the length of the semiminor axis which is equal, in these calculations, to R.

Since we can measure A directly, the expression for the volume of the particles is

$$V = 4/3\pi\, AR^2$$

A population of 34 particles was measured from a typical field, and the volumes of each of the particles were calculated. Five arbitrary classes of particle size were designated. The minimum, maximum, and mean values for the length ($=2A$), height ($=2R$), and volume were tabulated for each of the particle classes (Table 1).

A further analysis was performed as follows. It was postulated that the three smallest particles might be "monomers" or, more correctly, small integral polymers of a monomer of protein insoluble at *p*H 5. The average volume of these three particles was 1.75×10^6 A^3 and was designated as X. The hypothetical polymers of this assumed "monomer" were then calculated and are represented by the straight line in Fig. 2.

Figure 2 shows the linearity of the distribution of the particles of protein at *p*H 5 whose volumes were experimentally determined. Despite the errors inherently involved in the measurements and calculations of the volumes of the particles, the suggestion that they are small integral values of some "monomer" is correct. Class V particles (Table 1) were not included in Fig. 2 because the probability for linearity increases with larger multiples of X (*7*). Figure 2 also indicates that errors remaining in the calculation of volume are linear with respect to the actual volumes. The polymeric nature of the small particles of the protein strengthens the possibility that this material could have the dimensions of intracellular membranes and could contribute to their formation. We reason, too, that localized intracellular changes in *p*H likely exert a profound influence on the formation of membranes from the "soluble protein" fraction. The size classes of the particles, as presented in this report, have no definable biological reality, other than those implied above.

Table 1. Particle sizes of insoluble protein from soluble fraction of rat liver.

Particle class	Min.	Max.	Mean
	Height (A)		
I	68	145	105
II	148	194	164
III	171	194	184
IV	201	223	212
V	240	308	281
	Length (A)		
I	424	791	576
II	622	791	740
III	734	961	833
IV	828	993	883
V	960	1545	1184
	Volume (10^6 A^3)		
I	1.642	5.994	3.393
II	8.419	11.95	10.26
III	14.28	15.03	14.73
IV	18.47	22.76	20.58
V	28.95	72.75	50.02

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Transfer of Allergic Encephalomyelitis by Lymph Node Cells in Inbred Guinea Pigs

Abstract. Severe or lethal allergic encephalomyelitis was transferred between histocompatible guinea pigs by lymphoid cells capable of persistence in a viable state in the recipient. For optimum induction of passive disease, cells must be transferred before the day on which the disease becomes manifest in the donor.

Experimental allergic encephalomyelitis, induced in animals sensitized with brain or spinal cord preparations (*1*), was first passively transferred between parabiotic rats by Lipton and Freund (*2*). The disease has not been passively induced by transfer of anti-central nervous system serum or sensitized cells to normal random-bred animals, but has been transferred with lymphoid cells in "tolerant" rats by Paterson (*3*). Koprowski *et al.* (*4*) found histological evidence of passive sensitization in a small percentage of transfers between inbred rats (4 of 48 recipients showed damage in the central nervous system). The percentage of successful transfers was higher when the donors were splenectomized before lymph node cells were transferred (*5*). Since this disease is widely considered to be associated with hypersensitivity of the delayed type, and since the guinea pig is the animal of choice for studies in this area, in the experiments described in this report guinea pigs of the Wright (*6*) histocompatible (*7*) strain 13 were used for passive transfer of allergic encephalomyelitis. The facility with which transfers of this type of hypersensitivity are accomplished within this strain of guinea pig [Chase (*8*); Bauer and Stone (*9*)] is attributable to the viability of the lymphoid transplant in the recipient animal.

Except for cases otherwise recorded in Table 1, adult male strain 13 guinea pigs were sensitized by a single dose of strain 13 brain or spinal cord emulsified in complete Freunds adjuvant (*10*) injected intracutaneously into multiple sites in the nuchal region (0.25 ml of a 50 percent suspension of spinal cord or brain in 0.25 ml of Arlacel-Bayol containing 2.5 mg killed *Mycobacterium tuberculosis*). These donor animals were killed 5, 8, 11, or 12 days after injection, and the lymph nodes draining the nuchal region were removed; cell suspensions were then prepared and transferred by injection into the peritoneal cavity of normal recipients, as previously described (*9*). The donor : recipient ratio was roughly 2½ : 1. Some of the recipients were skin-tested with purified protein derivative (PPD) of tuberculin 14 to 23 days after transfer. Random-bred guinea pigs of the Hartley strain were used as control recipients. Donor and recipient animals were weighed each day to determine the onset of disease.

Table 1 shows that the allergic encephalomyelitis induced by isologous brain or spinal cord can be transferred between strain 13 guinea pigs and that the transfer results in severe or lethal disease in a large percentage of the recipients. In groups receiving cells 5, 8, or 11 days after active sensitization of donors, 17 of 20 strain 13 and none of 10 Hartley guinea pigs had the disease. In confirmation of Chase's prediction (*11*) and of Koprowski's results with inbred rats (*4*), the transfer was more likely to result in passive disease when the cells were taken before the symptoms were apparent in the donor (transfer at 5 and 8 days after active sensitization). Eleven days could elapse between sensitization and the successful transplantation of lymphoid cells from guinea pigs actively sensitized with brain preparations; but guinea pigs sensitized with the more potent spinal cord preparations were frequently manifestly ill by this time, and transfers at 11 or 12 days from these animals were not made under

optimum conditions. Early transfers insure that ample numbers of sensitized cells are still in the lymph node. Transfers made soon after the sensitizing injection are feasible in histocompatible guinea pigs because the cells are viable and the sensitization process can continue in recipients (*9*). The time interval between transfer and onset of disease in recipients was 7 to 10 days whether the transplant was made 5, 8, or 11 days after active sensitization of donor.

