epithelium 8 hours after inoculation. Fluorescence, at this stage, was mostly cytoplasmic, however both cytoplasmic and nuclear fluorescence occurred in a few cells. IF reached a peak on the second and third day in the bronchi and usually spread to alveoli by the second day post inoculation. After the fourth day post inoculation, IF diminished in the alveoli and bronchi and disappeared after the 7th day. IF indicated that both cytoplasm and nuclei of infected cells are involved in the synthesis of viral antigens. IF was limited to macrophages and epithelial cells of bronchi, alveoli, and turbinates. It was concluded that influenza virus progressively infects bronchi, bronchioles, alveolar ducts, and alveoli during the course of infection.

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Induction of Tolerance to Skin Homografts by Administering Splenic Cells to Pyridoxine-Deficient Mice.* (29202)

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Induction of tolerance to tissue homografts can be achieved in adult mice through parabiotic union or by suitable administration of appropriate viable splenic cells or cellular extracts. The extensive literature pertinent to this subject has been reviewed recently(1-3). Utilization of cellular extracts for production of tolerance possesses distinct advantages(1) and we have, accordingly, undertaken a series of investigations designed to ascertain the effectiveness of various cellular components derived from splenic cells syngeneic with those of the skin donor. In these experiments, the cellular components are administered to recipients in a temporary state of pyridoxine deficiency. Rationale for this experimental procedure derives from numerous demonstrations of immunological unresponsiveness produced by this deficiency(4). Since the utility of intact cells in induction of tolerance is well-documented, we considered it advisable to ascertain the value of such viable cells under our experimental conditions before embarking upon a prolonged study involving use of cellular components. The present paper records results of these initial experiments which demonstrate successful achievement of tolerance in mature mice which had received splenic cells while in a prior state of pyridoxine deficiency.

Materials and methods. Animals and diets. Male mice of the CBA/J, C3H/HeJ and A/HeJ strains, 4-5 weeks of age, were obtained from the Roscoe B. Jackson Memorial Laboratory. Animals of the recipient CBA/J strain received upon arrival the purified control (complete) diet described in Table I. The pyridoxine-deficient diet fed subsequent-

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TABLE I. Composition of Purified, Control Diet.

Component	Percent
Casein "Vitamin-free"	25
Sucrose	69
Salts (6)	4
Corn oil*	2
	mg/100g
Thiamin • HCl	.3
Riboflavin	.8
Calcium pantothenate	2.0
Pyridoxine • HCl	1.0
i-Inositol	100.0
Nicotinic acid	1.0
Choline chloride	200.0
Biotin	.05
Pteroylglutamic acid	.10
Vitamin B_{12}	.01
2-methyl-1,4 naphthoquinone	.10

* Each gram was fortified with 3 mg of dl-α-tocopherol acetate, 275 I.U. of vit A and 55 I.U. of vit D. Vit A and D were supplied by Natola (Parke, Davis and Co.)

ly to some of these animals was prepared by omitting pyridoxine from this control diet. Solutions of the pyridoxine antagonist, deoxypyridoxine, (100 μg/ml) were prepared in isotonic sodium chloride and adjusted to pH 7.2. Mice of the C3H/HeJ and A/HeJ strains received a commercial stock diet.† Animals were housed in individual metal cages with screen bottoms and fed *ad libitum*. *Preparation of splenic cells.* Spleens were removed under ether anesthesia and placed in ice-cold Hanks' solution. Splenic cells were isolated by scraping the spleen with the small prongs produced at the cut edges of a 60-mesh monel wire screen. The yield from spleens of isogenic donors was pooled and the resulting suspension centrifuged for 10 minutes at 140 × *g*. The supernatant was discarded and the splenic cells resuspended in Hanks' solution to give a final concentration of 1.6-2.0 × 10^8 cells per ml. Cell counts of the splenic suspensions were determined in a Neubauer hemocytometer after dilution in 3% acetic acid. *Grafting procedure.* Full-thickness skin grafts from the abdominal area of the donor, approximating 1.5 × 2.0 cm, were applied to the dorsal area of the recipient as described previously(5). Grafts were sewn in place by numerous interrupted silk

† Purina Laboratory Chow, Ralston Purina Co., St. Louis.

