spectively. The presence of supernumeraries cannot be detected in the polytene chromosomes. Most probably they will be incorporated into the chromocenter. The adaptive significance, if any, of these extra chromosomes is yet to be explored.

In the evolution of the *nasuta* subgroup, the karyotype of *D.n.albomicana* is thought to be the most recent product[10]. In the contemporary genetic system of *D.n.albomicana* there exists exuberant inversion polymorphism[11], and there are reports of centric fusion[12], pericentric inversion[13] and chromosome 4 variation[14].

To this gamut of chromosomal variation, the present findings add yet another pattern of karyotypic diversity. To the authors' knowledge, among *Drosophila, D.n.albomicana* is probably the only species which has revealed the occurrence of so many different types of chromosomal variations.

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1 Dobzhansky, Th., Genetics of the evolutionary process. Columbia University Press, New York and London 1970.
2 White, M.J.D., Animal Cytology and Evolution, 2nd edn. Cambridge University Press, London and New York 1977.
3 Appels, R., and Peacock, W.J., Int. Rev. Cytol. suppl. *8* (1978) 70.
4 John, B., and Miklos, G.L.G., Int. Rev. Cytol. *58* (1979) 1.
5 Baimai, V., Japan J. Genet. *55* (1980) 165.
6 Nirmala, S.S., and Krishnamurthy, N.B., Drosophila Inf. Serv. *49* (1972) 60.
7 Ranganath, H.A., and Krishnamurthy, N.B., J. Hered. *72* (1981) 19.
8 Lakhotia, S.C., and Kumar, M., Cytobios *21* (1979) 79.
9 Ranganath, H.A., and Hagele, K., Chromosoma *85* (1982) 83.
10 Ranganath, H.A., and Hagele, K., Naturwissenschaften *68* (1981) 527.
11 Mather, W.B., and Balwin, G., Drosophila Inf. Serv. *55* (1980) 99.
12 Hagele, K., and Ranganath, H.A., Drosophila Inf. Serv. *58* (1982) 70.
13 Wakahama, K., Kitagawa, O., and Yamaguchi, O., Drosophila Inf. Serv. *46* (1971) 44.
14 Clyde, M., Drosophila Inf. Serv. *55* (1980) 25.

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Pseudomonas lectin PA-I detects hybrid product of blood group AB genes in saliva

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Summary. Pseudomonas aeruginosa galactophilic lectin PA-I exhibits an outstanding affinity for soluble hybrid oligosaccharide products of human A and B genes in saliva of heterozygous AB individuals. Neither A nor B salivas, nor an artificial mixture of them, inhibit PA-I hemagglutinating activity to the same extent as saliva from heterozygotes. Other lectins examined do not exhibit this property.

Key words. Pseudomonas aeruginosa; lectin PA-I; saliva, AB genes.

Since the discovery of the ABO(H) blood groups by Landsteiner in 1900, the biochemistry and genetics of these antigens have been thoroughly investigated[1-4]. They have been found on blood cells, on various other cells and as soluble molecules in secretions[1-4]. Their presence in secretions facilitated the elucidation of their carbohydrate structure and mode of production. The A and B genes, which determine the respective antigens, are responsible for the production of specific enzymes which transfer the immunodominant sugar from UDP to the central precursor chain exhibiting H blood group specificity. N-acetyl D-galactosamine is the dominant sugar of A antigen and D-galactose is the dominant sugar of B antigen. Both are transferred from UDPX to form an α1–3 linkage with D-galactose, which already bears L-fucose as the immunodominant sugar of the H blood group[4]. The presence of ABO(H) blood antigens in secretions as well as on cell surfaces is further dependent on the Se gene[2]. This gene determines the presence of a fucosyltransferase (which forms the soluble precursor H antigen) in the secretory organs[5,6]. A large majority (80%) of the human population possess the Se gene and thus contain AB or H antigens in their secretions (always according to their blood group type). These are known as 'secretors'. The remaining 20% ('nonsecretors') are of the genotype sese. Their secreted glycoproteins or saccharides lack A, B or H specificity. The ABO blood groups are of interest for biochemical genetics since they are a result of single genes and because there is considerable knowledge about their chemical and serological properties. The distinct specificity of the A and B antigens could result in heterozygotes with AB antigens either on the same or on different molecules. Wiener and Karowe[7] suggested the assumption that in group AB individuals, the molecules would possess dual (A and B) specificity. They could not prove this. Their assumption was examined by Morgan and Watkins[8] using precipitation of the specific AB group substances from secretions of heterozygotes by anti-A or anti-B precipitating sera or lectins. Their results showed that the removal of precipitate with either anti-A or anti-B led to a complete loss of both A and B activity. When similar experiments were performed with artificial mixtures of A and B substances, only the compatible antigen was precipitated with the anti-A or anti-B reagent, while the activity of the other antigen was found in the supernatant fluid. They concluded that in secretions of the heterozygous AB individuals there is a hybrid molecule which results from the interaction of the A and B genes which differs

