subsequently slowly rejected over the 30 days following the rejection of the C57BL/6 grafts while the other four remained unaltered.

Finally, it has been previously shown that treatment with antiserum to lymphocytes prolongs the survival of second grafts in A/Jax mice which have rejected C57BL/6 or C3H/He skin grafts (*14*). Our experiments show, however, that thymectomy of mice previously sensitized to such grafts and then treated with the antiserum in doses already mentioned does not prolong the survival of second grafts beyond that seen in sensitized animals given the antiserum alone.

The data suggest that the adult thymus functions in the recovery from a state of immunologic depression associated with lymphocyte depletion induced by antiserum to lymphocytes. Of significance is the fact that recovery is delayed in most of the animals but not permanently prevented. In this regard the few mice with long-surviving C3H/He grafts are of interest. The possibility that specific tolerance of the C3H/He grafts was induced, perhaps maintained by release of antigen from the graft, is to be considered. More experimentation is required to clarify this point. No conclusion can be drawn as to the mechanism by which the adult thymus functions in the recovery from treatment with antiserum to lymphocytes. The thymus glands of intact mice treated with antiserum in these and other experiments (*14*) show a marked depletion of lymphoid elements, but epithelial-reticular components are preserved. This observation suggests to some extent that a humoral factor may be responsible for the action of the adult thymus. This is in keeping with the observations that implants of thymus in Millipore chambers restore immunologic competence in neonatally thymectomized mice (*18, 19*).

Adult thymectomy prior to hydrocortisone treatment in mice (*20*) or sublethal irradiation in rats (*8*) fails to augment skin allograft survival beyond that seen in unthymectomized animals. Neither treatment alone induces prolongation of skin allograft survival comparable to that achieved with antiserum to lymphocytes alone. The degree of immunosuppression induced may determine whether the adult thymus participates in restoration of immune responses. Lethally irradiated mice protected by isogeneic spleen cells recover immune responses at the same rate whether thymectomized or unthymectomized (*12*). Lethally irradiated thymectomized mice given bone marrow, however, fail to recover immune responses at the same rate as their unthymectomized litter mates. Apparently, bone marrow does not contain large numbers of immunologically competent cells, and bone marrow cells attain competence only in the presence of the thymus. Therefore, the degree to which a given immunosuppressive regimen destroys or depletes lymph node or spleen cells, with concomitant necessity for repopulation of lymphoid tissues dependent on cells from the bone marrow, may in turn determine the importance of the thymus in restoration of immunologic competence.

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Human Transferrins C and D_1: Chemical Difference in a Peptide

Abstract. *Peptide analyses have been carried out for chymotryptic digests of transferrin C and transferrin D_1. There is a difference in one peptide, and amino acid analyses indicate that an aspartic acid residue in transferrin C is replaced probably by a glycine residue in transferrin D_1.*

Transferrin (siderophilin) is an iron-binding protein. It has a molecular weight of about 90,000 and appears to be composed of a single polypeptide chain (*1*). It binds two atoms of ferric iron per molecule (*2*) and has four sialic acid residues in each molecule (*3, 4*).

Some 15 electrophoretic variants of human transferrins are known, each resulting from simple Mendelian inheritance. They are named according to their electrophoretic mobility in starch gel at alkaline *p*H (*5*). Transferrin C is the most frequent form, whereas transferrin D_1 is a slower-moving variant common in Negroes.

By analogy with hemoglobin variants, one or more amino acid exchanges would be expected to account for the differences among the transferrin variants, although no such exchanges have been reported. The object of our research was the elucidation of the structural differences between transferrins C and D_1.

Transferrins were isolated by a combination of rivanol precipitation and starch-block electrophoresis (*6*). About 500 ml of blood was collected from persons who were homozygous for transferrin C and for transferrin D_1. One part of plasma, to which $FeCl_3$ was added (about 1 mg/100 ml) to saturate the transferrin, was diluted with three parts buffer (tris-hydroxymethylaminomethane, 0.005*M*, *p*H 8.8), and an equal volume of 0.6 percent rivanol (2-ethoxy-6,9-diaminoacridine lactate, California Foundation for Biochemical Research) dissolved in the same buffer was added. The supernatant was passed through potato starch to remove the rivanol, and the proteins in the filtrate were concentrated by adsorption onto diethylaminoethyl-Sephadex (A-50, coarse). After elution with 0.1*M* NaCl and dialysis against water (*p*H 8.0), the material was lyophilized. The lyophilized material was dissolved in barbital buffer, *p*H 8.6, ionic strength 0.05, and subjected to