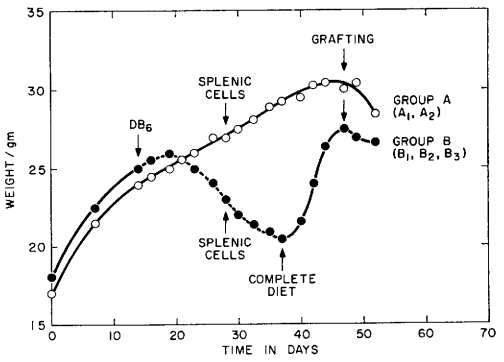

FIG. 1. Growth responses of recipient CBA/J mice. Periods during which the purified control (complete) and pyridoxine-deficient diets were fed are indicated by the unbroken and broken lines, respectively. DB_6 refers to deoxypyridoxine. A single injection of 0.5 ml of a suspension of splenic cells (0.8-1.0 × 10^8 cells) was administered intraperitoneally at indicated times.

sutures and no dressings were applied. Criteria used to judge survival of the grafts have been presented elsewhere(5).

Experiments and results. Growth curves of CBA/J recipient mice embodying the experimental design are shown in Fig. 1. Donors of splenic cells and skin grafts were always of the same age as the corresponding recipients.

CBA/J mice of group A, comprising subgroups A_1 and A_2, were fed the purified, control diet throughout the experiment and were grafted with skin of C3H/HeJ mice at the indicated time (day 47). Splenic cells derived from C3H/HeJ mice were administered on day 28 to animals of subgroup A_1. Except for omission of splenic cells, treatment of subgroup A_2 was identical with that of subgroup A_1.

CBA/J mice of Group B, comprising subgroups B_1, B_2 and B_3, were treated as follows. Following a 2-week period during which the purified, control diet was consumed, the animals were fed the pyridoxine-deficient diet and given daily intraperitoneal injections of 100 μg of deoxypyridoxine per 100 g of body weight for 3 weeks. The deleterious effect of this regimen upon growth of the animals is readily apparent. After 2 weeks of pyridoxine deprivation, animals of subgroup B_1 received splenic cells from C3H/HeJ mice while those of subgroup B_3 received a similar injection of cells derived from mice of the A/HeJ

TABLE II. Effect of Splenic Cells Administered to Pyridoxine-Deficient Mice Upon Viability of Skin Homografts.*

Exp	Sub group	Type	Source of splenic cells	No. of animals	No. of surviving grafts (weeks after grafting) 2	3	4	6	8	10	12	14	16	18	20
	A_1	Control	C3H/HeJ	17	8	1	0								
1–4†	A_2	"	—	21	10	1	0								
	B_2	Intervening pyridoxine deficiency	—	22	9	2	0								
1	B_1	*Idem*	C3H/HeJ	6	6	5	4	4	3	2	2	2	2	2	2‡
2	B_1	"	"	6	6	6	5	4	4	3	3	3	3	3‡	
3	B_1	"	"	8	8	7	7	7	6	6	6	6‡			
4	B_1	"	"	13	10	9	9	9	8	8‡					
3	B_3	"	A/HeJ	6	2	0									

* Recipients and skin donors were of the CBA/J and C3H/HeJ strains, respectively.
† Composite data of subgroups A_1, A_2, and B_2 of Exp 1 through 4.
‡ Animals possessing excellent grafts still under observation.

strain. Splenic cells were not administered to subgroup B_2. One week later, deoxypyridoxine treatment was discontinued and the animals resumed consumption of the purified, control diet. Growth response was immediate and 12 days later (day 47) each CBA/J mouse received a skin graft from a C3H/HeJ donor. The purified, control diet was fed to all animals for the remainder of the experiment. After a temporary weight loss subsequent to the grafting procedure, both growth and general appearance of the animals assumed the normal characteristics of stock CBA/J mice fed the commercial stock ration.

