sleep than during time-equivalent euthermic periods.

Torpor was usually terminated by increased wakefulness soon followed by an increase in T_{br}, but REM sleep was not present. Despite the suppression of REM sleep during torpor, compensatory REM rebounds did not occur in the 24-hour period after the return to euthermia (*9*) (Fig. 1) (Table 1). The occurrence of shallow torpor did not significantly affect the amounts of total sleep or SWS during subsequent euthermia when compared with pretorpor euthermia.

Daily shallow torpor occurring in some small mammals such as pocket mice is sometimes interpreted as an extension or magnification of the euthermic circadian rhythm of T_b. However, in this study some bouts of torpor occurred independently at times remote from the minor circadian decreases of T_b. Therefore, the decreased T_b of torpor appears to be associated more closely with the sleep state than with a preestablished circadian variation of T_b independent of sleep.

These results also point to the physiological identity of estivation and hibernation. Sleep patterns during shallow torpor in desert ground squirrels were qualitatively and quantitatively identical to those of shallow hibernation in alpine ground squirrels (*10*). Moreover, changes in EKG, EMG, and EEG activity during bouts of shallow torpor were similar to those of shallow hibernation.

The function of sleep is typically regarded as the restoration of one or more physiological processes degraded during prior wakefulness, in spite of a lack of concrete empirical support for such an interpretation (*11*). An alternative, but not necessarily exclusive hypothesis is that SWS evolved as an adaptation for energy conservation that partially offset the high costs of endothermy (*12-14*). Evidence for this hypothesis was until recently confined to correlative studies and included (i) strong positive correlations between metabolic rate and amount of SWS in mammals (*15*), (ii) the parallel ontogeny of SWS and thermoregulation (*14*), and (iii) the absence of SWS in ectotherms (*13*). The finding of a regulated decrease in T_b during SWS indicative of reduced metabolism provided direct support for this hypothesis (*5*). Since the electrophysiological patterns of SWS and shallow torpor are temporally continuous and essentially isomorphic, it is probable that thermoregulatory adjustments while entering torpor are an extension of those initiated during SWS. There can be little doubt about the bioenergetic adaptive value of torpor, and the physiological homologies between torpor and sleep described point to a unitary primordial function for both states.

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7. The initial weight of this animal was 143 g. After 12 days of food deprivation it weighed 108 g, and after 26 days, 85 g.
8. The first instance of torpor for three animals (torpor entrances at 10:00, 10:10, and 10:28 p.m.) and the second for the fourth animal (9:50 p.m.) served as the data base. Sleep-wakefulness patterns were determined for time-equivalent euthermic periods before the first period of torpor and after torpor. Comparisons between pretorpor, torpor, and post-torpor in this and subsequent comparisons were made by one-way analysis of variance for repeated measurements. Duncan's multiple-range test was used to compare specific means.
9. Euthermic sleep-wakefulness measures were determined in the 24-hour period (8 p.m. to 8 p.m.) prior to the first period of torpor. Although individual animals had experienced differing food deprivation with the varying times of occurrence of the first torpor, sleep-wakefulness measures were as homeogeneous as and within the range of normative data reported for another species of squirrel (*4*). Sleep-wakefulness patterns were measured in the 24-hour period that torpor occurred [same periods of torpor as in (*8*)] and in the subsequent 24-hour euthermic period.
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16. We thank L. Soholt and T. Gowdy for providing the animals and D. Ritter and E. Haskell for data collection and analysis. Supported by NIH grants GM-23694 awarded to R.J.B. and GM-23695 awarded to H.C.H., and by a UCSC faculty grant from the University of California, Santa Cruz, awarded to R.J.B.

9 February 1979

Membrane Potential Changes During Chemokinesis in *Paramecium*

Abstract. *Intracellular recordings show that (i) paramecia hyperpolarize slightly in attractants and depolarize in repellents that depend on the avoiding reaction (an abrupt change of swimming direction), and (ii) paramecia more strongly hyperpolarize in repellents and more strongly depolarize in attractants that depend on changes of swimming velocity. These membrane potential changes are in agreement with a hypothesis of membrane potential control of chemokinesis in* Paramecium.

Paramecia accumulate in or disperse from the vicinity of certain chemical stimuli (*1*). The animals do this by either of two mechanisms: modulation of frequency of changing swimming direction (the avoiding reaction) or modulation of forward swimming speed (*2-4*). Neither mechanism seems to involve orientation toward or away from the stimulus, and therefore the behavior is termed chemokinesis rather than chemotaxis (*5, 6*). Components of both of the mechanisms are under electrical control at the cell membrane: (i) each avoiding reaction corresponds to the generation of a calcium action potential, and (ii) the frequency and angle of ciliary beating, which determine swimming speed, are controlled by changes from the resting membrane potential (*7, 8*). Therefore, it is of interest to determine the electrophysiological correlates of the complex behavior of chemokinesis. I have made intracellular recordings from cells in attractants and repellents. The membrane potential (E_m) values from these recordings are presented here and are in agreement with a hypothesis of membrane potential control of chemokinesis (*2, 3*).

Accumulation of organisms is associated with decreased frequency of avoiding reaction (F_{AR}) or with decreased speed (V) in the area of attractant (*5, 9*). Conversely, dispersal is associated with increased F_{AR} or with increased V in the

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area of repellent (*5, 9*). However, in *Paramecium* one cannot separately affect the F_{AR} and the frequency of ciliary beating that determines V because both are under control at the cell membrane. For example, a small hyperpolarization will bring the membrane potential away from threshold and decrease F_{AR} and will also increase the frequency of ciliary beating and angle, increasing forward V. Note that the decreased F_{AR} accompanies accumulation, and increased speed accompanies dispersal of the animals. Therefore, not only are the components of chemokinesis inseparable, they appear to be opposed by the classical mechanisms outlined above.

Mutations and conditions that eliminate avoiding reaction have been used in determining the contributions of F_{AR} and V to chemokinesis (*2, 3, 10, 11*). Elimination of the avoiding reaction abolishes responses to only some attractants and repellents. In this manner, I have distinguished two groups of chemicals that cause chemokinesis by two different mechanisms, I and II (*2, 3, 10*). In mechanism I, attraction and repulsion are correlated with decreased and increased F_{AR}. The associated increase and decrease in velocity seem to be unimportant in determining net effect. Moreover, in "pawns" [mutants with no avoiding reaction (*12*)] agents of group I do not cause appreciable attraction and repulsion. In mechanisms II, it is the response swimming velocity that predominates. Repulsion is associated with increased V and a decrease of F_{AR} to zero, and attraction is associated with decreased V due to slow swimming and time spent in frequent turning in the avoiding reaction. Pawns are attracted and repelled by agents of group II. Attractants I and repellents II cause the same qualitative changes in behavior (decreased F_{AR} and increased V) but result in opposite chemokinesis behavior. Likewise, repellents I and attractants II cause qualitatively similar changes (increased F_{AR} and decreased V) but have opposite chemokinesis results. The attractants and repellents used for intracellular recordings were organized in these groups.

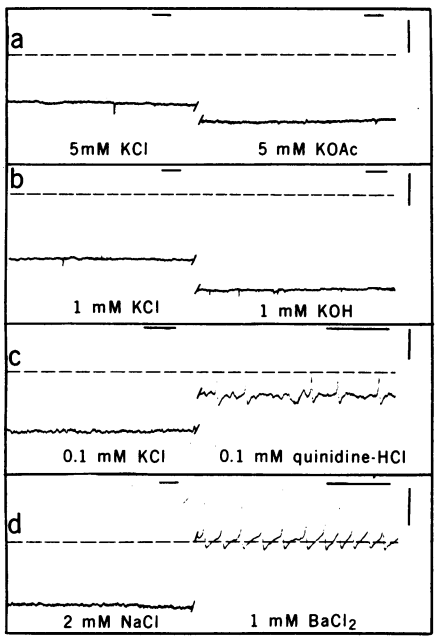

Fig. 1. Intracellular recordings from *Paramecium tetraurelia* in (a) 5 m*M* KCl control and 5 m*M* K acetate solutions; (b) 1 m*M* KCl (*p*H 7.0) control and 1 m*M* KOH (*p*H 8.7) solutions; (c) 0.1 m*M* KCl control and 0.1 m*M* quinidine-HCl solutions; and (d) 2 m*M* KCl control and 1 m*M* $BaCl_2$ solutions. Dashed lines indicate 0 mV. Horizontal lines in each panel are 2-second scales; vertical lines are 20-mV scales. Recording from paramecia was done by the methods of Naitoh and Eckert (*25*) and Satow and Kung (*26*). Hanging drops were eliminated by the use of an inverted microscope. Glass microelectrodes filled with 500 m*M* KCl (~ 70 to 120 megohms) were used. Tracings of E_m were used when the potential was again stable after a change of bath solution. It was estimated that 2 minutes was required for a complete change of bath solution. Cells were constantly bathed in a solution of 1 m*M* citric acid, 1 m*M* $Ca(OH)_2$, 1.3 m*M* tris, *p*H 7.0, with salt indicated above. The exception was the *p*H of 1 m*M* KOH solution (*p*H 8.7).

The E_m's of cells in attractants I, such as acetate (OAc^-), and repellents II, such as OH^-, measured by intracellular recording (Fig. 1) were more negative than those of the same cells in control solutions. The E_m's of cells in repellents I, such as quinidine-HCl, and attractants II, such as $BaCl_2$, were more positive than the E_m's in control solutions, and both repellents I and attractants II elicited frequent action potentials (Fig. 1). The membrane potentials of cells in several attractants and repellents and of controls are given in Table 1 along with measurements of the strengths of the attractants and repellents.

Variations in the measurements of E_m arise from differences in resting E_m between cells and possibly from gradual changes of electrode properties that alter apparent resting E_m. However, relative changes of E_m upon changing solutions were consistent and in the same direction in all cells. To demonstrate this constancy, the net changes of E_m upon changing solution from control to test and back were measured (Table 2). The changes of potential in attractants I and repellents II were always hyperpolarizing and were about twice as large for repellents II as for the strongest attractant I. The changes in repellents I attractant II were consistently depolarizations, and the depolarizations in attractant II were greater than those in any repellent I.

There are apparent contradictions in the chemokinesis behavior of animals in solutions that cause qualitatively similar changes in F_{AR} and V but cause opposite accumulation and dispersal results. (For example, attractants I and repellents II both decrease F_{AR} and increase V but have opposite chemokinesis results) (*2, 3*). A new mechanism of behavior control can be invoked for chemokinesis, or the observed behavior can be used to infer electrical events during chemokinesis based on the established membrane electrical control of *Paramecium* ciliary motion (*7*). I have taken the latter approach (*2, 3*). The resulting simple hypothesis of E_m control of chemokinesis (Fig. 2) predicts that attractants I will cause a slight hyperpolarization, causing the characteristic decrease in F_{AR} and small increase in V (Fig. 2a). As attractants I more strongly hyperpolarize the potential, the F_{AR} will drop toward zero, making the attractants less effective for accumulating animals. As the membrane potential is more strongly hyperpolarized, F_{AR} drops to zero and the velocity increases become important and cause repulsion. Hence, repellents II should strongly hyperpolarize the E_m. In this way, the same qualitative change of E_m and of behavior components can lead to two different chemoaccumulation results, depending on the magnitude of the membrane potential change.

The hypothesis also predicts a small positive shift in potential for cells in repellents I (Fig. 2a). The positive shift increases F_{AR} and decreases V. As the

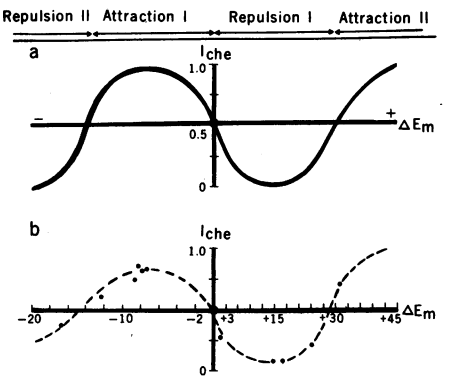

Fig. 2. (a) Graphical description of membrane potential control of chemokinesis. Change of membrane potential (ΔE_m) from control (at origin) is plotted against the index of chemokinesis; $I_{che} > 0.5$ indicates attraction; < 0.5 indicates repulsion (see Table 1 legend). As chemical stimuli change E_m relative to control, animals will be attracted or repelled, depending on the magnitude and direction of the E_m change. (b) Data from Tables 1 and 2 plotted as ΔE_m produced by the attractant or repellents versus I_{che}. Scale of ΔE_m is different for depolarizing and hyperpolarizing stimuli.

membrane potential is even more depolarized relative to control, the change in F_{AR} increases and V decreases until the animals no longer escape by changing direction, but instead are trapped by very slow movement and accumulate. Therefore, attractants II are expected to more strongly depolarize the membrane than repellents I.

The membrane potentials measured in attractants and repellents are in agreement with the hypothesis (Fig. 2b). Strong attractants I hyperpolarized the membrane slightly, about 8 to 10 mV. A weaker attractant, potassium folate, hyperpolarized the membrane even more, presumably to a point near the transition between the mechanisms of attraction I and repulsion II. The repellent II, OH^-, caused a stronger negative shift in potential than attractants I (−16 mV), which was well correlated with the decrease in F_{AR} to zero and the repulsion by the resulting increased V. Repellents I depolarized the E_m by up to +25 mV, while the attractant II depolarized even more (+31 mV).

Several observations can be made about the E_m data. Absolute E_m is probably not the determining factor in attraction and repulsion, but rather the magnitude of change of E_m from control determines attraction and repulsion (Fig. 2b). These changes of E_m are reversible (Table 2), and the strengths of attractants and repellents are not simply proportional to the E_m changes they produce (Fig. 2b).

Cells change E_m and behavior in response to new ionic environments. With time in the new solution, the cells accommodate their resting E_m, a new threshold for action potentials is established relative to the new E_m, frequency and angle of ciliary beating return to a resting level (*13, 14*), and F_{AR} and V return to basal levels (*2, 10*) despite the shift in E_m that has occurred. Although the eventual accommodation of electrical properties and accompanying adaptation of behavior are necessary for the classical mechanisms of chemokinesis (*5, 15*) and for the hypothesis of membrane potential control of chemokinesis (*2, 3*), the initial responses of the animals determine the direction (accumulation or dispersal) and strength of the response. Therefore, only initial membrane potential responses to attractants and repellents are reported at present.

Membrane electrical change may be a mechanism common to most chemoreception systems, while the nature of the change varies between systems: slime molds and macrophages hyperpolarize in response to attractants (*16, 17*), bacteria may hyperpolarize while adapting to either attractants or repellents (*18*) and require a Ca^{2+} flux across the plasma membrane for repulsion (*19*), and insects and vertebrates respond to food extracts and odors with increased frequency or bursts of membrane electrical activity (*20–23*). As more measurements of E_m in attractants and repellents are made, I would expect that ratios of F_{AR} or V in test and control solutions will modify the simple idea that changes in E_m control chemokinesis behavior.