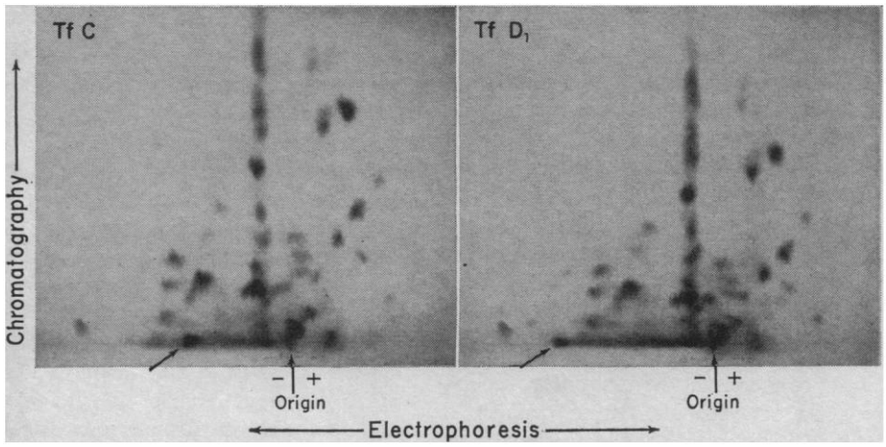

Fig. 1. Chymotrypsin digests of transferrins C and D_1. Electrophoresis was carried out at *p*H 6.4 in a buffer mixture of pyridine, acetic acid, and water buffer (25:1:224). This was followed by descending paper chromatography in a pyridine–isoamyl alcohol–water solvent (14 : 14 : 11). The peptide marked with an arrow is different in these two transferrins and is thought to account for their different electrophoretic mobilities.

starch-block electrophoresis in the same buffer. The colored band of transferrin can be eluted after its separation from contaminating proteins. From 500 ml of blood, approximately 350 mg each of essentially pure transferrins C and D_1 were isolated. Amino acid analyses and sedimentation studies gave results similar to those already reported for pure transferrin (*1*, *4*).

Both trypsin and chymotrypsin digestions of transferrin have been used for obtaining two-dimensional electrophoretic-chromatographic peptide patterns, called "fingerprints." Our results are based on chymotrypsin digests made at 37°C and *p*H 8.0. One milligram of enzyme (Worthington, crystallized three times) was used for 150 mg of heat-denatured transferrin. The transferrin-iron complex was heated at 100°C for 30 minutes to prevent renaturation of the transferrin to a form resistant to enzyme attack. The digestion is maintained at *p*H 8.0 with 0.1*N* NaOH in a *p*H-stat. Digestion is carried out for about 3 hours.

"Fingerprints" of transferrin C and transferrin D_1 (Fig. 1) indicate several differences. Only one difference was consistent for all separate digests of transferrin C and transferrin D_1 (marked with arrows). Other differences are presumably attributable to the facts that cystine residues were not stabilized by alkylation, that there were various degrees of sulfhydryl exchange, and that different digests showed different degrees of oxidation.

The peptide which is different has been arbitrarily designated as 1, and the two forms are 1C and $1D_1$. The homology of these two peptides rested initially on their strongly hydrophilic nature and their color reaction with ninhydrin, which starts as a yellow spot before turning purple.

The $1D_1$ peptides were isolated by paper chromatography and paper electrophoresis. The initial digest was subjected to paper electrophoresis at *p*H 6.4 in a mixture of pyridine, acetic acid, and water (25 : 1 : 224); 4500 volts for a strip of Whatman 3MM filter paper 125 cm long by 12.5 cm wide; 90 ma; 3 hours. Guide strips were cut from the paper and dipped in ninhydrin to locate the position of the band containing the $1D_1$ peptide. This region was then eluted with 1*M* acetic acid, and the eluate was evaporated to dryness. The residue was subjected to descending chromatography on Whatman 3MM paper with a solvent consisting of pyridine, isoamyl alcohol, and water (14 : 14 : 11). After 16 hours, the chromatogram was removed from the solvent and dried, and the $1D_1$ peptide was located with guide strips and eluted with 1*M* acetic acid. The chromatographic procedure was repeated by ascending chromatography in a solvent of ethanol, ammonia, and water (80 : 1 : 20) for 14 hours. Elution from this chromatogram yielded essentially pure peptide $1D_1$.

