The evidence presented provides support for the existence of two separate cell populations involved in recognitive and destructive phase of cell mediated immunity as studied in these in vitro systems. There is evidence that MLC responsive cells are largely thymus-derived (T) lymphocytes (*17*); investigations of monolayer-adherent sensitized cells and cells mediating cytotoxicity in systems similar to CML (*18*) also implicate T cells. Several lines of investigation suggest that more than one of type T cell exists (*19*). The separable cell populations reported here may thus represent two T cell populations. Alternatively, the CML active cell may be a bone marrow derived (B) lymphocyte (*20*) or a macrophage. One could argue that a single cell lineage is involved and that prior activation of LD antigens is required for that same cell to recognize SD and mediate CML. However, this interpretation is not consistent with the demonstration that adsorption of unsensitized cells on specific monolayers separates the cells responding in MLC from those mediating CML. This result, with evidence that MLC activation is required for CML (*9*), implies that cell-cell cooperation is involved in development of cells mediating CML.

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Dystrophic Chicken Muscle: Altered Synaptic Acetylcholinesterase

Abstract. *Individual motor endplates in the skeletal muscles of chickens genetically homozygous for muscular dystrophy have been compared with those in normal chickens. Measurements were made there, by specific autoradiographic techniques, of the numbers of total cholinesterase-like molecules and of acetylcholinesterase molecules. The acetylcholinesterase is distinctly decreased at the endplates in dystrophic muscles. The various data available on these muscles are compatible with the concept that a neural factor which determines the synaptic acetylcholinesterase, along with a number of other characters in the muscle cell, is defective in this disorder.*

Muscular dystrophy, in most of the types of this disorder in man and experimental animals, is inherited as a single Mendelian character (*1*); the molecular mechanisms, however, remain unknown. A major possibility that is consistent with several types of observation (*2*, *3*) is that regulation of functional systems in the muscle by a neural factor is genetically impaired. The synaptic junction is likely to be one of these regulated systems (*4*), and defects at this site in dystrophic muscles have been reported (*3*, *5*, *6*).

We report here on measurement of the cholinesterases (ChE) at the neuromuscular junctions in the muscles of normal chickens and of those homozygous for muscular dystrophy, revealing a difference there between the two types. The complement of cholinesterases [among which we include acetylcholinesterase (AChE)] is of especial interest here in view of the significant findings of Wilson and colleagues (*2*), that there are in these latter chicken muscles when dystrophy develops (soon after

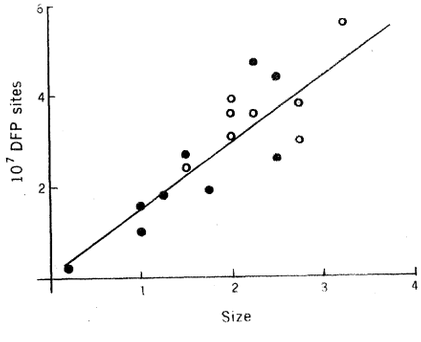

Fig. 1. The mean number of DFP-reactive active centers per endplate in the chicken muscles, measured by [^{32}P]DFP reaction and β-track autoradiography of endplates on individual fibers. These contents are shown as a function of the mean width of the fibers (in arbitrary units), both for normal (filled circles) and dystrophic (open circles) muscles: the increase in size of the fibers corresponds to the increasing age of the birds (to 14 months) or to dystrophy. Each point gives the mean value for the endplates of posterior latissimus dorsi or biceps muscle from one animal [a total of 254 fibers was employed, not including those for the lowest point (6-day chick) where 96 fibers in nondissected bundles were used]. A regression line was fitted by weighted least squares analysis, giving a correlation coefficient of $+.85$ ($P < .001$, for correlation).

hatching) some major ChE isoenzymes not present in the normal muscle. Those workers analyzed the soluble enzymes in extracts of whole muscle by electrophoresis. However, the motor endplate ChE is, in known cases (*7*), only 2 to 20 percent of the total ChE activity of the muscle, although this percentage appears to increase somewhat in smaller muscles (*8*); it is, moreover, membrane associated and insoluble (*9*). For these reasons, it is important to measure specifically the forms of ChE actually located at the junction. Methods developed in our laboratory (*10*) provide the required spatial and chemical selectivity. Radioactive diisopropylfluorophosphate (DFP) is reacted to saturate the ChE active centers, and the numbers of these at the endplates are determined by autoradiography. The different types of ChE at the endplate are then distinguished by their affinities for known specific ligands (*10*).

