To the Graduate Council:

I am submitting herewith a dissertation written by Kathy A. Vasa Thomas entitled "An Investigation of a Spontaneous Lymphoma Epizootic in Hamsters." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Radiation Biology.

Carl J. Wust, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

Thesis
T86
T486
cop. 2

AN INVESTIGATION OF A SPONTANEOUS LYMPHOMA EPIZOOTIC IN HAMSTERS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Kathy A. Vasa Thomas

August 1978

136613

ACKNOWLEDGEMENTS

The author is grateful for the support and editorial assistance of Dr. Carl J. Wust in preparing this dissertation, and for the advice offered by Drs. J. M. Holland, J. M. Woodward, and A. Brown. She would also like to thank Dr. J. H. Coggin, Jr., for the opportunity to study the phenomenon of apparently contagious tumors in hamsters.

The author would like to especially thank her husband, Wendell, for his unfailing support, patience, and encouragement during these investigations, and her parents for encouraging her curiosity in scientific pursuits.

ABSTRACT

The third of three outbreaks of lymphomas in hamsters was investigated with the objective of identifying the cause of the outbreaks. The recurrence of the outbreaks and young age of the animals involved suggested the tumors might be of viral origin, although the only common factor to be identified for all the epizootics was the use of a Simian Virus 40 (SV40) transformed hamster fibroblast cell line. A series of immunization and challenge experiments demonstrated a possible cross-protection between the SV40 transformed cells and an in vivo passaged lymphoma cell line, but the cross protection was not greater than that afforded by normal hamster muscle homogenate. In contrast, immunization and challenge experiments using lymphomas derived from three different animals resulted in protection against challenge and spontaneous lymphoma. This immunity could be transferred with peritoneal exudate cells from immunized animals, suggesting the common antigen on the lymphomas was a membrane antigen, possibly of viral origin. In the course of the immunization-challenge experiments, a number of hamsters were lost to enteritis or intussusceptions. This prompted an investigation into the pathology of the lymphomas and the background pathology of the hamster colony. Lymphomas accounted for 14 percent of deaths in the colony, and two other diseases, enteritis and pyelonephritis were also identified as causes of death. An increased incidence of lymphomas resulted when neonatal hamsters were

inoculated with cell-free preparations made from two different in vivo passaged lymphomas. Both lethal and oncogenic effects of the preparations could be neutralized with lymphoma-bearer serum. A theory is proposed that the induction of lymphomas involves an immunosuppressive virus and a virus inducing agent such as another virus.

TABLE OF CONTENTS

CHAPTER		PAGE
INTRODUCTION		1
	PART I. LITERATURE SURVEY	
I.	SPONTANEOUS LYMPHOID NEOPLASMS IN HAMSTERS	6
II.	INDUCED LYMPHOID NEOPLASMS IN HAMSTERS	14
III.	NATURAL TRANSMISSION OF TUMORS IN HAMSTERS	19
IV.	HISTORY OF THE UNIVERSITY OF TENNESSEE HAMSTER LYMPHOMA EPIZOOTICS	23
	PART II. EPIZOOTIOLOGY	
I.	INTRODUCTION	30
II.	MATERIALS AND METHODS	32
III.	RESULTS	35
IV.	DISCUSSION	45
	PART III. PATHOLOGY	
I.	INTRODUCTION	53
II.	METHODS	55
III.	RESULTS	57
IV.	DISCUSSION	79
	PART IV. IMMUNOLOGY	
I.	INTRODUCTION	83
II.	MATERIALS AND METHODS	85

CHAPTER PAGE

III. RESULTS 89

IV. DISCUSSION 100

PART V. VIROLOGY

I. INTRODUCTION 104

II. MATERIALS AND METHODS 106

III. RESULTS 109

IV. DISCUSSION 117

PART VI. GENERAL SUMMARY

GENERAL SUMMARY 124

LIST OF REFERENCES 129

APPENDICES 137

Appendix A 138

Appendix B 140

Appendix C 146

Appendix D 152

VITA 156

LIST OF TABLES

TABLE PAGE

PART I

I. Summary of Reports of Spontaneous Lymphomas in Hamsters . . 7

II. Lymphosarcoma Transmission Experiments 22

PART II

I. Causes of Death in the Lymphomatous Colony, December, 1974 to May, 1977 40

II. Mortality Data from 78 Hamsters and Their Progeny 42

III. Mortality in LSH and LVG Hamsters Exposed to the Lymphomatous Colony Between December, 1976 and May, 1977 43

PART III

I. Lymphomas in Nonexperimental Animals (January, 1975 to May, 1977) 59

II. Lymphomas in Experimental Animals (January, 1975 to May, 1977) 61

III. The Incidence of Lymphomas by Site in Animals of the Third Epizootic 62

IV. Length of Exposure of Animals Dying with Intussusceptions 68

V. Other Pathological Lesions 77

TABLE PAGE

PART IV

I. Results of Experiments to Demonstrate Presence or Lack of Cross-Protection Between Lymphomas (CL/LSH-2) and SV40 Transformed Cells (F5-1) 97

PART V

I. Incidence of Lymphomas in Hamsters Inoculated at 1 Day of Age with a Cell-Free Preparation Made from a Passaged Cervical Lymphoma 110

II. Lymphomas in Animals Inoculated with Cell-Free Preparations Made from a Passaged Cervical Lymphoma and Tested in 1 Day, 4 Day, and 10 Day Old Hamsters 112

III. Oncogenicity of a Cell-Free Preparation Made from a Passaged Inguinal Lymphoma 113

IV. Neutralization of Oncogenicity of a Cell-Free Preparation Made from a Passaged Inguinal Lymphoma 115

V. Lymphoma Incidence in Hamsters Used as Controls for Testing Oncogenicity of Cell-Free Preparations 119

APPENDICES

A-I. Number of Deaths in the Clean Facility from February, 1977 through September, 1977 139

B-I. Agents and Cells Used Prior to the Lymphoma Epizootics . . . 145

LIST OF FIGURES

FIGURE PAGE

PART III

1. Graphic Presentation of the Occurrence of Intussusceptions with Respect to the Length of Exposure to the Lymphomatous Colony 69

PART IV

1. Determination of the TPD_{100} of Three Spontaneous Lymphoma Lines 90
2. Effect of Lymphoma Immunization on Subsequent lymphoma Induction 92
3. Determination of Cross-Reactivity Between the Three Lines of Lymphoma 94
4. Determination of the Number of Immunizations That Would Provide Adequate Protection 95
5. Effect of Lymphoma Immunization on Subsequent Challenge with Lymphoma (a) or SV40-Transformed Cells (b) 96
6. Adoptive Transfer of Resistance to Lymphoma Challenge . . . 99

APPENDIX C

C-1. Intestinal Lymphoma (X 63) 147

C-2. Intestinal Lymphoma (X 950) 147

C-3. Spontaneous Thymic Lymphoma 148

C-4. Spontaneous Retroperitoneal Lymphoma (X 950) 148

FIGURE PAGE

C-5. Pyelonephritis 149

C-6. Spontaneous Liver Lymphoma (X 160) 149

C-7. Warts Around the Eyes and Under the Chin of an LSH Female . 150

C-8. Histological Section of the Warts 150

INTRODUCTION

One value of the Syrian hamster as an experimental animal is its relatively low incidence of spontaneous tumors. Incidences varying from less than 1 percent to 17 percent have been reported for different colonies (62), although it is not clear if these differences reflect the extent to which animals are evaluated histopathologically or whether they can be attributed to epizootiologic factors. Nevertheless, most of the tumors are benign, and most of the animals involved are at least one year of age. Differences in incidence with respect to sex have been noted, but again, this varies from colony to colony. There have been few reports of spontaneous tumors in animals less than one year of age although there have now been seven reports of sudden outbreaks of lymphomas in young hamsters (1,23,37,48,77). In no case has the etiologic agent been positively identified, although C-type virus has been implicated in six of the occurrences (1,23,37,48).

In November, 1972, epizootic lymphomatosis was detected in The University of Tennessee hamster colony. It involved experimental and breeding animals, all under one year of age, and was horizontally transmitted (contagious) to hamsters brought in from an outside source (Lakeview, Newfield, New Jersey). The colony was sacrificed and new animals purchased from Lakeview. A second epizootic was detected seven months later and included hamsters as young as one month of age. Again, the colony was sacrificed and new animals purchased. A third epizootic was detected four months later, and the third

epizootic is the subject of this investigation.

A spontaneous horizontally transmissible tumor affords an unusual opportunity to study horizontal transmission of an oncogenic agent. Epidemiologic evidence has been used to suggest the possibility of a transmissible element in various lymphoid tumors (35,60,78,86) and there is also recent in vitro evidence that suggests a contagious element for the human non-Hodgkin's lymphomas (13). The possibility that the hamster lymphomas might serve as model for the human lymphomas was considered. However, before the hamster lymphoma could become a useful model, it first had to be characterized and the cause of the tumors identified. This dissertation is concerned with the identification of epizootiologic characteristics of the outbreaks, with the histopathology of the tumors as well as the background pathology of the involved colony, with the detection of antigens associated with the tumors, and with attempts to isolate a causative agent.

The sudden onset of the tumors, their apparently equal distribution in experimental and breeding animals and the recurrence of the epizootics suggested that an agent was being induced in the hamsters or introduced into the colony possibly with some sort of experimental procedure. An analysis was made of the agents and procedures used prior to each epizootic and only one common factor was noted; the use of viable untreated and viable X-irradiated SV40 transformed hamster fibroblasts. Analyses were then made of the characteristics of the tumor bearing animals with respect to strain, sex and age to determine if a particular population was most susceptible. In addition, physical factors such as the source of food and bedding, the husbandry

procedures and other environmental variables were documented to determine if there were any differences between these factors in the lymphomatous colony and a clean colony used to supply animals for the lymphomatous colony. The procedures used and results obtained for these investigations will be presented under the topic of Epizootiology.

In order to characterize the nature of the lymphomas, the pathology of the spontaneous tumors was investigated. The incidence of tumors in different organs was assessed for the third epizootic and compared to the published values for the first two epizootics (1). The histopathology of tumors from all three epizootics was then evaluated and the results are presented in the section entitled Pathology. Pathological conditions other than lymphoma were also noted among hamsters in the lymphomatous colony and are presented in the Pathology section. Two of these conditions, enteritis and chronic pyelonephritis, had been seen prior to each epizootic and the possibility is discussed that both conditions are associated with presence of the lymphoma agent.

A number of investigations have shown that tumors have antigens which can be used to immunize animals against subsequent challenge with homologous tumor cells. In addition, it has been demonstrated that virally transformed cells exhibit a common antigen regardless of the cell type or species. A series of immunization-challenge experiments was devised to determine if animals could be protected against both spontaneous and challenge lymphomas, if the lymphomas exhibited a common antigen, and if the antigen had any cross-protective activity with antigens of the SV40 transformed cells. The results of these investigations are presented in the Immunology section.

The results of investigations to isolate the lymphoma agent are presented in the Virology section. These experiments were carried out against a background incidence of spontaneous lymphomas ranging from 0 to 50 percent in groups of control animals. Preliminary evidence was obtained that an oncogenic agent isolated from the lymphomas could induce lymphomas when inoculated into neonatal hamsters.

PART I

LITERATURE SURVEY

CHAPTER I

SPONTANEOUS LYMPHOID NEOPLASMS IN HAMSTERS

Spontaneous neoplasms in Syrian hamsters have been reported on occasion since 1945 (5). The incidence and type of tumor reported has varied from colony to colony and this variation has been attributed to unknown epidemiologic factors, to incomplete autopsy procedures, and to the source of the animals (62). Nevertheless, of the pathologic lesions reported, lymphoid neoplasms are among the most frequently cited.

The incidence of spontaneous lymphoid neoplasms in a number of Syrian hamster colonies has been collated in Table I. Most spontaneous lymphomas occurred in hamsters greater than one year of age and the histopathology was usually that of a reticulum cell sarcoma. Van Hoosier (82) surveyed reports of spontaneous tumors in hamsters and found an overall tumor incidence of 4 percent for all colonies, with lymphomas and leukemias accounting for 35 percent of the tumors reported (1.4 percent overall). An overall incidence for lymphomas of at least 3 percent can be calculated from Table I by totaling the number of animals with lymphomas and dividing by the number of animals evaluated. The higher incidence is due to several reports of outbreaks of lymphomas in various hamster facilities.

Since 1962, there have been seven occurrences of apparently spontaneous outbreaks of lymphomas in hamsters less than one year of age. In no case has the etiologic agent been identified, although

TABLE I. Summary of reports of spontaneous lymphomas in hamsters.

Year	Investigator	No. Animals	No. Lymphomas	Age	% Incidence
1945	Ashbel (5)	1000	2 polymorph sa	--	.2
1957	Fortner (18)	--	2 lymphoblastic sa	2 y	
1960	Strauli (77)	--	4 lymphoreticular sa	4-5 m	
1962	Strauli (76)	--	28 lymphoreticular sa	--	
1961	Brindley (7)	--	1 reticulum cell sa	732 d	
1967	Toth (80)	100	7 malignant lymphoma	76,82,82,91, 105,115,114 w	7
		100	2 malignant lymphoma	75,89 w	2
1968	Horn and Siewert (37)	2000	35 lymphosa, 1 lymphocytic, histiocytic leukosis	3-5 m	
1968	Graffi (28)				3
	Dunham and Herrold (14)	360	12 retic cell sa 3 plasma cell lymphoma 4 lymphocytic lymphoma	17-28 m 18, 26,30 m 18-36 m	
1972	Van Hoosier (83)	1671	3 lymphomas	391,472,772 d	
1973	Yabe (88)	1580	none prior to 6 m		
		75	3 lymphomas	490,582,852 d	
1975	Kistler (48)	245	malignant lymphomas	3-8 m	70
1975	Ambrose (1)	2000	malignant lymphomas		50
1976	Pour (63)	377	7 malignant lymphomas	30-109 w	
		500	6 malignant lymphomas	54-108 w	

NOTES: Numbers in parentheses refer to similarly numbered items in the List of Reference.
sa = sarcoma; y = years; m = months; w = weeks; d = days.

C-type virus has been detected in some of the tumors. Results of studies on these outbreaks are presented below.

Strauli and Haemmerli (77) reported in 1960 on the occurrence of four spontaneous lymphoreticular sarcomas in the University of Zurich colony. The origin of these animals was not determined, but the tumors occurred in four to five month old Syrian hamsters. This report was followed by a second in 1962 (76) that documented the occurrence of an additional 28 lymphomas. Horizontal transmission of the tumors was not discussed in either report although the sudden onset and young age of the animals suggested that they might have encountered some type of oncogenic agent. It was found that some of the tumors could be serially passaged to other animals via tumor minces, but the cause of the tumors was not identified.

Horn and Siewert (37) reported in 1968 on the appearance of spontaneous tumors in the golden hamster breeding colony started in 1960 at the Institut fur Krebsforschung, Berlin. This colony had been maintained separately from the experimental animals with an average stock of 1500 females and 500 males since 1962. No spontaneous tumors had been seen prior to November, 1966, however, in the eight months following that date, 35 lymphosarcomas, four papillomas, one adenocarcinoma of the liver, and one lymphocytic-histiocytic leukosis occurred. Thirty-four of the tumors appeared in females and more than two-thirds of the tumors were seen in animals no more than three months old. Twenty-eight of the lymphosarcomas were found in the abdomen, two were in the liver, one was cervical, three were axillary, and one was both ip and axillary. No mention was made of horizontal transmission to

animals from outside the colony, but the sudden onset and young age of the animals again suggested an oncogenic agent was involved.

Graffi et al. (28) discussed the possible sources and modes of transmission of these lymphomas in a second paper in 1968, and it is evident from their observations that the breeding colony had continued to have a background lymphoma incidence of approximately 3 percent. Spontaneous horizontal transmission was not reported although the failure of cell-free filtrates to produce tumors in progeny of the affected colony suggested the breeding stock had become infected and had passaged immunity to the progeny through the placenta or milk. Animals from outside the colony developed lymphomas following injection with cell-free filtrates of the spontaneous tumors; however, no mention was made of spontaneous horizontal transmission to animals brought in from outside the colony. Since no uninoculated controls were given, it is possible that horizontal transmission might have occurred, but was not detected. The lymphomas were attributed to activation of an endogenous hamster C-type virus since C-type virus was routinely seen in the induced lymphomas.

The third outbreak of lymphomas was recorded in a Telaco-gram in April, 1975 (48). According to Dr. G. S. Kistler, University of Zurich, Syrian hamsters in Switzerland were infested with C-particle virus, showed a high incidence of lymphomas at an early age, and high incidences of miscellaneous tumors later. Correspondence with Dr. Kistler provided the additional information that the source of the hamsters was Lakeview, Newfield, New Jersey, and that these animals were used to breed in the Zurich colony. A Professor F. Ott had purchased 80 males and 196

females in November, 1973 and of these animals, 245 were autopsied. Seventy percent had died with malignant lymphomas. The first tumors were seen at three months of age, and the peak incidence was by the eighth month of age. The incidence of tumors varied among different animal "sets" and it was apparent at the time of the letter (July 22, 1975), that a repeat experiment was going to yield fewer spontaneous lymphomas. No reference was made to the 1960 outbreak of lymphomas at the University of Zurich (77) so it was unclear if the 1975 lymphomas were a continuation of that problem or represented an entirely new occurrence. The high incidence of lymphomas in hamsters purchased from an outside source (Lakeview) suggests that horizontal transmission of an oncogenic agent occurred, or that the source itself was the common denominator.

The fourth, fifth, and sixth outbreaks were reported in 1975 by Ambrose and Coggin (1). This report documented the occurrence of three spontaneous epizootics of lymphomas in hamsters at The University of Tennessee. The first epizootic occurred in 1972, the second in 1973, and the third in 1974. New breeding stock was purchased from Lakeview (Newfield, New Jersey) after the sacrifice of the first and second colonies of hamsters affected with the lymphomas. The third colony was left for further study after the lymphomas were detected. The incidence of lymphomas was approximately 50 percent of all hamsters sacrificed for the first and second epizootics. Onset of lymphomas occurred as early as one month post-exposure to the infected facility and young animals appeared more susceptible than old. These observations were confirmed in the third epizootic which is the subject of this

dissertation. Details pertaining to the onset of the three epizootics will be presented in the section entitled "History of the Lymphoma Epizootics."

The seventh outbreak was communicated to Dr. J. H. Coggin, Jr., by Dr. M. Gardner, University of Southern California and is not yet in publication (23). Eight litters of Graffi Syrian hamsters had been inoculated with saline or with preparations of DNA from various human tissues. Four of the ten control hamsters inoculated with saline as neonates developed lymphomas between four and eight months of age. Four of 70 inoculated as neonates with cell-free DNA preparations also developed lymphomas. Onset of lymphomas in the control and experimental groups was essentially simultaneous: tumors were discovered in controls within two weeks of the date they were discovered in experimental hamsters. Lymphoma development appeared limited to the above groups of animals since the next set of animals introduced into the facility did not develop lymphomas. The only decontamination measure employed was autoclaving of the cages, whereas this was ineffective in the outbreaks reported by Ambrose and Coggin (1). The occurrence of lymphomas in the control animals was attributed to induction of endogenous HaLV in the experimental animals with subsequent horizontal spread of the HaLV. C-type virus was found by electron microscopic examination in only one of three tumors examined, and complement fixation tests for HaLV antigens were inconclusive due to the anticomplementary activity of the tumor homogenates. Normal tissues from tumor bearing animals were negative for HaLV antigens but this is not an unexpected result since Freeman et al. (20) demonstrated that C-type virus could be found in tumorous tissues but not in normal tissues of tumor-bearing hamsters.

Many reasons can be suggested for the occurrence of these seven outbreaks of lymphomas in Syrian hamsters, but one very likely possibility is that an endogenous hamster leukemia virus is activated and is responsible for the induction of lymphomas. Evidence for this theory is provided by the following:

1. C-type virus or viral antigens have been detected in tumors from six of the seven outbreaks.

2. Pregnant hamsters from the Telaco breeding facility were sent to Dr. Kistler at the University of Zurich. The progeny of the breeders housed in a lymphoma-free facility did not have C-type virus and did not develop lymphomas whereas the progeny of breeders housed in a facility where lymphomas had occurred developed lymphomas and had C-type particles in their tissues (48). This evidence indicates that the C-type virus may have been horizontally transmitted to the Telaco hamsters.

3. Lymphoma outbreaks have not, to my knowledge, been reported in the commercial breeding facilities, suggesting that experimental agents such as oncogenic viruses and chemical or physical carcinogens must be suspected as factors precipitating the lymphoma outbreaks. Freeman et al. (20) have shown that C-type virus can be induced by treating neonatal hamsters with oncogenic viruses or chemical carcinogens. One might theorize that the induced virus could be horizontally transmitted to virus-free animals and these animals would then develop lymphomas.

The theory that C-type virus is involved cannot be disproved until virus-free hamsters can be obtained and Freeman et al. (20) suggest that all hamsters may have endogenous HaLV. There is, however, some evidence to suggest that C-type virus may not be the only

agent involved in the lymphoma outbreaks:

1. Graffi et al. (28) found that cell-free filtrates of spontaneous epithelial tumors that were detected during the lymphoma outbreaks caused lymphomas when inoculated into neonatal hamsters. Only papova virus was seen in the epithelial tumors and DNase treatment of cell-free filtrates made from the pepithelial tumors destroyed the lymphomagenic activity. Cell-free filtrates of the lymphomas were oncogenic but DNA isolated from the lymphomas was not oncogenic. These findings suggest DNA induced a process which ended in lymphomagenesis.

2. Both Graffi et al. (17) and Gardner et al. (23) found cell-free preparations of DNA from various human tissues were lymphomagenic when inoculated into neonatal hamsters, again suggesting DNA was a necessary cofactor for lymphomagenesis.

3. There was some variation in the incidence of tumors and age of the animals in which tumors were detected: Gardner et al's. tumors appeared in 4-8 month old animals (23), Kistler's tumors were in animals under 3 months of age (48), Ambrose and Coggin's tumors were from 1 month of age on (1), Horn and Siewert's tumors were in 3 month old animals predominantly (37), and Strauli and Haemmerli's tumors were seen in 4-5 month old hamsters (77). Also, the outbreak described by Gardner et al. (23) was not reproduced in the next group of hamsters exposed to the facility whereas both Kistler (48) and Ambrose and Coggin (1) found transmission of tumors to subsequent groups of hamsters and Horn and Siewert (37) continued to have a tumor incidence of 3 percent. This variation suggests that unknown environmental factors may have influenced the occurrence of lymphomas in the seven outbreaks.

CHAPTER II

INDUCED LYMPHOID NEOPLASMS IN HAMSTERS

The relatively long latent period and low incidence of malignant tumors in most colonies of Syrian golden hamsters has allowed investigators to use the Syrian hamster as a model for induction of malignant disease. The following reports outline some investigations which have resulted in induction of malignant lymphomas in Syrian hamsters.

Two papova viruses have been shown to cause malignant lymphomas in hamsters. The first of these was described by Graffi et al. in 1968 (28). The virus was found in spontaneous papillomas and in epithelial tumors that occurred after inoculation of hamsters with hamster embryonic tissues carried in vitro. Cell-free preparations of the tumors caused lymphomas rather than papillomas when inoculated into neonatal hamsters, but only RNA C-type virus was seen in the induced lymphomas. Nevertheless, DNAse treatment of the cell-free preparations destroyed their lymphomagenic activity. These findings prompted the theory that the papova virus induced an endogenous hamster virus which then caused the lymphomas. The papova virus was thought not to be polyoma because it was not oncogenic in mice and was thought not to be SV40 because it did not cause fibrosarcomas in neonatal hamsters.

SV40 has, however, been shown to be lymphomagenic, but not for neonatal hamsters. Diamandopoulos (11) found that by inoculating 3 week old hamsters intravenously with $10^{8.5}$ $TCID_{50}$ SV40, lymphomas and

reticulum cell sarcomas resulted in up to 95 percent of the hamsters inoculated. Tumors were detected in as few as 3 months post inoculation with a peak tumor incidence during the fourth and fifth months. Further work revealed that variation of host age, virus dose, and route of inoculation all had an effect on the tumor incidence (12). The tumors were found to be positive for SV40 T antigen and SV40 was recovered when tumor cells were cocultivated with susceptible grivet monkey kidney cells. Tumors did not occur when hamsters were given SV40 treated with anti-SV40 antiserum and tests for HaLV were negative. It was concluded that SV40 was the agent responsible for induction of the lymphomas.

Adenovirus 7 (Adv 7) has been reported to induce undifferentiated sarcomas which have areas suggestive of lymphoma (49), but several in vivo passages of the tumors were required before sufficient differentiation occurred to allow the diagnosis of malignant lymphoma. Since the tumors arose at the injection site and no tumors were found in controls, Adv 7 was considered the etiologic agent of these tumors.

