is identified by the arrows. Below arrow 4 one can see the synaptic complex usually associated with chemical synapses; that is, the presynaptic cluster of synaptic agranular vesicles measuring 250 to 400 Å, synaptic cleft 200 to 300 Å wide, and a postsynaptic density or web. Following the synaptic membranes, one can see in the regions between arrows 3 and 4 and arrows 1 and 2 that the synaptic cleft has been obliterated and the synaptolemma has an appearance similar to tight junctions seen elsewhere. Between arrows 2 and 3 the intercellular space is again of conventional magnitude. Note the paucity of synaptic vesicles in the regions of the tight junctions. A further synaptic relationship is represented by the nerve fiber (F) which appears to end (judged by the presence of synaptic vesicles) upon the calyx. Perhaps this may be a mechanism for modulating the function of the calyx itself.

Of the 22 ganglia observed, I distinguished membrane tight junctions in 15, and in each calyx an F-type fiber was also observed, in agreement with the electrophysiological incidence of electrical transmission (*7*). It does not, however, preclude the possibility that the "spots" of pre- and postsynaptic membrane fusion were missed when not observed. In any event, the observations suggest that membrane fusions do occur in calyciform synapses and in the present study have been found only in the axon hillock region of the postsynaptic cell. Thus the synaptic membrane exhibits morphological characteristics of both electrical and chemical synapses.

A. J. DARIN DE LORENZO
Johns Hopkins University, School of Medicine, Johns Hopkins Hospital, Baltimore, Maryland 21205

References and Notes

1. A. J. D. de Lorenzo, *Biol. Bull.* **119**, 325 (1960); J. D. Robertson, *Ann. N.Y. Acad. Sci.* **94**, 339 (1961).
2. M. V. L. Bennett, E. Aljure, Y. Nakajima, G. D. Pappas, *Science* **139**, 330 (1963).
3. J. D. Robertson, T. S. Bodenheimer, D. E. Stage, *J. Cell Biol.* **19**, 159 (1963).
4. M. M. Dewey and L. Barr, *Science* **137**, 670 (1962).
5. H. E. Karrer, *J. Biophys. Biochem. Cytol.* **8**, 135 (1960).
6. A. R. Martin and G. Pilar, *J. Physiol. (London)* **168**, 443 (1963).
7. ———, *ibid.*, p. 464.
8. A. J. D. de Lorenzo, *J. Biophys. Biochem. Cytol.* **7**, 31 (1960).
9. A. Hess, *J. Cell Biol.* **25**, 1 (1965).
10. Aided by PHS grant NB 02173. I wish to thank D. S. Seiple and G. Barnett for technical assistance and L. M. de Lorenzo for help in collating the data.

21 January 1966

Increased Incidence of Lymphoma after Injections of Mice with Cells Differing at Weak Histocompatibility Loci

Abstract. *Newborns of two congenic strains of mice, in which the incidence of leukemia is low, differing only at the weak* H-1 *locus, were injected at birth with adult spleen cells of the parent and of the congenic strain. A marked increase in the incidence of lymphoma ensued in those mice of both strains injected with cells from the other strain. The experiment lends some support to the idea that transplantation immunologic mechanisms may play a role in the genesis of lymphomas.*

Injection of newborn C3H mice with adult spleen cells from a congenic C3H.K partner strain is associated with an increased absolute incidence of lymphoma and with a marked shift in the incidence of lymphoma toward a younger age (*1*). These strains differ from each other in histocompatibility only at the weak *H-1* locus. The C3H mice are $H\text{-}1^a$, and the C3H.K are $H\text{-}1^b$. As controls in this prior experiment C3H newborns were injected with adult C3H spleen cells. Thus test animals were those in which adult $H\text{-}1^b$ cells were injected into neonatal $H\text{-}1^a$, and controls were those in which adult $H\text{-}1^a$ cells were injected into neonatal $H\text{-}1^a$. This earlier work left almost entirely open the question whether induction of lymphoma was occasioned by oncogenic viruses, transplantation immune mechanisms, or other factors, although some inferential evidence was presented favoring the second possibility. We have now injected newborns of both strains with spleen cells of the strain differing only at the *H-1* locus and with cells of their parent strain as controls, with a resulting great increase in the incidence of lymphoma in each group receiving the differing cells.