Table 1. Inhibition of the lectin hemagglutinating activity (original titer: 128) by saliva from the different donors

Lectin	Saliva donors, blood groups and secretion (Se)					
	10*-A (sese)	14-A (Se)	6-B (sese)	7-B (Se)	8-0 (NE)	11-AB (Se)
Con A	8–16**	2–4	8–16	4	2–4	4
PA-II	4–8	8–16	8–16	8	4–8	2–4
SBA	64–128	64–128	64–128	64–128	64–128	64–128
ECA	64–128	64–128	64–128	64–128	64–128	64–128
PA-I	64–128	64–128	64–128	64–128	64–128	0–2

*No. of the saliva donors from each blood group. **Residual hemagglutination activity (last dilution^{-1}). NE, not examined.

Table 2. Inhibition of PA-I hemagglutinating activity (original titer: 128) by erythrocytes and salivas of A, B, 0 and AB blood types

Specimen	Blood types A	B	0	AB
Erythrocytes	16*	4	16–32	8
Salivas	64–128	64–128	64–128	0–2

* Residual hemagglutination activity (last dilution^{-1}). The number of the saliva and erythrocyte donors from each blood group as in table 1.

Table 3. Inhibition of PA-I hemagglutinating activity (original titer: 128) by salivas of A or B parents (unmixed or artificially mixed together) and of their AB siblings (undiluted and twofold diluted as a control for the mixture)

Saliva specimen	Blood type	Inhibition (residual activity, dilution^{-1})
Unmixed parent I	A	64–128
Unmixed parent II	B	64–128
Undiluted sibling I	AB	2
Undiluted sibling II	AB	0
2-fold diluted sibling I	AB	8
2-fold diluted sibling II	AB	4
Mixed parents I and II	A and B	64–128

from that produced by either of the genes in the homozygous state. Recently, different results have been reported by Viitala et al.[9] in polyglycosyl peptides isolated from human erythrocyte membranes[10,11]. Viitala et al., using A- and B-specific plant lectins, have shown that the A and B antigens of AB subjects are located in different polyglycosyl peptides (containing up to 70% of the total amount of the cell ABH antigenic sites)[10,11]. Approximately half of the chains carry A determinants; the other half carry B determinants. These researchers have pointed to a seeming discrepancy between their results and those of Morgan and Watkins[9] in this respect. The present report describes findings (reported in brief in Gilboa-Garber[14]) which indicate that the galactophilic lectin PA-I from *Pseudomonas aeruginosa* may be useful for explaining the apparent discrepancy and for the detection of the AB heterozygote molecules.

Fresh saliva samples from 58 healthy persons from various AB0 blood groups were collected and heated in boiling water for 5 min followed by centrifugation to remove insoluble material. Sugar determinations by the anthrone method[12] revealed no significant differences in total neutral hexose content between the salivas of secretors and nonsecretors of the different AB0 blood groups (approximately 1.4 μ mole/ml).

Human anti-A and anti-B sera were used for determinations of the donors' erythrocyte blood groups (by a hemagglutination test) and for the determinations of the donors 'secretion' status (by a hemagglutination inhibition test[2]). The following lectins were used: concanavalin A[13] (Con A, from Miles-Yeda, Rehovot) and PA-II of *Pseudomonas aeruginosa* (purified in our laboratory)[14] which share D-mannoside binding, and the lectins from soybean (SBA, from Miles-Yeda, Rehovot), *Erythrina corallodendron* (ECA) and PA-I of *Pseudomonas aeruginosa*[14] (both purified in our laboratory) which are specific for D-galactose and its derivatives. Papain-treated human erythrocytes were used for the hemagglutination and hemagglutination inhibition tests. 1 vol of the hemagglutinin preparation, exhibiting a hemagglutinating titre of $1/256$, was incubated for 30 min at room temperature with an equal volume of saliva or with ½ vol of thrice washed packed erythrocytes. The original as well as the residual hemagglutinating activity was assayed by titration in a series of 2-fold dilutions in 0.2 ml of 0.85% NaCl solution in tubes. 0.05 ml of a 5% suspension of papain-treated erythrocytes was added to each tube. After 1 h at room temperature, the tubes were centrifuged for 30 sec and the erythrocyte agglutination was determined.