Equine herpes virus 3 (EH3) has also been used to induce malignant lymphomas in hamsters but it was not known if EH3 was the causative agent since EH3 alone did not cause lymphomas (44). When EH3 was cultured with hamster embryonic cells and the infected cells were inoculated into neonatal hamsters, lymphomas developed. These lymphomas could be transmitted with cell suspensions or with cell-free preparations, suggesting a viral etiology. Natural transmission also occurred. In light of the report that a papova virus was apparently released by culturing hamster embryonic tissues (27), it is not clear if the lymphomas found after inoculation with EH3-infected hamster tissues were caused by

the EH3 or by a hamster virus released from the cultured tissue.

DNA from various normal and malignant human tissues has also been found to cause lymphomas in hamsters. Certain tissues appear to have more oncogenic activity than others; however, the reason for this is obscure. Both laboratories reporting the induction of lymphomas by human DNA attributed the lymphomas to activation of an endogenous hamster C-type virus since C-type viral particles were found in the lymphomas (23,26).

C-type or RNA tumor viruses are usually associated with lymphoid neoplasms and C-type viral particles or viral antigens have been detected in tumors from several lymphoma outbreaks. Hamsters have an endogenous C-type virus which can be detected in fetal tissues and which can be induced by treating the hamsters with several chemical or biological carcinogens (20). This virus was first isolated as a hamster specific variant of murine sarcoma virus, but was later shown to be of hamster origin (45). This HaLV is probably the C-type virus seen in the lymphomas.

C-type viruses from mice and cats appear not to be lymphomagenic for hamsters although the Moloney strain of murine sarcoma virus can cause reticulum cell sarcomas (58). C-type virus from cattle, however, apparently is oncogenic for hamsters. Lussier and Pavilanis (51) reported in 1969 that they were able to induce malignant lymphomas after inoculating neonatal hamsters with an extract of bovine leukemic tissue. About 70 percent of the animals developed a wasting disease after inoculation of the extract, and half of these animals died, but of the survivors, 5/31 (16 percent) developed malignant lymphomas at 8-9 months

of age. No tumors were seen in controls. It was suggested that the wasting disease and induction of malignant lymphomas was related and that the precipitating agent was the bovine leukemic tissue extract.

Lymphoid tumors in hamsters can evidently also be induced by immune mechanisms. Greene and Harvey (30) found that lymphomas could be induced by subcutaneously implanting adult hamsters with tumor tissues carried in other species. Hamster amelanotic melanoma carried in rabbit cornea, S-37 and S-180 mouse sarcomas and Brown-Pearce and V_2 rabbit carcinomas were implanted by trocar. Usually the tumors grew then regressed. Occasionally, however, persistent growth was seen and if this continued after the 18th day post transplant, lymphomatous transformation was found to have occurred. The resulting lymphomas could be serially passaged into other hamsters but not with cell-free filtrates, and karyotyping demonstrated that the lymphoid tumor cells were of hamster origin. Homologous transplants of other hamster tumor tissues never gave lymphomas, only tissues from heterologous species or hamster tissues passaged through heterologous species would induce the lymphomas and these lymphomas originated at the site of inoculation. Greene and Harvey suggested that the immunological stimulus provided by the temporary growth of the alien tumor cells may have been the stimulus for neoplastic transformation of the responding lymphoblasts.

A variety of lymphoid neoplasms have been induced by physical agents such as X-ray, urethan, DMBA and methylcholanthrene. These reports have been reviewed by Kirkman and Algard (47) and by Homburger (36). In general, tumors have been induced after latent periods of at least six months.

The preceding studies on the induction of malignant lymphomas allow the following conclusions concerning the outbreaks of spontaneous lymphomas:

1. The precipitating cause is probably not just a chemical carcinogen because the latent period for lymphoma induction with chemicals is far greater than that seen in most outbreaks of spontaneous lymphomas.

2. A viral agent is probably involved in the spontaneous lymphoma outbreaks since certain cell-free preparations were able to induce lymphomas in as short a latent period as that seen naturally.

3. SV40 is probably not the virus directly responsible for the lymphoma outbreaks since a high titer of virus is required and the route of inoculation must be intravenous. Another papova virus may, however, be involved.

4. An endogenous virus is probably involved since the reports which were able to reproduce the short latent period and high transmissibility had involved use of cultured hamster fetal cells.

5. Endogenous virus is not sufficient to explain the contagious element unless the endogenous virus can be naturally transmitted once it is induced.

CHAPTER III

NATURAL TRANSMISSION OF TUMORS IN HAMSTERS

An unusual aspect of the lymphoma outbreaks has been the probability that the tumors (or the tumor agents) are horizontally transmitted (contagious). Horizontal transmission of hamster tumors was first reported by Brindley and Banfield (7) who noted a contagious aspect of a spontaneous reticulum cell sarcoma that appeared on the lip of a 2 year old male hamster. They found that uninoculated hamsters caged with tumor-bearing hamsters developed the tumor in as few as 31 days, and that the disease could be transmitted even though the hamsters were separated by a screen. Feeding of tumors to five hamsters also resulted in transmission of the disease. Transmission via mosquito was also effective. Chromosome studies demonstrated only the male karyotype indicating the tumors had been transmitted by cells. This was further supported by the finding that cell-free preparations were ineffective.

Karpas and Samso (44) found both a horizontally and vertically transmissible aspect to the tumors induced by culturing EH3 with hamster embryos in vitro. In one case, the mother of an infected litter developed a subcutaneous lymphoid mass 65 days after her litter was inoculated indicating horizontal transmission of the agent from the pups. The tumor-bearing mother gave birth to a second litter 72 days after the first and one of the pups developed a large facial tumor at 3 months of age. This was called vertical transmission but there was

no evidence to show that the agent had not actually been transmitted through the milk or through the placenta which would be horizontal transmission.

Kirkman and Algard (47) also reported that a reticular cell sarcoma that occurred spontaneously in their colony gave results similar to those seen with Brindley and Banfield's contagious tumor when their tumor was deliberately tested for contagiousness.

Although horizontal transmission can be suspected for the lymphoma outbreaks reported by Horn and Siewert (37), Kistler (48), and Gardner (23), probably the most spectacular example occurred at The University of Tennessee. As reported by Ambrose and Coggin (1), an incidence of lymphoma as high as 50 percent occurred in hamsters born outside the University of Tennessee colony and exposed at various ages after birth. This held true for both the first and second epizootics and this dissertation will document its occurrence in the third epizootic.

Verification of the transmissibility of these tumors has also been confirmed by Dr. A. M. Jonas of Yale University and he documented it as follows:

> In order to confirm the findings at Tennessee and rule out local environmental factors, 18 hamsters from the colony were sent to Yale and immediately placed in infectious disease quarters. All animals appeared normal on arrival. 18 Lakeview weanling hamsters were placed in direct contact with the Tennessee hamsters for 11 days and then removed, housed 4-5 per cage, and examined daily for evidence of disease. After their removal, 24 weanling LSH/SSLAK Lakeview hamsters were placed in direct contact with the same Tennessee hamsters for 60 days, then removed and group housed. An additional 5 LSH/SS LAK weanling hamsters were set up in the same room under "filter caps" without direct contact with either Tennessee hamsters or those subsequently exposed.

> Tumor material from original and contact hamsters were minced in Eagles minimum essential medium with 3% fetal bovine serum and inoculated IP, SC or orally into recipient weanling hamsters. The results are shown in Table III [Table II of this study].
>
> The data indicates that the development of this tumor (incubation period) varies from as little as 2 weeks following ip administration to a maximum of 38 weeks following direct contact. The incubation periods were shortest for the ip route, followed by SC, oral and indirect and direct contacts. The incubation period for the contact experiments closely parallels the apparent incubation period in the natural outbreaks.

Further verification of horizontal transmission was also obtained from the National Cancer Institute where the use of hamsters as experimental animals was discontinued due to contamination of the stock by the lymphoma agent (R. Heubner, personal communication).

TABLE II. Lymphosarcoma transmission experiments.

Inoculation Route	Mean Incubation Period (wks)	Range (wks)	No. With Lymphoma / No. Exposed
i.p.	3.9	2-6	9/12
s.c.	7.5	4-12	8/14
Oral	14.4	6-20	5/5
Contact 11 days	19.3	9-38	10/18
Contact 60 days	24.1	15-34	11/11
Indirect contact	14.5	12-15	2/5

CHAPTER IV

HISTORY OF THE UNIVERSITY OF TENNESSEE HAMSTER LYMPHOMA EPIZOOTICS

First Epizootic

The hamster colony involved was kept in two areas at The University of Tennessee. Breeding animals were kept in room 410B of Hesler Biology Building, and experimental animals were kept in room 427 in a newer section of the same building. Monthly inventories of the two rooms averaged 3000 animals total. The colony was composed of randombred LVG/ssLAK (LVG) and inbred LSH/ssLAK (LSH) Syrian golden hamsters supplied from Lakeview (Newfield, New Jersey) or bred in the U. T. colony. LVG's had been used for experiments and breeding without incident since 1966. However, LSH's had not been present until late 1971 when they were purchased from Lakeview with the intent of developing a breeding colony to supply inbred animals for experimental use.

Late in 1972, it became evident that there was something wrong with a number of the animals. This was noted by K. Ambrose and described as follows:

> First lesions (were) noted in November 1972, approximately a month after a "wet tail" epidemic in one of the two hamster rooms. By the end of December, hamsters in both rooms began showing masses tentatively diagnosed as lymphosarcomas. In the mouse room adjoining the hamster rooms which did not experience the "wet tail" problem, all attempts to breed mice were unsuccessful and a considerable number of Balb/C mice died. (No pathology was done on these mice.) When hamsters from terminated experiments were examined approximately 50% of these animals had lymphosarcomas. We therefore decided to kill all the hamsters in our colony (approximately 3000 animals).

Documentation of the onset of the first epizootic is also available in the pathology reports written by Dr. Bruce Bellomy, Fort Sanders Presbyterian Hospital. The first sample was evaluated on December 12, 1972, and given the diagnosis of malignant lymphoma. Subsequent samples were remarkably similar despite the source of the tissue examined and it was evident that an outbreak of lymphomas had occurred.

The kill of the colony was completed by late February, 1973, and the rooms were cleaned and disinfected. From the animal inventory records, it is evident that the kill and cleaning was a two-step process. First, the breeding room was emptied and cleaned, then the experimental room was emptied and cleaned. Cleaning at this point was limited to scrubbing down walls, floors and racks, and to elimination of all hamsters so that new animals from the supplier would hopefully not be exposed to the cause of the lymphomas. By April, experiments were in progress and the animals were maintained as before. At the termination of each experiment, all animals were checked for the presence of lymphomas.

Second Epizootic

The onset of the second epizootic was described by K. Ambrose:

> By February, 1973, we had killed our entire colony and disinfected the rooms as best we could. In March, 1973, we received shipments of LSH and LVG hamsters and began again our breeding and experimental use of hamsters. In July and August, two unusual situations arose in the animal rooms: a high incidence of spontaneous abortions (15-25%) in the inbred breeders, and the development of hind end paralysis in several of the 8-10 week old inbred animals. Lymphosarcomas similar to those noted in December were evident by September, 1973.

Later in the same report, the following was included:

Miscellaneous (possibly related) information:
1. HaL virus was once used as a vaccine - July 1972.
2. A rat facility and a mouse facility were next door to the normal hamster room. The rats and mice were not sacrificed in December when the hamster colony was killed off.
3. A mild epidemic of "regional enteritis" in the experimental room preceded by two months the detection of lymphosarcomas.
4. The lymphosarcomas can be transplanted by cells. Cell-free transmission has not been attempted since the whole colony is "infected" (lymphoma seen as early as 1 month after birth).
5. Involvement of the thymus has been noticed in the "second round" of the lymphosarcoma epidemic.

From an analysis of individual notebooks and the pathology reports it was possible to document the following concerning the onset of the second epizootic:

1. A breeder with a mesenteric node mass was noted August 29, 1973.
2. The first diagnosis of lymphosarcoma was made on a mesenteric node taken September 14, 1973.
3. A liver sample taken from a 1-1/2 month old LSH male on September 21, 1973, was given the diagnosis "extensively involved by lymphosarcoma."
4. Additional tumors were noted in both strains of normal and experimental hamsters.

Thus, it became evident that a second epizootic of lymphomas had occurred almost seven months after animals were put in the facility.

A decision to sacrifice the colony was made in December, 1973, and it is evident from the animal inventory that again, it was a two-step process. By January 31, 1974, the experimental room was empty of animals. However, experimental animals were available through February 19, so it is likely that animals had been moved into the breeding room while the experimental room was being cleaned. The animals in the breeding room were sacrificed by the end of February and 10 pregnant females shipped from Lakeview were placed in the experimental room.

The breeding room was cleaned and renovated into laboratory space and thus was not used again as an animal facility.

There is no written record of the procedures used to clean out the experimental room during the month it was empty prior to receiving new animals from the supplier. However, it has been possible to determine from interviews with lab personnel present at the time that virucidal agents were used for scrubbing down the walls and floors. The door of the animal room was also taped shut so that the animal room could be fumigated with a quaternary ammonia compound and the entire wing of the building containing the experimental room was fumigated over one weekend. A decision was made against using a formaldehyde bomb since the room had a false ceiling of acoustic tile and it would have been impossible to contain the fumes. The clean-up procedures, in general, were far more stringent after this second epizootic.

Third Epizootic

No synopsis is available as to the onset of the third epizootic, even though a much closer watch was kept on all the animals in the third colony. New animal caretakers had been hired, and personnel were expected to wear masks, gloves, lab coats, hats and boots when in the animal facility to insure against the possibility that the lymphoma agent was carried by lab personnel.

Samples of tissue were sent for pathology by June 11, 1974, three months after the first animals were received from Lakeview. These samples were all given the diagnosis of nonspecific hyperplasia and not lymphosarcoma. On July 22, 1974, a diagnosis was given of an extra-osseous myeloma with the comment that the lesion had an entirely

different appearance from the previously identified epidemic lymphosarcoma. Then, on July 27, a pathology report made by Dr. Emilio Machado was summarized: "lymphoproliferative disorder with lymphosarcoma transformation." Information as to the sex, strain and age of the hamster autopsied is not available. On August 13, a number of hamsters were agina autopsied and several samples sent to Dr. Bellomy for pathological evaluation. These samples were diagnosed as lymphosarcoma, but there was no information as to the sex, strain, age or experimental treatment (if any) of these hamsters. Nevertheless, it was evident that lymphosarcomas were again present and the colony was left intact for further study.

Fourth Epizootic

In August, 1974, a set of trailers approximately 100 yards north from the lymphomatous colony was made available as an animal facility. New animal personnel were hired, new animals were sent from the supplier, and an attempt was made to never introduce anything into the trailers that might have been a cause or an associated cause of the lymphosarcoma. SV40 virus and SV40 transformed cells were used routinely, but only those pools of virus and cell stocks that were frozen prior to onset of the first epizootic in 1972 were used. Experiments were carried out as before, but the experimental animals were kept in separate rooms from the breeding and holding stocks. The LSH hamsters were also kept in their own room and these hamsters were used as a supply of "clean" animals for experiments in the lymphomatous colony.

The trailers remained free of lymphosarcoma until May, 1977, when atypical lymphoid accumulations were diagnosed in animals killed due to the presence of a "wet tail" outbreak. These samples were not given a definite diagnosis of lymphosarcoma, but exhibited stages of lymphoproliferation so atypical that a diagnosis of lymphosarcoma could not be ruled out.

On June 9, 1977, 37 six-month-old female LVG hamsters were sacrificed to provide normal spleens for an experiment. Three of the females had abnormalities which were noted on autopsy and which were submitted for histopathology. These abnormalities were:

1. a plaque on the cecum which appeared lymphoid
2. a very prominent Peyer's patch
3. a round white mass centrally located in the left dorso-caudal lobe of the liver.

The samples were evaluated by Dr. Bellomy and a diagnosis of lymphosarcoma was made on sample 3. Sample 1 was not lymphosarcoma but was too atypical to rule out a diagnosis of early lymphosarcoma while sample 2 was hyperplastic. Additional animals with lymphomas were subsequently detected in all rooms of hamsters kept in the trailers. The chronology of animals dying with lymphomas in the fourth epizootic is presented in Appendix A.

PART II

EPIZOOTIOLOGY

CHAPTER I

INTRODUCTION

The value of epidemiologic techniques in the characterization and prevention of disease was established in the classic work of Snow on cholera (72). These techniques have been used in the study of both human and animal diseases (epizootiology) to identify etiologic agents and their mode of transmission (70). Epidemiologic-epizootiologic evidence has also been obtained to demonstrate that environmental influences affect the induction of cancer (31,8,62) and to show that there are oncogenic agents in several species that are transmissible by contact (35,60,66,79). Transmission of an oncogenic agent has yet to be demonstrated conclusively in humans although some investigators have raised this possibility (13,59,22,86).

The occurrence of three spontaneous horizontally-transmissible outbreaks of lymphomas in hamsters maintained at The University of Tennessee (1) prompted an investigation into the epizootiologic aspects of the disease. The objectives of the investigation were to determine: (1) the presence of a common factor(s) associated with each outbreak; (2) the occurrence of tumors as related to the sex, strain, and age of the animals; and (3) the mode of transmission of any putative agent responsible for initiation of lymphomas. During these studies, an epizootic of lymphomas was detected in a hamster facility that had been considered lymphoma-free since its establishment three years previously.

Data from this fourth epizootic (detected in June, 1977) are included in the present report.

CHAPTER II

MATERIALS AND METHODS

Animals

Two colonies of Syrian golden hamsters were evaluated. They were housed in separate facilities with independent maintenance. Three epizootics of lymphomas had been observed over a five year period in one colony and it is referred to as the lymphomatous colony. The other colony is referred to as the "clean" colony and served as the source of lymphoma-free hamsters for almost three years. (The fourth epizootic of lymphomas was detected in this colony in June, 1977.) Animal husbandry procedures and physical features of the two facilities were documented and these records were compared to determine what factors may have contributed to the lymphomatous or clean status of the respective colonies.

Two strains of Syrian golden hamsters were used in both facilities: the inbred LSH/ssLAK strain (LSH) and the randombred LVG/ssLAK strain (LVG). The two strains were housed separately in the clean colony, but were kept in adjacent cages in the lymphomatous colony. The LVG stock was originally purchased from Lakeview (Newfield, New Jersey), and used to establish a breeding colony at The University of Tennessee. The university colony then served as the source of pregnant LVG females for the lymphomatous facility. A similar breeding program was attempted with LSH stock purchased from Lakeview, but was

not as successful. Therefore, the pregnant LSH used in the lymphomatous facility were obtained directly from Lakeview.

Data Sources

Notebooks had been kept in which were recorded the schedule of animal immunization, the sources of cells and virus used to immunize the animals and the findings noted when palpations of experimental animals were made. These records served as a source for analysis of data that would correlate any possible common factors with the outbreaks of lymphomas. Data from notebooks containing the inventory of cells and virus pools frozen at -80°C were also evaluated.

Daily death records were kept of the deaths of animals in the lymphomatous colony in order to assess the incidence of lymphomas. Animals had been previously coded on master records as to their location in the facility, location with respect to other hamster cages on a rack, their sex, strain, age at exposure, and parentage (location where the dam had been bred, and where possible, the specific dam and sire). By combining this information with the daily death records, it was possible to calculate mortality rates (number of animals dying/number of animals in the colony) for the colony as a whole, for animals of a given sex, strain, age and parentage, for animals at different locations within the facility, and for animals dying at different seasons of the year.

Autopsies were not routinely performed on animals that died in the clean facility. However, when large numbers of animals from the clean facility were sacrificed to provide tissues for various experiments, the animals were given cursory autopsies to detect the presence of any

abdominal or thoracic masses. In addition, any animals with masses or with an unusual appearance were sacrificed and autopsied. Owing to the high turnover rate of animals in the clean facility, master records detailing the location of animals within the facility had not been kept. However, when hamsters with lymphomas were noted in June, 1977, master charts detailing the location of animals were made. As more lymphomas were detected, these charts were evaluated in an effort to correlate any parameters to the appearance of lymphomas.

CHAPTER III

RESULTS

The lymphomatous and clean hamster facilities were located in separate buildings with independent maintenance. Physical features of both facilities are presented in Appendix B. Although there are numerous differences in the two facilities, one factor that may have been significant in the clean facility was the isolation of experimental, normal, and breeding hamsters in separate rooms of the clean facility with each room having its own air intake and exhaust. In addition, the two strains of animals were kept in separate rooms and this isolation minimized the possibility of transmission of any agents between the different groups of hamsters.

Husbandry procedures for each colony are detailed in Appendix B. The same sources of food (Purina Lab Chow), bedding (Absorb-Dri), cages (Maryland Plastics), and water (City of Knoxville) were used in both facilities. However, cages and water bottles for the lymphomatous colony were not autoclaved either prior to or after washing while the cages in the clean colony were autoclaved with each washing. The water bottles of the clean colony were autoclaved on occasion. Since cages, cage tops, and water bottles were upon occasion exchanged between the two facilities and were autoclaved prior to their use in the clean facility it seems possible that autoclaving of all equipment might have been a significant factor in the prevention of lymphomas in the clean facility.

Separate animal care personnel were hired to maintain the two facilities so that there would be no contamination from agents carried from one facility to the other by the animal care personnel. However, several investigators who had worked with hamsters prior to and during the third epizootic subsequently worked with hamsters in the clean facility. Only one investigator carried out experiments in both facilities and she took care not to work in the clean facility and the lymphomatous facility on the same day. Her research in the lymphomatous facility was completed within one year and lymphomas were not detected in the clean facility until two years later.

Common Factors

A list of cells and viruses used prior to the first three epizootics is presented in Appendix B. These cells and virus were noted in analyses of individual laboratory notebooks and palpation records for the various experiments conducted between January, 1972 and August, 1974. One common factor was identified, and that was the use of SV40 transformed hamster fibroblasts. It was not clear if SV40 virus itself was used prior to the onset of the third epizootic. The agents and procedures used in the clean facility are listed in Appendix B, and again, SV40 transformed cells and SV40 virus were used prior to the detection of lymphomas.

Only the F5-1 line of SV40 transformed hamster fibroblasts, a non-virus-shedding cell line explanted from the tumor of an LVG hamster infected with SV40 virus at birth (24) had been used in hamsters before the onset of all four epizootics. However, these cells had also been used in LVG hamsters at The University of Tennessee from 1966-1971

without the detection of lymphomas, so it is not clear if the cells promoted lymphoma occurrences.

A second SV40 transformed hamster fibroblast cell line, SV/LSH-2, had been used prior to the onset of the first three epizootics. This line was explanted in February, 1972 from the tumor of an LSH male inoculated with SV40 virus at birth. The occurrence of the lymphoma epizootics prevented the complete characterization of this cell line, but it is interesting to note that experiments to characterize the line were initiated before each of the first three epizootics.

Records were re-checked after the identification of the common factors of the epizootics to determine if a specific passage level of cells or a specific pool of virus might be implicated. No common passage level of SV40 transformed cells was found. However, the cells had all been derived from the same frozen stock, carried in vitro for varying numbers of passages, then re-frozen; so the lack of a common passage level may not be significant. No common pool of SV40 virus was found nor was there one common seed stock from which the SV40 virus pools were made.

The following other common factors have been identified for at least three of the epizootics:

Personnel:

One investigator (CMB), had been involved in taking care of animals prior to the first and second epizootics and had been conducting her own experiments prior to the onset of the third and fourth epizootics. A number of lymphomas were identified in her experimental animals. One might speculate that this person carried an agent capable of inducing

lymphomas in hamsters or that the type of experiments this investigator was doing was capable of inducing lymphomas in hamsters.

A second investigator (KRA) had conducted experiments prior to the first three epizootics and had worked with hamsters in the clean facility during the first two years of its use. Lymphomas were not detected in the clean facility until the end of the third year of use.

Experimental design:

The highest incidence of lymphomas in a single cage of animals in the fourth epizootic was found in a group of LVG hamsters given SV40 virus sc as neonates to cause tumor formation, then given SV40 transformed cells (F5-1) ip as weanlings to interrupt oncogenesis. A similar experiment had been executed in the summer of 1976 with loss of a number of animals to enteritis, but no lymphomas had been detected. Enteritis was seen in the former group of animals as well, but this time, lymphomas were also detected. This type of experiment, referred to as an interruption of oncogenicity experiment, had also been conducted before the first two epizootics.

Pathological findings:

These will be discussed in greater detail in the Pathology section, but it is pertinent to note here that a "wet tail" or "hamster enteritis" (68) outbreak preceded the detection of all four lymphoma epizootics by one to two months, and severe pyelonephritis was seen concomitantly with the detection of lymphomas.

Animal characteristics:

Possibly the most obvious correlation made is that the lymphomas were not seen until after LSH hamsters were purchased for experimental

use in 1971. For five years previously, LVG hamsters had been bred and used experimentally without the detection of lymphomas. The LSH hamsters were purchased from the same supplier and housed in the same facility as the LVG hamsters. Lymphomas were detected within one year of receiving the LSH strain. The two strains were housed separately in the clean facility for two and one-half years and lymphomas were not detected during that time. However, in December, 1976, a shortage of space for normal hamsters necessitated housing normal LVG weanlings with the normal LSH. Lymphomas were detected six months later in both the LSH and LVG (fourth epizootic).