The plan of the experiment is indicated in Table 1. For purposes of exposition, the two congenic strains are referred to by their *H-1* histocompatibility genes as $H\text{-}1^a$ or $H\text{-}1^b$, respectively. The two strains were obtained initially from Snell at the Jackson Memorial Laboratory, and have been further inbred here for about 5 years. The mice are considered to be essentially genetically identical at all histocompatibility loci except the *H-1* locus. About 2.5×10^6 spleen cells from 2- to 3-month-old apparently normal male mice were injected into recipients less than 24 hours old by the intracardiac route. A small population of noninjected mice was also retained. The females were discarded at time of weaning because of the high incidence and early deaths from mammary carcinoma in female C3H mice. Five or six mice were housed in a cage. They were fed commercial mouse chow, and all were allowed to live out their natural life spans. The total weights of body and spleens were determined at death, and a gross necropsy was performed on all animals. Microscopic examination of liver, spleen, and other organs, as seemed appropriate, was accomplished for all animals except when advanced autolysis interfered.

The incidence of lymphoma was markedly increased by injection of the newborns with spleen cells differing only at the *H-1* locus (Table 1). This increase occurred in both directions (that is, when $H\text{-}1^b$ cells were injected into $H\text{-}1^a$ or when $H\text{-}1^a$ cells were injected into $H\text{-}1^b$) as compared to the controls who received cells from the parent strain. Also, injection of $H\text{-}1^b$ cells into $H\text{-}1^b$ newborns yielded no increase in lymphoma incidence as compared with noninjected $H\text{-}1^b$ controls. There was, however, an intermediate augmentation of lymphoma incidence in the $H\text{-}1^a$ set treated similarly. In separate experiments, it was found that injection of viable liver or kidney cells in either differing strain did not increase the lymphoma incidence over that in noninjected mice.

The lymphomas in all mice were characterized by marked splenomegaly, liver infiltration by lymphoid cells, and frequent lymphadenopathy. The thymus was rarely involved. The histologic pattern for animals that received the differing spleen cells has been described (*1*), and the same pattern was seen in all the present combinations. The lymphomas resembled that designated as lymphocytic neoplasm (*2*).

In C3H mice in general, and in both of the present strains as well, reticuloendothelial neoplasm is quite uncommon. Indeed, C3H strains are commonly used in studying virus-induced lymphomas or leukemias in mice precisely

because of their low spontaneous incidence of this disease. The induction of lymphoma in 50 percent or more of the animals after injection of immunologically competent cells differing from the recipients' cells only at the weak *H-1* histocompatibility locus, together with the fact that cells of mice having a low incidence of leukemia were used, suggests the possibility that transplantation disease mechanisms might be involved. Tyler (*3*) postulated that cancer may be due to inactivation or loss in the precancer cell of a gene or genes which determine histocompatibility antigens and that the antigens, still present in the host but lost in the precancer cell, would stimulate the precancer cell to malignant proliferation. Cancer might thus be considered as a type of graft-versus-host reaction. Ideas roughly similar to those of Tyler have also been set forth by others (*4, 5*).

A theoretically analogous situation might be considered to obtain in the mice in our experiment. The newborns injected at birth would be rendered tolerant to the foreign injected cells. However, the injected cells in the continuous presence of histocompatibility antigen of a "foreign" host might be expected to undergo proliferative and eventually malignant changes. Owing to the weakness of the histocompatibility differences (which I believe to be a fundamental point in the experiments), the injected cells would probably not suffer the "allergic" death that frequently supervenes when cells are continuously exposed to an excess of strong, foreign histocompatibility antigens.

The occurrence of a moderately elevated incidence of lymphomas when *H-1*^{a} spleen cells of adult mice were injected into the *H-1*^{a} newborns does not correlate with the above interpretation. This augmentation of lymphoma could, however, be due to residual or developing heterozygosity in the *H-1*^{a} mice. The fact that one is specifically dealing with very weak differences in histocompatibility, which perhaps act over a long period, would be in accord with this possible interpretation. It is also of note that the lymphomas in the *H-1*^{a} mice injected with *H-1*^{a} spleen cells developed much later in life than in the *H-1*^{a} mice injected with *H-1*^{b} cells (Table 1). The lack of effect on lymphoma incidence of injection of *H-1*^{b} mice with *H-1*^{b} cells is, on the other hand, in accord with an interpretation based upon the Tyler-Schwartz-Dameshek hypothesis. It is of fundamental importance to determine whether lymphomas induced by these mechanisms in the mice injected with the differing cells will grow as malignancies in the parental strains, and to determine by appropriate technics the *H-1* composition of the lymphoma cells.

Table 1. Age at death, and incidence of lymphomas, in test and control mice injected at birth with cells differing at a weak histocompatibility locus.