Several recipients receiving the cells from 2½ donors sensitized with the less potent brain preparation had a more severe form of the disease than actively sensitized animals from the group used for donors. Allergic encephalomyelitis in the recipients was not induced by active sensitization caused by traces of adjuvant-antigen removed with the lymph nodes, as is shown by the absence of signs of the disease in the Hartley recipient controls. Hartley guinea pigs have been shown to be of highest susceptibility to actively induced allergic encephalomyelitis (*12*); furthermore, this line is as susceptible as, or more susceptible than, strain 13 animals whether sensitized by intradermal or intraperitoneal route (*13*). When transfers were made at 5 days, although the disease was not apparent in control Hartley recipients, sensitization to tuberculin, probably of the active type, did occur, as was shown by strong reactions to purified protein derivative in the Hartley controls. In Hartley guinea pigs, 0.02 mg of *Mycobacterium tuberculosis* is a threshold dose in the emulsion used for induction of allergic encephalomyelitis, but only 1 μg of the killed and dried mycobacteria is required to induce strong sensitivity to purified protein derivative (*14*). Transfers at 8 to 12 days conferred weak active or possibly weak passive sensitivity to purified protein derivative to Hartley recipients (also see *9*). The eliciting of a tuberculin reaction in recipients of lymph node cells harvested 8 to 12 days after active sensitization, which would otherwise serve as a control for the successful transfer and persistence of viable lymphoid cells (*9*), was complicated by probable impairment of the skin reactivity of animals sick with encephalomyelitis.

The problems which come to the fore concerning lymphoid transfers between histocompatible guinea pigs were discussed previously (*9*, *15*).

Table 1. Transfer of allergic encephalomyelitis. PPD = purified protein derivative of tuberculin; ND = not done; ? = doubtful reaction. Braces ({) encompass animals receiving a given pool of cells.

Cell pool	Strain of recipient	Day of transfer	Cells (ml)*	Disease		Day of death	PPD test	
				Day of onset†	Degree‡		Day	Square of radius at 24 hr (mm²)
				Brain antigen				
I	13	11	2.0	10	++		22	72
I	13	11	2.0	10	++		22	61
I	Hartley	11	2.0		0		22	30
I	Hartley	11	2.0		0		22	?
II	13§	11	2.0	8	++		22	106
II	13§	11	2.0	9	++		22	121
III	13§	11	2.5		0		20	64
III	13§	11	2.5	10	++++	17	ND	
				Spinal cord antigen				
IV	13	12	2.0	10	+		14	68
IV	13	12	2.0		0		14	64
V	13	11	1.8	9	++		ND	
V	13	11	1.8	10	+		23	25
VI	13	8	1.7		0		17	42
VI	13	8	1.7	9	+++		17	?
VI	13	8	1.7	8–9	++++	12	ND	
VII	13	8	2.0	8	++++	14	ND	
VII	13	8	2.0	10	+++		17	49
VIII	13	8	2.0	8	++		16	28
VIII	Hartley	8	2.0		0		16	36
VIII	Hartley	8	2.0		0		16	49
IX	13	8	2.4		0		20	40
IX	13	8	2.4	9	++++	16	ND	
IX	Hartley	8	2.4		0		20	25
IX	Hartley	8	2.4		0		20	69
X	13	5	1.3	8	++++	13	ND	
X	13	5	1.3	7	++++	10	ND	
XI	13	5	0.8	7	++++	10	ND	
XI	13	5	0.8	8	++++	11	ND	
XI	Hartley	5	0.8		0		20	117
XI	Hartley	5	0.8		0		20	100
XII	Hartley	5	1.0		0		16	105
XII	Hartley	5	1.0		0		16	46
				Egg albumin antigen				
	13	8	1.8		0		18	110
	13	8	1.8		0		18	110
	13	8	1.8		0		18	106

* Packed lymph node cells. † Day after transfer that weight loss began. ‡ ++++, death; +++, paresis or paralysis; ++, impacted feces, wet mouth, loss of weight; +, loss of weight only. § Received 5 mg of *M. tuberculosis* and 0.5 ml of 50 percent brain suspension in 0.5 ml of Arlacel-Bayol (2× dose).

The problems which come to the fore concerning lymphoid transfers between histocompatible guinea pigs were discussed previously (*9*, *15*).

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15. A description of the results of histological examination of the brains of guinea pigs with passively induced allergic encephalomyelitis is in preparation. These experiments were undertaken with the technical assistance of Julius H. Goode. This report is dedicated to Dr. Jules Freund, late Chief of the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases. Full-scale production of inbred guinea pigs at N.I.H. was encouraged by Dr. Freund in the hope that important questions could be answered relating genetics to reactions of hypersensitivity.

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