The 1C peptides were isolated on a Dowex 50-X2 column (1.9 cm by 36 cm) with a buffer of pyridine, acetic acid, and water, beginning at *p*H 3.1 and ending at *p*H 5.0. The fractions containing the 1C peptide were pooled and dried, and the residue was subjected to paper electrophoresis as in the $1D_1$ peptides, but for 5 rather than 3 hours. Elution from the paper strip gave essentially pure 1C peptide. Two separate isolations, designated I and II, were carried out for each of the 1C and $1D_1$ peptides.

The isolated peptides were hydrolyzed with 6*N* HCl under reduced pressure at 110°C for 16 hours. The resulting free amino acids were analyzed (*7*) on the Spinco automatic amino acid analyzer (Table 1). The absence of aspartic acid in $1D_1$ as compared to 1C is readily apparent. Correspondingly, there is a consistent increase in glycine in $1D_1$. There is also an increase in the amount of lysine, but the increase in lysine is smaller and less consistent. The increase is probably an artifact, caused perhaps by consistently greater

Table 1. Amino acid analysis of the 1C and $1D_1$ peptides. Each run (I and II) was made on independently isolated material. Analysis was on the Spinco automatic amino acid analyzer. Blank spaces indicate that the amount was too small for a reading.

Amino acid	1C Peptide					$1D_1$ Peptide				
	μMole		Mole fractions		Residues (No.)	μMole		Mole fractions		Residues (No.)
	I	II	I	II		I	II	I	II	
Lysine	0.131	0.179	.29	.26	2+	0.047	0.136	.34	.37	2+
Histidine	.009		.02							
Arginine	.007	.020	.02	.03			trace			
Aspartic acid	.059	.087	.13	.13	1	.004		.03		
Threonine	.010	.021	.02	.03						
Serine	.056	.072	.12	.11	1	.017	.048	.12	.13	1
Glutamic acid	.062	.089	.14	.13	1	.020	.054	.14	.15	1
Proline										
Glycine	.055	.098	.12	.14	1	.031	.085	.22	.23	2
Alanine	.010	.024	.02	.04						
Half cystine										
Valine	.006	.014	.01	.02						
Methionine	trace									
Isoleucine	trace						trace			
Leucine	.005	.008	.01	.01		.002		.01		
Tyrosine										
Phenylalanine	.045	.072	.10	.11	1	.017	0.040	.12	.11	1

contamination of lysine-containing peptides in the more-electropositive $1D_1$ peptide.

The replacement of aspartic acid by glycine would explain the difference in electrophoretic behavior of transferrin C and transferrin D_1 at alkaline *p*H. Transferrin C would have a greater negative charge and therefore would migrate more rapidly to the anode. In view of the great genetic variability in human transferrin, it is possible that other amino acid changes also exist between transferrin C and transferrin D_1. However, the one reported here is sufficient to explain the observed difference in mobility.

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Molt and Intermolt Activities in the Epidermal Cells of an Insect

Abstract. *In the larva of the butterfly* Calpodes ethlius, *molt and intermolt syntheses by the epidermis are each preceded by a phase of RNA synthesis. Endocuticle deposition and the secretion of wax are not controlled only by the molting hormone when they take place during the intermolt period.*

In the fifth-instar larva of *Calpodes ethlius* (Lepidoptera, Hesperiidae), two molting activities of the epidermis, wax secretion and endocuticle deposition, recur during the intermolt period. Some endocuticle is deposited throughout the stadium, but the rate of deposition is low until about 2½ days after the fourth to fifth molt, 2 days after feeding has begun. The fifth stadium lasts about 7 days, during which time an endocuticle about 7 to 8 times as thick (70 to 100 μ) as that in the fourth stadium (10 to 14 μ) is laid down. This massive reserve of material is resorbed at the end of the stadium during the construction of the pupa (*1*). The deposition of endocuticle after feeding is not unusual in insects (*2*).