Age range at death (week)	Genetic combination: adult splenic cells → newborns											
	H-1^b→*H-1*^{a}		*H-1*^{a}→*H-1*^{a}		*H-1*^{a}→*H*^{b}		*H-1*^{b}→*H-1*^{b}		Noninjected			
									H-1^b		*H-1*^{a}	
	D*	L*	D	L	D	L	D	L	D	L	D	L
30–35	1											
35–40							1		1			
40–45												
45–50	1	1										
50–55	1	1									2	
55–60			1				1					
60–65	1	1										
65–70	2	1					1					
70–75	3	2					1	1				
75–80	5	2			2	1			3	1		
80–85	1						2		1			
85–90							4				1	
90–95					4	3	3		2		1	
95–100	3	3	4	1	2	1	6		1		2	
100–105	3	2	4				3	1			1	
105–110	4	1	1		1		5	1	1			
110–115	2		4	1	2	1	3				3	
115–120	1	1	2	1	1	1	3				1	
120–125	2		2	1			4					
125–130							1		1			
130–135					1				1		1	
135–140							1					
Lymphoma incidence (%)												
	50		22		54		7.7		9		None	

* D = deaths in interval; L = lymphomas in interval.

Several other interpretations of the experiment are possible. Injection of immunologically competent cells into animals with even a very weak histocompatibility barrier might lead to slight injury to the immunological system of the recipient, and later developing lymphomas might arise because of this injury. There is no evidence at present for such injury in terms of the classical signs of "runt" or transplantation disease. In separate experiments of the type reported here, no differences in rate of weight gain or eventual weights between test and control mice were detected. Thymic and splenic weight indices were normal and no pathologic changes in these organs could be detected by microscopic examination. These findings do not rule out the possibility of unobserved minimum changes.

Particularly in injection of newborn mice, the possibility of viral oncogenesis must be considered. Even mice of a strain with low incidence of leukemia, such as the mice used in this study, may nevertheless harbor a virus capable of inducing leukemia under proper conditions. It does seem somewhat unlikely that *H-1*^{b} mice would carry a virus transmissible to *H-1*^{a}, but not to *H-1*^{b} newborns, and that correspondingly *H-1*^{a} mice would carry a virus showing a much greater transmissibility to *H-1*^{b} than to *H-1*^{a} newborns (both mouse strains being so closely related genetically), and that finally both viruses would produce identical gross and histologic lymphoma. Despite these surmises, viral oncogenesis, particularly if coupled with a very mild or subclinical transplantation disease reaction, cannot be entirely excluded on present evidence as an inductive factor in these experiments.

The foregoing results seem related to the recent experiments of Schwartz and Beldotti (*5*), who injected adult C57B1/6 spleen cells into 4- to 6-week-old (C57B1/6 × DBA/2) F_1 hybrid recipients, and partially averted the severe transplantation disease and death that ordinarily obtains with this strong (*H-2*) difference in histocompatibility antigens by treatment with amethopterin. In their experiments a significant number of lymphomas developed in the test animals as compared to controls. Whereas my experiment is complicated

by the as yet unresolved possible role of viral oncogenesis, that of Schwartz and Beldotti (*5*) was complicated by the frequent occurrence of transient mild-to-severe runting, which would indicate considerable injury to the lymphoid system. The studies are thus complementary, one obviating the runting syndrome and the other probably obviating viral oncogenesis. Together they would seem to present the strongest experimental evidence yet available that reactions intimately concerned with histocompatibility difference might play a role in the genesis of lymphomas.

ROY L. WALFORD
Department of Pathology, University of California School of Medicine, Los Angeles

References and Notes

1. R. L. Walford, W. H. Hildemann, *Am. J. Pathol.* **47**, 713 (1965).
2. T. B. Dunn, *J. Nat. Cancer Inst.* **14**, 1281 (1954).
3. A. Tyler, *ibid.* **25**, 1197 (1960); ———, *Biological Interactions in Normal and Neoplastic Growth*, M. J. Brennan and W. L. Simpson, Eds. (Little, Brown, Boston, 1962), pp. 533–576.
4. W. Dameshek and R. S. Schwartz, *Blood* **14**, 1151 (1959); H. S. Kaplan and D. W. Smithers, *Lancet* **1959-II**, 1 (1959); I. Green, M. Inkelas, L. Allen, *ibid.* **1960-I**, 30 (1960).
5. R. S. Schwartz and L. Beldotti, *Science* **149**, 1511 (1965).
6. Supported by USPHS grant HD-0534.