The Population at Risk

Valuable information can be gained from analyses of the mortality rates of hamsters in the lymphomatous colony. Calculating these rates with respect to the sex, strain, and age of the hamsters can give an indication of the role that host variables play in the occurrence of lymphomas.

It was determined from the daily death records that over 5,000 animals died or were killed in the lymphomatous colony since December, 1974. Of these, approximately 700 animals died from sc passage of tumor cells, approximately 150 died from unknown causes, and approximately 500 were lost to cannibalism. A total of 662 animals dying with lymphomas was found by May, 1977, accounting for approximately 12 percent of the total deaths. Other causes of death were kidney disease, enteritis, and termination of experiments (Table I).

TABLE I. Causes of death in the lymphomatous colony, December, 1974 to May, 1977.

Cause	No. Animals	Percent Total Deaths
Lymphoma	662	12
Subcutaneous tumor passage	696	13
Enteritis/Intussusception	325	6
Kidney disease	42	.1
Unknown but not lymphoma*	155	2
Preweaning (unknown)	443	8
Cannibalized	512	9
Sacrificed (end of an experiment)	2614	49
TOTAL	5449	

*Animals were autopsied, but no cause of death was identified.

In order to evaluate the incidence of lymphomas attributable to natural occurrence, groups of animals were simply exposed to the lymphomatous facility as neonates and monitored for the presence of palpable masses. Moribund animals were sacrificed and autopsied. Data from a representative group of 78 LVG hamsters born in November, 1975 are presented in Table 2. Several of the animals were allowed to breed and the incidence of deaths and lymphomas in the progeny are also presented. It is evident that the progeny have a lower incidence of lymphomas than the parents. This observation held for all animals allowed to breed in the lymphomatous colony, making parentage a variable influencing the incidence of lymphomas in the colony.

It was of interest to determine if the incidence of lymphomas also differed with the strain of animals. In order to evaluate this possibility, the pooled data from several groups of LVG and LSH hamsters exposed to the lymphomatous facility between December, 1976 and May, 1977 were compared. The causes and ages of animals at death were compared for the two groups (Table III). It is evident that the LSH hamsters have a higher mortality rate and a higher incidence of lymphomas than the LVG hamsters (14/93 v. 18/190, $X^2 = 1.4$). The occurrence of lymphomas with respect to the age of the animals differs most during the interval of 61-70 days; otherwise, the distribution of lymphomas with respect to age of the animals seems comparable.

Finally, it was of interest to determine if the sex of the animal might be a determinant in the occurrence of lymphomas. A simple chi-square test was performed on the total number of lymphomas in each sex using the two groups of animals evaluated above. A significant

TABLE II. Mortality data from 78 hamsters and their progeny.

Generation	Natural Deaths	Lymphomas	Known Non-Lymphoma	Unknown
Parental (exposed as adults, 2 d prior to parturition)	0/9	1 d 225		
First (exposed 2 d prior to birth)	38/78	25 (67-316 d)	7	6
Second (bred and born in lymphomatous colony)	6/49	3 (175-235)	3	0
Third (bred of second generation in the lymphomatous colony)	1/25	0	1	0

NOTE: Each generation was killed when the animals reached one year of age.

TABLE III. Mortality in LSH and LVG hamsters exposed to the lymphomatous colony between December, 1976 and May, 1977.*

Age of Animal at Death Intervals in Days	LSH Lymphoma Deaths	LSH Total Deaths	LSH Lymphoma Mortality+	LVG Lymphoma Deaths	LVG Total Deaths	LVG Lymphoma Mortality+
0-30	0	176	0/269	0	125	0/315
31-40	1	4	1/93 (1%)	1	3	1/190 (.5%)
41-50	3	5	3/89 (3%)	4	15	4/187 (2%)
51-60	2	8	2/84 (2%)	1	5	1/172 (.6%)
61-70	0	2	0/76 (0%)	4	7	4/167 (2%)
71-80	0	3	0/74	2	6	2/160 (1%)
81-90	1	0	1/71 (1%)	0	2	0/154
91-100	2	2	2/70 (3%)	2	4	2/152 (1%)
101-110	3	4	3/68 (4%)	2	5	2/148 (1%)
111-120	2	3	2/64 (3%)	2	3	2/143 (1%)

*Animals received no experimental treatments and were simply palpated weekly to check for the presence of any growing masses. Moribund hamsters were killed and autopsied.

+Lymphoma mortality is calculated as the percentage of hamsters dying with lymphoma during an interval. The denominator is the number of hamsters alive at the beginning of the interval. Animals were exposed two days prior to delivery, but age is determined from the date of birth.

NOTE: Total lymphoma incidence in LSH by 120 days after birth was 14/93 or 15 percent; total lymphoma incidence in LVG by 120 days after birth was 18/190 or 9 percent. Incidence is calculated on the basis of the number of animals surviving to weaning since lymphomas were not seen before 30 days of age.

difference was found for males as compared to females in the LSH strain (7/37 male, 4/56 female, X^2 = 1.9) but not for the LVG strain (7/90 male, 10/94 female, X^2 = .17). Initial results using pooled data from non-experimental animals observed up to 200 days of age had suggested that females of both strains were more susceptible to lymphomas than males (30/412 female LVG, 22/430 male LVG; 30/396 female LSH, 16/239 male LSH) but the incidence in these groups at 120 days of age suggested male LVG and female LSH were most susceptible. Since these pooled groups were not matched for parentage, it is conceivable that the variation in results in comparison to the results for the matched groups used for Table III is not due to sex alone.

Mode of Transmission of the Lymphomas

Data from the three epizootics demonstrated that the lymphomas were contagious, although the mode of transmission of the tumors was not evident. In order to assess the possibility that the tumors might be transmitted via spread of contaminated litter or by the handling of contaminated animals, the master records detailing the location of each tumor bearing animal were analyzed. The chronology of animals dying with lymphomas was assessed for each of eight racks of non-experimental animals, and the occurrence of lymphomas appeared to be entirely random. Runs tests (73) were performed to see if vertical or horizontal patterns of tumor spread were evident; again, the patterns were those that would be expected from a random occurrence of lymphomas. Thus, it does not appear that mechanical transmission plays an obvious role in the spread of the lymphomas.

CHAPTER IV

DISCUSSION

Epizootiologic techniques have as a primary objective, the control or prevention of future disease occurrences (70). By determining the frequency and patterns of disease, information can be gained concerning the nature of the agent, its mode of transmission and the population of animals at risk. This information may then serve in a predictive and preventive capacity.

Data from the third of three spontaneous epizootics of lymphomas in hamsters were assessed to determine variables that may have had a role in the occurrence of the lymphoma epizootics. These data were sorted according to the characteristics of the place, time, and animals involved.

An analysis of the characteristics of place involved documenting and comparing the physical characteristics and husbandry procedures used in the lymphomatous facility and a nearby clean (lymphoma-free) hamster facility. Numerous differences were found, but the ones that might have been significant in preventing or slowing the occurrence of lymphomas in the clean facility were the autoclaving of equipment and the separation of breeding, experimental, and normal hamsters.

The choice of these variables as possibly being of significance was based on the observations that equipment was exchanged between the two facilities but was autoclaved prior to its use in the clean facility, and that lymphomas were detected in the clean facility six months after the common exposure of the two strains of hamsters. The lymphoma agent may have been horizontally transmitted in the lymphomatous facility

via contaminated equipment. Autoclaving of the equipment might destroy the agent, thus, although equipment was exchanged between the two facilities, lymphomas would not be seen in the clean facility. This theory necessitates a fairly thermostable agent since the cages and water bottles were washed and rinsed in 180°F water.

It may prove difficult to assess the role of the common exposure of the two strains of animals until the agent is recovered. Possibly one strain of hamster carries an endogenous agent capable of inducing lymphomas in the other strain. Once the two strains were exposed to each other, the altered agent might have been horizontally transmitted back to the original strain, resulting in the detection of lymphomas in both strains.

A number of intriguing possibilities were raised from an analysis of common factors among the epizootics. Common factors included the use of an SV40 transformed line of hamster fibroblasts (F5-1) prior to all four of the epizootics, the use of SV40 virus grown in primary monkey kidney cells prior to three of the epizootics, and the use of a second SV40 transformed line of hamster fibroblasts (SV/LSH-2) prior to three of the epizootics. SV40 and SV40 transformed cells have been used in other laboratories without the occurrence of lymphomas (11,24) and were used in LVG hamsters at The University of Tennessee for almost six years without the occurrence of lymphomas. According to Ambrose and Coggin (1) the lymphomas do not exhibit an antigen common to SV40 transformed cells. The lymphomas also do not protect against SV40 transformed cell challenge in immunization-challenge experiments (85). Yet, the sudden onset of the lymphomas, the involvement of predominantly young hamsters, and the recurrence of the epizootics

suggests some sort of precipitating event or factor is required. The only one that has been identified for all four of the epizootics is the use of a non-virus-shedding line of SV40 transformed hamster fibroblasts, F5-1.

The F5-1 cell line was also used in experiments to interrupt the oncogenicity of SV40 virus prior to three of the epizootics, and one of the highest incidences of lymphomas in a single cage of hamsters in the fourth epizootic was found in a group of hamsters infected with SV40 virus as neonates, then immunized three weeks later with irradiated F5-1 cells (Appendix A). Greene and Harvey (30) demonstrated that a persistent antigenic stimulus could result in neoplastic conversion of a hamster's lymphocytes in the location of the stimulus in as few as 18 days. One could speculate that the battle being waged between the hamster's immune system and the virus-transformed cells might have sufficiently stimulated the immune system to neoplastic conversion. Or, the stimulus might have been sufficient to induce an endogenous virus which was then horizontally transmitted and infected other animals, thus causing lymphomas. It has been shown that endogenous virus can be immunologically activated in the murine system (4) and a number of investigators have shown the presence of endogenous C-type virus in hamsters (6,9,20,75,83). Thus, the role of the SV40 transformed cells may have been to activate an endogenous virus.

Another common factor noted was the presence of a specific person who handled hamsters prior to all four of the epizootics. One might speculate that this person carried an agent which was capable of causing lymphomas in hamsters. Hamster to human transmission of agents

such as lymphocytic choriomeningitis virus has been shown (3), but there have been no reports of human to hamster transmission of agents that I am aware of. Some human agents have, however, been shown to be oncogenic in hamsters (49,81).

A final common factor was the occurrence of a "wet tail" outbreak one to two months prior to the detection of lymphomas. Wet tail is the most frequently mentioned disease of hamsters (68) but has not been reported in conjunction with lymphoma outbreaks. It is particularly interesting that two investigators working with SV40 virus in hamsters reported loss of hamsters to enteritis (11,21). The wet tail epizootics may have been a precipitating factor in the occurrence of lymphomas at The University of Tennessee, or the enteritis may have been a reflection of altered susceptibility to disease in hamsters exposed to the lymphoma agent.

A number of host factors were investigated in analyses of the lymphoma incidence with respect to the sex, strain, age and parentage of animals in the lymphomatous colony. It was found that the incidence of lymphomas was higher in the inbred strain of hamsters although the distribution of tumors with respect to age was similar for the two strains. The LSH strain had a higher mortality rate than the LVG strain for lymphomas and other causes of death. Male LSH appeared more susceptible to lymphomas than female LSH, and male and female LVG appeared equally susceptible when a group of hamsters matched for age at exposure and parentage was evaluated. However, the increased susceptibility of LSH males was not noted when a larger group of non-experimental animals of unknown parentage was compared at 120 days and

200 days of age. Thus, the sex of the animal does not appear to be a consistent factor governing the animals' susceptibility to lymphoma.

There was a definite decrease and delay in the occurrence of lymphomas and other causes of death among hamsters that were progeny of animals bred in the lymphomatous colony as opposed to hamsters bred outside the lymphomatous colony. Whether this resistance is genetically transmitted is not yet known, but it may be similar to that proposed by Graffi et al. (28) who found that progeny of hamsters from a strain that had a 3 percent incidence of spontaneous lymphomas were more resistant to the oncogenic effects of cell-free preparations of lymphomas than progeny of hamsters from an outside source. The increased resistance to other causes of death in the lymphomatous colony progeny suggests that the enteritis and pyelonephritis may well have been related to the outbreaks of lymphomas. Finally, lymphomas were found in hamsters of all ages greater than 26 days, but predominated in hamsters less than six months of age. An overall lymphoma incidence of 14 percent can be calculated for the colony based on the data in Table III, page 43, considering only those animals not given direct inoculations with viable tumor cells (662/4753).

The mode of transmission of the tumors was not obvious. Determining the order of occurrence of lymphomas in a given group of animals suggested that the initial exposure was random. Transmission might then occur to other hamsters in the same cage, but cage to cage transmission was not evident. These data do not rule out mechanical transmission of some type, but suggest that airborn transmission of an agent is also possible.

No association could be made with the time of year and the outbreaks of lymphomas, but it was interesting to note that the outbreaks reported by Strauli and Haemmerli (77), by Horn and Siewert (37) and by Ambrose and Coggin (1) were first detected in October/November. Subsequent outbreaks at The University of Tennessee were detected in September, July, and June, so it is not clear if the time of year association is significant.

In conclusion, the data from the Epizootiology section suggest the following:

1. There is a precipitating factor. The factor may well involve SV40 transformed cells and may be a combination of events and endogenous host factors.

2. Host factors have a role. The incidence of lymphomas varies in the two strains with the LSH strain being more susceptible; sex may play a variable role in influencing the incidence of lymphomas with respect to age of the animals; there do not seem to be any great differences in the susceptibility of the two strains with respect to age; age probably has some role in the induction of lymphomas since most lymphomas are seen in hamsters less than six months of age; and the LSH strain is more susceptible to other causes of death than the LVG strain.

3. Other factors may have a role. Wet tail outbreaks were seen prior to the detection of each lymphoma epizootic, and severe pyelonephritis was seen concurrently. Both are associated with bacterial infection (2,40,69). These diseases may serve as a predisposing condition to the development of lymphomas or may reflect an altered

susceptibility of the hamsters to disease following infection with the lymphoma agent.

4. In terms of prevention of future epizootics, the following measures may be considered: careful autoclaving of all equipment; separation of the two strains of hamsters and of experimental, breeding and normal hamsters; use of masks and gloves by all personnel handling the hamsters to prevent contamination with human agents; isolation of hamsters treated with SV40 virus or SV40 transformed cells.

PART III

PATHOLOGY

CHAPTER I

INTRODUCTION

Tumors of lymph nodes are collectively referred to as lymphomas, and are the predominant neoplastic lesions found in a number of species (43,32,53,52,74,71,65,60). The histopathology of lymphomas includes the range of lymphocyte differentiation from poorly differentiated lymphoblastoid cells to well differentiated lymphocytes and also encompasses reticulum cell/histiocytic lymphomas and other more unusual lesions such as the starry-sky histiocytosis of Burkitt's lymphoma and the pathognomonic Reed-Sternberg cells of Hodgkin's disease. Hodgkin's lymphomas differ from other lymphomas because of the predictable pattern of contiguous spread and the sensitivity to radiation therapy; also, no natural animal model has been found for the Hodgkin's lymphomas. Therefore, different classification schemes have been devised for the Hodgkin's and the non-Hodgkin's lymphomas. The classification scheme for the non-Hodgkin's lymphomas devised by Rappaport (67) has found widespread application although other classification schemes are now being proposed to encompass the functional characteristics of lymphocytes as well as the structural morphology of the tumors (50).

Tumors taken from each of four apparently spontaneous outbreaks of lymphomas in hamsters at The University of Tennessee were all lymphocytic or lymphoblastic lymphomas rather than the reticulum cell sarcomas found in most hamster colonies (84). In addition, a large number of animals were lost to diseases such as enteritis and

pyelonephritis. Since other investigators have reported low incidences of lymphomas and other diseases (62) the histopathology of the lymphomas and other diseases occurring in hamsters at The University of Tennessee was evaluated to determine if there were any differences from colonies in which lymphoma outbreaks did not occur.

CHAPTER II

METHODS

Autopsies were routinely performed on all animal remains found in the lymphomatous colony; however, since hamsters are cannibalistic, it was not possible to autopsy all animals that died. Animals were checked for the presence of any abnormalities and suspicious tissues were coded, fixed in buffered acetic acid-formalin-alcohol, then sent to Dr. Bruce Bellomy, Fort Sanders Hospital, to be sectioned, stained and evaluated. Diagnoses were recorded and compared to the diagnosis suggested by gross autopsy. Discrepancies were re-evaluated.

It was of interest to know if the histopathology of the lymphoma differed between the epizootics and whether or not it differed with respect to the location of the tumor in the animal. This possibility was investigated by comparing the histopathology of all slides given the diagnosis of lymphoma on the high dry (40X) and/or oil immersion objectives. Each sample was evaluated for density of cells, amount of cytoplasm and variety of cell types. The results for each sample were recorded with the code on the slide. These results were then decoded and compared to determine if all the lymphomas had the same histopathology, and if not, if there were patterns of cellularity that correspond with certain types of lymphomas (i.e., cervical, liver, ip).

In order to determine the background pathology of the colony, data from all the autopsies were evaluated and the major causes of

death were noted. Other lesions noted on autopsy were also included and it was found that enteritis and kidney disease, inflammatory lesions of the skin and lymph nodes as well as most of the internal organs and several types of tumors other than lymphomas had occurred. The histopathology of these lesions is presented along with pertinent data from other investigators.

CHAPTER III

RESULTS

Gross Pathology of the Lymphomas

Animals with advanced lymphomas are distinguished readily from healthy animals. If the tumor is cervical or inguinal, the animal has a large mass which continues to increase in size until the animal can no longer walk or feed. The surface of the tumor often becomes ulcerated and the animal usually dies from blood loss, starvation, or cannibalism by cage mates. Animals with large intestinal or liver masses have large bellies and often a proportionately shrunken head and shoulder area. In younger animals, less than 60 days, a large firm belly is the conspicuous symptom and the animal often resembles a pregnant female late in the gestation period. If not sacrificed, these animals become progressively debilitated as the tumor mass increases and die from renal failure, hemorrhage, intestinal obstruction, or a variety of other causes.

Animals with thymomas are thinner than their cage mates and have a characteristic deep breathing and rounded chest that makes the diagnosis obvious before the animal is sacrificed and autopsied. These animals generally die within 48 hours of detection and so, deep breathing animals were sacrificed when found to prevent cannibalism.

On gross autopsy, lymphomas are soft, round, creamy-white masses, often with some central necrosis. Cervical, axillary and

inguinal lymphomas are generally localized tumors; no visible metastases are present in the abdomen although the spleen is enlarged. Liver lymphomas are seen most frequently in the dorso-caudal lobes and frequently are associated with metastases to the kidneys and the formation of bloody ascites. Intestinal lymphomas run a gamut from hyperproliferation of a single Peyer's patch (considered a pre-lymphomatous condition) to multiple nodules along the small intestine, to a single large mass involving intestine, liver, kidney and connective tissue. Metastases to the omental tissue are frequently seen with intestinal and liver lymphomas as thin, red veining in the normally grey-white fat. On occasion, this omental tissue is completely pink and firm, encasing the intestine and adhering to the peritoneal wall. This extent of lymphoma as well as those lymphomas involving several abdominal organs almost equally are referred to collectively as the "ip" lymphomas. Those tumors which are localized to a single organ are categorized under that organ: e.g., liver thymic, intestinal. Those tumors which appear to involve only two organs unequally (usually liver and kidney) are classified under the category of the organ that had the largest tumor mass.

The incidence of lymphomas in various tissues is shown in Tables I, II, and III. These tables were derived from daily death records of all animals dying with lymphoma between January, 1975 and May, 1977. As can be seen from the data in Tables I and II, intestinal and liver tumors account for most lymphomas in both nonexperimental and experimental groups. There seems to be a disproportionate number of liver lymphomas in the LVG strain, but this may be due to the almost

TABLE I. Lymphomas in nonexperimental animals (January, 1975 to May, 1977).

IP	Intest.	Liver	LVG Kidney	Other	Total	Days	Total	Other	LSH Kidney	Liver	Intest.	IP
					0	0-30	2			1	1	
	3	3		1t	7	31-40	6	1i	1	3	1	
1	2	10		1c	14	41-50	9	2tt		5	1	1
1	2	6		1p	10	51-60	3	2tt				1
	3	13	1		17	61-70	5	1c		1	2	1
	1	4	1	1p	7	71-80	9		2	1	5	1
	2	4	1		7	81-90	7	3ttt	1	1	2	
2	2	2		1s	7	91-100	10	2tt		4	2	2
	1	3	1		5	101-110	10	5tttt		2	2	1
1	3	4	3		11	111-120	13	7ttttpp		3	2	1
2	7	10			19	121-130	9	4tttt	1	1	2	1
	8	4	1	1e	14	131-140	14	2ct	1		10	1
	7	1		2c	10	141-150	4			1	1	2
		1	1	2s	4	151-160	18		3	1	14	
2	4			2ec	8	161-170	6			2	4	
1	1		1	3	3	171-180	2				2	
2	3	3			8	181-190	9	1c		1	7	
	5	1		1c	7	191-200	4	1c		1	2	
	1				1	201-210	1	1i				
		2		1c	3	211-220	7				6	1
	1	1		1c	3	221-230	2				2	
1	3	4		1i	9	231-240	3	1t			2	
1	1				2	241-250	1				1	
	1				1	251-260	1			1		
1	3				4	261-270	2			1		1
		1		1ic	2	271-280	1				1	
1		1			2	281-290	1	1c				
	1				1	291-300	0					
					0	301-310	0					
	2			2pt	4	311-320	1				1	

TABLE I. (continued)

IP	Intest.	Liver	LVG Kidney	Other	Total	Days	Total	Other	LSH Kidney	Liver	Intest.	IP
	1				1	321-330	0					
	8	1	2	1c	12	331-340	1	1s				
1	1				2	341-350	0					
	6	2	1	1c	10	351-360	0					
	4	3	2		9	361-370	1				1	
	3				3	371-380	0					
		1			1	381-390	0					
17	141	32	15	22	226	TOTAL	162	35	9	30	74	14
7%	39%	37%	14%	9%		% OF TOTAL		22%	6%	18%	46%	9%

NOTE: t = thymoma; p = perirenal; c = cervical; i = inguinal; s = stomach; e = eye.

TABLE II. Lymphomas in experimental animals (January, 1975 to May, 1977).

IP	Intest.	Liver	LVG Kidney	Other	Total	Days	Total	Other	LSH Kidney	Liver	Intest.	IP
2					2	0-30						
1	1	32		1	35	31-40	3		1	2		
1	3	15			19	41-50	5	3		2		
1	1	12		2	16	51-60	3			1	2	
1	1	21	1	1	25	61-70	3	1	1			1
3	1	4	3	1	12	71-80	4	1			3	
2	5	6	1		14	81-90	1					1
6		2		1	9	91-100	2	1			1	
2		1			3	101-110	4			1	3	
1	7	2	1	1	12	111-120	5				2	3
	1		1		2	121-130	12				10	2
2	4	2	3	1	12	131-140	3				2	1
	1				1	141-150	0					
	1				1	151-160	2	1			1	
1					1	161-170	6				5	1
	1				1	171-180	0					
					0	191-200	0					
					0	191-200	0					
	2			3	5	201-210	0					
23	29	97	10	11	170	TOTAL	53	7	2	6	29	9
14%	17%	57%	6%	6%		% OF TOTAL		13%	4%	12%	55%	17%

TABLE III. The incidence of lymphomas by site in animals of the third epizootic.

Site	LVG Number	(%)	Site	LSH Number	(%)	First Epizootic Site	(%)
IP	17	7	IP	14	9		
Intestinal	88	39	Intestinal	74	46	Intestinal	45
Liver	85	37	Liver	30	18	Liver	11
Kidney	15	7	Kidney	9	6	Kidney	10
Thymic	2	1	Thymic	23	14		
Cervical	9	4	Cervical	5	3	Cervical	6
Perirenal	3	1	Perirenal	1	.6	Spleen	6
Stomach	3	1	Stomach	3	2	Mesenteric Node	19
Eye	2	1	Eye	1	.6		
Inguinal	2	1	Inguinal	2	1	Inguinal	Infrequent
Total	226			162			

exclusive use of LVG neonates for testing cell-free preparations. Liver lymphomas account for 82 percent (80/97) of the LVG tumors seen within 70 days of birth in experimental animals (Table II), but account for only 66 percent (32/48) of the tumors seen in the same time period in nonexperimental animals. This is, however, not a significant difference by the chi-square test, so it is unclear if the preponderance of liver lymphomas in the LVG strain hamsters is a result of experimental manipulation only.

The incidence of lymphoma by location for the first epizootic is included in Table III for comparison. According to Ambrose and Coggin (1), similar data were obtained for the second epizootic; however, in addition to the other types of tumors, thymomas were also noted in the second epizootic. In the third epizootic, primary spleen lymphomas were seen in only one animal. Only infrequent mesenteric lymphomas were seen in the third epizootic, although a number of mesenteric nodes had benign reactive hyperplasia. Lymphomas involving the orbital region were noted in the third epizootic in two animals. It is possible that some facial tumors might have occurred and were not noticed in the earlier epizootics since these tumors resembled facial abscesses.

