THE EFFECT OF NEONATAL THYMECTOMY AND X-IRRADIATION OF THE CHICKEN ON THE PERCENT OF BLOOD LYMPHOCYTES

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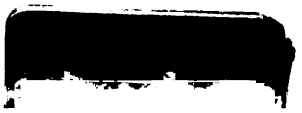

THE EFFECT OF NEONATAL THYMECTOMY AND X-IRRADIATION OF THE CHICKEN ON THE PERCENT OF BLOOD LYMPHOCYTES

By

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INTRODUCTION

The chicken is a model in which the immunologic aspects of immediate hypersensitivity and delayed hypersensitivity can be studied singly. Removal of the bursa of Fabricius, one of the central lymphoid organs, prevents the development of the chicken's capacity to respond to primary antigenic stimulation by production of humoral antibodies. Removal of the thymus, the other central lymphoid organ, prevents the capacity to develop delayed hypersensitivity of the tuberculin type.

The following is a description of the techniques employed to thymectomize one day old chickens. The $LD_{50/35}$ (lethal dose to 50 percent of the chickens 35 days after treatment) for three day old chickens was determined to investigate if there is a need to follow thymectomy with X-irradiation.

Because the lymphocyte is the cell type postulated to be under thymic influence, differential blood counts were performed to determine the effect of X-irradiation and/or thymectomy on the percent of lymphocytes in the peripheral blood. It was postulated that a prediction could be made by the percent of lymphocytes as to the effectiveness of thymectomy prior to necropsy.

Total white blood cell counts were performed to demonstrate the effect of X-irradiation in lowering the total number of leukocytes/mm^3.

The effect that an infection with Mycobacterium avium and tuberculin testing had on the percent of lymphocytes in chickens belonging to the following four treatment groups: thymectomized, thymectomized-X-irradiated, normal, and X-irradiated was determined.

LITERATURE REVIEW

In man and many animals, the thymus is primarily responsible for the maturation of humoral and cellular immunity. The study toward an understanding of the immune mechanism has received great impetus with the discovery that morphologically compartmentalized and separable immune systems exist in chickens. The report by Glick in 1956 as summarized in 1964 that the bursa of Fabricius functioned in the development of humoral immunity was an indication of the morphologic separability of the central lymphoid tissue of the chicken. The bursectomized chicken cannot respond to primary antigenic stimulation (Jankovic et al., 1966). That the bursa of Fabricius and the thymus are two separable immunologic systems, functionally as well as morphologically, was postulated by Warner et al., 1962 and confirmed experimentally by Cooper et al. in 1966.

In the chicken the development of the cell system, which includes the plasma cells and the large lymphocytes in the red pulp of the spleen, and Peyer's patches, is largely bursa-dependent (Cooper et al., 1965; Cooper et al., 1966; Woods et al., 1965). Relatively few bursa-dependent lymphocytes are found in the peripheral circulation (Lucas et al., 1961). The thymus is responsible for the development of the

small lymphocytes of the circulation, either indirectly or directly. A humoral factor of thymic origin may affect cells in the peripheral lymphoid tissue or thymocytes (lymphocytes of the thymus) may migrate to the peripheral lymphoid tissue (Larsson, 1966; Nossal, 1964; Toro, 1967; Owen et al., 1969). The small lymphocytes are believed to be the mediators of delayed hypersensitivity, homograft rejection, and possibly for the graft versus host reaction. The small, thymus dependent lymphocytes found in the spleen are thought to be confined to the white pulp in the peri-arteriole zone. The white pulp develops shortly after hatching; the red pulp develops 4-5 weeks post hatching (Cooper et al., 1965).

Functionally, the two systems are not completely independent. The thymus-dependent system may be necessary for the recognition of a substance as foreign which precedes the inititation of an immunologically specific response by either the thymus or bursa dependent systems (Peterson, 1965).

The thymus in the chicken is reported to be the first lymphoid organ to function in the embryo. Lymphocyto-poiesis begins sooner in the thymus than in any other lymphoid organ. Thymic lymphocytes are well developed prior to hatching, but bursal lymphocytes are not (Peterson et al., 1965).

Cooper et al. (1966) recommended X-irradiation together with thymectomy in the newly hatched period to establish an experimental model in which the thymus dependent system is nonfunctional. The purpose of X-irradiation after thymectomy was to destroy any thymus dependent peripheral lymphoid tissue present at hatching. The use of X-irradiation is a two-fold disadvantage. First, an LD_{50} is used to insure sufficient irradiation of the surviving chickens and therefore, half of all X-irradiated chickens are lost due to X-irradiation. The cost is slight but thymectomies are tedious and delicate. The second disadvantage is that another variable in the experimental model is introduced.

The assumption under which X-irradiation is used is the following: Lymphoid and myeloid tissues are most easily damaged so that a dose of radiation can be found which destroys every cell in these tissues without irreparable damage to the rest of the body (Dresser et al., 1959). Stearner et al. (1951) reported that the amount of tissue destruction by X-irradiation is dependent on dose as well as upon the length of time during which the dose is administered. The manner in which X-irradiation destroys cells is not known (Stearner et al., 1956).