As may be seen from table 1, Con A and PA-II were inhibited by all the salivas independently of their blood group or secretion status. The other three lectins which exhibit a D-galactophilic activity (SBA, ECA and PA-I) were only slightly inhibited by the same, except for the special phenomenon of the marked inhibition of PA-I by AB salivas. These results led us to a further investigation of PA-I. This lectin agglutinated A, B, 0 and AB erythrocytes without distinction and was adsorbed to all of them due to the presence of D-galactose and its derivatives on their surface. Highest adsorption was observed with type B erythrocytes possibly due to the higher affinity of this lectin for D-galactose (the dominant sugar of B antigen), as compared to N-acetyl D-galactosamine (the dominant sugar in A antigen). AB erythrocytes were less active than B in adsorption of PA-I. With saliva of the same donors, essentially different results were obtained (tables 1, 2). PA-I hemagglutinating activity was most strongly inhibited by salivas of AB individuals compared to a low inhibition by B and other types. The same pattern was also observed with families of the heterozygotes AB (table 3). An artificial mixture of A and B salivas even from parents of the AB individuals could not inhibit PA-I hemagglutinating activity as did the AB salivas (table 3).

The results obtained with PA-I using both erythrocytes and salivas from the same donors bridge over the seeming discrepancy[9] between the results of Viitala et al.[9] with the erythrocyte polyglycosyl peptides and those of Morgan and Watkins[8] with the salivas. They indicate that while the erythrocytes from AB individuals behave as though they bear a mixture of A and B antigens, the saliva AB antigens of heterozygotes cannot be replaced by an artificial mixture of A and B antigens. The different interrelationships of the A and B antigens in the erythrocytes and saliva of AB individuals may be related to the nature of the molecules which carry them and the mode of incorporation of the antigens into them. The A and B dominant sugars are added, each to a single polyglycosyl-peptide of the red blood cells, by independently functioning transferases (coded by the A and B genes), after the organization of the cell membrane. In the AB-bearing soluble molecules the A and B antigens are incorporated in to the secreted molecule structure in an interdependent synthesis which leads to the coexistence of both antigens in the same molecule. PA-I is suggested as a useful lectin for the study of blood group antigens and for the elucidation of complex saccharide-bearing structures.

1 Kabat, E.A., Structural concepts in immunology and immunochemistry, 2nd edn. Holt, Rinehart and Winston, New York 1976.
2 Race, R.R., and Sanger, R., Blood groups in man, 5th edn. Davis, Philadelphia 1968.
3 Wu, A.M., Kabat, E.A., Pereira, M.E.A., Gruezo, F.G., and Liao, J., Arch. Biochem. Biophys. *215* (1982) 390.
4 Ginsburg, V., Adv. Enzymol. *36* (1972) 131.
5 Shen, L., Grollman, E.F., and Ginsburg, V., Proc. natn. Acad. Sci. USA *59* (1968) 224.
6 Chester, M.A., and Watkins, W.M., Biochem. biophys. Res. Commun. *34* (1969) 835.
7 Wiener, A.S., and Karowe, H.E., J. Immun. *49* (1944) 51.
8 Morgan, W.T.J., and Watkins, W.M., Nature *177* (1956) 521.
9 Viitala, J., Karhi, K.K., Gahmberg, C.G., Finne, J., Järnefelt, J., Myllylä, G., and Krusius, T., Eur. J. Biochem. *113* (1981) 259.
10 Finne, J., Krusius, T., Rauvala, H., Kekomaki, R., and Myllylä, G., FEBS Lett. *89* (1978) 111.
11 Finne, J., Krusius, T., Rauvala, H., and Järnefelt, J., Blood Trans. Immunohaemat. *23* (1980) 545.
12 Spiro, R.G., in: Methods in Enzymology, vol. 8, p. 3. Eds S.P. Colowick and N.O. Kaplan. Academic Press, New York 1966.
13 Goldstein, I.J., and Hayes, C.E., Adv. Carbohyd. Chem. Biochem. *35* (1978) 127.
14 Gilboa-Garber, N., in: Methods in Enzymology, vol. 83, p. 378. Ed. V. Ginsburg. Academic Press, New York 1982.

0014-4754/85/050681-02$1.50 + 0.20/0