Liver, cervical, axillary, ip and intestinal lymphomas were seen in a fourth epizootic that occurred in hamsters at The University of Tennessee (Appendix A). The final distribution of tumors in that colony was not determined.

Observations on the occurrence of tumors within single groups of animals exposed on the same day suggested that the first tumors to be seen were thymomas and liver lymphomas, then intestinal/ip lymphomas,

and finally, cervical and axillary tumors. Essentially the same distribution was noted in both strains except that thymomas occurred more frequently in the LSH strain. This phenomenon can be seen to a certain extent from the data in Tables I and II, pages 59 and 61, respectively: liver tumors appear early, intestinal/ip tumors occur at all ages without any particular increase in frequency at a given age, and cervical/inguinal/axillary tumors are well represented after 120 days.

Microscopic Pathology of the Lymphomas

On scanning power, the lymphomas appear to be densely cellular monotonous accumulations of fairly well-differentiated lymphocytes. Nucleoli are prominent, cytoplasm is often scanty, and mitoses may be frequent. Examination on higher power reveals a variety of degrees of lymphocytic differentiation between tumor sites and even within the same tumor. This range does not include seeing both well-differentiated and poorly-differentiated cells within the same tumor--the cells tend to represent stages within the same classification. Follicular architecture was evident only rarely.

Several patterns of mitoses and cell types were seen, but the only consistent cellular pattern with respect to location of the tumors was the finding of plasmacytoid cells in primary liver lymphomas. Plasmacytoid cells were found in varying numbers in kidney, lymph node, and intestinal lymphomas also, but were not seen in thymic or orbital lymphomas. Giant cells with eosinophilic inclusions were noted in most tumor sections although their number was never sufficient to suggest a diagnosis of histiocytic lymphoma. Occasional bizarre cell

types were also noted such as the large, apparently multinucleate deeply basophilic cells with no visible cytoplasm seen in one of the thymic lymphomas, and the Reed-Sternberg like cells seen in an intestinal lymphoma. However, the predominant cell type in sections of most tumors was the small or medium lymphocyte. Photomicrographs of representative sections of lymphomas are included in Appendix C.

A variety of other types of tumors and pathological lesions were noted and are presented below with pertinent findings by other investigators.

Enteritis:

Hamster enteritis or "wet tail" appears to be a universal problem. Incidence in a given colony may vary from loss of a few animals to loss of the entire colony. In a survey of hamster diseases, Renshaw et al. (68) found wet tail was the most important source of animal loss post-weaning in 71 percent of their respondents. Suggested etiologies of hamster enteritis include stress, bacterial infection with Clostridium sp., E. coli, and Proteus morganii, heavy parasitic infestations, neoplasms, dietary deficiencies, and virus. Recently, there have been two reports suggesting that a slow lactose fermenting E. coli is a causative factor (2,40). The bacterium has been found within atypical ileal mucosa cells, is found in bacterial flora isolated from the lesions, and has caused the acute enteritis syndrome in animals inoculated orally with the bacterial suspension. The possibility of an associated viral infection or synergy with other bacteria has not yet been ruled out since inoculation with the slow lactose fermenting E. coli alone does not cause the atypical ileal mucosa

proliferations seen in the "chronic" form of the disease.

A variety of enteritis syndromes have been seen in the third epizootic. Until 1977, the predominant form of enteritis was what I have called "bloody intussusception." Animals with this form of enteritis were noted to be huddled in the corner of the cage, were extremely irritable, appeared dehydrated and had dried and/or fresh blood on the mouth, paws, and perianal region. On autopsy, blood could be found throughout the gi tract including in the esophagus and mouth. The cecum was purple and firm due to extensive intussusception of the ileum and jejunum, or was flaccid and filled with dark purple blood. If no intussusception was seen in the ileum, one could be found in the descending colon and rectum with extrusion of the intussuscepted area out of the anus, as though the animal were trying to extrude a foreign body. The few Peyer's patches that remained visible had a reddish halo and "cheesy" white center. The other abdominal organs showed signs of severe blood loss and/or irritation; the liver appeared to have a follicular architecture delineated in red and yellow; the spleen was a pale red, and the kidneys appeared dull brown instead of red. No gastric ulcers were noted, nor was there any thickening of the ileum other than that caused by the intussusception. In only two cases was the focus of the intussusception identified. In one case, an enlarged Peyer's patch appeared responsible; in the other, a small lymphoma was responsible. E. coli was the only organism cultured from these animals.

Young animals, less than 12 weeks of age, were most susceptible to the disease, with onset between 8 and 30 days post exposure to the

facility (Table IV and Figure 1). The inbred LSH strain hamsters seemed most susceptible although the comparison is not conclusive since adult randombred LVG hamsters were not used in the lymphoma colony. Filter topping the cages did seem to delay the onset, but did not prevent the disease.

One other type of bloody enteritis was seen and this was found infrequently and in older animals. The same bloody mouth, paws, and perianal region were noted, although these animals did not have any intussusceptions. The prominent feature was the purplish fluid-filled flaccid intestine and cecum. Gastric ulcers were not apparent.

Only one type of non-bloody enteritis was seen in the lymphomatous colony prior to 1977 and this was characterized by flakes of brownish yellow fecal material on the paws, mouth, eyes, and perianal region of pre-weaning animals. On autopsy, the intestines were gassy with minimal soft greenish fecal material and the cecum was transparent; or, the intestines were collapsed, translucent, and had minimal yellowish fluid. Occasional intussusceptions were detected by gross examination although lymphomas were not seen on gross or microscopic examination.

A second type of non-bloody enteritis was detected in the lymphomatous facility after its appearance in a clean facility which was used to supply animals for the contaminated facility. In March, 1977, an enteritis that appeared to be the classical wet tail with watery diarrhea and yellow-mucus filled intestines was detected. Peyer's patches were prominent with red halos and "cheesy" white centers. Occasional ileal intussusceptions were noted, although they were not bloody and many appeared agonal, with no damage to the intussuscepted

TABLE IV. Length of exposure of animals dying with intussusceptions.*

Length of Exposure (Days)	No. of Animals Dying	Length of Exposure (Days)	No. of Animals Dying	Length of Exposure (Days)	No. of Animals Dying
0-1	0	30-31	4	101	1
2-3	1	32-33	5	102	1
4-5	0	34-35	6	106	1
6-7	0	36-37	6	117	1
8-9	7	38-39	2	136	1
10-11	15	40-41	1	137	1
12-13	9	42-43	4	152	1
14-15	16	44-45	2	161	1
16-17	5	46-47	0	162	1
18-19	9	48-49	0	192	1
20-21	12	50-59	4	197	1
22-23	5	60-69	3	204	2
24-25	5	70-79	7	222	1
26-27	4	80-89	2	295	1
28-29	1	90-99	0	298	1

*Includes animals that received experimental treatments since no difference could be seen between the susceptibility of normal and experimental animals. The age of the animals is not included. This was the "bloody intussusception syndrome."

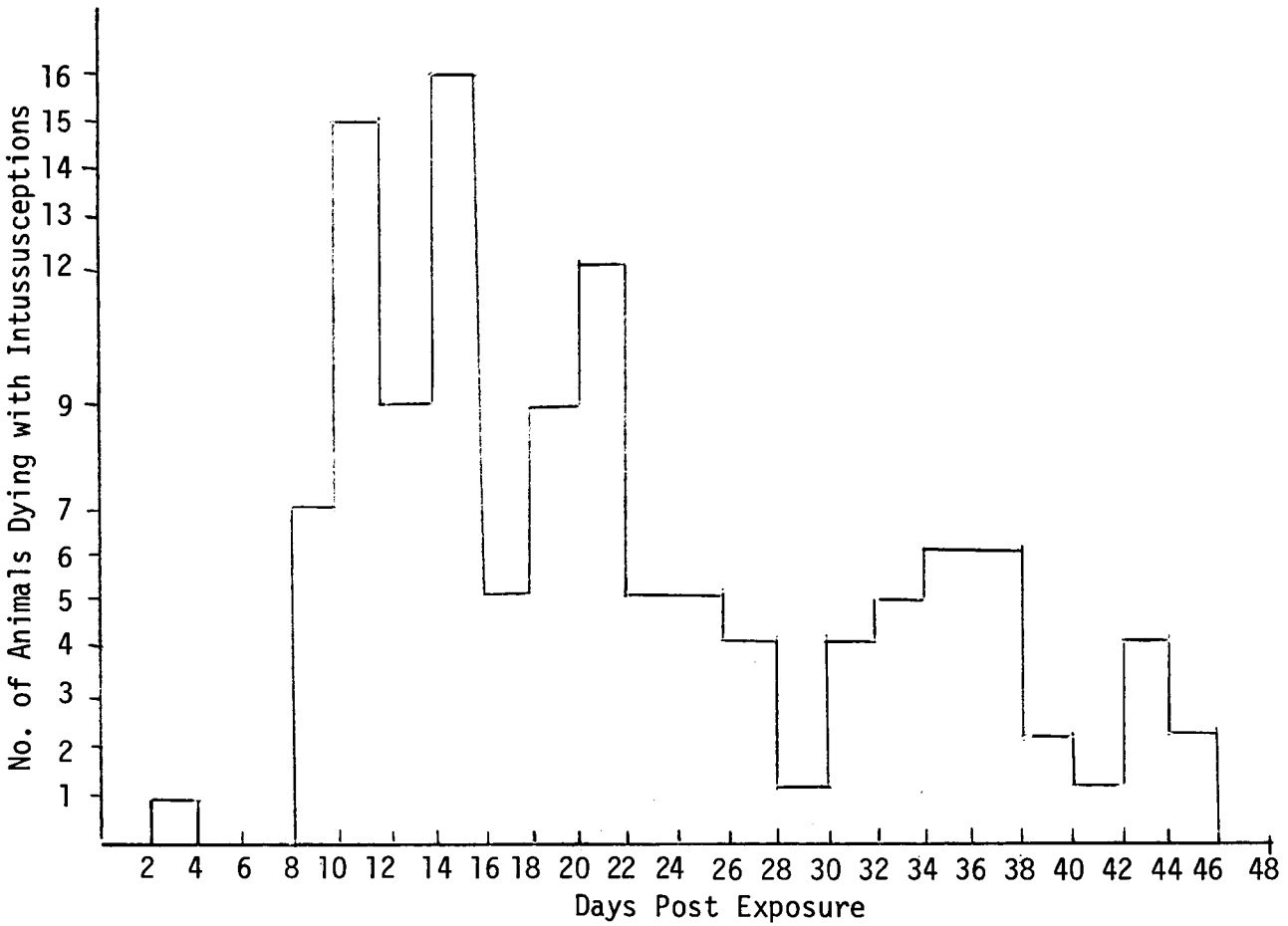


FIGURE 1. Graphic presentation of the occurrence of intussusceptions with respect to the length of exposure to the lymphomatous colony.

section. In only one animal was the thickened ileum noted by other investigators (2,40) found. Gastric ulcers were seen occasionally.

The bloody intussusception syndrome was not seen in the clean facility until August, 1977, more than two months after detection of lymphomas in the facility, and more than six months after detection of a wet tail epidemic in the facility. The bloody intussusception was seen in only one animal, an uninoculated control for an experiment evaluating the oncogenicity of some cell-free preparations of lymphomas.

Although several syndromes of enteritis have been reported in the literature, the following seem to represent the syndromes seen in the lymphomatous colony most closely. The non-bloody enteritis appears to be the same as the acute disease described by Amend et al. (2) as atypical ileal hyperplasia (AIH) and as the subacute AIH syndrome described by Jacoby et al. (40), although the thickening of the ileal mucosa was a rare finding in The University of Tennessee hamsters. The bloody intussusception and bloody enteritis syndromes seem identical to Jacoby et al's. (40) acute syndrome. Only occasional instances of the chronic syndrome have been seen, generally in older animals. At the time those animals were autopsied, the rope-like quality of the ileum was thought to be due to the advancing age of the animals and was not associated with enteritis. Histopathology was not done.

The association of enteritis and lymphomas is interesting as they both appear to be horizontally transmissible diseases and enteritis has been seen prior to all four of the lymphoma epizootics. A viral etiology has been suggested for the ileal hyperplasia, and neither Amend (2) nor Jacoby (40) is able to rule the possibility out.

Intussusception and enteritis in children has been shown to be associated with Reovirus (10) and Reovirus type 3 has been found in some hamster colonies (83). However, it does not appear to be enzootic in either of The University of Tennessee hamster colonies (55). Nevertheless, the possibility exists that the lymphoma agent and the enteritis agent act synergistically in producing both disease states. Such a possibility is suggested in the feline leukemia virus system where it has been shown that infection with the virus can result in tumorigenesis (41) but infection with the virus is also associated with other diseases such as a nonregenerative anemia, a panleukopenia-like syndrome and a thymic atrophy or fading kitten syndrome (33,34,64).

Also of interest is a report of the induction of malignant lymphomas in hamsters following a wasting disease produced with an extract of bovine lymphoma (51). The wasting disease was characterized by cessation of growth, inactivity, ruffled, damp fur, mincing gait and diarrhea. The outstanding histological finding was the almost complete absence of lymphocytes in the lymphoid organs and cortical zone of the thymus. The disease was seen in 70 percent of the animals given the extract, and half of these died. Of the survivors, about 16 percent later developed lymphomas that arose from the mesenteric lymph nodes, Peyer's patches and retroperitoneal nodes and tended to envelop the abdominal viscera. The description of the acute wasting disease is strikingly similar to the description of acute enteritis given by Amend (2) and, in some respects, to the chronic pyelonephritis syndrome seen in The University of Tennessee hamsters (cessation of growth, mincing gait, enteritis, thymus inconspicuous). In addition, the description

of the lymphomas is similar to the ip lymphoma found in The University of Tennessee hamsters.

Kidney Disease

Although from the daily death records kidney disease appears to account for less than 1 percent of the total deaths in the lymphomatous colony, it is a frequent autopsy finding in LSH hamsters. In addition, it was not seen in the "clean" colony until after the lymphomas were detected, so there is a possibility that it, like the enteritis, may be a lymphoma associated disease.

A number of kidney samples have been evaluated for the presence of lymphomas by Dr. Bellomy. These samples have invariably been described as severe subacute and chronic pyelonephritis. A survey of the literature has shown that only two investigators mention pyelonephritis as a significant finding in their hamster colonies. Data are not available from the first investigator (47). The second investigator (62) reported incidences of 10 percent and 9 percent in two unrelated colonies of Syrian hamsters. The average age of the animals with pyelonephritis was 73 weeks or greater. In contrast, pyelonephritis has been seen in the lymphomatous colony in animals exposed as neonates in as few as 41 days after birth, suggesting some sort of infectious etiology.

The cause of chronic pyelonephritis in humans is not clear. Acute disease is seen with bacteremia, but most frequently is found as an ascending infection from the lower urinary tract or adjacent lymph nodes. Unresolved acute disease may progress to a chronic form in which the kidneys are sterile, but the tubular necrosis and scarring

progresses. However, a previous history of acute disease is frequently not found in many cases of chronic pyelonephritis. Kimmelstiel (46) has shown that by sensitizing animals to certain strains of bacteria, then injecting the killed organisms into the renal pelves, it is possible to produce a totally noninfectious and progressive pyelonephritis. Thus, the insult to the kidney may be acute bacterial infection or a noninfectious immunologic insult. Drug abuse has also been associated with chronic pyelonephritis, particularly the phenacetin compounds, although it is an unlikely mechanism for the hamster pyelonephritis.

Clinically, hamsters with severe pyelonephritis are smaller than their cage mates and tend to become thinner and more dehydrated with time. In the terminal stages, animals may breathe deeply in the same manner as the animals with thymomas, although no thoracic lesions have been found.

On gross examination, the abdominal organs are small and pale. The kidneys range from pale rose to yellow brown in color and may show pitting and scarring varying in extent from one or two cortical indentations to a complete destruction of the normal shape resulting in kidneys that resemble small golden raisins. The severe disease is frequently associated with a mild enteritis: no fecal material is seen; the small intestine appears to have a minimal amount of yellow fluid, is reddened and translucent; and the cecum appears congested. The spleen is generally small, pale, and fibrotic, and the thymus and thoracic nodes are inconspicuous.

Microscopically, the kidney tubules show varying degrees of necrosis and fibrosis. Glomeruli are generally spared unless there is extensive scarring. The cellular infiltrate varies in extent from a few foci of small lymphocytes, plasma cells and neutrophils to a densely cellular infiltrate which causes the entire kidney to appear blue in stained section. Photomicrographs are included to show some of the lesions and the histopathology of these kidneys is described in Appendix C.

Most of the kidneys submitted for histopathological diagnosis were sent because it was not possible to determine the presence of lymphoma by gross diagnosis alone. Indeed, some specimens were equally hard to evaluate in histological section because of the dense accumulations of lymphocytes. Pleiomorphism of cell types, invasiveness of the infiltrate and scarcity of neutrophils were considered indicative of neoplasia. Interestingly, of more than 80 kidneys evaluated, 67 diagnoses of pyelonephritis were given and only 7 diagnoses of lymphoma. In no case was lymphoma seen in a kidney with chronic pyelonephritis. What this observation means in terms of the etiology of either disease is not clear. It may indicate that characteristics of the individual hamsters may be significant factors in determining the susceptibility to either the lymphoma or the pyelonephritis.

Several other kidney lesions have been noted. They include an angiosarcoma of unknown origin which seemed to be invading the kidney rather than arising from within it, two infarctions, two kidneys with chronic disease of uncertain type (kidney architecture compressed, extensive cast formation in tubules) one kidney with amyloid

degeneration, one kidney with necrosis secondary to renal obstruction, and one kidney with diffuse enlargement but otherwise normal anatomy.

Liver Lesions

Liver lymphomas account for one-third of the liver histopathology evaluated, cholangitis accounts for another 44 percent, and benign cysts, tumors, and degenerative lesions account for the remainder. However, not all liver lesions have been examined in histologic section, and so, these percentages do not represent the true incidence of any of the above lesions.

Liver lymphomas are usually distinctive lesions on gross autopsy as whitish accumulations of lymphocytes contrasting with the liver tissue. However, because this distinction cannot be made for early lesions, livers that showed any sort of unusual coloration, bulge, cyst, or other abnormality were submitted for histopathology. Most of these specimens had focal infiltrations of lymphocytes in the portal triads and bile stasis suggestive of cholangitis rather than lymphoma. In addition, the livers with cholangitis were frequently from animals with severe pyelonephritis. However, liver lymphoma was also seen in animals with severe pyelonephritis, so the significance of the cholangitis-pyelonephritis association was not clear.

A number of other liver lesions were seen. These included two benign hemangiomas, four specimens with benign, possibly congenital cysts, two specimens with cirrhosis, one specimen with fatty infiltrations, one with degenerative liver disease, and one specimen with a dystrophic calcified mass attached.

Lesions in Other Tissues

A variety of lesions other than lymphoma were seen. These are simply presented in tabular form to demonstrate the multiplicity of findings (Table V).

According to Kirkman (47), tumors of the skin are infrequent: four instances of basal cell carcinoma have been reported, three instances of squamous cell carcinoma, one benign papilloma, and one sweat gland adenoma. Subsequent to Kirkman's report came Graffi et al.'s report of papillomas associated with lymphomas in the Berlin colony of Syrian golden hamsters (28) and then Pour's (62) mention of the skin tumors in a few hamsters in the two colonies he studied. The total incidence of skin tumors detected by each of these investigators was less than 5 percent of each colony studied, and the same has been found in The University of Tennessee hamster colony.

One skin lesion with a wart-like appearance on gross autopsy bears further discussion as it resembles the Graffi papillomas (28) and is transmissible with a cell-free saline homogenate. The warts have been seen in the inbred LSH strain hamsters even though transmission has been attempted in the randombred LVG strain. Microscopically, the lesions are benign proliferations of the pilosebaceous apparatus and most animals have only a few. From the photomicrographs available (28) the warts appear more benign than the Graffi tumors, and only one hamster had warts all over like the Graffi hamsters. Graffi was not able to transmit the warts with cell-free preparations; instead, cell-free preparations caused lymphomas when inoculated into neonatal hamsters (26). In contrast, cell-free saline homogenates of the U.T. warts have

TABLE V. Other pathological lesions.

Organ System	No. Animals	Description of Lesion
Ovary	2	Tumor of probable granulosa cell origin
	1	Thecoma
	2	Benign cysts
Skin	4	"Wart-like" lesions on gross autopsy, benign adenomas on microscopic examination
	1	Basal cell/squamous cell tumor (mixed)
	2	Squamous cell carcinomas
	1	Benign cyst
	1	Mesothelioma-like nodules
	1	Teratoma-like nodule
	1	Benign fibroma
	1	Benign hair-follicle tumor
	1	Scarring and alopecia
Testis/Epididymis	5	Epididymitis
	1	Testicular hypertrophy
Spleen	3	Fibrosis
	6	Infarctions
	1	Focal hemorrhage
	1	Hemangioma
Pancreas	2	Pancreatic adenomas
All organs		Inflammatory lesions
All lymphoid tissue		Benign reactive hyperplasia

been used to serially passage the warts three times and do not appear to cause lymphomas (Appendix C), although the number of animals involved is too small to be conclusive.

CHAPTER IV

DISCUSSION

Other than the lymphomas and the high incidence of pyelonephritis and cholangitis, the pathology seen in The University of Tennessee hamster colony is not unusual. Once Fortner (17) clearly demonstrated that hamsters developed spontaneous tumors, a number of investigators reported tumors in low incidence in essentially every organ system in the hamster (for review, see 62). A surprisingly high incidence of tumors of the endocrine system was found prompting the theory that hamsters are extremely susceptible to an endocrine imbalance which may result from the stress of being confined (17). Endocrine tumors were not looked for in the U.T. hamsters.

The other most commonly cited lesions have been those of the lymphoreticular system. There have now been reports by a number of investigators on the occurrence of spontaneous lymphocytic tumors in hamsters. Almost all were reticulum cell sarcomas in animals over one year of age (85). Only four other facilities have reported lymphomas in hamsters younger than one year of age (23,28,48,77) and these tumors have been classified as lymphocytic lymphomas and reticulum cell sarcomas. Graffi et al. (28) have been able to induce lymphomas, primarily in the liver, by inoculating neonatal hamsters with cell-free extracts of spontaneous papillomas. Both from the gross and microscopic pathology, some of these tumors appear similar to those seen in

The University of Tennessee hamster lymphoma epizootics. Graffi et al. (28) also reported a total 3 percent incidence of spontaneous lymphomas arising primarily from the mesenteric nodes in young hamsters in their colony. Lymphomas of the mesenteric nodes have been seen in the U.T. epizootics, although the most frequently seen tumors are the intestinal and liver lymphomas. Papillomas have also been seen, but these tumors can be transmitted with cell-free preparations and are not lymphomagenic.

Pour (63) has also reported lymphomas arising from the mesenteric nodes and suggested an infectious etiology since the hamsters also had a mild enteritis. This is a particularly interesting theory in light of the strong association seen between enteritis epizootics and the subsequent detection of lymphomas at The University of Tennessee. Enteritis was seen within one to two months of the onset of each of the four lymphoma epizootics. Data from experiments testing the oncogenicity of cell-free preparations of the hamster lymphomas also suggest the enteritis and lymphomas may be related (84).

The histopathology of the tumors was investigated with the objective of gaining information about the etiology or pathogenesis of the lymphomas. The lymphomas were all found to be lymphocytic or lymphoblastic lymphomas rather than the histiocytic lymphomas or reticulum cell sarcomas seen by most investigators. Even the lymphomas induced by Graffi et al. (28) with cell-free preparations were occasionally histiocytic, so it is possible that the etiology of the U.T. lymphomas is that of a highly lymphotropic agent. The number of thymic lymphomas that occurred was also unusual since thymic lymphomas are rare in other hamster colonies (63) and most of the thymic lymphomas occurred in the inbred LSH strain of hamsters. If the lymphoma agent involves

an endogenous virus, inbred animals might be expected to have more of the virus and possibly more tumors than randombred animals.

Although only lymphoblastic and lymphocytic lymphomas were seen, there was no clear pattern of cellular differentiation with respect to the organ in which the lymphoma was found nor did there appear to be any difference in the histopathology of the lymphomas in animals inoculated with cell-free preparations of the lymphomas as compared to animals given no inoculations. There was a difference in the occurrence of certain tumors with respect to age, since tumors of the viscera were seen in younger animals than were tumors of the peripheral nodes. Also, most of the lymphomas induced with cell-free preparations occurred in young animals and were tumors originating predominantly in the liver. Thus, the data suggest that the same agent caused both the apparently spontaneous lymphomas and the lymphomas transmitted with cell-free preparations.