There are several reports that total body X-irradiation of the chicken destroys lymphocytes (Lucas et al., 1957; Murray et al., 1948; Cooper et al., 1966). The dose and age

at which X-irradiation was administered differed in all the reports and the results varied with the age and dose.

The rapid destruction of lymphocytes in the peripheral blood, internal tissues and organs by total body X-irradiation in the 3 week old chicken has been described most extensively by Murray et al., 1948. The lymphocytes throughout the body were severely affected by X-irradiation. A reduction of 50 percent in the total white blood cell count occurred within the first hour after X-irradiation, and was due to the sudden destruction of lymphocytes. The bone marrow was among the first tissues to show extensive damage. Destruction of almost all lymphocytes of the cortex and medulla of the thymus was followed by regeneration of lymphocytes, but never more than a third of normal. Damage to the liver included reduction of lymphatic areas followed by partial restoration. Many lymphocytes of the lymphatic tissue of the lamina propria were destroyed, but damage to the lymphatic tissues was not as extensive as in the spleen or bone marrow. It was emphasized that in 3 week old chickens the small lymphocytes are more susceptible to damage by total body X-irradiation than large lymphocytes, although destruction occurs to both.

A reduction in the percent of lymphocytes in the peripheral blood of thymectomized chickens has been reported by several workers (Warner et al., 1964; Jankovic et al, 1964; Isakovic et al., 1964; Jaffee, 1966). None of these workers made a comparison of the decrease in lymphocytes in

thymectomized and in thymectomized X-irradiated chickens. Cooper et al. (1966) reported a decrease in the numbers of small lymphocytes in thymectomized X-irradiated chickens but in their earlier work had found no reduction in thymectomized chickens. Whether thymectomy only causes a significant depletion in the percent of lymphocytes in the peripheral circulation or whether thymectomy must be followed by X-irradiation should be resolved. Because an LD_{50} irradiation dose is used, one half of the chickens die. Omission of the irradiation would reduce the number of chickens to be thymectomized necessary to provide for a statistically significant number to be used in subsequent studies (Pearson et al., 1951). Besides this practical consideration, the additional variability in results due to the added experimental condition of X-irradiation would be avoided.

Determination of the role of the lymphocyte in the delayed hypersensitivity reaction has potential diagnostic importance in the identification of antigens to which an animal or individual is hypersensitive (Benezra et al., 1967). The lymphocyte has been postulated as being the cell type either mediating or effecting the delayed hypersensitivity reaction (Mills, 1966; Kay et al., 1963). McFarland et al. (1966) have demonstrated that in a mixed leukocyte reaction, the lymphocytes interact with macrophages, cell debri and lymphoblasts. The reaction is by the uropod, suggesting that stimulatory material may be acquired through the appendage. In vitro studies to determine the specificity of the delayed

immune response have been reported (George et al., 1962; Thor, 1967; David et al., 1964). In vitro, lymphocytes from individuals hypersensitive to tuberculin increase in blast formation when cultured with Purified Protein Derivative (PPD) (Gell, 1967). Migration in capillary tubes of peritoneal exudate cells, consisting of macrophages and lymphocytes, from guinea pigs with delayed hypersensitivity to PPD, ovalbumin and diphtheria toxoid is markedly inhibited only by the antigen to which the animal had been sensitized (David, 1964). Lymphocytes from sensitive guinea pigs release a factor, the migration inhibition factor (M.I.F.) which inhibits the migration of monocytes in vitro (Bloom et al., 1966). This factor inhibits the migration of monocytes from sensitive or normal guinea pigs, the latter acting as an in vitro passive sensitization (Bloom et al., 1966). If in fact the migration inhibition phenomenon of in vitro studies is a manifestation of the cellular hypersensitivity residing in the sensitive animal, the peritoneal exudate cells from sensitized thymectomized chickens would not be capable of displaying migration inhibition when exposed to the antigen used in sensitizing. The chicken model provides the opportunity to study whether the origin of the capacity to develop delayed hypersensitivity as detected in skin testing and by in vitro studies depends on a continued thymic function.

MATERIALS AND METHODS

Chickens

Fertile eggs from single comb white Leghorns were incubated at dry bulb 99°-100° F, wet bulb 85°-86° F in an International incubator (automatic rotation, controlled humidity).

Surgical Procedures

Thymectomy of chickens was performed one day after hatching. After anesthetization by 0.05-0.08 cc Combuthal (R)* administered intraperitoneally, a dorsal incision approximately 4-6 cm long was made on the neck to expose the lobes of the thymus. Each lobe and the surrounding connective tissue and fat deposits were removed by blunt dissection. Sham thymectomies were performed in the same manner, removing only fat deposits and connective tissue. The incisions were closed with clamps. Aseptic technique was used and no antibiotics were administered.

X-Irradiation

Three days after hatching, irradiation was administered with the General Electric Maxitron 300 X-ray machine.

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*Diamond Laboratories, