

The radioactive material tentatively identified as UDPGal (R_F , 0.18) was eluted from the paper in 1*N* HCl, boiled 10 minutes, and rechromatographed in an isopropanol-water (4:1) solvent (10) which separates galactose from glucose (R_{glucose} of galactose, 0.80). Over 70 percent of the radioactivity was then associated with the galactose area.

In the L-cell extract prepared according to the above procedures, 88 percent of the radioactivity was found to be associated with galactose, 8 percent with Gal-1-P, and the remaining 4 percent migrated with a R_F characteristic of UDPGal. As much as 6 μmole Gal-1-P and 3 μmole UDPGal accumulated per milliliter of packed cells.

When L-cells were incubated in the presence of lower concentrations of galactose in the medium (5 $\mu\text{mole/ml}$), approximately 10 percent of the total intracellular radioactivity was associated with UDPGal (0.59 $\mu\text{mole/ml}$ of packed cells). Appropriate experiments indicated that less than 0.05 μmole of UDPG was present per milliliter of packed cells. In the presence of still lower concentrations of galactose in the medium (0.05 $\mu\text{mole/ml}$), 54 percent

of the total intracellular radioactivity was associated with UDPGal (0.42 $\mu\text{mole/ml}$ of cells).

The presence of 28 μmole of galactose per milliliter of medium did not significantly inhibit the growth of L-cells when glucose served as a source of carbon. A slight increase in the lag period prior to the onset of exponential growth was observed.

The presence of Gal-1-P in extracts from L-cells incubated with galactose was also demonstrated by the reduction of triphosphopyridine nucleotide (TPN) (2) in the presence of an extract from yeasts adapted to galactose (Fig. 1). Attempts to measure galactokinase activity in crude L-cell extracts by the method of Horecker *et al.* (11) were unsuccessful. Transferase activity was determined by the method of Kurahashi and Anderson (12). Instead of the glycine buffer employed by these authors a histidine-HCl (0.006*M*) buffer at pH 7.5 was used. L-cell extracts prepared by freeze-thawing were found to be rich in phosphoglucomutase and glucose-6-phosphate dehydrogenase activity, and hence it was not necessary to add these enzymes to the assay preparation. Since UDPGal was not available to us, we could not measure epimerase activity directly by the method of Kalckar *et al.* (4). However, by limiting the amount of UDPG in the assay mixture to catalytic, rather than substrate, concentrations, the relative activity of epimerase could be measured: in the absence of epimerase, TPN reduction is dependent upon the concentration of UDPG, whereas in the presence of epimerase, TPN reduction requires only catalytic amounts of UDPG.

Comparison of curves 1 and 3 (Fig. 2) shows that the L-cell extracts had transferase activity (0.10 μmole UDPGal formed in 2 hours) but no detectable epimerase activity. The effectiveness of the assay procedure was demonstrated by the finding that, under identical assay conditions, freshly excised mouse liver displayed epimerase as well as transferase activity.

The results reported here suggest strongly that the inability of L-cells to use galactose as a source of carbon reflects the absence of epimerase activity in this cell strain (13).

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Relationship of Blood Type to Histocompatibility in Chickens

Abstract. Evidence shows that the B blood group locus in chickens, which controls red cell antigens, is associated with tolerance of skin homografts. Three other blood group loci studied did not show this effect.

The various factors involved in transplantation biology have been studied by workers in several scientific areas. Investigations have been most fruitful in those species where inbred lines are available.

In the case of chickens, tolerance to skin homografts is associated with degree of kinship between donor and host (1) as well as with age at grafting (2). Early workers, using heteroimmune hemagglutinins, reported that homograft tolerance and blood type were independent (3). Experiments involving induced homograft tolerance by embryonic transfusion of whole blood or its separate components have led to contradictory conclusions. Red blood cells were held to be ineffective in inducing tolerance in one case (4) but were found to be the most effective cell fraction in another (5). High incidence of homograft acceptance has been observed in one inbred line of chickens known to be segregating at the A blood group locus (6).

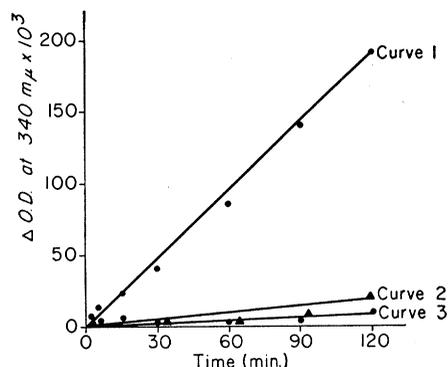


Fig. 2. Demonstration of transferase activity in L-cell extracts. The composition of the assay mixtures was similar to that used in the assay for Gal-1-P (Fig. 1). However, 0.01 μmole of diphosphopyridine nucleotide (14) was added to all reaction vessels in addition to the TPN; the yeast enzyme preparation was omitted. L-cell extracts were prepared by freeze-thawing and contained 8 mg of protein per milliliter: 0.05 ml of this extract was added to the cuvettes, and the total volume in all cuvettes brought to 1 ml with H_2O . Curve 1, L-cell extract incubated in the presence of 0.1 μmole of Gal-1-P. Curve 2, L-cell extract incubated in the presence of 0.4 μmole of Gal-1-P only. Curve 3, extract incubated in the presence of 0.01 μmole of UDPG and 0.4 μmole of Gal-1-P. No TPN reduction occurred when extracts were incubated with 0.1 μmole of UDPG only.