Effect of treatment with splenic cells upon viability of C3H/HeJ skin grafted to CBA/J recipients is presented in Table II. Many skin grafts in control mice fed the purified, complete diet throughout the entire experiment were rejected within 2 weeks and none survived beyond 4 weeks (subgroup A_2). A comparable rejection pattern was seen in controls injected with C3H/HeJ splenic cells (subgroup A_1) and in animals subjected to an intervening pyridoxine deficiency (subgroup B_2). Ineffectiveness of A/HeJ cells administered during pyridoxine deficiency was also observed (subgroup B_3). In striking contrast to these negative results were those we noted when pyridoxine-deficient recipients were treated with splenic cells from C3H/HeJ mice (subgroup B_1). In each of 4 experiments, survival of the grafts was markedly prolonged. Thus, 58% of the grafts were in excellent condition 10 weeks post-grafting. Many of the grafts are still under observation 10-20 weeks after grafting and their current excellent condition affords a favorable prognosis for continued survival. Appearance of representative surviving grafts is shown in Fig. 2.

The following experiment demonstrated the passive transfer of transplantation immunity or adoptive immunization. Donor splenic cells

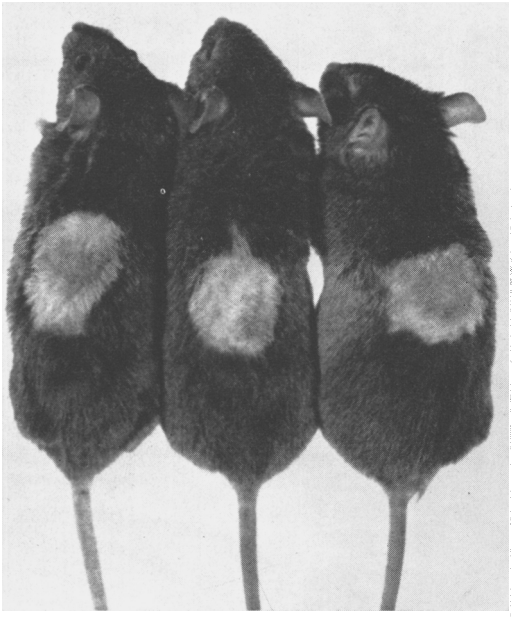

FIG. 2. Surviving grafts in CBA/J mice treated with C3H/HeJ cells while in a state of pyridoxine deficiency and grafted with C3H/HeJ skin subsequent to pyridoxine therapy (Subgroup B_1 of Table II). From left to right, age of grafts is 132, 125, and 96 days, respectively.

prepared as described above were obtained from control CBA/J mice grafted with skin from C3H/HeJ mice. Spleens were removed on the 11th day post-grafting when signs of rejection were apparent. Such sensitized cells (2.0×10^8) obtained from 2 donor CBA/J mice were then injected intraperitoneally in one dose into each of 4 CBA/J mice bearing skin grafts in excellent condition at 50, 64 and 71 days post-grafting. Tolerance to C3H/HeJ skin grafts had been produced in these recipients by a procedure identical to that described for subgroup B_1 (Table II) although these animals are not included among those described in this Table. In all 4 recipients, successful passive transfer of immunity was evidenced by appearance of inflammation and ulceration 4-7 days after injection of splenic cells followed by complete necrosis of the graft a few days later.

Discussion. Data presented here have demonstrated that tolerance to skin grafts can be induced by administering splenic cells syngeneic with those of the skin donor to pyridoxine-deficient mice. The ineffectiveness of allogeneic cells from A/HeJ mice attested to the essential specificity of donor cells. It should be noted that the grafting procedure was performed *after* recovery with pyridoxine therapy and that the graft recipients continued to receive a complete diet providing adequate nutrition for the duration of experiment. The absence of graft prolongation in control mice receiving syngeneic splenic cells emphasized the requirement for a pyridoxine deficiency at time of splenic cell administration. However, the mere existence of a prior state of pyridoxine deficiency did not affect graft survival.