The principal method used in this system for the discrimination of the AChE active centers among all of those reacting with the isotopic DFP is the removal of the label from the DFP-inhibited AChE by the reactivator highly selective (*11*) for this active center, pyridine-2-aldoxime methiodide (PAM). This inference from biochemical data on AChE has been upheld in studies on endplates in mouse muscles (*9*), in which the AChE-specific reversible inhibitor BW284C51 was shown to protect from [^{3}H]DFP reaction the same number of sites that was assigned to AChE by the PAM-reactivation criterion. These PAM-sensitive molecules have been shown by electron microscope autoradiography to be specifically associated with the synaptic membranes or cleft at a high surface density (*12*). Further, the PAM-reactivable (or BW284C51-binding) sites have been shown, by microspectrophotometric enzyme assays, to be responsible for all of the measurable AChE activity when studied in mammalian endplates (*7, 13*). Histochemical evidence (*14*) on chicken endplates is in line with this.

Chickens (*15*) homozygous for the dystrophy gene, and the corresponding control line, were used. All of the hatched chicks from this dystrophic stock exhibited the first symptoms (*1, 2*) of the disorder about 10 days after hatching. From freshly dissected muscles, fragments of endplate-rich regions were treated (in turn) with 10 percent formalin (4°C for 2 hours), *p*H 7.4, buffer (two baths), and $1 \times 10^{-5}M$ [^{3}H]-DFP (Amersham Searle, 3.23 c/mmole) for 20 minutes at room temperature, and then washed twice with DFP ($10^{-3}M$) and buffer; this procedure was followed by grain density autoradiography of frozen sections. All other details have been described (*10*), as have the procedures used on parallel specimens with [^{32}P]DFP instead of [^{3}H]DFP, and β-track autoradiography.

The total number of the active centers at each endplate that reacted rapidly and to completion with DFP was determined (*10*) by the absolute β-track autoradiographic method (Fig. 1). This number increases linearly with the fiber size [as we have found, too, in mammalian muscles (*13*)], but is *not* significantly changed at the endplates of dystrophic, as compared to normal, muscle fibers of the same size. The percentage of these DFP-reactive endplate sites that represent ChE of all types was determined by the blockade of the [^{3}H]DFP reaction by eserine (*10*), which carbamylates ChE (*16*). This showed (Table 1) that about 80 percent of the DFP-reactive sites are ChE active centers, and that there is, again, no significant difference in this proportion between normal and dystrophic muscles.

A highly significant decline in AChE (by one-third to one-half) was seen (by the PAM-reactivation method) in every case in the endplates of dystrophic, as compared to normal, chicken posterior latissimus dorsi (Table 1) or pectoral or biceps muscles (Table 2). There was no significant decrease in endplate AChE when, instead, the anterior latissimus dorsi, or the biventer muscle of the same dystrophic birds, was used (Table 2). In these last two types, unlike the first three, the fibers are predominantly red and are refractory to the dystrophy (*1, 2*).

In view of the evidence referred to above on the properties of the PAM-reactivable molecules at the motor endplate, we conclude that there is a decrease in the number of AChE molecules at the dystrophic junction. They appear to be replaced by the same number of ChE molecules (by the eserine-binding criterion) of some other type. Relevant evidence on mammalian muscles can also be mentioned. In dystrophic mice (*17*) and humans (*18*) it has been reported that the slow twitch (red) muscles are, again, relatively resistant to the pathogenesis. Hypersensitivity to anticholinesterases in the response to nerve stimulation has been found in the affected muscles of dystrophic mice (*5*), and similar observations have been made on these dystrophic chicken muscles (*13*).

In conclusion, either there are at least two isoenzymes in the endplate AChE of the normal muscle, one of which is suppressed or inactive in dystrophy, *or* the quantitative expression of a single AChE type which becomes bound at the endplate is reduced. Either possibility is consistent with a

Table 1. Cholinesterase types in endplates of posterior latissimus dorsi muscles of paired normal (N) and dystrophic (D) chickens. The percentage of the DFP-reactive sites that corresponds (i) to ChE active centers of all types and (ii) to AChE active centers, was determined (i) by the blockade of the [^{3}H]-DFP reaction by $1 \times 10^{-5}M$ eserine, present for 30 minutes before, during, and in the washing after the reaction and (ii) by the reduction in labeling due to treatment with PAM ($10^{-3}M$ for 30 minutes) given immediately after the washing with DFP that followed the [^{3}H]DFP reaction. Autoradiographic exposures were on the same slides for all specimens, for each pair of birds, for periods of 24 to 39 days. The mean grain count in 100 to 900 fields over endplates was taken for each animal, this always being in the range 8 to 16 grains per 36 μm^2; the standard error of the mean was always in the range 3 to 8 percent of the mean.

Age	ChE (%)		AChE (%)	
	N	D	N	D
8 days	77	79	57	39
4 weeks	86	88	56	38
6 weeks			53	39
12 weeks	78	88	54	36
14 weeks			56	31
Means	80	85*	55	37†
Leghorn adult‡			54	28†

* Difference from normal not statistically significant. † Difference from normal significant, at $P < .001$. ‡ A second experiment was performed using another line of dystrophic chickens, that is, the Connecticut stock dystrophic chickens, and White Leghorn controls; each mean is for the total of 600 fields from the muscles of three animals.

