TABLE I

HBsAg titres of two sera tested by CIE and PBA

Number	The titre* of HBsAg in sera by	
	CIE	PBA
1.	16	512
2.	128	4096

* The reciprocal of the highest dilution found positive.

TABLE II

Comparison of 100 *sera tested for HBsAg by CIE and PPA*

	CIE positive	CIE negative	Total
PBA *positive*	1	6	7
PBA negative	0	93	93
Total	1	99	100

highly sensitive method using Cowan I strain of *Staphylococcus aureus* for passive agglutination. This organism is rich in protein A which is a receptor for the Fc portion of immunoglobulin molecules. After the bacteria are coated with immunoglobulins of the desired specificity, they form a convenient reagent for the detection of antigen as it will agglutinate the bacteria[7].

The bacteria were grown in Todd-Hewitt broth at 37° C for 18 hours, harvested, washed in phosphate buffered saline (PBS, pH 7·4, 0·03M) and suspended in PBS (10% v/v). It was treated with formaldehyde (0·3% final concentration) washed again and resuspended as above and heated at 56° C for 30 minutes[8]. The cells were again washed and resuspended in PBS containing 0·1% sodium azide, and stored at 4° C as stabilized bacteria. Human antibody to HBsAg was collected from suitable donors and used to coat the bacteria[8]. Coating was done by mixing 1 volume of antiserum with 10 volumes of the 10% stabilized bacterial suspension and incubating at 30–33° C for 30 minutes. The coated cells were resuspended (10% v/v) in PBS with 0·1% sodium azide and stored at 4° C.

Sera to be tested for the presence of HBsAg were first absorbed with stabilised bacteria to remove any antistaphylococcal antibody. Twenty microlitres of each of the absorbed sera and equal volumes of 1% suspension of bacteria coated with anti-HBs were placed within wax circles of 2 cm diameter, on clean glass slides. After 2 minutes of continuous shaking, the mixtures were examined under appropriate lighting conditions for visible bacterial agglutination. This method is termed passive bacterial agglutination (PBA).

Two sera positive for HBsAg by CIE were serially diluted and the dilutions were tested by both CIE and PBA. The results are presented in Table I. The new method is about 30 times more sensitive than CIE.

Sera from 100 blood donors were also tested simultaneously by both methods, and the results are presented in Table II. While only one serum was positive by CIE, 7 were found to be positive by PBA.

This method would be suitable for routine diagnostic testing of human sera and other body fluids for the presence of HBsAg. It is simple, rapid, specific and highly sensitive.

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BONNET MONKEY ERYTHROCYTE ROSETTING: A MARKER FOR 'B' LYMPHOCYTES

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ROSETTING techniques have been widely employed for identifying cell surface markers of human lymphocytes[1–3]. Lymphocytes modulated by thymic influence (T-cells) rosette with sheep erythrocytes (SE) and thymus-independent lymphocytes (B cells) rosette with SE coated with antibody and complement (SEAC)

Recently we have observed that a proportion of human peripheral lymphocytes (HPL) rosette with erythrocytes (ME) of bonnet monkey (*Macaca radiata*). These lymphocytes appear to belong to the B cell population.

Materials and Methods

Isolation of HPL : Venous blood was collected using heparinized syringes from 15 healthy subjects of 20–46 years. HPL were isolated on Ficoll-Hypaque density gradient[5], washed thrice and adjusted to the required concentration. Physiologic saline was used for washing and suspending the cells[6]. Viability was checked by dye exclusion using 0·2% nigrosine.

Preparation of erythrocytes : Sheep and monkey blood was collected in Alsever's solution and used within 2 weeks. The erythrocytes were washed thrice and adjusted to 1% (v/v). SEAC was prepared as described previously[2]. For some experiments SE and ME were conjugated with fluorescein isothiocyanate (FITC)[2].

SE and SEAC rosettes : These were prepared as described by Jondal and Bianco[2,5].

ME rosettes : The optimal conditions required for HPL to form stable rosettes with ME were first established. The optimum ratio of HPL to ME was found to be 1 : 30–40. The number of rosettes obtained was closely similar when prepared in saline, Hank's balanced salt solution or Eagle's minimal essential medium. There was no significant difference in the number of rosettes when they were read immediately or after incubating them at 4° C for 2 hours. The rosettes did not dissociate when they were kept at 37° C for 15 minutes. Foetal calf serum did not enhance ME rosette count at 2 hours. Therefore rosetting was done in further experiments as follows : 100 µl of HPL suspension (3×10^6/ml) was mixed with 100 µl of 1% ME, centrifuged at 200 g for 5 min and kept at 4° C for 2 hr. The cell mixture was suspended 5 times with a fine pasteur pipette and loaded on a haemacytometer. HPL attached with 3 or more ME were considered as rosettes. A total of 200 HPL were counted for the calculation of percentage of rosette forming cells.

Mixed rosettes : The assay of mixed rosettes using FITC conjugated erythrocytes was done as described earlier[2,8].

Results and Discussion

The ME rosettes were easily identifiable under the microscope. While HPL from persons with A, B, O and AB blood groups were found to rosette with ME, their erythrocytes did not rosette with monkey lymphocytes. Erythrocytes from several monkeys were found to be suitable for rosetting.