The degree of successful tolerance attained in the present experiments with mice was greater than that observed by Fisher and Schewe(7) who utilized a similar procedure in rats. It was also more pronounced than that seen in our previous experiments in which viability of rat skin homografts was prolonged by administration of microsomal ribonucleic acid(8). In agreement with present observations, successful graft prolongation in the latter studies could only be achieved when a specific donor ribonucleic acid preparation was injected into pyridoxine-deficient rats(9).

The mechanism underlying the induction of tolerance in our experiments is not clear. Of relevance is the observation that pyridoxine deficiency limits the availability of one carbon unit required for biosynthesis of DNA and thereby depletes the number of splenic cells(unpublished observations). An accompanying decreased RNA synthesis with a presumed involvement of messenger RNA is also noted in this deficiency state. A combination of these two effects leads to an impairment of antibody synthesis which would be expected to interfere with the normal rejection of donor splenic cells and thus produce a situation favorable to survival of such cells in the host. An analogy with the cellular chimeric state induced by administering splenic cells to X-irradiated animals can thereby be drawn(10-13). The successful production of adoptive immunization indicates that the tolerance observed in our experiments is due to a central failure of the mechanism of immunological response. Various mechanisms of immunological tolerance have been discussed(1-3,10-13) which may relate to the type of tolerance described here.

Summary. 1. Tolerance of CBA/J mice to skin grafts from C3H/HeJ mice has been achieved by injection of C3H/HeJ splenic cells into pyridoxine-deficient CBA/J recipients. Skin grafting was performed subsequent to pyridoxine therapy. 2. Splenic cells obtained from A/HeJ cells were ineffective under otherwise identical conditions. 3. Grafts of tolerant CBA/J mice were rejected following injection with syngeneic cells sensitized to C3H/HeJ skin.

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Studies in Demyelination, II. Enzymatic Patterns in Central Nervous System and Sciatic Nerve in Experimental "Allergic" Encephalomyelitis. (29203)

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We have studied the enzymatic patterns of the sciatic nerve in Wallerian degeneration (1), which was considered to be an experimental condition suitable for study of multiple sclerosis and for the demyelination process in general. We then turned our attention to experimental allergic encephalomyelitis, since demyelination produced by resection of the sciatic nerve cannot be strictly compared to demyelination produced by infections and metabolic disturbances. Many authors(2,3,4) consider experimental allergic encephalomyelitis to be a better experimental model for multiple sclerosis. The clinical picture and the occurrence of remissions and relapses in some of the animals show a striking analogy to multiple sclerosis. We, therefore, studied the changes in the enzymatic patterns of the central nervous system as well as those of the sciatic nerve and looked for an analogy between the changes in the enzymatic patterns observed in Wallerian degeneration(1, 5) with those occurring in the demyelination due to experimental allergic encephalomyelitis.

The enzymatic pattern in Wallerian degeneration showed an increase in the acid and alkaline phosphatases, in adenosinase and in β-glucuronidase(1,5). Roizin(6) tried to correlate the demyelination with the activity of oxidases, peroxidase and acid phosphatase of white matter in some multiple sclerosis cases and in experimental allergic encephalomyelitis in *Macacus rhesus* monkeys by histochemical techniques. He found increased enzyme activity in the demyelinated areas.

A 10-fold increase of β-glucuronidase has been found in Wallerian degeneration by determination of the enzymatic activities of the homogenized sciatic nerve(1). This high β-glucuronidase activity was also observed in the sciaticus of the rat by histochemical methods in Wallerian degeneration(7). Ten days after transection there was an increase in β-glucuronidase in the myelin sheath, but none in the nuclei of the axis cylinder cells or the connective tissue(7).

No alkaline phosphatase, adenosinase nor β-glucuronidase determinations have been reported in experimental allergic encephalomyelitis.

The encephalitogenic activity of nervous tissue is mainly exerted by preparations rich in white matter(8,9) and is absent from fetal or unmyelinated brain(9,10). Experimental allergic encephalomyelitis (EAE) can be produced by nervous tissue of foreign species as well as by homologous tissue(9-13), and the antigenicity is considered to be organ-specific rather than species-specific. It is for this reason that we decided to study the enzymatic patterns of the spinal cord in EAE. We divided the spinal cord into 4 main sec-

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