PART IV

IMMUNOLOGY

CHAPTER I

INTRODUCTION

Brindley and Banfield (7) reported the horizontal transmission of a spontaneous reticulum cell sarcoma in hamsters in 1961. The disease was transmitted by cannibalism, a mosquito vector, and feeding of intact tumor cells. An oncogenic agent was not suspected, since cell-free filtrates did not produce tumors. Horizontal transmission of an oncogenic agent, however, has been reported in other animal systems (35,60,78). No reports demonstrating horizontal transmission of an oncogenic agent in hamsters have been made until recently, when Ambrose and Coggin (1) reported a horizontally transmissible epizootic of lymphosarcomas that arose spontaneously in a colony of hamsters kept in The University of Tennessee animal facility. The epizootic occurred in the facility three times, despite extensive cleaning with virucidal agents, UV irradiation of the facility, and the adoption of other control measures prior to the introduction of new animals from the supplier. The new shipments of animals were divided; half were housed in the infected colony while the other half were housed in a separate disease-free animal facility. Only those hamsters placed in the infected colony developed lymphomas. By adopting stringent containment procedures, the epizootic was successfully limited to a single animal facility, and a new colony was established at another site at the University. The new colony was maintained free of lymphomas for three years.

Several years of investigation of the lymphoma epizootic by researchers at three different laboratories have been completed. The reaponsible agent has yet to be isolated, although cell-free transmission has been reported (R. Heubner, personal communication). The agent involved is quite oncogenic, routinely producing greater than 50 percent tumor incidence in animals exposed to the infected colony as neonates. Infected animals from this colony can transmit the disease horizontally simply by being placed in a room with normal hamsters, and the epizootic has been successfully reproduced in several different laboratories by this means. Direct contact with the infected hamsters is not required (A. Jonas, personal communication).

In continuing efforts to isolate the agent, the antigens of the lymphomas were evaluated to determine whether the tumors carried tumor-associated transplantation antigens (TATA), cross-reacting antigens common to all lymphomas that occurred in an epizootic, or possible tumor-associated antigens common to SV40, the only known oncogenic agent used in the colony prior to the onset of each of the three epizootics. In addition, the effect of direct immunization of hamsters to produce immunity to the infectious agent was assessed.

CHAPTER II

MATERIALS AND METHODS

Animals

The LSH strain of inbred Syrian hamsters (Lakeview Colony, Newfield, New Jersey) was used. Hamsters used for tumor immunity studies were between three and twelve weeks of age and age-matched within each experiment.

Cells

Three lines of lymphoma cells have been developed from spontaneous tumors occurring in the third epizootic. These lines have been maintained by in vivo subcutaneous (sc) passage of tumor cell suspensions. IL/LSH was developed from an inguinal mass composed of well-differentiated lymphosarcoma cells in a 28 week old LSH male. CL/LSH-1 and CL/LSH-2 were developed from spontaneous cervical lymphomas; CL/LSH-2 occurred in a 28 week old LSH male and CL/LSH-2, a 30 week old LSH female. These cells have been used for immunizations and challenges up to the 30th passage in vivo. For these procedures, tumors were removed aseptically, minced in medium without serum to give single cell suspensions, counted for viability (trypan blue dye exclusion) and then diluted appropriately.

A line of SV40 transformed hamster fibroblasts maintained in tissue culture, F5-1 (25), was used for specificity experiments. Cells were grown in Eagle's basal medium with Hanks' salts (HBME) with 10 percent calf serum (CS) penicillin, streptomycin and amphotericin B

supplements. For vaccination with irradiated cells and live cell challenges, cells were harvested with trypsin, washed once with HBME with 10 percent CS, washed three times with HBME without serum, counted for viability, and diluted to the appropriate concentration.

Vaccines

All vaccines made with whole cells were prepared as single cell suspensions, counted for viability (trypan blue dye exclusion), and adjusted to 5×10^6 to 10^7 viable cells/ml. The cell suspensions were then given 5,000 R of X-irradiation to prevent cell division and subsequent tumor development.

Muscle Vaccine

Immunizations with muscle homogenates were performed as normal tissue control vaccines. Muscle was removed from the legs, arms, and backs of adult LSH hamsters, minced in HBSS, and disassociated in a blender until a uniform suspension was formed. This suspension was forced through a 1 x 1 mm mesh to remove sinew and then centrifuged at 2,000 rpm for 10 minutes. The washed pellet was weighed, 4 volumes of medium were added to give approximately 0.2 g/ml, the suspension was forced through a 22-gauge needle and irradiated (5,000 R) and 0.5 ml was injected ip (intraperitoneally) into each hamster.

Irradiations

All irradiations were done with a General Electric Maxitron 300 at 250 kilovolt peak, 20 mA, with 0.42 mm copper and 0.5 mm aluminum filters. All samples were irradiated 22 cm from the source.

Oncogenicity of LSH Lymphomas

Each line was titrated for oncogenicity by the sixth passage in vivo. Tumors were removed aseptically, minced, washed once in HBSS (Hanks' Balanced Salt Solution) and diluted to give from 2×10^6 to 2×10^1 viable cells/ml. Groups of 10 hamsters were inoculated sc in the right subscapular region with 0.5 ml of a cell suspension and then palpated weekly for tumors.

Determination of Tumor Resistance

Natural exposure: for these experiments, animals were divided into two groups. One group received four weekly ip immunizations with at least 5×10^6 irradiated lymphoma cells. The other group was held as normal controls. On the date of challenge, half of each group was challenged sc with 10^4 CL/LSH-2 cells. The other half was given no challenge and was placed in the infected colony for room exposure.

Direct challenge: in all these experiments, animals were given four weekly ip immunizations with at least 5×10^6 irradiated lymphoma cells. Ten days after the last vaccine, animals were challenged sc with a homologous or heterologous live lymphoma cell challenge and were palpated weekly for tumors. Heterologous challenges were used to determine the specificity of the animal's response to immunization.

Determinations of Specificity of Tumor Resistance

Animals were immunized four times ip with either spontaneous lymphoma or SV40 transformed cells, irradiated muscle, or nothing. Ten days after the final immunization, animals were given an sc challenge of lymphoma cells, or SV40 transformed cells and were palpated weekly.

Adoptive Transfer of Tumor Resistance

For these experiments, animals received either four weekly ip immunizations of 5×10^6 irradiated CL/LSH-2 cells or no immunization. Ten days after the last vaccine, peritoneal exudate cells (PEC) or spleen cells were collected from immunized or normal animals, washed three times, mixed with tumor cells at a ratio of 200:1 effector cells: tumor cells, incubated 30 minutes at room temperature, and then injected sc into the right subscapular region. Animals were palpated weekly for tumors.

CHAPTER III

RESULTS

Oncogenicity

All in vivo derived lines were highly oncogenic at passage six in vivo (Figure 1). The IL/LSH had a TPD_{100} (tumor producing dose that gives 100 percent tumor incidence) of 10^5 cells; the CL/LSH-1, a TPD_{100} of 10^4 cells; and the CL/LSH-2, a TPD_{100} of 10^2 cells. With subsequent in vivo passage, these lines became increasingly oncogenic with the TPD_{100} of the IL/LSH dropping to 10 cells. Tumors appeared at the challenge site within a week following the sc injection of 10^6 or more lymphoma cells. Rapid metastasis and death followed in these animals within 3-4 weeks. Lymphomas induced by challenge were histologically indistinguishable from those originally derived from spontaneous tumors.

Resistance to Lymphoma Induction

To determine whether lymphoma induction could be prevented, a series of experiments was done. The first was to determine whether induction of spontaneous lymphomas could be prevented by the immunization of animals with irradiated lymphoma cells. Animals were given either no immunization or four ip immunizations with at least 5×10^6 irradiated CL/LSH-2 cells. Ten days after the last injection, all animals were either challenged sc with 10^4 viable CL/LSH-2 cells or given no challenge but exposed to the lymphomatous colony.

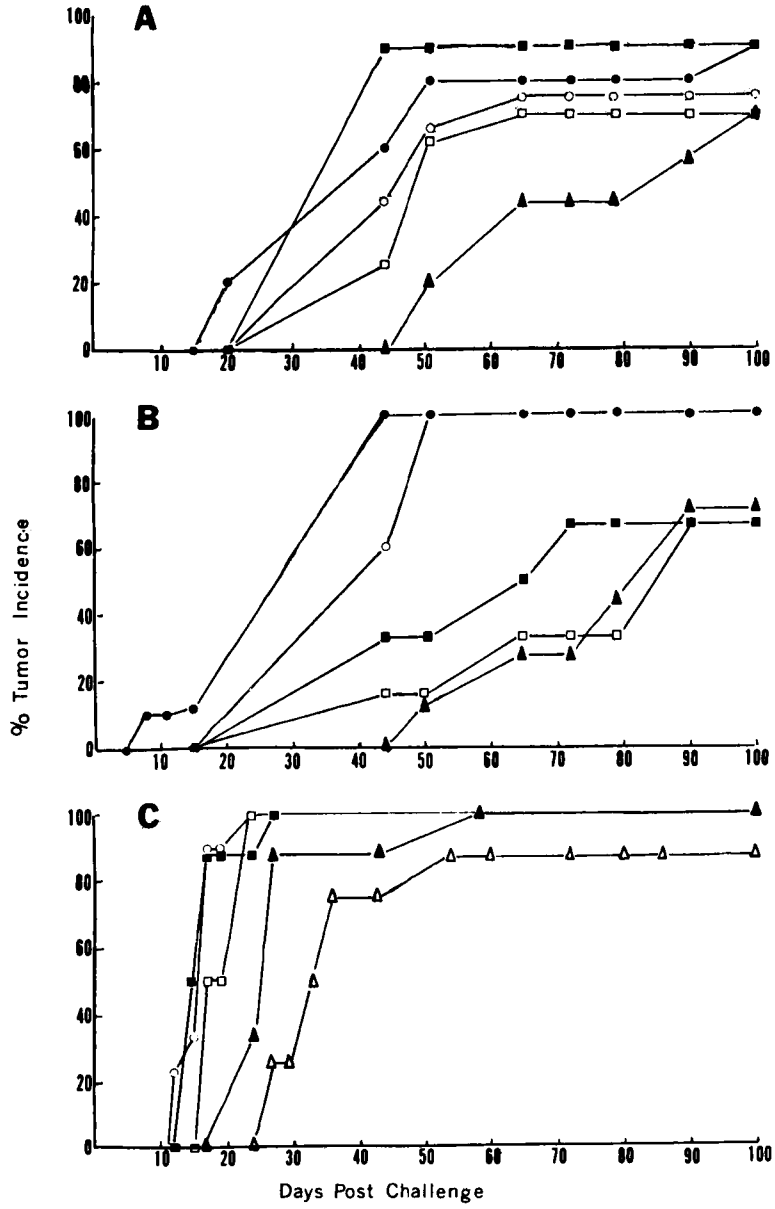


FIGURE 1. Determination of the TPD_{100} of three spontaneous lymphoma lines: (a) CL/LSH-1, (b) IL-LSH, and (c) CL/LSH-2. Animals were challenged sc with 10^1 (▽), 10^2 (▼), 10^3 (□), 10^4 (■), 10^5 (○), or 10^6 (●) viable cells.

A control group receiving no immunization or challenge was maintained in the "clean" facility for the duration of the experiment to verify that lymphoma induction was a phenomenon confined to the lymphoma colony.

As seen in Figure 2, 100 percent of the animals receiving no immunization followed by a live cell challenge developed lymphomas at the challenge site. Only 2 of 11 immunized and challenged animals developed tumors at the challenge site and both of these tumors quickly regressed and never recurred.

Among the 10 animals immunized and given no challenge other than exposure to the lymphomatous colony, only two spontaneous lymphomas were detected, one at 70 days post exposure and one at necropsy 180 days post exposure. All control hamsters that were not immunized developed spontaneous lymphomas by 120 days post exposure. Subsequent experiments have not always given 100 percent tumor incidence among controls; however, the total incidence of spontaneous lymphomas among animals immunized with irradiated lymphoma cells has always been less than 20 percent.

The second experiment was designed to determine whether the three lines of lymphoma carried in vivo would protect against each other with a direct challenge and to indicate whether some common antigen might be detected. Animals were divided into four groups of 30 each. One group was held in the clean facility until the date of challenge and then groups of 10 hamsters were challenged sc with one of the three spontaneous lymphoma lines. The other three groups were immunized with one of the three lines of lymphoma and were cross-challenged with the

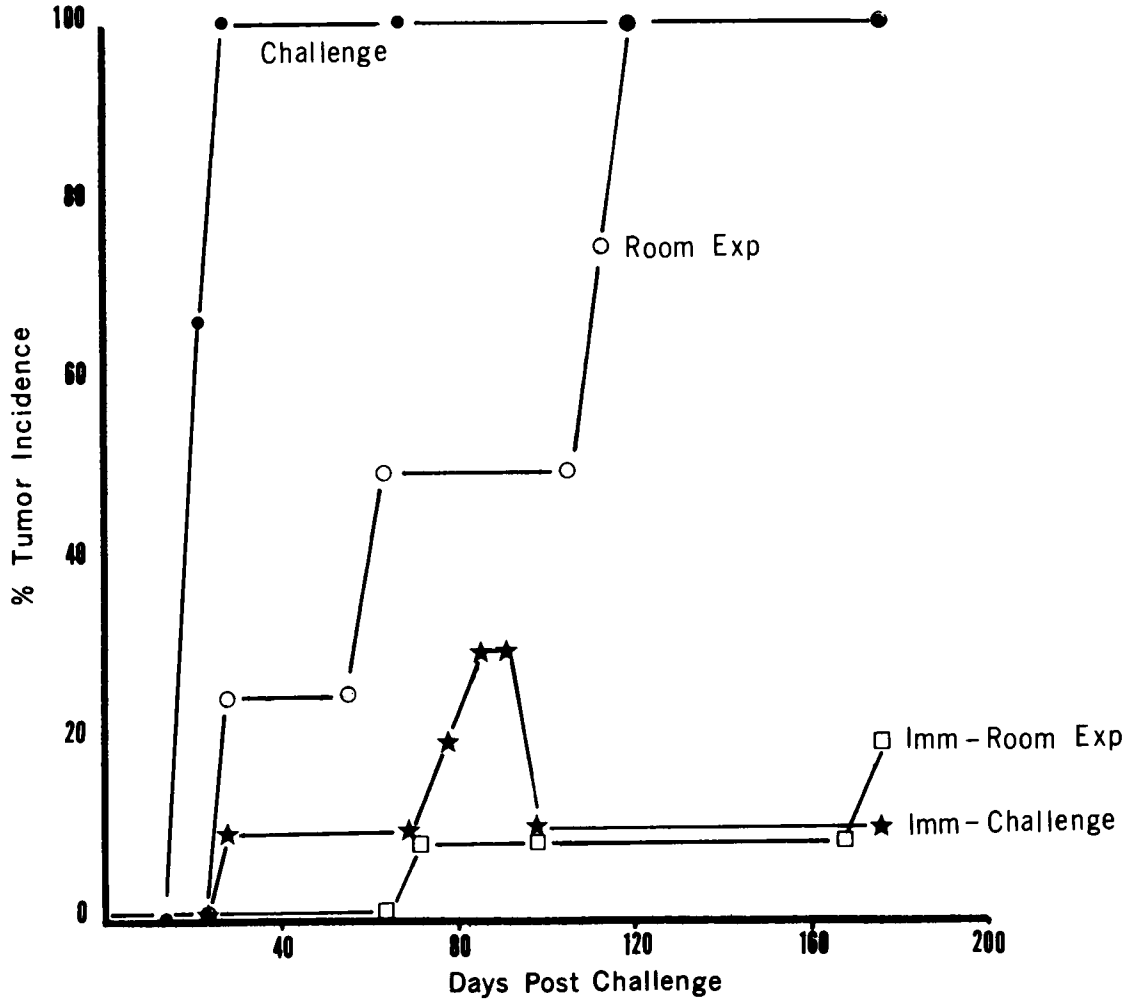


FIGURE 2. Effect of lymphoma immunization on subsequent lymphoma induction: animals were immunized with lymphoma and challenged with 10^4 cells sc (*) or left for "natural exposure" (□). Controls were either challenged sc with 10^4 lymphoma cells (●) or left for natural exposure (○).

other two lines of lymphoma. As seen in Figure 3, all three lines appeared to cross-protect, although immunization with IL/LSH did not give 100 percent protection against the homologous challenge, even though it did protect against heterologous challenge. Of the immunized animals that did not develop sc tumors, none had abdominal lymphomas at necropsy.

In the third experiment, groups were given one to five ip immunizations with irradiated CL/LSH-2 cells and were challenged 10 days after the final immunization (Figure 4). A dose-dependent relationship was observed: one immunization gave some protection against challenge, whereas four immunizations routinely gave excellent protection.

Specificity of Resistance

The specificity of protection afforded by immunization with irradiated lymphoma cells was evaluated by immunizing animals with irradiated SV40 transformed cells, irradiated CL/LSH-2 lymphoma cells, or normal hamster muscle. Controls were not vaccinated. As seen in Figure 5, neither immunization with SV40 transformed cells nor immunization with muscle homogenate gave protection against lymphoma challenge. There was also no significant protection as compared to controls when lymphoma immunized animals were challenged with SV40 transformed cells. Table I includes the data from a number of other experiments in which hamsters were immunized with either SV40 transformed cells, CL/LSH-2 lymphoma cells, muscle, or nothing. Again, some slight protection against SV40 transformed cell challenge is seen in animals immunized with irradiated lymphoma cells, but it is no greater than that afforded by immunizing animals with normal muscle tissue.

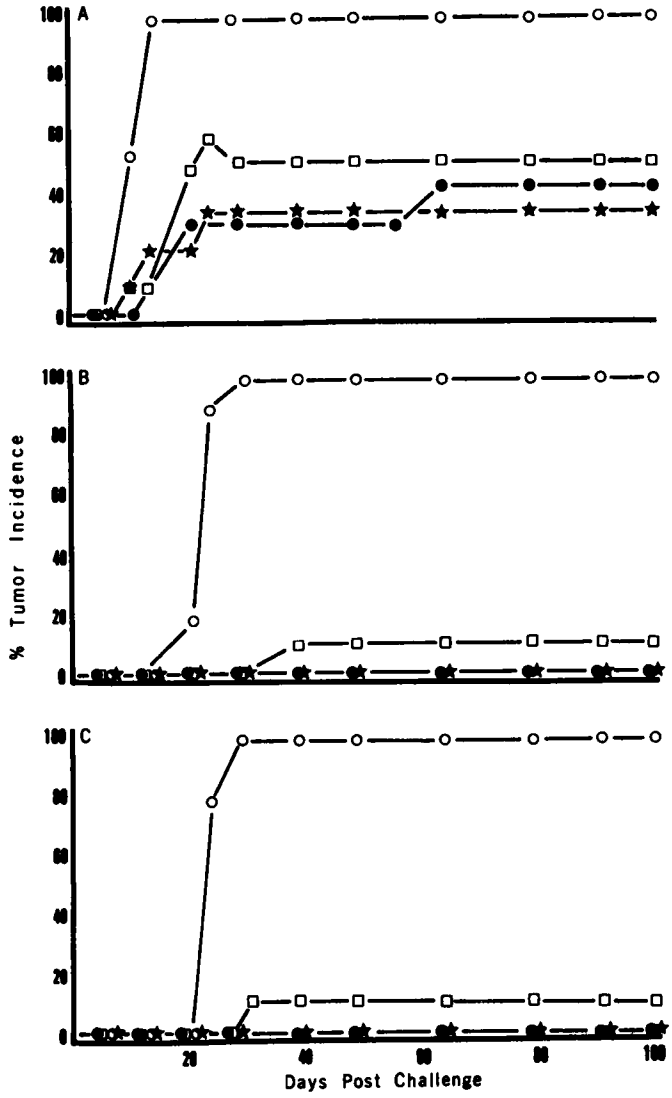


FIGURE 3. Determination of cross-reactivity between the three lines of lymphoma. Animals were immunized ip four times with IL/LSH (*) CL/LSH-1 (□), or CL/LSH-2 (●), or received no immunization (O). Ten days after the last immunization, animals were challenged as follows: (a) 10^4 IL/LSH cells, (b) 10^4 CL/LSH-1 cells, and (c) 10^3 CL/LSH-2 cells.

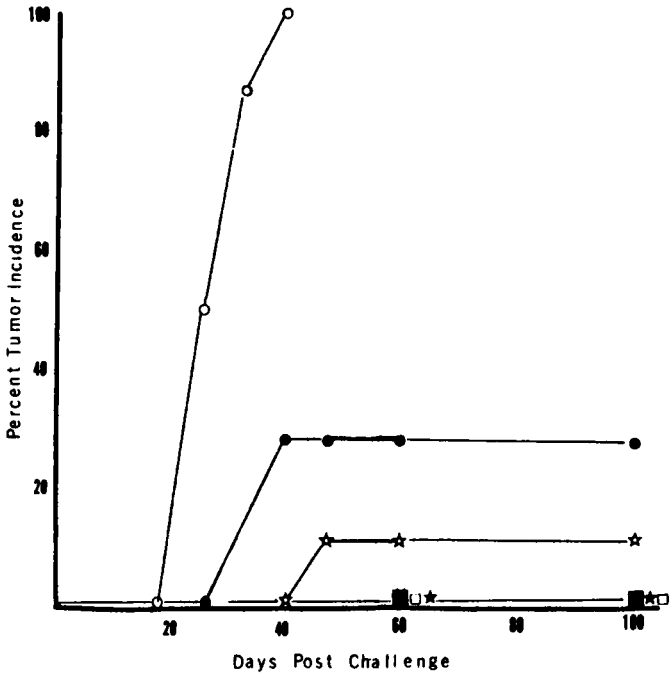


FIGURE 4. Determination of the number of immunizations that would provide adequate protection. Animals were given 1 (⋆), 2 (■), 3 (●), 4 (□), or 5 (*) ip immunizations with irradiated cells and were challenged with 10^3 CL/LSH-2 cells. Controls received no immunization (o)

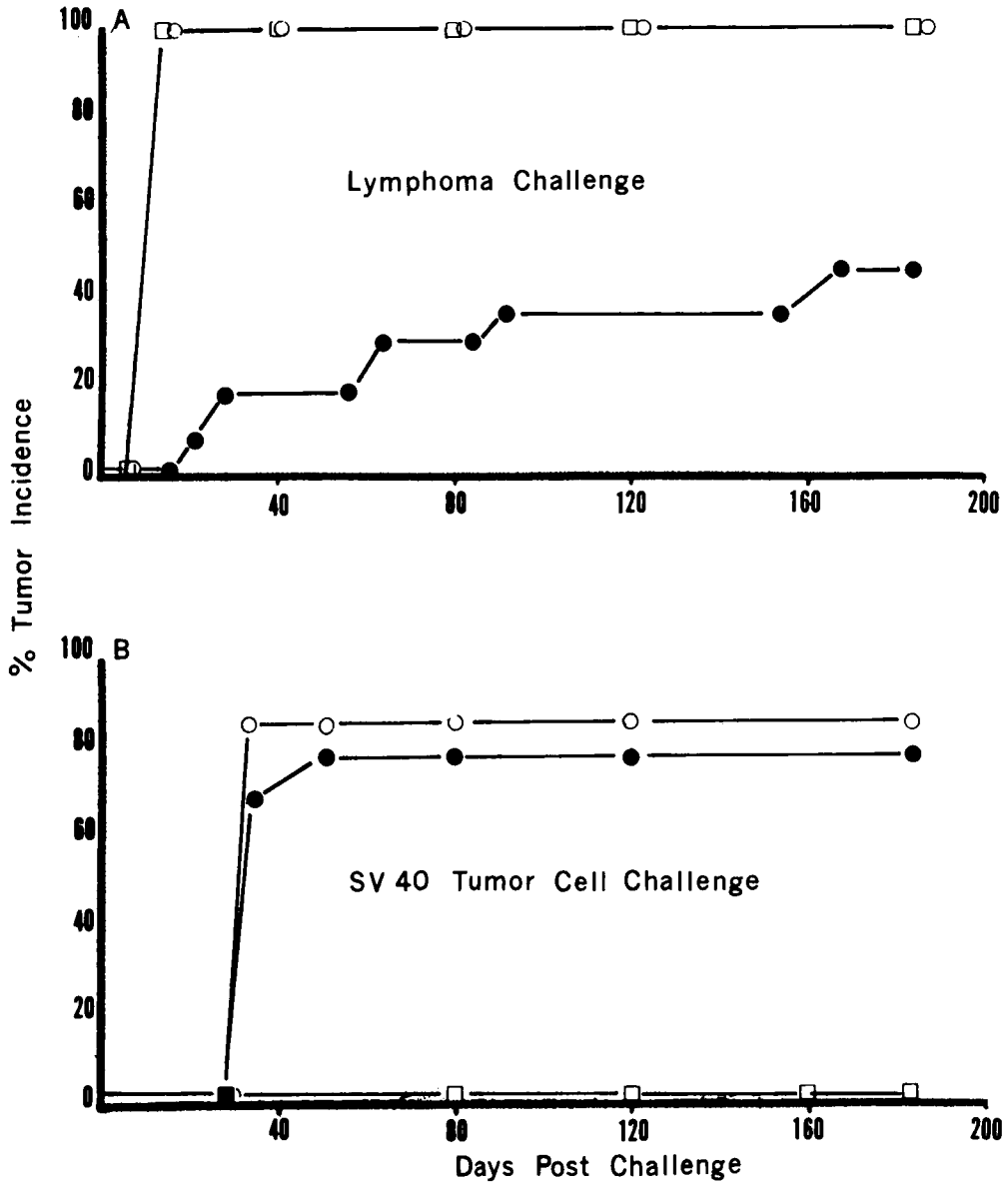


FIGURE 5. Effect of lymphoma immunization on subsequent challenge with lymphoma (a) or SV40-transformed cells (b). Animals were immunized with either lymphoma cells (●), SV40-transformed cells (□), or nothing (o).