Table 1. Membrane potentials (E_m) of cells in test and control solutions. Cells were bathed in control or test solution of *p*H 7 (see Fig. 1 legend) except in acetic acid (*p*H 5.3) and KOH solution (*p*H 8.7). The E_m data are averages of N measurements ± 1 standard error (S.E.). The E_m's were measured 6 minutes after the bath solution was changed because potentials were again stable by then. An exception was the E_m when the solution was changed from quinidine-HCl to KCl, in which case cells could take more than 8 minutes to recover a stable potential. The E_m's in quinidine were difficult to measure accurately because cells in quinidine sometimes showed action potentials with plateaus prolonged for 2 to 3 minutes. The response of paramecia to chemicals was measured by a T-maze assay designed to present a test and control solution to a population of animals (*24*). The index of chemokinesis (I_{che}) is defined as the number of animals swimming into the arm of the T containing test solution divided by the number of animals swimming into both arms, containing test and control solutions. An $I_{che} > 0.5$ indicates attraction to the test solution; $I_{che} < 0.5$ indicates repulsion from the test into the control solution. The I_{che} data are averages of four or more experiments ± 1 standard deviation.

Control solution	E_m (mV)	Test solution	E_m (mV)	N	I_{che}
Attractants I					
KCl, 5 m*M*	−31.3 ± 0.8	K acetate, 5 m*M*	−38.8 ± 1.6	50, 16	0.84 ± 0.07
KCl, 5 m*M*	−31.3 ± 0.8	K lactate, 5 m*M*	−37.4 ± 1.4	50, 16	0.83 ± 0.06
NaCl, 5 m*M*	−27.0 ± 0.8	Na acetate, 5 m*M*	−37.7 ± 1.1	22, 19	0.75 ± 0.11
NaCl, 5 m*M*	−27.0 ± 0.8	NH_4Cl, 5 m*M*	−36.8 ± 2.4	22, 10	0.86 ± 0.11
KCl, 2 m*M*	−33.8 ± 0.9	K_2 folate, 1 m*M*	−45.6 ± 2.8	10, 6	0.61 ± 0.06
Repellents I					
KCl, 5 m*M*	−31.3 ± 0.8	NaCl, 5 m*M*	−27.0 ± 0.8	50, 50	0.27 ± 0.13
KCl, 0.1 m*M*	−44.1 ± 1.3	Quinidine-HCl, 0.1 m*M*	−30.1 ± 3.3	7, 12	0.08 ± 0.04
K acetate, 1 m*M*	−39.9 ± 1.4	Acetic acid, 1 m*M*	−19.2 ± 2.6	18, 11	0.09 ± 0.07
KCl, 5 m*M*	−26.9 ± 1.3	$BaCl_2$, 2.5 m*M*	0.5 ± 2.4	27, 16	0.21 ± 0.11
Repellent II					
KCl, 1 m*M*	−36.8 ± 1.1	KOH, 1 m*M*	−52.3 ± 1.5	15, 19	0.38 ± 0.05
Attractant II					
NaCl, 2 m*M*	−32.9 ± 1.5	$BaCl_2$, 1 m*M*	− 0.9 ± 2.6	12, 8	0.72 ± 0.22

Table 2. Net changes of E_m (ΔE_m) when the solution around the cell was changed from control to test solution or vice versa. Values are averages of N measurements ± 1 S.E. The ΔE_m was measured as the difference between the E_m of the cell in control solution just before the solution change and the E_m of the cell 6 minutes after the solution change, except when the solution was changed from quinidine to KCl (see Table 1 legend). The E_m changes were measured after a change of solution that should have simulated the diffusion gradients in the T-mazes. Gradients were not identical in the electrical recording and T-maze, and therefore the time course of ΔE_m was not analyzed.