Table 1. Influence of *B* locus genotype with respect to homograft tolerance.

Family designation No.	Birds (No.)	Genotype of host	No. of grafts accepted (+) and rejected (-) from donor of genotype shown					
			B ¹ /B ¹		B ¹ /B ²		B ² /B ²	
			(+)	(-)	(+)	(-)	(+)	(-)
3168	3	B ¹ /B ¹	4	0	0	8		
	4	B ¹ /B ²	8	0	6	0		
3159	4	B ¹ /B ¹	7	0	0	6		
	3	B ¹ /B ²	6	0	6	0		
19	2	B ¹ /B ²					6	0
	3	B ² /B ²			0	6	5	0

The purpose of the experiment reported here (7) was to determine genetic relationships between different blood group genotypes and histocompatibility in an inbred line of Leghorn chickens. Nineteen chicks from three females but having a common sire were used. Inbreeding coefficients ranged from 0.45 to 0.49. Two alleles were segregating at each of four independent loci determining red cell antigens. This included the *A*, *B*, and *L* loci in family No. 3159, the *B*, *D*, and *L* loci in family No. 3168, and the *B* and *L* loci in family No. 19. Prior to grafting, the chicks were blood typed by tube agglutination with isoimmune sera. These sera, considered specific for this line of birds, were used to identify antigens designated A₁, A₂, B₁, B₂, D₁, D₂, L₁, and L₂. Reference reagents, supplied by a commercial firm, were used to establish that the blood group systems studied corresponded to the systems ascertained by other workers (8).

A total of 70 whole thickness skin grafts were made 16 days after hatching occurred. Tissue was exchanged between full sibs only and also reciprocally so that each chick was both donor and host. Chicks received from two to four 8-mm-square grafts, but not more than one graft from the same donor. Location of the graft was randomly assigned to one of four positions on the back. Grafts were exchanged such that the donor had 0, 1, or 2 identifiable red cell antigens not in common with the host. Beginning on the 6th day after grafting began, grafts were observed daily for 1 week and on alternate days thereafter. The grafts were scored for degree of vigor by a series of arbitrary grades (2) which provided an accurate determination of graft rejection time.

Two of the homografts were lost because of faulty technique soon after grafting. In 38 of the remaining 68 grafts, the donor possessed one or more red cell antigens not possessed by the

host. Twenty such donor-host red cell incompatibilities were, with respect to *B* antigens, either alone or in combination with antigens of the other three systems. These 20 grafts exhibited the typical pattern of an immunological reaction by showing signs of rejection on the 7th postoperative day; all had been sloughed off 5 days later. The remaining 48 grafts appeared healthy and were considered to have been accepted by the host at this time.

Thus, rejection occurred only when B₁ or B₂ donor red cell antigens were not also present in the host. The results presented in Table 1 indicate that the *B* locus in chickens not only determines red cell antigens but is also a major histocompatibility locus. This would suggest that red cells may share antigens with the skin which are directly involved in histocompatibility. Studies with birds of known blood type should help to explicate the contradictory results obtained where erythrocytes have been used to induce skin graft tolerance.

The number of remaining donor-host differences involving loci *A*, *D*, and *L* were 8, 4, and 13, respectively. These differences, as well as differences in sex, were not found to influence histocompatibility to the 40th postoperative day. However, five grafts were rejected during the 5th week after grafting. A gradual and less violent reaction was observed in these cases than that previously noted. Thus, it appears that alleles at other loci determining comparatively weak histocompatibility antigens may be segregating in this inbred line. It is well known that sloughing often occurs even after long periods of graft tolerance. Alleles and loci other than those examined in this study may well have variable differences in their effects on histocompatibility.

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Electrophoretic Analysis of the Serum Proteins of Neurological Mutations in Mice

Abstract. The serum proteins of three neurological mutations, tremulous, quivering, and waddler, were studied. The albumin level rose while the globulin level, especially the level of alpha fraction, fell in tremulous mice, but the trend was reversed in quivering mice. In waddler mice the changes were observed only in females.

During the past few years a large number of neurological mutations have been reported in mice by various investigators. These mutations, as briefly reviewed by Yoon (1), may be classified into two large categories: the waltzer-shaker type and the trembler-waddler type. A rapid circular movement is a common characteristic of the waltzer-shaker type, but this movement is absent in the trembler-waddler type. Instead, the latter shows various combinations of the following traits: tremor, paralysis of front or hind legs or of both, muscular incoordination, loss of straightening reflex, locking hindlegs when picked up by the tail, priapism, epileptic form of convulsion, and reduction or loss of fertility. In the search for a biochemical basis for these genetic abnormalities, the serum protein patterns of three trembler-waddler type mutations, quivering (gene symbol, *qv*), waddler (gene symbol, *wd*), and tremulous (gene symbol, *tm*) (2) were studied. Electrophoresis was carried out in a Durrum-type electrophoresis cell with Schleicher and Schuell 2043-A mg/l paper strips (Spinco No. 300-846) and