TABLE I. Results of experiments to demonstrate presence or lack of cross-protection between lymphomas (CL/LSH-2) and SV40 transformed cells (F5-1).

Immunization	Challenge	No. sc Tumors / No. Animals Challenged+		No. ip Lymphomas	Protection*
CL/LSH-2	F5-1	7/9	(78%)	1 day 50	Slight
None	F5-1	6/7	(86%)	1 day 126	
CL/LSH-2	F5-1	6/8	(75%)	none	Slight
Muscle	F5-1	5/10	(50%)	1 day 99	Definite
None	F5-1	8/9	(88%)	2 days 37, 99	
CL/LSH-2	F5-1	8/9	(88%)	none	None
None	F5-1	6/7	(86%)	none	
CL/LSH-2	F5-1	5/11	(45%)	none	Slight
Muscle	F5-1	8/11	(73%)	none	None
None	F5-1	6/9	(67%)	2 days 120	
CL/LSH-2	F5-1	7/9	(78%)	none	Slight
Muscle	F5-1	4/5	(80%)	1 day 120	Slight
None	F5-1	9/9	(100%)	none	
F5-1	CL/LSH-2	8/8	(100%)	none	None
None	CL/LSH-2	9/9	(100%)	none	
F5-1	CL/LSH-2	7/9	(78%)	none	Slight
None	CL/LSH-2	8/8	(100%)	none	
F5-1	CL/LSH-2	12/12	(100%)	none	None
Muscle	CL/LSH-2	10/10	(100%)	none	None
None	CL/LSH-2	11/11	(100%)	none	

*Protection: Slight = a difference of 8 to 22 percent when compared to controls given no immunization.
Definite = a difference of greater than 30 percent when compared to controls given no immunization.

+Does not include animals lost to intercurrent disease.

Transfer of Resistance

A final test of the effectiveness of immunization against lymphoma was to adoptively transfer immune effector cells mixed with tumor cells to normal animals. Donors were given either no immunization or five ip immunizations with CL/LSH-2 lymphoma cells. PEC and spleen cells were harvested from both immune and normal animals ten days after the last immunization, mixed with tumor cells at a 200:1 ratio of effector: tumor cells, and then injected into normal recipients. Animals were given a 10^3 tumor cell challenge. As shown in Figure 6, all animals receiving effector cells from nonimmunized donors developed tumors within 38 days. Of those receiving effector cells from immunized donors, the first tumor was seen in 38 days, and the total incidence was only 10 percent by 100 days.

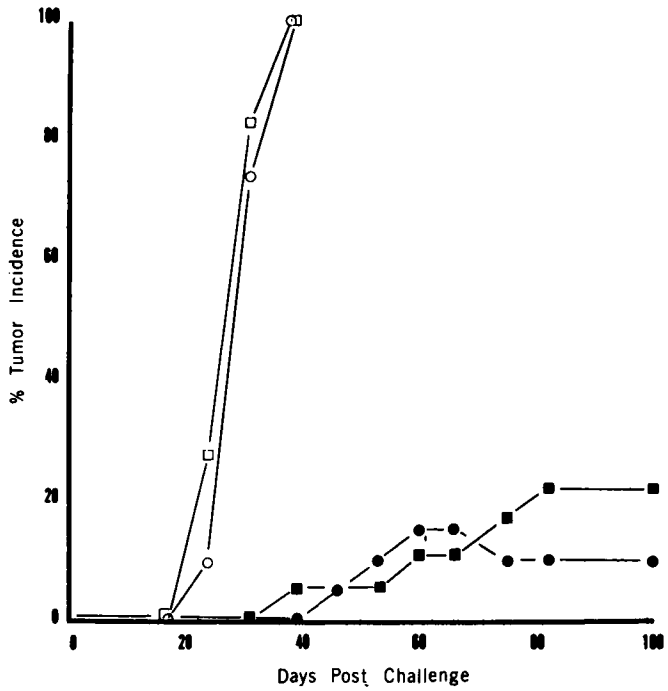


FIGURE 6. Adoptive transfer of resistance to lymphoma challenge. PEC (o) and spleen cells (□) were harvested from immune (solid symbols) and normal (open symbols) animals, mixed with tumor cells (10^3), and injected into normal recipients.

CHAPTER IV

DISCUSSION

The occurrence of a third epizootic of lymphomas in The University of Tennessee hamster colony prompted an investigation to determine whether hamsters could be protected against the lymphoma by immunization with irradiated lymphoma cells. Simultaneously, a study was undertaken to investigate whether the lymphoma was related to any agent that had been used in the colony prior to the epizootics.

Immunization with irradiated lymphoma cells has proved to be an effective means of protecting animals against subsequent challenge with viable tumor cells. It was found that hamsters immunized with irradiated lymphoma cells not only were resistant to challenge with viable lymphoma cells (Figure 3, page 94) but also did not develop spontaneous lymphomas after being exposed to the contaminated facility (Figure 2, page 92). Since the causative agent of the lymphoma has not been identified, it is not yet possible to say if the immunity detected in the above studies is in response to an agent antigen alone, an agent-specified antigen at the lymphoma cell surface, or pure nonvirion TATA. Adoptive transfer assays show that significant protection against lymphoma challenge results when immune effector cells are transferred to normal recipients (Figure 6, page 99). This finding suggests that cell-mediated immunity is responsible for tumor resistance, indicating that the lymphoma agent's antigens may be on the surface of the lymphoma cells and that the response is

not limited to a response against an agent released from the lymphoma cells.

Olsen et al. (61) recently reported that immunization of kittens with a killed oncornavirus tumor cell vaccine prevented induction of tumors after challenge with an oncogenic feline sarcoma virus. Production of virus-neutralizing antibody was not seen nor was viremia prevented; however, their data indicate that immunity to a membrane antigen was sufficient to induce immunity to a disease that has been horizontally transmitted in the natural environment. Thus it is possible that the immunity against spontaneous lymphomas in hamsters was also directed against a membrane-bound antigen.

For most tumors induced by viruses, the transformed cells express a virally specified antigen that is found on all tissues transformed by that virus. The fact that immunization with irradiated lymphoma cells will protect against induction of spontaneous lymphomas as well as against direct challenge with viable lymphoma cells again suggests that an agent-associated antigen is on the lymphoma cell membrane. In addition, the ability of animals immunized with the three lines of lymphoma, which have been passaged up to 30 times in vivo, to resist direct challenge with viable lymphoma cells indicates that the antigen is stable and is common to cells transformed by the lymphoma agent. Possibly the antigen being detected is virally specified; however, it has not been conclusively shown that the lymphoma agent is a virus.

Virally-transformed cells may also possess nonvirion tumor-associated antigens in addition to the virally specified antigens. Possibly the antigen detected is not the only antigen on the lymphoma

cells, since the IL/LSH line does not give 100 percent protection against itself even though it does protect against challenge with the other lymphoma lines (Figure 3, page 94). This asymmetry of protection suggests that another antigen may be required in conjunction with the agent-associated antigen for complete resistance to challenge.

SV40 was the only oncogenic agent known to have been used by this laboratory prior to two of the epizootics and SV40 transformed cells were used prior to all three epizootics. The CL/LSH-2 line does not seem to share any transplantation rejection antigens with SV40 transformed cells, as suggested by the results obtained from the direct challenge experiments shown in Figure 5 and Table I, pages 96 and 97, respectively. Animals immunized with SV40 transformed cells developed spontaneous lymphomas in the same proportions as controls, whereas lymphoma immunized animals from the same experiments remained free of spontaneous lymphomas. Indeed, the presence of a separately maintained, disease-free facility only 100 yards away in which SV40 and SV40 transformed cells were used continuously for three years further indicates that SV40 is not the responsible agent.

These data taken together clearly show that it is possible to prevent the induction of spontaneous lymphomas in hamsters exposed to the third epizootic. Current work centers on isolating the agent involved and determining how it is transmitted. It is hoped that this information will then resolve the question of whether the antigen(s) that were detected are TATA (nonvirion) or membrane-associated agent-specified antigens on the surface of the lymphoma cells.

PART V

VIROLOGY

CHAPTER I

INTRODUCTION

It has been suggested that most, if not all, lymphoid neoplasms in animals have a viral etiology (28). Virus has been demonstrated in tumorous tissues by electron microscopy and by a variety of immunologic tests, and oncogenic virus has been recovered from tumor bearing animals. Most of the viruses recovered have been RNA viruses (C-type or oncornaviruses) (32,56,58,87) although some DNA viruses have also been found (15,29,54,60).

C-type virus has been seen in the tumorous tissues of hamsters involved in the spontaneous lymphoma outbreaks reported by Horn and Siewert (37), Strauli and Haemmerli (76,77), Kistler (48), and Gardner et al. (23) and has been considered to be the cause of the last four outbreaks. However, Freeman et al. (20) found that hamster C-type virus could also be seen in association with a variety of non-lymphoid tumors induced by the chemical carcinogens dimethylbenzanthracene and methylcholanthrene and by the oncogenic viruses SV40 and polyoma. The role of the C-type virus in the induction of these non-lymphoid tumors was not clear, but the data raise the possibility that the C-type virus seen in the hamster lymphoma outbreaks was not necessarily the oncogenic factor.

Three spontaneous outbreaks of lymphomas occurred in hamsters kept at The University of Tennessee (1). The outbreaks were unusual in

that natural horizontal transmission of the tumors occurred. The cause of the outbreaks was not readily identified, but an antigen common to the hamster C-type viruses was detected in four of twelve tumors examined. Therefore, cell-free preparations were made from tumors of hamsters sent to the National Cancer Institute. These preparations were tested for oncogenicity in neonatal hamsters and the results suggested that an oncogenic virus was recovered and transmitted (39). Data from The University of Tennessee further suggested that the lymphoma agent was an oncogenic virus since a common antigen was detected on the tumorous lymphoid cells (85). This antigen was probably associated with the lymphomagenic agent since hamsters immunized with irradiated lymphoma cells were resistant to the induction of lymphomas following exposure to the lymphomatous colony.

Cell-free preparations were made at The University of Tennessee in order to recover and characterize the lymphoma agent. These experiments were conducted in the facility where horizontal transmission of lymphomas occurred, but controls were included in each experiment to assess the incidence of spontaneous lymphomas. Oncogenic preparations were those that yielded a higher incidence of lymphomas at an earlier time than controls. Two of 24 cell-free preparations tested were oncogenic and the results are described.

CHAPTER II

MATERIALS AND METHODS

Animals

Randombred LVG strain Syrian golden hamsters (Lakeview, Newfield, New Jersey), were bred at The University of Tennessee in a hamster facility in which no spontaneous lymphomas had been detected. Pregnant females from this lymphoma-free facility were transferred two days prior to parturition to a facility in which horizontal transmission of lymphomas occurred (lymphomatous) (1). Neonates were inoculated with cell-free preparations at 1, 4, or 10 days of age and were weaned between 21 and 28 days of age. Both the progeny and the dams were palpated weekly. The LVG strain hamsters were used in preference to the inbred LSH strain since the latter had poor survival rates (84).

Tumors

Both spontaneous lymphomas and lymphomas passaged subcutaneously (sc) (85) were used. Tumor bearing animals were sacrificed with CO_2 and exsanguinated by cardiac puncture. Non-necrotic areas of tumors were excised, rinsed in HBME (Eagle's Basal Medium with Hank's Salts) and cleaned of extraneous tissue and fat before further treatment.

Normal Tissues

Spleens of normal animals were processed as control tissue since the spleen was the largest, most readily obtained source of normal

hamster lymphoid tissue. Adult LVG hamsters from the lymphoma-free facility were sacrificed with CO_2 and the spleens removed aseptically. They were then rinsed in HBME and trimmed of fat before further processing. Approximately four grams of spleens were obtained from 40 hamsters.

Cell-Free Preparations

Information provided by Dr. R. J. Heubner (National Cancer Institute) had suggested that a modification of the Moloney virus isolation procedure (57) be used to obtain the cell-free preparations. This modified procedure is detailed in Appendix D. Briefly, tumor tissue was finely minced and homogenized intermittently in an Oster blender at room temperature for one hour in 0.153 M potassium citrate containing .01 mg/ml protamine sulfate (Nutritional Biochemicals, Cleveland). Cellular debris was removed after two centrifugations at 1500 x g, each for 20 minutes and one at 17,000 x g for 1 minute. Finally, centrifugation at 40,000 x g yielded a pellet that contained the oncogenic agent. The pellet was resuspended in 0.02 M sodium citrate, pH 6.7, sonicated, then centrifuged at 1500 x g for 20 minutes. The supernatant fluid was removed and filter-sterilized using a Millipore Swinnex apparatus with a 0.45 mu filter. The pellet was mixed with 1 ml sodium citrate, pH 6.7. Both pellet and supernatant were then aliquotted separately and frozen at -80°C.

Evaluation of Oncogenicity

Frozen cell-free preparations were quickly thawed and 0.05 ml was injected ip or sc into at least two litters of neonatal hamsters.

Animals were observed daily and palpated weekly. Moribund animals or animals with palpable tumors were sacrificed and autopsied. Tissues with abnormalities were removed, fixed in acetic acid-alcohol-formalin buffer and submitted to Dr. Bruce Bellomy (Fort Sanders Presbyterian Hospital) for histopathological evaluation.

Controls for most of these experiments included at least two litters of hamsters given no inoculation, two litters of hamsters inoculated with sodium citrate alone, and two litters of hamsters inoculated with a cell-free preparation of normal hamster spleen.

Data Presentation

Results of experiments to test the oncogenicity of cell-free preparations are presented in tabular form. Included are the number of animals inoculated, the number of animals weaned and the number of animals lost to unknown causes, to known causes other than lymphoma, and to lymphoma. The days after inoculation on which animals died are also included. Experiments were terminated 120 days after inoculation of neonates and the animals were autopsied. Hamsters with lymphomas at autopsy were grouped with those dying with lymphoma prior to the end of the experiment.

The lymphoma incidence in each group is calculated as the ratio of hamsters with lymphoma to the number of hamsters weaned. A chi square test was used to test for significant differences in lymphoma incidence between control and experimental groups.

CHAPTER III

RESULTS

Two of 24 cell-free preparations of tumors tested were found to be oncogenic. Both preparations were tested in at least eight litters of hamsters.

The first of the oncogenic "cell-free" preparations was made from a cervical lymphoma carried in vivo, CL/LSH-2 (85). Tumors were taken 16 days after sc passage and prepared according to the modified Moloney virus isolation procedure described in Materials and Methods and Appendix D with the following changes: 0.01 mg/ml hyaluronidase was used for the homogenization step rather than 0.01 mg/ml protamine sulfate; the final resuspension was in 0.05 M sodium citrate, pH 6.7 rather than 0.02 M sodium citrate, and the pellet from the 40,000 x g centrifugation was simply resuspended, sonicated, aliquotted and frozen without a filter sterilization step.

The oncogenicity of the preparation was assayed by inoculating neonatal hamsters either sc or ip with 0.05 ml of the thawed preparation. The incidence of lymphomas in these hamsters is presented in Table I. Lymphomas were found in 11 of 16 (69 percent) hamsters inoculated ip and in 10 of 14 (71 percent) inoculated sc. Tumors were found only in the peritoneal cavity, even in hamsters inoculated sc, and most appeared to originate in the liver.

TABLE I. Incidence of lymphomas in hamsters inoculated at 1 day of age with a cell-free preparation made from a passaged cervical lymphoma.

Treatment	Born	Weaned	Number of Deaths and Age Post-Weaning[a]					
			Non-Lymphoma	Percent	Unknown	Percent	Lymphoma	Percent
0.05 ml sodium citrate ip	19	9	2 (54,109)	22	0		2 (54,131)	22
0.05 ml cell-free normal spleen ip	19	8	2 (40,126)	25	0		0	
0.05 ml cell-free cervical lymphoma ip	22	16	1 (36)	6	0		11 (31,131)	69
0.05 ml cell-free cervical lymphoma sc	20	14	0		1(120)	7	10 (43,131)	71

[a]Numbers in parenthesis indicate the day of death after inoculation.

The oncogenicity of the preparation was then tested in hamsters 1 day, 4 days, and 10 days old to determine if the age at which the hamsters were inoculated was important. The data in Table II indicate that hamsters inoculated at 1 day of age were most susceptible to the oncogenic effects of the cell-free preparation: 8 of 15 hamsters inoculated at 1 day of age developed lymphomas (53 percent); 8 of 20 hamsters inoculated at 4 days of age developed lymphomas (40 percent); 5 of 17 hamsters inoculated at 10 days of age developed lymphomas (29 percent). Lymphomas were detected as early as 30 days post inoculation.

A second oncogenic preparation was made from an inguinal lymphoma that had been passaged in vivo, IL/LSH (85). The modified Moloney procedure was used, but only the final filtered supernatant was tested for oncogenicity. Data from this initial test are presented in Table III. There was a pre-weaning loss of approximately 30 percent in all groups. Each group originally consisted of two litters of hamsters, but three litters died or were killed by the mother within two weeks of inoculation. The small number of survivors makes the incidence of spontaneous lymphomas very high in animals simply exposed to the lymphomatous colony. However, the group with the highest incidence of lymphomas at the earliest date is the group inoculated with the cell-free preparation.

This cell-free preparation was tested in another group of neonates after diluting it with an equal volume of 0.02 M sodium citrate, pH 6.7, or with an equal volume of presumably normal hamster serum or tumor bearer hamster serum. The tumor bearer serum was collected by cardiac puncture from a hamster inoculated 21 days previously with

TABLE II. Lymphomas in animals inoculated with cell-free preparations made from a passaged cervical lymphoma and tested in 1 day, 4 day, and 10 day old hamsters.

Treatment (Age)	Born	Weaned	Deaths Post-Weaning[a]			Percent[b]
			Non-Lymphoma	Unknown	Lymphoma	
None (1)	16	12	3 (62)	1 (38)	4 (43-118)	33
(4)	33	27	3 (66-92)	3 (68-78)	4 (35-118)	15
0.05 ml sodium citrate ip (1)	27	15	2 (39,80)	0	3 (75-102)	20
0.05 ml normal spleen cell-free ip (1)	27	15	0	1 (77)	4 (50-131)	27
0.05 ml cervical lymphoma cell-free ip (1)	28	15	0	0	8 (36-118)	53
(4)	23	20	0	0	8 (30-118)	40
(10)	19	17	0	0	5 (80-118)	30

[a]Numbers in parentheses indicate the days post inoculation on which animals died or were killed.

[b]Percentages are calculated on the basis of the number of animals weaned.

TABLE III. Oncogenicity of a cell-free preparation made from a passaged inguinal lymphoma.

Treatment	Born	Weaned	Deaths Post Weaning[a]			Percent[b]
			Non-Lymphoma	Unknown	Lymphoma	
None	10	6	0	0	3 (48,73,107)	50
0.05 ml sodium citrate ip	12	9	1 (94)	0	1 (79)	11
0.05 ml normal spleen cell-free ip	21	15	3 (45-79)	0	3 (36,45,113)	20
0.05 ml cell-free inguinal lymphoma ip	16	7	0	0	4 (35,56,61,61)	57

[a]Numbers in parentheses indicate the days post inoculation on which animals died or were killed.

[b]Percentages are calculated on the basis of the number of animals weaned.

IL/LSH cells. Normal serum was collected by cardiac puncture from a normal LVG from the clean facility. Serum was collected on the day the neonates were to be inoculated with the cell-free preparations, allowed to clot two hours at room temperature, centrifuged at 1500 x g for 20 minutes to remove red blood cells, filter-sterilized, then heat inactivated for 30 minutes at 56°C. Once the serum had cooled, an equal volume was mixed with the cell-free preparation and was inoculated into the neonates.

Results from the above experiment are presented in Table IV. There was a pre-weaning death of 46 percent in control hamsters and 55 percent in hamsters inoculated with the preparation diluted with buffer. Treatment of the preparation with normal hamster serum lowered the pre-weaning mortality to 28 percent and treatment of the preparation with tumor bearer hamster serum lowered the preweaning mortality to 5 percent. Treatment with hamster serum also affected the oncogenicity of the preparation: neonates treated with the preparation alone had a lymphoma incidence of 44 percent (4 of 9 survivors), hamsters inoculated with the preparation treated with normal hamster serum had a lymphoma incidence of 27 percent (4 of 15 survivors), while hamsters inoculated with the preparation treated with tumor bearer hamster serum had no lymphomas. Additionally, treatment of the preparation with tumor bearer hamster serum may have resulted in protection of the hamsters against intercurrent disease since no hamsters were lost to causes of death other than lymphoma.

This oncogenic cell-free preparation was sent to the Frederick Cancer Research Center for electron microscopic examination. According

TABLE IV. Neutralization of oncogenicity of a cell-free preparation made from a passaged inguinal lymphoma.[a]

Treatment	No. Born	No. Weaned	Deaths Post Weaning[b]			
			Non-Lymphoma	Unknown	Lymphoma	Percent[c]
None	62	33	3 (41-102)	5	3 (32-109)	9
0.05 ml sodium citrate ip	20	13	1 (79)	2 (79,108)	2 (158)	15
0.05 ml cell-free inguinal lymphoma ip	20	9	1 (52)	0	4 (32-104)	44
0.05 ml cell-free inguinal lymphoma ip + normal serum	21	15	1 (151)	0	4 (32-158)	27
0.05 ml cell-free inguinal lymphoma ip + tumor bearer serum	20	19	2 (81, 140)[d]	0	0	0

[a]Prior to inoculation of the hamsters, the preparation made from the passaged inguinal lymphoma was mixed with an equal volume of either sodium citrate, normal hamster serum or tumor bearer serum from hamsters bearing the passaged inguinal lymphoma.

[b]Numbers in parentheses indicate the days post inoculation on which animals died or were killed.

[c]Percentages are calculated on the basis of the number of animals weaned.

[d]These two hamsters escaped and were killed and autopsied upon recapture.

to Dr. Cora Bucana and Ms. Suzanne Breesman, spoked particles and papova/picodna virus-like particles were seen in the preparation. No C-type viral particles were noted (personal communication).

Twenty-two other cell-free preparations of passaged and spontaneous tumors were made and tested for oncogenicity. Preparations tested included cell-free preparations of spontaneous ip and liver lymphomas, passaged cervical lymphomas and liver lymphomas occurring after hamsters were inoculated with a cell-free preparation. None were as oncogenic as the two described above.

CHAPTER IV

DISCUSSION

Hamsters have endogenous virus of two types: a spoked or radial particle first detected by Bernhard and Tournier (6) for which oncogenicity has been suggested (9) but has not been demonstrated (42); and C-type virus which is oncogenic for neonatal hamsters (45) and which can be demonstrated in normal hamster fetal tissues (83) as well as in association with non-lymphoid tumors induced by the oncogenic viruses SV40 and polyoma and by the chemicals dimethylbenzanthracene and methylcholanthrene (20).

Graffi et al. (29) have suggested some hamsters may have a third type of endogenous virus that resembles a papova virus. This virus was discovered in epithelial tumors that occurred after inoculation of neonatal hamsters with cultures of hamster embryonic tissue grown in vitro (27). Cell-free extracts of the epithelial tumors induced lymphomas rather than epithelial tumors when inoculated into neonatal hamsters, and only C-type virus was seen in the lymphomatous tissues (28). The lymphomas could be passaged cell-free, but the lymphomagenic activity of the cell-free preparations was seen only in hamsters from a colony free of spontaneous tumors and rarely in more than 50 percent of the hamsters inoculated. Hamsters from a colony in which lymphomas occurred spontaneously were resistant to the oncogenic activity of the cell-free preparations. DNA from the epithelial tumors was also

lymphomagenic in the susceptible strain of hamsters and the lymphomagenic activity was destroyed by treatment with DNase but was resistant to treatment with pronase. DNA from a variety of human tumors was also lymphomagenic (26) and the tumors could be transmitted with cell-free preparations. Graffi attributed all the lymphomagenic activity to induction of an endogenous hamster C-type virus since only C-type virus was seen in the lymphomas.

Three outbreaks of lymphomas occurred in a hamster facility at The University of Tennessee (1). C-type viral antigens were demonstrated in 4 of 12 hamster tumors examined and the lymphomas shared a cellular antigen which could be used to immunize hamsters against challenge lymphomas (85). Cell-free preparations of the tumors were made in order to recover an oncogenic agent since the epizootiologic and immunologic data indicated an oncogenic virus was probably responsible for the lymphoma outbreaks.

