Antibody Formation in First and Second Generation Offspring of Nutritionally Deprived Rats

Abstract. *Prolonged partial restriction of calorie intake in young rats results in reduced growth, involution of lymphoid organs, and lymphopenia. After immunization with sheep red blood cells, the number of antibody-forming cells in the spleen and serum hemolysin titers are markedly reduced. The effect is more pronounced on the primary response and on immunoglobulin G antibody-forming cells. Significant impairment of antibody formation is detectable in the first (F_1) and second (F_2) generation offspring of starved F_0 mothers.*

Nutritional deficiency in man can alter several parameters of immunocompetence, including cell-mediated immunity, thymus-dependent lymphocytes in the blood and tissues, metabolic and bactericidal functions of polymorphonuclear leukocytes, macrophages, complement, and secretory antibody response (*1*). It is recognized that intrauterine growth retardation due to maternal malnutrition, placental insufficiency, and other causes during pregnancy produces a profound adverse effect on postnatal immunocompetence (*2, 3*). However, the duration for which such an abnormality may persist in the offspring is not known. It is suspected that reduced physical and mental development due to fetal malnutrition may last for a long time after birth (*4*). If the defects are lifelong, it may have significant implications for the progeny. This may also apply to impaired immune capacity. I have used an animal model to evaluate the effect of nutritional deprivation on antibody-forming cell response in the first and second generation offspring.

A batch of inbred young rats received a calorie-restricted diet for 6 weeks and were immunized with sheep red blood cells. Antibody-forming cells in the spleen were counted by the Jerne's technique as modified by Dresser and Wortis (*5*). As shown in our earlier experiments (*6*), starved animals had lower body weights with marked involution of the thymus and the spleen; there was a significant depletion of the lymphocytes in the blood and tissues. The data for primary immune response to sheep red blood cells in three generations of animals are shown in Table 1. The nutritionally deprived group showed a significant reduction of antibody-forming cells of both immunoglobulin classes and of hemolysin titers. The effect was more marked on developed plaques representing immunoglobulin G–producing antibody-forming cells. Impaired antibody response was seen in the F_1 and F_2 offspring of starved female rats, even though the litters in both generations had free access to food. The differences between each batch of matched experimental and control groups were significant statistically ($P < .01$). In some animals, the secondary immune response to a booster dose of 2×10^8 sheep red blood cells given after an interval of 3 weeks was evaluated in a similar fashion. A significant reduction in antibody-forming cells was seen in the experimental group ($P < .05$), but the impairment was less marked as compared with that of the primary response.

Our observations in nutritionally deprived F_0 female rats confirm the data of others (*7*) and extends them to show that reduced antibody-forming cell response may be seen in the F_1 (*8*) and F_2 offspring of such starved F_0 mothers.

The pathogenesis of reduced antibody formation in the progeny who had free access to food is not clear. Malnutrition acquired during fetal life is difficult to reverse completely. Poor lactation of F_0 nursing animals in the experimental group is an additional contributory factor. Protein restriction prior to and during pregnancy retards the development of several organs and physiological processes (*9*). In the case of DNA content of neonatal brain, the adverse effect of reduction in maternal protein intake can be carried over to the F_2 offspring (*10*).

Malnutrition causes a reduction in the weight of lymphoid organs and depletes the lymphocytes, especially the thymus-dependent ones (*1*). Lymphoreticular shrinkage is pronounced if malnutrition occurs during fetal life. Thymus-dependent lymphocyte proportion in the peripheral blood of human infants who were small for their gestational age may continue to be pathologically low for several months after birth despite attempts at nutritional rehabilitation (*2*). The findings in our experimental study may have a clinical bearing, in that impaired immunocompetence as a result of

Table 1. Immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody-forming cells in the spleen and serum hemolysin titer in starved and control rats, and in their progeny. Three-week-old rats were subjected to partial starvation for 6 weeks by restricting the food to 25 percent of the optimal amount fed to control animals. One batch from each experimental and control group (F_0 generation) was immunized with 2×10^8 sheep red blood cells (SRBC) intraperitoneally. The animals were killed after 4 to 14 days and studied for the above-mentioned data. Another batch of female rats from the starved and control groups were mated with healthy male animals. On weaning, the litter (F_1 generation) was given free access to food for 6 weeks. Some of these animals were immunized with SRBC and studied as above. Others were mated with healthy males and the progeny (F_2 generation) were evaluated at age 4 weeks. Albino rats from the stock colony of our institution inbred for eight generations were used in all experiments. S.E.M., standard error of mean.

Interval after SRBC injection (days)	Animal group	IgM-forming spleen cells per 10^6 mononuclears (mean ± S.E.M.)	IgG-forming spleen cells per 10^6 mononuclears (mean ± S.E.M.)	Reciprocal hemolysin titer (mean)
		F_0 generation		
4	Starved	151 ± 19	Not done	8
4	Control	1283 ± 167	Not done	16
6	Starved	371 ± 33	0	128
6	Control	945 ± 64	91 ± 21	1024
10	Starved	136 ± 17	79 ± 8	128
10	Control	677 ± 49	520 ± 37	512
14	Starved	79 ± 9	60 ± 9	64
14	Control	201 ± 13	195 ± 13	512
		F_1 generation		
4	Experimental	290 ± 31	Not done	4
4	Control	1476 ± 125	Not done	16
6	Experimental	480 ± 29	0	128
6	Control	1013 ± 92	0	1024
10	Experimental	310 ± 18	211 ± 19	256
10	Control	603 ± 37	689 ± 45	1024
14	Experimental	111 ± 11	106 ± 13	128
14	Control	235 ± 26	271 ± 32	512
		F_2 generation		
4	Experimental	473 ± 31	Not done	8
4	Control	1019 ± 114	Not done	32
6	Experimental	389 ± 37	0	128
6	Control	761 ± 42	46 ± 15	512
10	Experimental	230 ± 19	147 ± 20	64
10	Control	401 ± 32	446 ± 51	512
14	Experimental	92 ± 13	61 ± 8	32
14	Control	135 ± 11	153 ± 16	256

