated which showed consistent patterns, *i.e,* positives for several tubes followed by negatives, were retitrated in conjunction with an hemagglutination inhibition (HAI) test. For the inhibition test 0.1 ml of solution containing a total of 0.025 mg of egg albumin N was added to the serially diluted sera. The tubes were shaken and allowed to stand for 15 minutes at 22°C before adding the coupled cells. Control experiments showed that addition of the extra 0.1 ml volume did not alter *per se* the end point for a standard titration.

Sera which showed good repeat HA and HAI titrations were once more titrated and neutralized. There were, therefore, usually 3 titration (HA) and 2 neutralization (HAI) values for each patient with a positive serum; 4 had one less of each due to insufficient serum. The figures presented are the geometric mean titers (GMT) from all titrations and neutralizations.

Preliminary studies indicated that the HA antibody was essentially stable to heating to 56°C for 30 minutes.

Controls. These were carried out daily and consisted of 1) cell controls containing only PS and EA cells set up to duplicate, 2) a standard negative serum from a 23-year-old woman, diluted 1:20 plus EA cells and 3) anti-egg albumin serum from a rabbit which had received 40 mg of alum-precipitated EA I.V. followed in 2 months by 10 mg I.V. with bleeding one week later. This antiserum had an HA titer ranging between 100,000 and 200,000 with EA cells. The titer was decreased to between 100 and 320 in the HA inhibition test.

TABLE I. Hemagglutination Titers to Egg Albumin.

Age	Sex	HA	HAI
17	♀	2190	10
68	♂	226	20
6	♂	160	<10
77	♂	133	10
71	♂	113	10
33	♂	80	<10
74	♂	80	<10
16	♀	80	<10
21	♀	64	<10
26	♂	57	<10
35	♀	57	10
18	♂	40	<10
13	♀	28	<10
21	♀	28	10
21	♂	25	<10

Microprecipitin Tests. 70 x 0.7 mm capillary tubes were used. A solution of EA (0.1 mg N/ml in H_2O) was drawn up followed by undiluted serum. Tubes were read on a 0 to 4+ basis after standing overnight at 22°C. Positive and negative controls were run. The standard antiserum gave a 2+ precipitate on a basis of 1 mm = 1+.

Results. The sample consisted of sera from 1124 individuals of which 526 were males and 598 females. All age groups were represented the median being in the fifth decade.

Microprecipitin tests were done on the first 443 of the sera and on all sera giving positive reactions for HA antibody. All were negative. Hemagglutination tests were positive in only 15 patients out of 1124. The geometric mean HA titers to EA and the inhibited titers are presented in order of decreasing titer in Table I.

To check the specificity of inhibition by EA, the standard antiserum and the serum of the patient with the highest titer were titrated with both EA and bovine-γ-globulin (BGG) used as inhibiting agents. EA reduced the titer of the standard antiserum to 0.2% of the original titer and reduced the patient's titer to 0.3% of the original titer. In contrast, BGG had no effect on either serum, thus indicating the specificity of the inhibition by EA.

The patients with antibody could be divided into 3 general groups: 1) Five patients

with complaints involving abdominal discomfort (median HA 160). 2) Four pregnant patients (median HA 68). 3) Six patients with miscellaneous diseases (median HA 60).

Discussion. The hemagglutination test is an extremely sensitive test and false positives can be obtained easily. We believe that false positives are not represented in these results and that we have avoided this problem by 1) thorough acid cleaning of tubes prior to use, 2) positive and negative controls with each series, 3) repeated titrations, and 4) repeated hemagglutination inhibition tests.

György *et al.*(2) reported that one eczematous child had CF antibodies to egg white while a normal child did not. Two normal children developed CF antibodies following injection with egg white. Strobl and Wasitzky (3) studied 224 infants and children. Nineteen or 8% had CF antibodies to egg white. Lippard(4) fed cooked egg to both normal and eczematous infants. One in 20 (5%) of the normals developed CF antibodies to egg white. The antibody titers are hard to determine since these workers used antigen dilutions rather than serum dilutions or box titrations. Their finding that high dilutions of antigen were required to demonstrate antibodies suggest that quantities of antibody present were very small.

The first hemagglutination study appears to have been done by Grabar *et al.*(5). Three patients clinically sensitive to egg albumin gave positive HA tests while 4 controls were negative. Coombs *et al.*(6), reported that 23 normal persons (ages not given) were tested for HA antibodies. Although 11 gave positive reactions, many were too low to be significant in an HA test; in only 2 (9%) were titers over 20, one being over 256 and the other over 2500. No inhibition data were reported.