The experiments to isolate an oncogenic agent were carried out in a facility with a spontaneous lymphoma incidence that averaged 17 percent of all hamsters that survived to weaning (Table V). Results of experiments were confused by high pre-weaning mortality rates and post-weaning losses to intercurrent diseases. Nevertheless, it was possible to identify two cell-free preparations which had oncogenic activity.

Preliminary experiments to define the cell-free preparations demonstrated that neonatal hamsters were most susceptible to the oncogenic effects of the preparations and that the susceptibility decreased by four days post partum. This finding is similar to that reported by Girardi et al. (25) for SV40 virus and by Friedman and

TABLE V. Lymphoma incidence in hamsters used as controls for testing oncogenicity of cell-free preparations.[a]

Treatment	No. Born	No. Weaned	Percent	No. Lymphomas	Percent[b]
None	313	190	61	26	14
0.05 ml ip sodium citrate	234	137	59	26	19
0.05 ml ip normal spleen cell-free preparation	233	167	72	26	16

[a]Pregnant LVG females were exposed to the lymphomatous colony two days prior to parturition. Neonates were inoculated ip and checked frequently for palpable masses or evidence of disease. Animals were weaned and sexed by four weeks of age and then were palpated weekly for tumors.

[b]Percentage is calculated on the basis of the number of animals weaned.

Goldner (21) for SV40 and polyoma. The greater susceptibility of neonates was suggested to be due to the immaturity of the immune response. However, adult hamsters were susceptible to SV40 tumorigenesis when inoculated with large doses of virus (11) and adult hamsters were susceptible to spontaneous lymphoma induction following exposure to the lymphomatous facility (1). The susceptibility of both neonatal and adult hamsters suggests that either the adult hamsters are being spontaneously exposed to large inoculums of the lymphoma agent, that the agent is immunosuppressive, or that the agent is active regardless of the immune status of the host.

Electron microscopic examination of an oncogenic cell-free preparation revealed only papova/picodna virus-like particles. It is interesting to note that the papova virus described by Graffi et al. (28) was lymphomagenic. Further experimentation should reveal whether or not the papova virus seen in the cell-free preparations described previously is oncogenic.

It is possible that the lymphomas were induced subsequent to an interaction of the papova virus and an endogenous RNA virus. Evidence for the interaction of herpes viruses and oncornaviruses has been reported for poultry (19) and guinea pigs (38) and Freeman et al. (20) have provided evidence that this type of interaction might even occur in hamsters inoculated with SV40 virus. Since most hamsters appear to have endogenous C-type virus it may prove difficult to demonstrate whether or not C-type virus is involved in the lymphoma outbreaks. However, preliminary evidence provided by Dr. R. J. Heubner indicates that immunizing hamsters against HaLV will not provide immunity against

horizontally transmitted University of Tennessee hamster lymphomas (39).

Animals bearing virally induced tumors usually develop immune reactivity against their tumors. This reactivity is directed against antigens of the transformed cells but may also be directed against any viral agents shed by the cells. The protection against lymphoma induction seen in hamsters given an oncogenic cell-free preparation treated with heat-inactivated tumor-bearer hamster serum indicates that the tumor-bearer serum contained an anti-lymphoma agent activity. The anti-lymphoma activity is probably not due to complement since the serum was heat-inactivated at 56°C for 30 minutes prior to use. The activity may well be antibody since serum from animals bearing spontaneous or passaged lymphomas is anti-complementary (55) and anti-complementary activity may be attributed to circulating antigen-antibody complexes.

The reason for loss of oncogenicity of a cell-free preparation after treatment with normal hamster serum is not known. Since lymphomas were detected in the facility from which the normal hamster was obtained three months after the serum was taken, the possibility exists that the donor hamster had already developed a mild reactivity against the lymphoma agent. Alternatively, the lymphoma agent may involve endogenous virus to which the donor already had reactivity.

The transmission of lymphomas with cell-free preparations that can be inactivated with treatment with tumor-bearer serum does indicate that a viral agent is responsible for the induction of lymphomas. However, the lack of oncogenicity of a number of cell-free preparations made from both spontaneous and in vivo passaged tumors is puzzling.

The most likely explanations are that the tumors were not shedding virus or that the recipient hamsters were either immune to or lacked an endogenous virus necessary for lymphoma induction. It has been shown in the SV40 system that virally transformed cells do not necessarily shed virus (25) and for years, investigators were unable to transmit Marek's disease using cell-free preparations of tumors, although the infectious herpes virus was eventually recovered from the feather follicle epithelium (60).

Graffi et al. (28) suggested that the lack of oncogenicity of cell-free preparations may be a result of infection of the stock with passage of resistance to the progeny. Since lymphomas were eventually detected in the facility which was the source of hamsters for the oncogenicity experiments, there is a possibility that transmission of protection occurred. However, the fact that preparations that were tested were oncogenic in duplicate experiments indicates the lack of oncogenicity in other preparations was probably due to lack of agent in the preparations, rather than increased resistance of test stock.

None of the cell-free preparations was oncogenic in all the neonates inoculated. It is conceivable that oncogenicity of the preparations might be enriched by serial in vivo passage of cell-free preparations as was accomplished in the murine system (4). However, if the enrichment procedure is unsuccessful, it may be necessary to conclude that host factors such as the genetic background of the animal have a role. Since random-bred hamsters were used, variation in susceptibility within a litter is not unreasonable.

The high pre-weaning mortality rate in some experiments remains to be explained. Some preparations were both oncogenic and lethal, yet one of these preparations, when treated with tumor-bearer serum lost both its oncogenic and lethal effects. Precedent has been set in other systems for using lethality as a criterion for activity of an oncogenic agent (19). Until the pre-weaning mortality can be alleviated and demonstrated not to affect the incidence of lymphomas, it may be concluded that the two are related.

In conclusion, these data indicate an agent (or agents) is responsible for the induction of lymphomas since the tumors can be transmitted with cell-free preparations. This agent which is most oncogenic for neonatal hamsters can be neutralized by serum from a tumor-bearing animal. Electron microscopy of an oncogenic cell-free preparation reveals a spoked particle or a papova/picodna-like particle. Either or both of these particles may be the oncogenic agent; further work should resolve this question. However, it may not be possible to assess the role of endogenous virus in the induction of lymphomas until either hamsters free of endogenous virus can be obtained, or the role of the endogenous virus can be minimized, perhaps by immunizing hamsters to the virus.

PART VI

GENERAL SUMMARY

GENERAL SUMMARY

This dissertation has been concerned with the identification of factors responsible for precipitating outbreaks of lymphomas, with a description of the lymphomas, with the demonstration that the lymphomas have a common antigen (possibly membrane bound) and with the recovery of two cell-free preparations that caused an increased incidence of lymphomas when inoculated into neonatal hamsters.

The purpose of these investigations has been to determine the cause of the lymphomas and one possible explanation is that a C-type virus was induced in the LSH strain of hamsters subsequent to experimental manipulations involving SV40 transformed cells or virus. Horizontal transmission of the virus would then occur through use of contaminated equipment, mechanically through spread of contaminated litter by animal caretakers or the animals themselves, or by an airborne route on dust particles or as aerosols.

The common, possibly membrane bound antigen detected on the in vivo passaged lymphomas suggests the lymphomas are of viral origin. A viral origin is also suggested from the young age of the involved animals (from one month old on) and from the fact that progeny of hamsters bred in the lymphomatous colony apparently acquire immunity to the lymphomas from their dam. Neither the early onset of the tumors, nor the acquisition of immunity would be expected if the lymphomas were chemically induced and there have been no reports of bacterially induced lymphomas in hamsters. The ability to cause an increased incidence of

lymphomas with a filtered cell-free preparation and to neutralize this effect with tumor bearer serum further suggests a viral origin for the lymphomas as opposed to a chemical or bacterial origin.

The lymphoma agent does appear related to two other disease processes of probable bacterial origin: enteritis and pyelonephritis. Both diseases were seen prior to or concomitantly with the lymphoma outbreaks and animals resistant to lymphomagenesis were also resistant to enteritis and pyelonephritis. If the lymphoma agent were an immunosuppressive virus, increased susceptibility to disease would occur with viral infection and an apparent resistance to disease would be evident with acquisition of immunity to the virus. Histopathology of diseased animals should also indicate an immunosuppressed state and it was noted that animals with pyelonephritis had an inconspicuous thymus. Thus it would appear quite possible that the lymphoma agent is an immunosuppressive virus, similar, perhaps to the immunosuppressive feline leukemia C-type virus.

The immunosuppressive virus is possibly not the only agent involved. Warts similar to the Graffi papillomas (28) occurred in a very few LSH hamsters, but unlike the Graffi papillomas, were transmissible with cell-free preparations and did not appear to increase the incidence of lymphomas when inoculated into neonatal LVG hamsters. Although the warts were not examined for virus, cell-free transmission did suggest a viral origin. Graffi found only papova virus in the spontaneous papillomas occurring in his colony, but only C-type virus was seen in the lymphomas induced with cell-free preparations of the papillomas. DNAse treatment of the preparations destroyed the

lymphomagenicity (26). Since papova/picodna virus-like particles were seen in a lymphomagenic cell-free preparation of the in vivo passaged IL/LSH lymphoma, the virus-like particles might be similar to Graffi's papova virus and capable of either inducing an endogenous virus which would cause lymphomas or of causing lymphomas itself.

Involvement of two viruses might help explain the fact that lymphoma outbreaks first occurred after purchase of the LSH strain and subsequent to the housing of the two strain in close proximity. One might surmise the LVG strain was resistant to the agents induced by experimental procedures used at The University of Tennessee. The LSH strain, on the other hand, would not have been exposed to these agents at the supplier and thus would develop lymphomas when brought to the University. The amount of virus released by the LSH might be of sufficient quantity to overcome the resistance of the LVG strain, resulting in lymphomas but in lower incidence than in the LSH strain.

Another explanation is that the LSH hamsters were already infected with an agent when sent from the supplier. Development of lymphomas would simply be a matter of time, and with their development, horizontal transmission would occur with subsequent infection of all the stock. Use of experimental agents such as SV40 transformed cells and virus might speed up lymphoma development and thus make the agents appear causative when in reality they were only promotive. This theory would be consistent with the lack of significant cross-protection against lymphoma using SV40 transformed cells, but would not explain the lack of lymphoma outbreaks in other facilities using Lakeview hamsters. The requisite involvement of a second agent capable of inducing the endogenous agent would explain the lack of lymphomas in other facilities.

An effective evaluation of these theories necessitates recovery of an oncogenic agent. However, unless the experiments to evaluate oncogenicity are carried out in an uncontaminated facility, the results will be obscured by the possibility that the preparation is simply promoting an ongoing process and is not actually causative. Until such a facility becomes available, the following possibilities can be explored:

(1) DNAse, RNase and protease sensitivity of apparently oncogenic preparations.

(2) Effect of these preparations in vitro on normal hamster cells.

(3) Involvement of HaLV using a radioimmunoassay.

(4) Similarities of the warts to the Graffi papillomas.

(5) Susceptibility of hamsters from other suppliers.

By pursuing the immunology of the tumors it may be possible to develop an assay for the lymphoma agent. Tumor bearing animals have serum reactivity against an agent and immunized animals have effector cells which can be used to transfer immunity to other animals. Since there appears to be an agent antigen, development of an agar immunodiffusion assay for the agent should be straightforward. Similarities to the FeLV FOCMA antigen (33) could also be pursued.

The areas of investigation of the hamster lymphomas are greater in number now than when this investigation started, but the areas are also better defined and more amenable to exploration than before. The information in this dissertation should be of use to other investigators in either preventing or helping to characterize further outbreaks of lymphomas in hamsters.

LIST OF REFERENCES

LIST OF REFERENCES

1. Ambrose, K. R., and Coggin, J. H., Jr. An epizootic in hamsters of lymphomas of undetermined origin and mode of transmission. J. Natl. Cancer Inst. 54:877-880, 1975.

2. Amend, N. K., Loeffler, D. G., Ward, B. C., et al. "Transmission of enteritis in the Syrian hamster." Lab. Animal Sci. 26:566-572, 1976.

3. Anonymous. "The Not-so-harmless hamster." Lancet 2:216, 1975.

4. Armstrong, M. Y. K., Ruddle, N. H., Lipman, M. B., et al. "Role of endogenous murine leukemia virus in immunologically triggered lymphatic tumors. I. Development and use of oncogenic cell-free preparations serially passaged in vivo." J. Natl. Cancer Inst. 58:67-72, 1977.

5. Ashbel, R. "Spontaneous transmissible tumors in the Syrian hamster." Nature 155:607, 1945.

6. Bernhard, W., and Tournier, P. "Infection virale inapparente des cellules de hamsters decelee par la microscopie electronique." Ann. l'Institut Pasteur 107:447-459, 1964.

7. Brindley, D. C., and Banfield, W. G. "A contagious tumor of the hamster." J. Natl. Cancer Inst. 26:949-957, 1961.

8. Burkitt, D. P. "Etiology of Burkitt's lymphoma--an alternative hypothesis to a vectored virus." J. Natl. Cancer Inst. 42:19-28, 1969.

9. Cesarini, J. P., and DeMicco, C. "Studies on Type-H virus-like particles in hamsters: their role in oncogenesis." Int. J. Cancer 10:174-185, 1972.

10. Davidson, G. P., Bishop, R. F., Townley, R. R. W., et al. "Importance of a new virus in acute sporadic enteritis in children." Lancet 1:242, 1975.

11. Diamandopoulos, G. T. "Induction of lymphocytic leukemia, lymphosarcoma, reticulum cell sarcoma and osteogenic sarcome in the Syrian golden hamster by oncogenic DNA Simian Virus 40." J. Natl. Cancer Inst. 50:1347-1365, 1973.

12. Diamandopoulos, G. T., and McLane, M. F. "Effect of host age, virus dose and route of inoculation on tumor incidence, latency and morphology in Syrian hamsters inoculated intravenously with oncogenic DNA simian virus 40." J. Natl. Cancer Inst. 55:479-482, 1975.

13. Drewinko, B., Mavligit, G. M., Ambus, U., and Hersh, E. M. "Inhibition of human lymphoma cell-line colony formation by lymphocytes from patients with lymphoma and cancer hospital employees." Cancer 37:2929-2937, 1976.

14. Dunham, L. J., and Herrold, K. M. "Failure to produce tumors in the hamster cheek pouch by exposure to ingredients of Betel quid; histopathologic changes in the pouch and other organs by exposure to known carcinogens." J. Natl. Cancer Inst. 29:1047-1067, 1962.

15. Eddy, B. E., Stewart, S. E., Young, R., et al. "Neoplasms in hamsters induced by mouse tumor agent passed in tissue culture." J. Natl. Cancer Inst. 20:747-756, 1958.

16. Fortner, J. G. "Spontaneous tumors, including GI neoplasms and malignant melanomas, in the Syrian golden hamster." Cancer 10:1153-1156, 1957.

17. Fortner, J. G. "The influence of castration on spontaneous tumorigenesis in the Syrian (golden) hamster." Cancer Research 21:1491-1498, 1961.

18. Fortner, J. G., Mahy, A. G., and Cotran, R. S. "Transplantable tumors of the Syrian (golden) hamster." Cancer Research 21:199-234, 1961.

19. Frankel, J. W. "Experimental models for DNA-RNA viral interactions: a brief review." Cancer Research 36:670-673, 1976.

20. Freeman, A. E., Kelloff, G. J., Vernon, M. L., et al. "Prevalence of endogenous type-C virus in normal hamster tissues and hamster tumors induced by chemical carcinogens, simian virus 40 and polyoma virus." J. Natl. Cancer Inst. 52:1469-1476, 1974.

21. Friedman, H., and Goldner, H. "Relationship between immunologic maturation and viral oncogenesis in hamsters." J. Natl. Cancer Inst. 44:809-817, 1970.

22. Fudenberg, H. H. "Dialyzable transfer factor in the treatment of human osteosarcoma: an analytic review." Ann. N. Y. Acad. Sci. 277:545-557, 1976.

23. Gardner, M. B., Roy-Burman, P., Rongey, R. W., et al. "An outbreak of hamster lymphomas associated with activation and probable horizontal spread of endogenous hamster leukemia virus." Personal communication to J. H. Coggin, Jr. Manuscript in preparation.

24. Girardi, A. J., Sweet, B. H., Slotnick, V. A., et al. "Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus, SV40." Proc. Soc. Exp. Biol. Med. 109:640-660, 1962.

25. Girardi, A. J., Sweet, B. H., and Hilleman, M. R. "Factors influencing tumor induction in hamsters by vacuolating virus, SV40." Proc. Soc. Exp. Biol. and Med. 112:662-667, 1963.

26. Graffi, A., Bender, E., and Schramm, T. "Induction of transmissible lymphomas in Syrian hamsters by application of DNA from viral hamster papovavirus-induced tumors and by cell-free filtrates from human tumors." Proc. Natl. Acad. Sci. 64:1172-1175, 1969.

27. Graffi, A., Schramm, T., Bender, E., et al. "Uber einen neuen virushaltigen Hauttmumor beim Goldhamster." Archiv fur Geschwulstforschung 30:277-283, 1967.

28. Graffi, A., Schramm, T., Bender, E., et al. "Cell-free transmissible leukoses in Syrian hamsters probably of viral etiology." Brit. J. Cancer 22:577-581, 1968.

29. Graffi, A., Schramm, T., Graffi, I., et al. "Virus-associated skin tumors of the Syrian hamster: preliminary note." J. Natl. Cancer Inst. 40:867-873, 1968.

30. Greene, H. S. N., and Harvey, E. K. "The induction of lymphomas in hamsters by heterologous tumor transplants." Amer. J. Pathology 51:447-469, 1967.

31. Hammond, E. C. "The epidemiological approach to the etiology of cancer." Cancer 35:652-654, 1975.

32. Hardy, W. D., Jr. "Feline lymphosarcoma: a model of viral carcinogenesis and significance related to human neoplasia." In Animal Models for Biomedical Research. IV. National Academy of Sciences, Washington, D. C., 1971.

33. Hardy, W. D., Jr., Geering, G., Old, L. J., et al. "Feline leukemia virus: occurrence of viral antigen in the tissues of cats with lymphosarcoma and other diseases." Science 166:1019-1021, 1969.

34. Hardy, W. D., Jr., Hess, P. W., MacEwen, E. G., et al. "Biology of feline leukemia virus in the natural environment." Cancer Research 36:582-588, 1976.

35. Hardy, W. D., Jr., Old, L. J., Hess, P. W., et al. "Horizontal transmission of feline leukemia virus." Nature 244:266-269, 1973.

36. Homburger, F. "Chemical carcinogenesis in Syrian hamsters." In Progress in Experimental Tumor Research 16:152-175, 1972.

37. Horn, K. H., and Siewert, R. "Uber das plotzliche Auftreten von Spontantumoren in einer Goldhamster Koloniezucht." Acta Biol. Med. Germ. 20:103-110, 1968.

38. Hsiung, G. D., and McTighe, A. H. "An animal model for DNA-RNA virus interaction based upon virological and histological findings." Cancer Research 36:674-677, 1976.

39. Heubner, R. J. National Cancer Institute. Personal communication.

40. Jacoby, R. O., Osbaldiston, G. W., and Jonas, A. M. "Experimental transmission of atypical ileal hyperplasia of hamsters." Lab. Animal Sci. 25:465-473, 1975.

41. Jarrett, W. F., Martin, W. B., Crighton, G. W., et al. "Leukemia in the cat. Transmission experiments with leukemia (lymphosarcoma)." Nature 292:566-567, 1964.

42. Kaaden, O., and Dietzschold, B. "Isolation and characterization of extracellular virus-like particles from BHK cells." Archiv. Virusforsch. 35:317-321, 1971.

43. Kaplan, H. A. "Hodgkin's disease and other human malignant lymphomas: advances and prospects--G.H.A. Clowes Memorial Lecture." Cancer Research 36:3863-3878, 1976.

44. Karpas, A., and Samso, A. "A new malignant lymphoma induced in the Syrian hamster." Europ. J. Cancer 8:231-237, 1972.

45. Kelloff, G. J., Huebner, R. J., and Gilden, R. V. "Isolation and characterization of the hamster C-type viruses." Unifying Concepts of Leukemia, Bibl. Haemat. 39:61-68, 1973.

46. Kimmelstiel, P. Cited in Pathology, Stanley L. Robbins, ed. W. B. Saunders Co., Philadelphia, 1967, p. 1022.

47. Kirkman, H., and Algard, R. "Spontaneous and nonviral-induced neoplasms." In The Golden Hamster, R. A. Hoffman, P. F. Robinson, and H. Magalhaes, eds. Iowa State University Press, 1968, pp. 227-240.

48. Kistler, G. Personal communication to F. Homburger. Cited in Telacogram, 10/30/75 and 4/2/76, from Telaco, RFD 1, Bar Harbor, Maine 04609.

49. Larson, V. M., Girardi, A. J., Hilleman, M. R., et al. "Studies of oncogenicity of adenovirus type 7 viruses in hamsters." Proc. Soc. Exp. Biol. Med. 118:15-24, 1965.

50. Lukes, R. J., and Collins, R. D. "Immunologic characterization of human malignant lymphomas." Cancer 34:1488-1503, 1974.

51. Lussier, C., and Pavilanis, V. "Malignant lymphomas in hamsters following a wasting disease produced with bovine leukemic extract--a preliminary report." Can. J. Comp. Med. 33:81-82, 1969.

52. Manning, J. R. S., and Griesemer, R. A. "Spontaneous lymphoma of the nonhuman primate." Lab. Animal Sci. 24:204-210, 1974.

53. McKercher, D. G., Wada, E. M., Straub, O. C., et al. "Possible viral etiology of bovine and equine leukemia." Annals New York Acad. Sci. 108:1163-1172, 1963.

54. Melendez, L. V., Daniel, M. D., Hunt, R. D., et al. "Herpesvirus saimiri. V. Further evidence to consider this virus as the etiological agent of a lethal disease in primates which resembles a malignant lymphoma." J. Natl. Cancer Inst. 44:1175-1181, 1970.

55. Microbiological Associates Testing Service. Personal communication; and H. Charmin, M.D., Frederick Cancer Research Center.

56. Miller, L. D., Miller, J. M., and Olson, C. "Inoculation of calves with particles resembling C-type virus from cultures of bovine lymphosarcoma." J. Natl. Cancer Inst. 48:423-428, 1972.

57. Moloney, J. B. Biological studies on a lymphoid-leukemia virus extracted from Sarcoma 37. J. Natl. Cancer Inst. 24:933-951, 1960.

58. Moloney, J. B. "The murine leukemias." Federation Proc. 21:19-31, 1962.

59. Moser, R. H. "The bramble bush, the transmissibility of Hodgkin's disease." J. Am. Med. Assoc. 233:452-455, 1975.

60. Nazerian, K., and Witter, R. L. "Cell-free transmission and in vivo replication of Marek's disease virus." J. Virol. 244:266-269, 1973.

61. Olsen, R. G., Hoover, E. A., Mathes, L. E., et al. "Immunization against feline oncornavirus disease using a killed tumor cell vaccine." Cancer Research 36:3642, 1976.

62. Pour, P., Kmoch, N., Greiser, E., et al. "Spontaneous tumors and common diseases in two colonies of Syrian hamsters. I. Incidence and sites." J. Natl. Cancer Inst. 56:931-935, 1976.

63. Pour, P., Mohr, U., Althoff, J., et al. "Spontaneous tumors and common diseases in two colonies of Syrian hamsters. IV. Vascular and lymphatic systems and lesions of other sites." J. Natl. Cancer Inst. 56:963-974, 1976.

64. Priester, W. A., and Hayes, H. M. "Brief communication: feline leukemia after feline infectious anemia." J. Natl. Cancer Inst. 51:289-291, 1973.

65. Priester, W. A., and Mantel, N. "Occurrence of tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine." J. Natl. Cancer Inst. 47:1333-1344, 1971.

66. Rabin, H., Neubauer, R. H. Pearson, G. R., et al. "Spontaneous lymphoma associated with herpesvirus saimiri in owl monkeys." J. Natl. Cancer Inst. 54:499-502, 1975.

67. Rappaport, H. Tumors of the Hematopoietic System. Armed Forces Institute of Pathology, Washington, D. C., 1966.

68. Renshaw, H. W., Van Hoosier, G. L., Jr., and Amend, N. K. "A survey of naturally occurring diseases of the Syrian hamster." Laboratory Animals 9:179-191, 1975.

69. Robbins, S. L. Pathology. Third Edition. W. B. Saunders Co., Philadelphia, 1967, pp. 1020-1025.

70. Schwabe, C. W., Riemann, H. P., and Franti, C. E. Epidemiology in Veterinary Practice. Lea and Febiger, Philadelphia, 1977.

71. Smith, H. A. "Malignant lymphomas in animals." Am. J. Clinical Pathology 38:75-87, 1962.

72. Snow, J. On the Mode of Communication of Cholera. Second Edition. Churchill, London, 1955. Reproduced in Snow on Cholera. Commonwealth Fund, New York, 1936.