Table 2. The proportion of AChE active centers in the DFP-reactive sites at the endplate in four other muscles. The procedure was the same as that described for the experiments in Table 1. The two red muscles in the dystrophic chickens did not show the disease symptoms. Adult Connecticut stock dystrophic chickens and White Leghorn controls were used, except for the anterior latissimus dorsi (ALD), when lines 304 and 200 (as in Table 1) were used, their ages being 1 to 14 weeks (with no significant variation with age of these percentages).

Muscle	Animals (No.)	AChE (%)	
		Normal	Dystrophic
	White		
Pectoral	6	54	37*
Biceps	6	61	36*
	Red		
ALD	10	48	41†
Biventer	4	48	49†

* Difference from parallel normal muscle is significant, at $P < .001$. † Difference is not significant, $P > .1$.

change in regulation by a neural factor, an interpretation which is suggested by the association of this decrease in AChE at the endplate with the large increase in the amount of certain AChE isoenzymes in the soluble fraction of these muscles, and the evidence that there is a parallel change of the latter type in denervated normal muscle (*2*). From this, combined with the foregoing evidence, it appears that those AChE isoenzymes which are increased in dystrophy must be sarcoplasmic. Whether these new soluble AChE types in the dystrophic muscles are the same molecular species as are localized at the endplates in the normal muscle is not yet known.

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Host Immunoglobulin G and Complement Deposits in the Choroid Plexus during Spontaneous Immune Complex Disease

Abstract. *Hybrid (NZB × W)F_1 mice spontaneously develop antibodies to nuclear antigens (ANA) and DNA (ADNA) and are an animal model of human systemic lupus erythematosus. Immunofluorescent and electron microscopic observations of the choroid plexus and renal glomeruli of (NZB × W)F_1 mice reveal deposits of host immunoglobulin G (IgG) and the third complement component which appear shortly after the development of ANA and ADNA in the circulation. Additionally, enhancement of ADNA responses accelerates the appearance and severity of IgG deposits in the choroid plexus. The choroid plexus may be a favored site for the deposition of immune complexes and the neuropsychiatric findings in patients with systemic lupus erythematosus and some patients with acute or chronic infections may be related in part to immune complex disease of the choroid plexus.*

Hybrid (NZB × W)F_1 mice consistently develop a spontaneous disease that closely resembles human systemic lupus erythematosus (SLE) (*1*). Within 1 year, most of the females die of a glomerulonephritis that is characterized mainly by deposits of DNA-antibody to DNA (ADNA) and nuclear antigen–antibody to nuclear antigens (ANA), and to a lesser extent by C type virus associated antigens–antibody to C type virus and red blood cell antigens–antibody to red blood cells in glomerular basement membranes and mesangia (*2*). Immunofluorescence studies reveal that host immunoglobulin G (IgG) and the third component of complement (C3) have begun to accumulate in the glomeruli of female mice at 3 to 5 months of age subsequent to the appearance of ADNA and ANA in their circulations (*2*).

In human SLE other organs besides the kidneys are involved producing a multitude of clinical manifestations. Nervous and mental disorders have been recorded in 75 percent of patients with SLE, but neuropathologic correlations are frequently difficult to establish (*3*). Since the vascular endothelium of the choroid plexus is fenestrated and thus morphologically similar to the endothelium in renal glomeruli, the choroid plexus may be another favored site for the deposition of immune complexes. Deposits of such complexes might play a role in the pathogenesis of nervous and mental disturbances.

We now describe the spontaneous accumulation of IgG and C3 in the choroid plexus of (NZB × W)F_1 mice and its relation to the formation of ADNA and ANA. Additional experiments which enhanced ADNA or ANA responses in the circulation also accelerated the development and increased the severity of choroid plexus deposition.

Female (NZB × W)F_1 mice in groups of ten mice at 1, 2, 3, 4, 5, 7, 8, and 9 months of age were killed by exsanguination. The choroid plexus from one-half of the brain and a small slice from the kidney were fixed in 5 percent phosphate-buffered glutaraldehyde for electron microscopy. The remaining brains and kidneys were frozen in liquid nitrogen for immunofluorescence studies. For electron microscopy, the tissue was again fixed in 1 percent phosphate-buffered osmium tetroxide, dehydrated, and embedded in Araldite. Sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 101 electron microscope. For direct immunofluorescence, frozen sections were stained with fluorescent labeled antibodies against mouse IgG, C3, fibrinogen, and albumin (*2*). Plasma was collected from all animals for the determination of ADNA and ANA. Antibodies to DNA were quantitated by means of [^{3}H]thymidine labeled DNA in a modified Farr antigen binding assay as reported (*4*), and ANA determinations were done on normal mouse kidney sections by the indirect immunofluorescent technique (*2*).

The results showed no IgG deposits in the choroid plexus until the animals were 4 months old. When the animals were 5 and 9 months of age, IgG was