Control solution	Test solution	ΔE_m, change from control to test solution (mV)	N	ΔE_m, change from test to control solution (mV)	N
Attractants I					
KCl, 5 m*M*	K acetate, 5 m*M*	− 7.3 ± 0.7	14	6.9 ± 1.4	13
KCl, 5 m*M*	K lactate, 5 m*M*	− 7.9 ± 1.3	13	6.2 ± 0.8	13
NaCl, 5 m*M*	Na acetate, 5 m*M*	− 8.5 ± 1.2	10	8.3 ± 1.2	9
NaCl, 5 m*M*	NH_4Cl, 5 m*M*	− 8.3 ± 2.5	6	12.7 ± 3.0	5
KCl, 2 m*M*	K_2 folate, 1 m*M*	−12.4 ± 3.6	6	10.1 ± 2.2	6
Repellents I					
KCl, 5 m*M*	NaCl, 5 m*M*	1.6 ± 0.6	3	− 2.6 ± 0.9	3
KCl, 0.1 m*M*	Quinidine-HCl, 0.1 m*M*	14.8 ± 2.7	10	−13.7 ± 4.6	6
K acetate, 1 m*M*	Acetic acid, 1 m*M*	21.9 ± 1.6	11	−16.9 ± 1.6	9
KCl, 5 m*M*	$BaCl_2$, 2.5 m*M*	25.3 ± 3.5	6	−24.0 ± 3.0	5
Repellent II					
KCl, 1 m*M*	KOH, 1 m*M*	−16.8 ± 1.9	10	14.1 ± 1.9	11
Attractant II					
NaCl, 2 m*M*	$BaCl_2$, 1 m*M*	31.6 ± 1.5	7	−31.0 ± 1.4	6

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4. The term speed is differentiated here from velocity, meaning a directed vectoral quantity. Here speed denotes the time required to traverse a given distance. It is not yet clear whether true velocity (determined by frequency and angle of ciliary beat) or translation time (speed) will be the important parameter in the hypothesis of E_m control of chemokinesis behavior. Previous measurements of V have approached a true velocity measurement, but in some conditions where animals make frequent stops, reversals, and turns the measured quantity V more closely appproximates speed.
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9. Decreases in F_{AR} are often associated with accumulation of organisms (*5*) but have not been shown to be a sufficient cause of accumulation. There are intuitive arguments that decreasing F_{AR} causes accumulation (*5*) and that decreasing F_{AR} causes dispersal (as in an analogy with diffusion of gases). In the case of *Paramecium*, I have shown that with the elimination of the avoiding reaction (AR), one type of attraction is eliminated. Therefore, the AR is necessary and the change in its frequency is probably sufficient to cause attraction. At present, there is one computer simulation of accumulation dependent on either decreased F_{AR} or decreased F_{AR} with increased speed (*15*). An even more rigorous computer simulation or mathematical model should provide firmer evidence concerning modulation of F_{AR} as a cause of chemokinesis.
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27. I thank J. Gudaitis for excellent technical assistance; Y. Satow, D. Oertel, D. M. J. Quastel, and C. Kung for discussions of this work; and M. McLaverty for preparation of the manuscript. Supported by PHS fellowship 5 F32 NS 05587-02.

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12 September 1978; revised 9 February 1979

Experimental Allergic Neuritis Induced by Sensitization with Galactocerebroside

Abstract. *Thirteen of 31 rabbits immunized repeatedly with bovine brain galactocerebroside developed experimental allergic neuritis, manifested by flaccid paresis and hypesthesia of four limbs, 2 to 11 months after the initial inoculation. Electrophysiological studies revealed multifocal conduction block of peripheral nerves. Perivenular demyelinative lesions associated with phagocytic mononuclear cells occurred in spinal ganglia, roots, and less frequently in distal nerves.*