We have studied the control of the deposition of this endocuticle autoradiographically, using tritiated glucose as a marker for chitin and tritiated tyrosine for the protein component. Glucose is incorporated in clearly defined bands, presumably in the chitin component of the lamellae (Fig. 1*a*). Larvae in the middle of the stadium continue to deposit cuticle next to the epidermis even when the nerve cord has been cut to induce starvation (Fig. 1*b*). Larvae with the head ligated go on to pupate but do not deposit layered endocuticle (Fig. 1*c*). The nutritional state and the amount of ecdysone are thus adequate for pupation, but some stimulus for the deposition of endocuticle during the intermolt period is missing. The deposition of endocuticle during the intermolt period may thus be similar to the secretion of wax in this period, which has been found to require the presence of both the prothoracic gland and some factor from the head for its continuation (*3*). We may conclude that the epidermis is influenced in its syntheses during the intermolt period by factors in addition to the molting hormone and nutrition.

If the molt and intermolt phases of synthetic activity in the epidermal cells are causally independent, one might expect two periods of preparation on the part of a cell. We obtained evidence for this hypothesis from autoradiographic studies of the rates of RNA synthesis at different times in the stadium, using tritiated uridine (10 μc/gram-weight of *Calpodes* larva, in 0.1 ml Ringer solution) as a marker. The solution was injected into the haemocoel; 4 hours later the larvae were fixed and sections were prepared for autoradiography. Control sections incubated in ribonuclease showed little incorporation of tritium. Most of the tissues showed that some tritium was incorporated, maximum incorporation being related to molting, but the epidermal cells which secrete endocuticle and the cells which later also secrete wax showed two peaks of activity in each stadium. Figure 2 shows the changes in rate of RNA synthesis in the molt-intermolt cycle. There is a peak just before the molt toward the end of the fourth stadium, a peak just before the intermolt period at about a day after ecdysis, and another peak shortly before pupation. The pupa deposits little if any intermolt endocuticle and it shows no increase in RNA synthesis before the intermolt period. We shall refer to the peak before molting as the premolt peak and the peak preceding the intermolt period as the preintermolt peak. The pattern of RNA synthesis is not attributable to the synthetic activity associated with nuclear division. The number of labeled nuclei after the incorporation of tritiated thymidine for 4 hours reaches a broad maximum toward the end of the stadium. These results suggest that the preparation on the part of a cell for the syntheses involved in molting is not

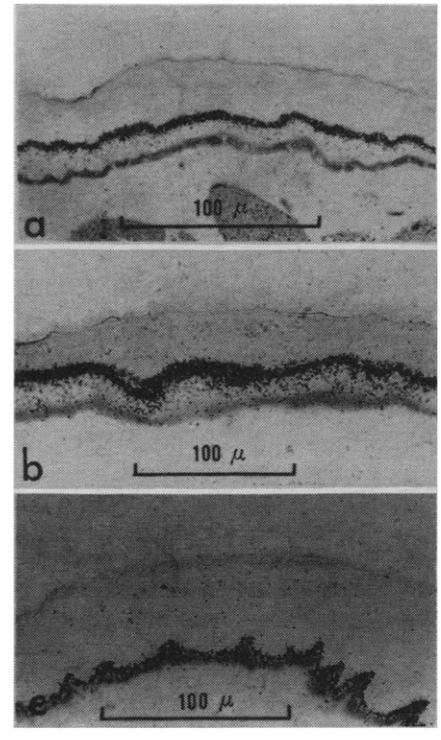

Fig. 1. Autoradiographs showing the uptake of tritiated glucose (dose, 10 μc per gram live weight of larva) and its deposition in the endocuticle after a 12-hour period of incorporation (fixed in 4 percent formaldehyde at *p*H 7). (*a*) Control larva; the silver grains extend in a dense band at a distance from the epidermis. (*b*) Larva starved and quiescent after the nerve cord had been cut between the first and second thoracic segments 24 hours before the beginning of the incorporation period. The incorporation of labeled glucose still occurs at a distance from the epidermis. (*c*) Larva which had had the head ligated 24 hours before the incorporation period. The silver grains lie next to the epidermis and there has been little deposition of endocuticle, although this larva would have gone on to pupate.