Larose *et al.*(7). studied 13 patients with clinical sensitivity to egg. Using the tanned-cell technic 6 were negative (<10) and the remainder had titers ranging from 80 to 5120. These results with 54% positive are in reasonable agreement with the values obtained by Lippard in his eczematous group. However, Larose *et al.* studied 9 apparently normal laboratory workers and found 80% positive with titers ranging from 40 to 2560. One explanation for the large number of positive reactors in this small group could be that the group of laboratory workers which Larose *et al.* used as controls had received parenteral immunizations with egg products either as vaccines or skin tests. The latter appears more likely in that all controls were given an intracutaneous injection of egg white (skin test) apparently prior to drawing of sera for the HA antibody determinations.

Information concerning the allergic history in our series of patients is scanty. The 26-year-old man with a titer of 57 had received 3 injections of egg-grown typhus vaccine and had a febrile reaction to the third injection. In this case, it is quite likely that the antibody to EA represents active parenteral immunization. All of the hospital sera were collected in the spring of 1960. It is almost certain that a large number of these patients had received influenza virus vaccine between 1958 and 1960. Such vaccine is prepared from allantoic fluid and contains very little egg albumin. The low incidence of positive sera makes it unlikely that the anti-EA antibody detected was due to influenza vaccine.

Summary. 1) Sera from 1086 patients admitted to a general hospital and from 38 medical students were examined for antibodies to egg albumin using both a microprecipitin technic and a very sensitive hemagglutination technic. 2) Of 450 sera examined by the microprecipitin technic, all were negative. 3) Only 15 patients of the 1124 or 1.3% were found to have antibodies by the hemagglutination technic. Their HA titers ranged from 25 to 2190 with a median of 80. None of these HA positive sera contained precipitins. 4) Five of these patients with circulating antibody had abdominal complaints, 4 were pregnant and the remaining 6 had miscellaneous illnesses.

We wish to thank Sister Josina, SCN, supervisor of St. Joseph's Hospital Clinical Laboratory, and the staff for obtaining serum samples. (J. T. McClellan, M.D., Director).

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Received February 1, 1963. P.S.E.B.M., 1963, v113.

Effects of Phytohemagglutinin on Rat and Normal and Leukemic Human Blood Cells.* (28317)

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Nowell(1) found that phytohemagglutinin (PHA) initiated mitosis in cultures of normal human leukocytes and produced cells which were undifferentiated or blast-like. He stated (2) that the undifferentiated cells were presumably derived from monocytes and possibly young lymphocytes, while other studies(3,4) indicated that the cells represented transformed lymphocytes.

In the present work, the effect of PHA was studied on 1) leukocytes of normal blood, 2) lymphocytes, concentrated and purified, from normal blood, 3) blood cells from patients with chronic lymphocytic leukemia, and 4) leukocytes from rat blood. The objectives of this work were to study the origin and morphology of the living blastlike cells produced by PHA, and to compare them with macrophages.

Methods. The slide chamber method to study white blood cells has been described (5). The cells were suspended in a mixture of equal parts of homologous serum and TC 199 (Difco). Human blood was obtained from non-leukemic patients with normal hemograms and from 10 patients with chronic lymphocytic leukemia. The phytohemagglutinin used was PHA-P of Difco Laboratories. The contents of a vial were dissolved in 5 ml of distilled water. The dilutions were expressed as μl of stock solution per ml of final suspension.

* Aided by grants from Nat. Cancer Inst., U. S. Public Health Service.

Results. Addition of PHA, 0.5 to 10 μl/ml, to suspensions of normal human blood cells resulted in small and large clumps of white and red blood cells, platelets and debris. Large blast-like cells developed after 2 to 4 days of incubation at 37°C and these were called "PHA-cells." The number of PHA-cells in 4 days was usually 2 to 20% of the original number of mononuclear cells. In suspension 3787 (Table I) an unusually large number of PHA-cells developed.

The living PHA-cell as seen in the slide chamber was 12 μ or more in diameter (Fig. 1). Usually the cell was irregular in shape or elongated with pseudopod formation. The nucleus was large and vesicular and comprised about half the cross sectional area of the cell. The pale, rather homogeneous nucleoplasm was enclosed in a sharply defined nuclear membrane. Each cell had one or two large, dark, usually irregularly shaped nucleoli which often appeared to be attached to the nuclear membrane. The cytoplasm was homogeneous with a few coarse dark granules, presumably mitochondria. Pseudopods were large and free of granules.

The viable PHA-cells could be readily differentiated from macrophages. Viable macrophages, as shown previously(6), were much larger, had numerous granules and tended to flatten on the glass slide (Fig. 2). The PHA-cells resembled somewhat lymphosarcoma-cells (Fig. 4) observed in blood of patients with lymphosarcoma cell leukemia(7). Both types