intrauterine malnutrition may persist for a very long time, and perhaps be permanent and affect the next generation as well, with the implication that pathological disorders known to be associated with immunodeficiency, such as autoimmunity, frequent infections, and neoplasia, may be commoner in individuals with such perinatal history and in their children.

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Response Plasticity of Lateral Geniculate Neurons During and After Pairing of Auditory and Visual Stimuli

Abstract. *Neurons of the lateral geniculate nucleus, responsive only to visual stimulation, show response plasticity during and after pairing of auditory and visual stimuli. Modal response histograms reveal a gradual decrement in the number of spikes at interstimulus pairing intervals of 0 and 100 milliseconds but not 500 milliseconds. This plasticity effect, limited to tonic units, may persist for 2 to 3 minutes after termination of click-flash pairing.*

Few studies have described plasticity or response modifiability of single visual units in the central nervous system of vertebrates as a result of either unimodal or cross-modal pairing of sensory stimuli (*1, 2*). We have determined the "conditionability" (plasticity or response modifiability) of units in the lateral geniculate nucleus of the cat as a result of pairing of auditory and visual stimuli. These units were differentiated by type of response (tonic or phasic) to visual stimuli. None were responsive to auditory stimuli before, during, or after stimulus pairing. Unit response modifiability was not of the classical conditioning type in which a response to an effective (unconditioned) stimulus is transferred to an originally ineffective (conditioned) stimulus. A period of pairing of auditory and visual stimuli did not cause lateral geniculate units, originally unresponsive to auditory stimulation, to become responsive; instead, the response to visual stimulation was gradually modified, and, in some instances, this response modification persisted for an appreciable period of time after cessation of the pairing of the auditory and visual stimuli. The response modification during and after stimulus pairing consisted in a decrease of responsivity, as shown by a reduction of the spike count of the modal peak of the peri-stimulus-time histogram (PSTH).

These results have a twofold importance, one, in demonstrating that unit response modifiability can be induced gradually in a specific sensory system during intermodal pairing of stimuli, and two, in showing that response plasticity may persist beyond the period of pairing of the auditory and visual stimuli.

Four cats prepared for repeated recording were employed in this study. Initially, each animal was anesthetized with halothane and an opening was made in the skull over which a metal cylinder 1 cm in diameter was mounted in dental acrylic. A microelectrode could be inserted via this cylinder into the lateral geniculate body. Three bolts mounted in the acrylic pedestal were inserted in a metal plate attached to the stereotaxic instrument and held the animal's head rigidly and painlessly without the use of ear and eye bars. After recovery from surgery, each animal was used for several 3- to 5-hour recording sessions, spanning a 2- or 3-week period. Before each recording session the animal was anesthetized with halothane, the trachea was intubated with an endotracheal tube coated with lidocaine (Xylocaine), and the animal was placed on artificial respiration after infusion of gallamine triethiodide (Flaxedil) via an indwelling venous catheter. Thereafter, halothane anesthesia was discontinued. Pupils were dilated with 2 percent homatropine hydrochloride, and the nictitating membrane was retracted by topical application of 10 percent Neosynephrine.

Single unit activity was recorded from the lateral geniculate nucleus with stainless steel microelectrodes coupled with an emitter follower, the output of which was amplified by a Grass P-5 a-c preamplifier and recorded on FM tape by an Ampex SP-300 tape recorder. Unit responses were monitored on a Tektronix 565 oscilloscope and photographed by a Grass kymograph camera.

The animals were in an electrically shielded, dark, quiet room, facing a white, translucent, plastic hemisphere 30 cm in diameter. The cat's eye was 20 cm from the front surface of the hemisphere. The light flash (20 μsec) was produced by a Grass PS-2 photostimulator, encased in a sound-attentuating box, which illuminated uniformly the entire surface of the plastic hemisphere. Miniature insert earphones in each auditory meatus, driven by square-wave pulses from a Grass S-4 physiological stimulator, furnished a 75-db click stimulus.

Once a single lateral geniculate unit giving a stable response to light flash was located by audiomonitor and observed on the oscilloscope, it was classified as a tonic or phasic unit by its response to a steady spot of light, projected by an ophthalmoscope. After these preliminaries a "conditioning" procedure was initiated which consisted of three consecutive phases: (i) flashes alone (one per 2 seconds for 10 minutes; (ii) click-flash pairing (interstimulus intervals of 0, 100, or 500 msec) for 5 minutes; and (iii) flashes alone (one per 2 seconds) for 10 minutes. Seven successive PSTH's, each based on 40 stimulus presentations, were computed in each of phases 1 and 3 of the experiment, and three in phase 2. The PSTH's were computed by a Mnemotron computer of average transients (model 400B) with a bin width of 2.5 msec, covering a 500-msec epoch, and were plotted with a Moseley *x-y* plotter.