27 December 1965

Interferon: Lack of Detectable Uptake by Cells

Abstract. *No interferon, or at most a small fraction of that applied, is taken up by cells during the period of induction of antiviral activity. The lack of detectable adsorption was not influenced either by the concentration of interferon or the volume. The results were similar in both chicken and mouse systems.*

Many of the concepts of the mechanism of antiviral action of interferon are based on the assumption that cells rapidly adsorb the major portion of the applied interferon (*1*, *2*). In view of experiments which did not show disappearance of interferon from fluid, we have investigated the reaction of interferon with cells. Our results do not support the suggestion that interferon is rapidly adsorbed by cells, but they indicate that interferon can render cells resistant to virus in the absence of detectable adsorption.

Chick and mouse embryo tissue cultures and vesicular stomatitis virus were prepared, as described (*3*). Interferons from mouse serum and from chicken allantoic fluid were produced, characterized, and assayed (*3*). In the interferon transfer experiments, tissue culture plates were thoroughly drained by aspiration with capillary pipettes. This technique of thorough aspiration was also used to transfer interferon from one group of plates to another. Appropriate controls demonstrated that dilution by residual fluid on plates, evaporation during incubation, and the experimental procedure itself had no significant influence on the results.

In the first experiment, the conditions were similar to those reported for studies of interferon adsorption (*1*). Interferon (1.7 units) in 0.1-ml volume was added to ten thoroughly drained plates of chick embryo culture and incubated at 37°C. After 30 minutes, the interferon from five plates was recovered and assayed for residual activity. The interferon from the remaining five plates was recovered at 60 minutes and assayed (Fig. 1). The slope was determined by statistical analysis and indicates no significant decrease in residual interferon activity after a 1-hour incubation period with cells. For comparison, the slope of the reported consumption rate of 90 percent per hour is also shown (*1*).

If a subdetectable amount of interferon is actually taken up by cells then it might be detected if there was an increase in the number of cells exposed to the same amount of interferon. Such an increased ratio of cells to interferon was achieved by serial transfer of interferon onto cell cultures as follows. Chick interferon (30 units per milliliter) was incubated with chick embryo cell cultures for 3 hours, and then the culture fluid containing residual interferon was recovered. A portion of the recovered fluid was stored for later assay of interferon content, and the remaining fluid was transferred to new cell cultures. This was repeated for a total of eight serial transfers. In this way, the quantity of interferon adsorbed in 3 hours should be magnified eightfold. Three hours was sufficient to stimulate maximum antiviral activity in the plaque-reduction assay. Figure 2 shows the residual interferon titer as a function of the transfer number. The slope of the line as well as its 95 percent confidence limit is shown. The results

Table 1. Effect of serial transfer of 0.5 and 2.0 units (U) of mouse interferon on residual antiviral activity. Percentages determined from the mean value of five to eight plates per point.

Transfer No.	Cumulative (hr)	Inhibition of plaques by 0.5 U (%)	Inhibition of plaques by 2.0 U (%)
1	3	34	66
2	6	10	53
3	9	32	59
4	12	42	59

indicate that 0 ± 7.2 percent of the interferon was taken up by the cells during each transfer. Also shown are slopes representing a theoretical 50 percent uptake per passage and a theoretical 90 percent uptake per passage, which can be ruled out by comparison of the observed residual titer. Within the limits of these assays, it is not possible to detect any change in the amount of residual interferon remaining after eight serial adsorptions. If the uptake of interferon by cells were only 1 or 2 units per passage, then, in experi-

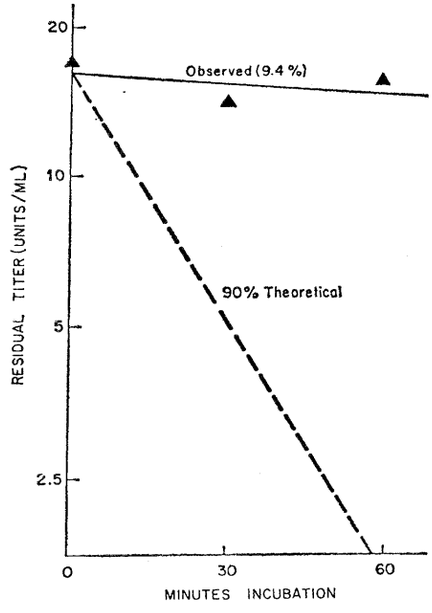

Fig. 1. Residual titer of a preparation of chicken interferon after incubation with chicken embryo cell cultures. Plaque counts from dilution assays of incubated preparations by the method of regression analysis (*12*) were found to be linear functions (with a common slope) of the log dilutions. Residual titers (50 percent inhibitory dilutions) were calculated from the individual regressions and the logarithms plotted as a function of the incubation time. The slope (b) of the line fitted to these values by least squares was expressed as: consumption (%)/hr = $100 (1 = 10^b)$.