73. Sokal, R. R., and Rohlf, F. J. Biometry. W. H. Freeman and Co., San Francisco, 1969, p. 624.

74. Squire, R. A. "Spontaneous hematopoietic tumors of dogs." National Cancer Institute Monograph 32:97-109, 1969.

75. Stenback, W. A., Van Hoosier, G. L., Jr., and Trentin, J. J. "Virus particles in hamster tumors as revealed by electron microscopy." Proc. Soc. Exp. Biol. Med. 122:1219-1223, 1966.

76. Strauli, P. "Morphogenetische Untersuchungen an einem lymphoreticulocytaren sarkom des Goldhamsters." Path. et Microbiol. 25:301-305, 1962.

77. Strauli, P., and Haemmerli, G. "Uber ein spontanes transplantables lymphoreticulocytares Sarkom des Goldhamsters." Z. Krebsforschg. 63:503-510, 1960.

78. Theilen, G. H., Appleman, R. D., and Wixom, H. G. "Epizootiology of lymphosarcoma in California cattle." Ann. New York Acad. Sci. 108:1203-1213, 1963.

79. Theilen, G. H., Dungworth, D. L., Lengyel, J., and Rosenblatt, L. S. "Bovine lymphosarcoma in California. 1. Epizootiologic and hematologic aspects." Health Lab. Science 1:96-106, 1964.

80. Toth, B. "Studies on the incidence, morphology, transplantation and cell-free filtration of malignant lymphomas in the Syrian golden hamster. Cancer Research 27:1430-1442, 1967.

81. Trentin, J. J., Van Hoosier, G. L., Jr., Boren Ferguson, D., et al. "Oncogenic evaluation of human picorna-, paramyxo- and herpesviruses." Proc. Soc. Exp. Biol. Med. 132:912-915, 1969.

82. Van Hoosier, G. L., Jr., Spjut, H. J., and Trentin, J. J. "Spontaneous tumors of the Syrian hamster: observations in a closed breeding colony and a review of the literature." In Defining the Laboratory Animal. National Academy of Sciences, Washington, 1971.

83. Van Hoosier, G. L., Jr., Stenback, W. A., Parker, J. C., et al. "The effects of Cesarean derivation and foster nursing procedures on enzootic viruses of the LSH strain of inbred hamsters." Lab. Animal Care 20:232-237, 1970.

84. Vasa Thomas, K. A. "An investigation of a spontaneous lymphroma epizootic in hamsters." Unpublished Doctoral dissertation, The University of Tennessee, Knoxville. In preparation. Parts I, II, and IV.

85. Vasa Thomas, K., Ambrose, K. R., Bellomy, B. B., et al. "Characterization of immune responses to spontaneous hamster lymphomas. J. Natl. Cancer Inst. 58:1287-1293, 1977.

86. Vianna, N. J. "Is Hodgkin's disease infectious?" Cancer Research 34:1149-1155, 1974.

87. Weyl, K. G., and Dougherty, R. M. "Contact transmission of avian leukosis virus." J. Natl. Cancer Inst. 58:1019-1025, 1977.

88. Yabe, Y., Kataoka, N., and Koyama, H. "Spontaneous tumors in hamsters: incidence, morphology, transplantation and virus studies." Gann 63:329-336, 1972.

APPENDICES

APPENDIX A

TABLE A-I. Number of deaths in the clean facility from February, 1977 through September, 1977.

Month	Nonexperimental LVG			Nonexperimental LSH			Experimental LVG		
	Lymphoma	Enteritis	Other	Lymphoma	Enteritis	Other	Lymphoma	Enteritis	Other
February		several							
March		15						several	
April		2						2	
May		3	174 ok 3 fibrosa					16***	116 ok 1 A.L.H.
June	1	7	34 ok 1 A.L.H. 1 mastitis	1		1 kd	1*	1	1*epider-moid ca
July	2	6	3 ok	3	1	2 kd	3*		
August	3	0	1 ok 1 kd	5		2 kd	2*	4	
September	3	2	3 pelts	6	2	4 pelts	5**	4	3 kd

* = animals from an interruption of oncogenicity experiment; each * indicates one animal.

NOTE: A.L.H. = atypical lymphoid hyperplasia; ok = no abnormalities on autopsy; pelts = animal cannibalized; kd = kidney disease; ca = carcinoma; and sa = sarcoma.

APPENDIX B

PHYSICAL CHARACTERISTICS OF THE ANIMAL FACILITIES

	Lymphomatous Facility	Clean Facility
Location	Rm. 427, Hesler Biology Bldg. The University of Tennessee, Knoxville	Approximately 100 yards north of Hesler Biology Bldg., in a set of trailers
Dimensions	One main room 27.5 x 18 x 10 ft with an anteroom 4 x 6 ft and an adjacent surgery 6 x 12 ft	Six individual rooms 12 x 12 ft, all serviced by a common corridor
Description	Animals were housed in one large room with a painted concrete floor and central drain, tile walls, and suspended acoustic tile ceiling	Animals were housed in individual rooms with vinyl floors (no drain), aluminum walls and aluminum ceiling
	10-15 racks of animals kept in the room, 24-30 cages of animals per rack	3-4 racks of animals kept in each room, 24-30 cages of animals per rack
	LSH and LVG hamsters housed on the same rack but never in the same cage	LSH kept in room 7, LVG breeding stock kept in room 8, LVG experimental hamsters kept in rooms 6, 7, 9, 10, and LVG normal hamsters (received and holding) kept in room 5
	No other species kept adjacent	Cats, rats, mice housed in same facility, in other rooms; room 10 was used for both mice and hamsters but at different times
Average Temperature	70°F	70°F
Humidity	No humidity control	No humidity control

	Lymphomatous Facility	Clean Facility
Air Circulation	Effected by 2 fresh air vents in ceiling, 2 wall air conditioning units, 1 ceiling air conditioning unit, 1 exhaust fan running continuously capable of 2 complete air exchanges per hour	Effected by individual Fedders air conditioning units in each room and a continuously running exhaust fan in each room. Specifications called for five air turnovers per hour
Light Source	12 150 watt light bulbs in ceiling; 3 large windows	2 sets of fluorescent lights in ceiling of each room; no windows
Supplies	Cages, water bottles, food supplied from a facility on sixth floor of Hesler Biology Bldg.; food supply also kept in lymphomatous facility	Cages, water bottles supplied from an adjacent facility in South College; food kept in bins in the trailers

HUSBANDRY PROCEDURES AND SUPPLIES USED

IN THE HAMSTER FACILITIES

Lymphomatous Facility	Procedures/ Supplies	Clean Facility
Polypropylene (Maryland Plastics)	Cages	Polypropylene (Maryland Plastics)
Stainless steel (Maryland Plastics)	Cage tops	Stainless steel (Maryland Plastics)
Absorb-Dri (chipped hardwood)	Bedding	Absorb-Dri (chipped hardwood)
Purina Hamster Chow	Food	Purina Hamster Chow
10 hamsters or fewer per cage	Care	10 hamsters or fewer per cage
cages changed twice weekly food and water ad libitum water bottles washed twice weekly, filled with city water		cages changed twice weekly food and water ad libitum water bottles washed twice weekly, filled with city water
cages scraped and washed in sixth floor facility; washed 5 min in Better Built Washer, 180°F water, Impact Detergent (Economy Labs); rinsed twice for 3 minutes in 180°F water clean cages filled with bedding and held in sixth floor facility until needed	Cage Turnover	cages scraped in the individual hamster rooms, washed in South College facility; washed 5 min in Girton 664 washer, 180°F water, Impact Detergent (Economy Labs); rinsed twice for 3 minutes in 180°F water clean cages autoclaved, then filled with bedding and held in South College until needed
same procedure as for cages; filled in sixth floor facility and refilled as necessary from sink in lymphomatous facility	Water Bottle Turnover	same procedure as for cages, but not autoclaved with each washing (intermittently); filled in supply room of clean facility, refilled as necessary from reservoir; rolled into the individual hamster rooms

Lymphomatous Facility	Procedures/ Supplies	Clean Facility
12 hours dark, 12 light 6 A.M.-6 P.M.	Light Cycle	12 hours dark, 12 light 5:30 A.M.-5:30 P.M.
Betsey McDonald Larry Sanderson Glen Dixon Sam and Cathy Beckman	Caretakers	Doug Davis Mark Parry Larry Trent

TABLE B-I. Agents and cells used prior to the lymphoma epizootics.

Agent or Cell	Epizootic			
	First	Second	Third	Fourth
SV40 virus	X	X		X
F5-1 (SV40 transformed hamster fibroblasts)	X	X	X	X
SV/LSH (SV40 transformed hamster fibroblasts)	X	X	X	
Hamster fetal cells	X	X		X
Adenovirus 7	X	X		
Adenovirus 7 transformed cells				X
Adenovirus 31	X	X		X
OBT (chemically transformed hamster fibroblasts)	X			
2952 (SV40 transformed hamster cells)	X			
Hamster Leukemia Virus	X			
Hamster kidney	X			
333-89 (Herpes transformed hamster fibroblasts)				X
GD-36 (SV40 transformed lymphoid cells)				X
GD-248 (SV40 transformed lymphoid cells)				X
F5-1, antigenically altered				X
Dimethylbenzanthracene				X
MKSA (SV40 transformed mouse cells)				X

NOTE: Both untreated and viable, X-irradiated transformed cells and fetal cells were used to immunize and challenge animals. Only the F5-1 cells were used prior to each epizootic.

APPENDIX C

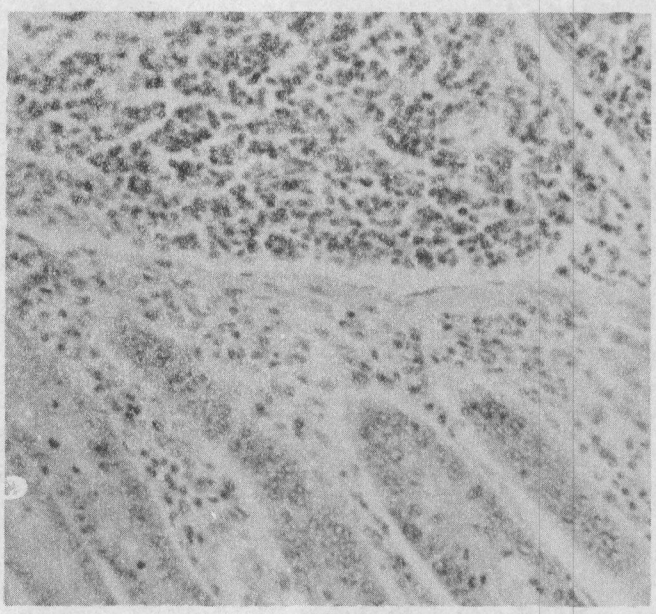


FIGURE C-1. Intestinal lymphoma (X 63).

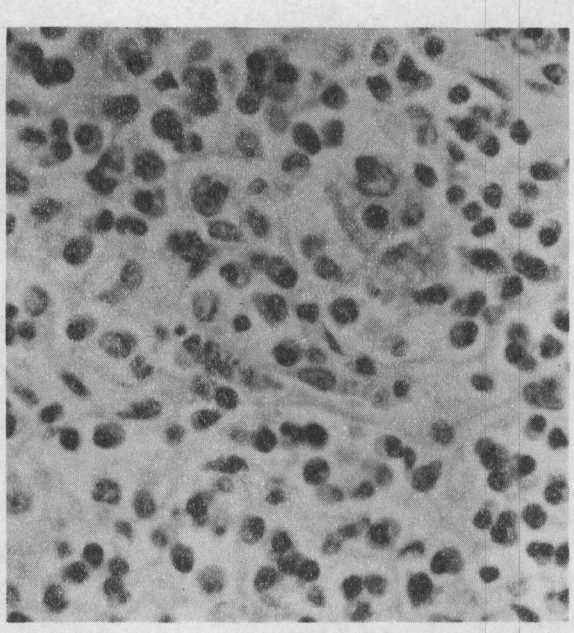


FIGURE C-2. Intestinal lymphoma (X 950). Cells are plasmacytoid.

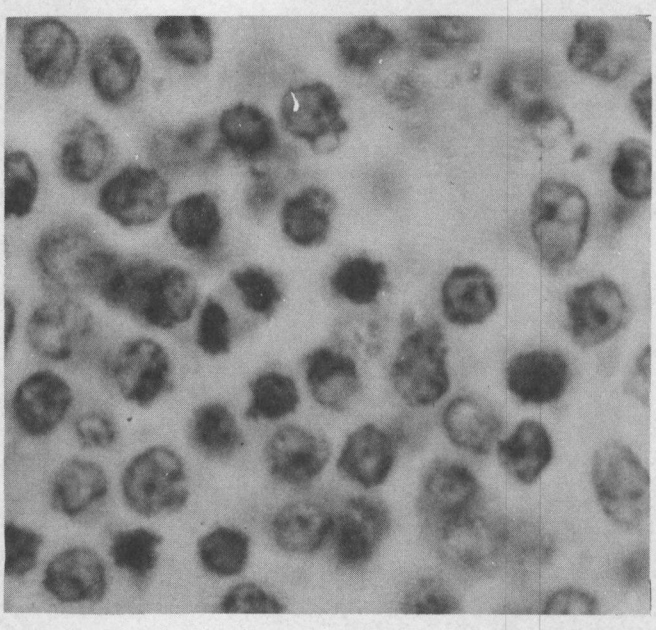


FIGURE C-3. Spontaneous thymic lymphoma. Highly mitotic (X 950).

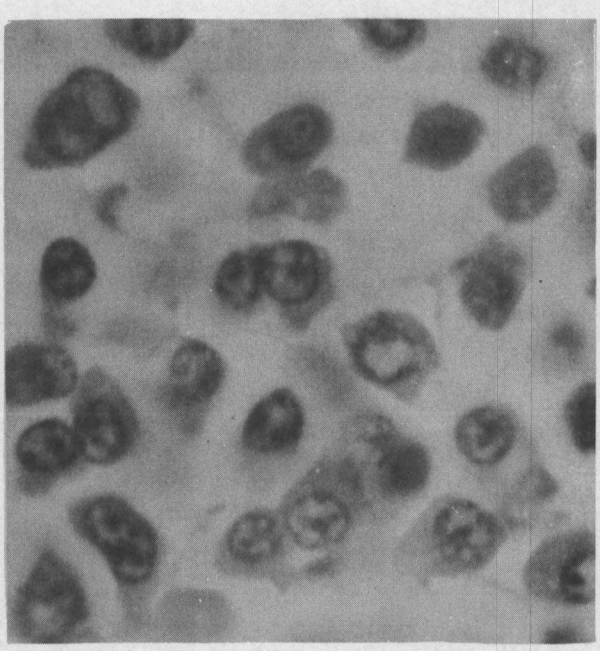


FIGURE C-4. Spontaneous retroperitoneal lymphoma (X 950).

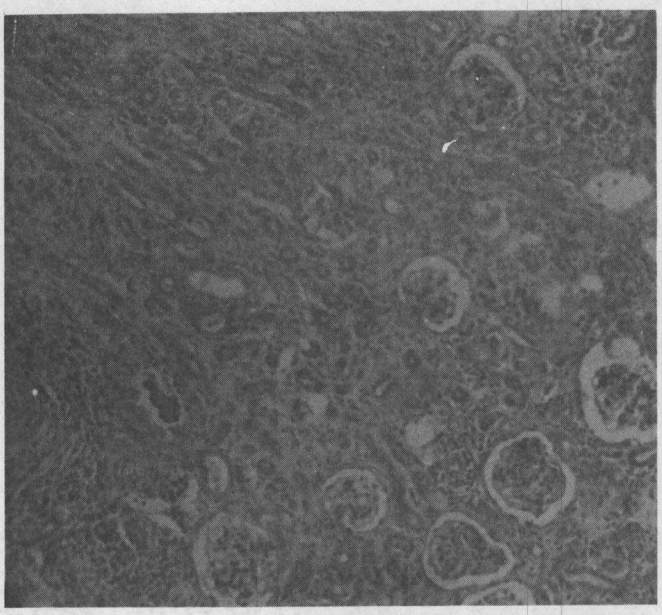


FIGURE C-5. Pyelonephritis. Note relative sparing of glomeruli (X 63).

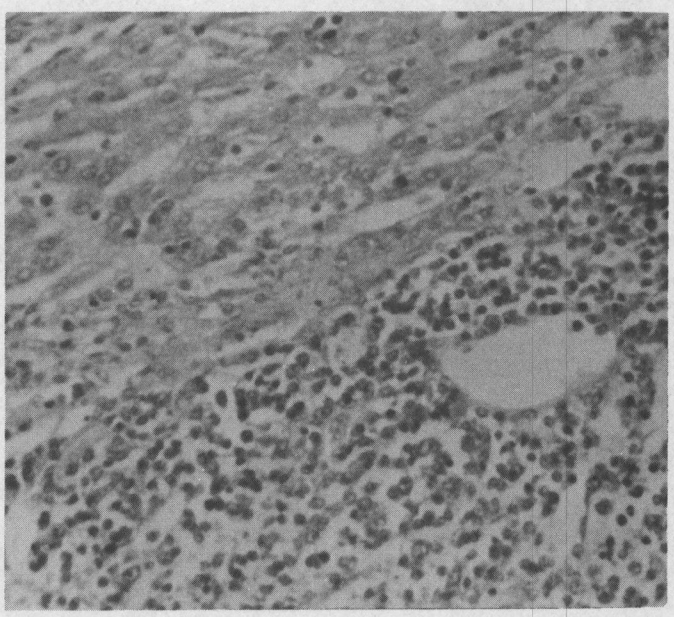


FIGURE C-6. Spontaneous liver lymphoma (X 160).



FIGURE C-7. Warts around the eyes and under the chin of an LSH female.

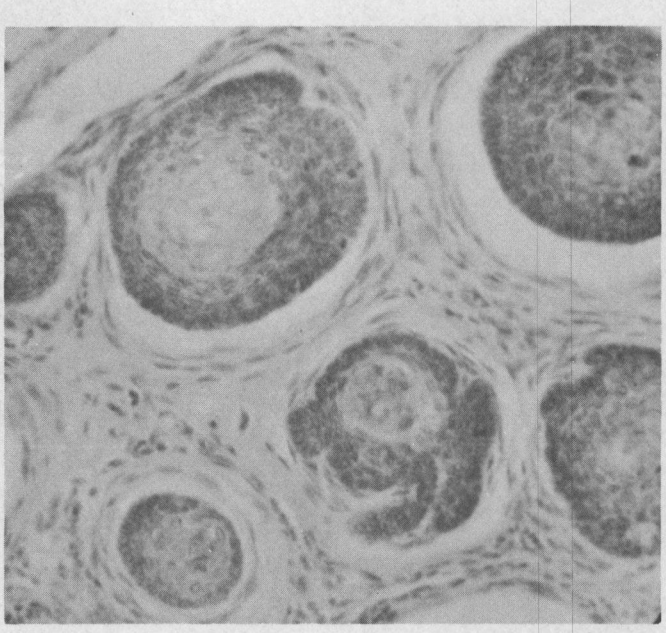


FIGURE C-8. Histological section of the warts. Described as a pilosebaceous adenoma (X 63).

TRANSMISSION OF "WARTS" IN LSH HAMSTERS

Procedure:

(1) Remove "warts."
(2) Homogenize in saline.
(3) Centrifuge twice for 10 minutes at 2,000 rpm, discarding the pellet.
(4) Filter-sterilize supernatant through an 0.45 mμ Millipore filter.
(5) Inoculate into 6-8 week old LSH males around the eye and on the back.

Results

(1) Latent period of 5.5 months for the first passage. 2/9 animals had lymphomas at necropsy, nine months post inoculation.

(2) Latent period of 4 months for the second passage. Actual wart lesions were not seen until 5 months, but a subcutaneous mass on the back was noted before then. 0/5 had lymphomas at necropsy, 10 months after inoculation.

(3) Latent period of 4.5 months for the third passage. Transmission into neonatal LVG hamsters resulted in one lymphoma out of 13 animals inoculated, and no "warts." None of the LSH neonates inoculated survived to weaning. 6/6 LSH inoculated as adults developed warts.

(4) Development of "warts" is not limited to the site of inoculation. Animals inoculated on the back develop "warts" under the chin, on the forearms, and even in the ears.

APPENDIX D

REVISED MODIFIED MOLONEY PROCEDURE

Step No. 1 Sacrifice and exsanguinate animals.
Remove tumors (small and non-necrotic) to Petri dish of sterile medium. Before mincing, wash tissue several times with BME containing 250 units penicillin, 250 ug streptomycin per ml to remove any fur or fatty tissue. Prepare a finely minced suspension of tissue in 0.153 M potassium citrate containing 1 mg protamine sulphate (Nutritional Biochemicals, Cleveland) per 100 cc. (Prepare a stock solution of 100 mg protamine sulphate in 100 ml potassium citrate, aliquot in 1 ml quantities and freeze).
The suspension of tissue should be 6.6 to 10 percent.

(Example--1 gm of tumor mince + 15 cc of potassium citrate containing the protamine sulfate, or 1 gm of tumor mince + 9 cc of potassium citrate, containing the protamine sulfate. Weigh before mincing.)

Digest for 1 hour at room temperature with occasional mixing in the Waring blender (every 15 minutes).

Step No. 2 Homogenize intermittently at room temperature for 3 minutes, making sure blender vessel does not heat up.

Step No. 3 Centrifuge at 2,500 rpm for 20 minutes at 4°C in the Lab Line refrigerated centrifuge using rotor #253. (Tubes should be precooled also.) Carefully pipette off middle supernate between top layer scum and bottom layer debris and cells. Cotton swabs (sterile) may be used to pull off the top layer scum. (Supernate = S_1)

Step No. 4 Recentrifuge S_1 exactly as in Step No. 3: carefully pipette off supernate (S_2). This step may be omitted if the first centrifugation has yielded a clear supernatant. (I have found additional debris after the second spin, so I do not recommend leaving it out.)

Step No. 5 Centrifuge S_2 at 17,000 g (12,000 rpm) for 1 minute in Spinco Model L Ultracentrifuge using type 30 rotor. Pour off supernate (S_3). The 1 minute should be timed after the 12,000 rpm is reached.

Step No. 6 Centrifuge S_3 at 40,000 g (19,000 rpm) for 1 hour in Spinco Model L Ultracentrifuge using type 30 rotor. Pour supernate and discard. This, like all other centrifugations should be allowed to coast down.

Step No. 7 Centrifuge tube containing pellet placed in wet ice. Pellet rinsed 3 times with 0.02 M sodium citrate buffer, pH 6.7 containing 250 U/ml penicillin, 250 ug/ml streptomycin. This procedure done very carefully in order not to disturb the pellet. Centrifuge tube containing pellet is further cleaned with sterile applicator swab not disturbing pellet to get rid of any materials attached to the centrifuge tube walls. Rinse pellet 1 more time as above. Resuspend pellet in 0.02 M sodium citrate buffer pH 6.7 so as to make 1 gram of original tumor tissue equivalent to 1 cc. Agitate the pellet and 0.02 M sodium citrate buffer while on wet ice using a 1 cc tuberculin syringe with 19 ga, 1-1/2 inch needle for 5-10 minutes to give an even suspension of virus material.

Sonicate about 3 fast blips.

Step No. 8 Centrifuge the suspension at 2,500 rpm for 20 minutes as before. Carefully remove the supernate with a cannula attached to a syringe and filter sterilize using an 0.45 mu Millipore Swinnex apparatus. Aliquot the sterilized

supernate. Store in the Revco. Estimate the volume of pellet (it will be very soft and easily disturbed--really not a pellet) and add an equal volume of 0.02 M sodium citrate buffer. Resuspend the pellet in the buffer and aliquot as above (0.5 cc per vial). Screw-cap vials were used. Store in the Revco.

All centrifuge tubes should be sterile and pre-cooled. All centrifugation steps should coast to a stop. The buffers should all be treated with penicillin and streptomycin. (It may be necessary to omit it from the final step since hamsters may react adversely to it.) Step No. 8 is to allow filtration of the preparation. It may be omitted if sterility is not required or if other methods for eliminating the possibility of cells in the final supernate are employed.

VITA

Kathy A. Vasa Thomas was born in Great Lakes, Illinois, on May 14, 1951. She attended elementary and secondary schools in Kensington, Maryland, and in Memphis, Tennessee. She was graduated from White Station High School in June, 1969. The following September, she entered The University of Tennessee and in June, 1972, she received a Bachelor of Arts degree with a major in Chemistry and a minor in Zoology.

After a summer as a research laboratory technician, she accepted a fellowship to study General Medical Sciences at Washington University in Saint Louis, where she passed the preliminary examination for her Doctorate in June, 1974.

She transferred to The University of Tennessee, Knoxville, in August, 1974 and accepted a research assistantship under the direction of Dr. J. H. Coggin, Jr. She was awarded the Doctor of Philosophy degree with a major in Radiation Biology in August, 1978.

The author is a member of the American Society for Microbiology and the Tissue Culture Association.