Experimental allergic neuritis (EAN) and experimental allergic encephalomyelitis (EAE) are autoimmune, demyelinating diseases of the peripheral nervous system (PNS) and central nervous system (CNS); they are classically produced in animals by injection of homogenates of PNS or CNS tissue, respectively, with complete Freund's adjuvant (*1*). Experimental allergic neuritis can be induced by injection of P2 basic protein (BP) of peripheral nerve myelin. A peptide from myelin P2 BP is at least one of the neuritogenic determinants (*2*). In EAE, the encephalitogen is CNS myelin BP (*3, 4*). Another major component of CNS and PNS myelin (*5*), galactocerebroside (β-D-galactopyranosyl ceramide) (GC), is a glycolipid hapten (*6, 7*) and binds specific antibodies in isolated myelin (*7-9*). Antiserum to GC binds GC in both central and peripheral myelin in sections of rat optic and sciatic nerve, as well as in CNS myelin of unfixed spinal cord cultures as demonstrated by indirect immunofluorescence or immunoperoxidase techniques (*10*). Galactocerebroside can serve as a cell-surface antigenic marker for oligodendrocytes in culture (*10*). Further, rabbit antiserum to GC demyelinates organotypic CNS cultures and inhibits myelination and sulfatide synthesis in immature CNS cultures (*11-13*). However, immunization with one or two injections of GC has not been encephalitogenic (*6*). We describe here the first successful production of EAN in rabbits by repeated immunization with GC. The distribution of demyelinative lesions seems to correspond to areas known to have a defective blood-nerve barrier (*14, 15*).

Thirty-one male New Zealand albino rabbits, weighing 2.3 to 2.7 kg, were sensitized with GC up to seven or eight times following one of three schedules (I to III) (Table 1). The immunizing inoculum contained 1 or 2 mg of bovine brain GC (lower spot cerebrosides, 98 percent with hydroxylated fatty acids) (Sigma) and bovine serum albumin or egg albumin (Sigma) as a carrier protein (5 mg per milligram of GC), in complete Freund's adjuvant (Difco) or, for booster injections in schedules II and III, without adjuvant. Galactocerebroside was checked for purity by thin-layer chromatography on silica gel G plate developed with chloroform, methanol, and water (65:25:4, by volume) using six standard sphingolipids and phosphoglycerides (*16*). Two spots were obtained. The major spot (> 99 percent) had an R_F of 0.85 and the minor spot (< 1 percent) had an R_F of 0.88, corresponding, respectively, to cerebrosides with longer (24 carbon) or shorter (16 or 18 carbon) length fatty acids. Analysis by thin-layer and gas chromatography after hydrolysis revealed that galactose was the only carbohydrate moiety detectable (> 99.9 percent). Sixteen control rabbits were similarly immunized but without GC. Since results with the three schedules did not vary significantly, we will describe the clinical, pathological, and serological results as grouped data.

Thirteen of 31 rabbits immunized with GC developed a neurological disorder, with onset ranging from day 44 to day 314 (135 ± 21 days, mean ± standard error) after the initial inoculation (Table 1). Rabbits were maintained for a maximum of 1 year. Subacute onset of weight loss, tremulousness, ataxia, flaccid paresis, and hypesthesia of four limbs were the main features of the clinical illness (Fig. 1A). Progress was sometimes rapid; quadriplegia and respiratory paresis were terminal events in three animals less than 2 weeks after onset of signs of illness. None of the control rabbits showed neurological abnormalities.

Animals immunized following schedule I were subjected to electrophysiological studies prior to terminal histological examination. The characteristic abnormality was multifocal conduction block (Fig. 1, E and F). In animals examined from 2 to 24 weeks after onset of weakness, motor conduction velocities were diffusely slowed (11 m/sec; normal is 50 m/sec), suggesting widespread peripheral nerve demyelination. These electrophysiological abnormalities were indistinguishable from those found in human multifocal demyelinative neuropathies such as Guillain-Barre syndrome (*17*).

Twenty-three GC-immunized rabbits, including all 13 paralyzed rabbits at various clinical stages, and 12 control rabbits were killed at corresponding intervals between 1 month to 1 year after immuni-

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