In 15 normal subjects, the SEAC rosettes and ME rosettes were counted and the results are presented in Table I. The mean counts were 17·1% and 18·7% respectively, the difference being not statistically significant ($t = 1·141$). There was good correlation, between the results, $r = +0·611$, $p < 0·02$. Thus it appears that SEAC and ME rosetting identified the same population of lymphocytes.

The results of the mixed rosetting tests are presented in Table II. Lymphocytes mixedly rosetting with SE and ME were very few (2·5 ± 1·7%), confirming that the receptors for ME and SE were mutually exclusive. The number of null cells remaining after SE and SEAC rosetting and SE and ME rosetting were 12·1% and 9·9% respectively (Table III).

TABLE I

Comparison of HPL rosetting with sheep erythrocytes, SEAC and monkey erythrocytes

Sl. No.	SE rosettes (%)	SEAC rosettes (%)	ME rosettes (%)
1	55·7	10·0	11·2
2	68·0	19·3	20·0
3	57·9	10·5	18·8
4	75·5	18·7	26·1
5	68·6	24·1	26·0
6	61·3	11·3	10·8
7	68·7	11·0	16·5
8	68·7	26·3	14·6
9	67·2	24·5	20·5
10	64·0	8·1	10·4
11	51·8	12·4	22·5
12	61·4	22·0	18·2
13	62·9	25·5	27·6
14	68·3	16·5	16·2
15	62·0	17·3	22·5
Mean ± SD	64·1 ± 6·0	17·1 ± 6·1	18·7 ± 5·3

TABLE II

HPL showing double markers

Sl. No.	% HPL rosetting with both SE and ME
1	1·0
2	5·2
3	4·0
4	0·9
5	1·8
6	2·4
Mean ± SD	2·5 ± 1·7

TABLE III

Comparison of number of null cells after combined rosette assays

Sl. No.	% null cells after combined SE and SEAC rosetting	% null cells after combined SE and ME rosetting
1	14·4	13·8
2	7·8	5·4
3	17·0	12·9
4	9·2	7·6
Mean±SD	12·1±4·3	9·9±4·0

The rosetting of HPJ. with erythrocytes from other species of monkeys has been reported earlier[7–10]. Lohrmann and Novikos reported that rhesus (*Macaca mulata*) erythrocytes behave like SE and rosette with T cells[7]. However more recently Chiao *et al* have shown that rhesus erythrocytes rosette with null cells, contradicting the above report[10] Pellegrino *et al* described that Japanese ape (*Macaca speciosa*) erythrocytes form rosettes with human B cells[8]. Patel has shown that variable proportions of both B and T lymphocytes obtained from different sources such as thymus, tonsils, spleen, peripheral blood and lymphoblastoid cell lines form rosettes with erythrocytes of African green monkey (*Cercopithecus aethiops*) and rhesus monkeys[9]. Therefore green monkey or rhesus monkey erythrocyte rosetting assay cannot be reliably used to identify lymphocyte sub-populations. On the other hand B lymphocytes may be reliably identified by rosetting with bonnet monkey erythrocytes. ME rosetting is a simpler procedure than SEAC rosetting which is more cumbersome and requires anti-erythrocyte antibody and complement.

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NITRATE ABSORPTION BY CORN: INDEPENDENT OF ROOT NITRATE REDUCTASE ACTIVITY

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RAO AND RAINS[1] have noted that inhibiting the development of nitrate reductase activity (NRA) by tungstate decreased nitrate (NO_3^-) absorption. They presented indirect evidence to show that at least a part of the total NO_3^- absorption is closely correlated with NRA. Benzioni *et al.*[2] suggested that NO_3^- uptake by roots is regulated by NO_3^- reduction products of the shoot, *i.e.*, malate production. These data do not indicate whether root NO_3^- absorption *per se* is dependent on root NRA or not.

Materials and Methods

Cultivar XL-81 corn seeds were germinated in glass trays. After 2–3 days the seedlings were transferred to nutrient culture containers and grown as described by Raghuveer[3]. The plants were grown in controlled growth chambers. The environmental conditions were 14 hour photoperiod (2,500 foot candles supplied by fluorescent and incandescence bulbs) and 30°/20°C day/night temperature regime. Initially 1/4th the strength Hoagland solution was used as medium for growth and later (after the 7th day) full strength Hoagland solution complete with micronutrients was used as described by Hoagland and Arnon[4]. The plants were grown in 4·5 litre containers with 15 mM NO_3^- N per litre nutrient solution. The NO_3^- concentration was maintained at high level (by changing nutrient solution as required) so that NO_3^- uptake is independent of concentration. pH control was achieved using carboxy cation exchange resin IRC–50. NO_3^- samples 0·5 ml aliquot each was taken as described by Raghuveer[3] and NO_3^- nitrogen was determined enzymatically by the method of McNamara *et al.*[5] Though NO_3^- uptake was measured everyday from 9th day onwards, cumulative NO_3^- uptake and average NO_3^- uptake per day was given for sake of concise statement.

Plant roots were harvested at the end of the experiment, *i.e.*, at 23 days after sowing and *in vitro* NRA was estimated as described by Hageman and Hucklesby[6]. Casein (1%) was used in the extraction medium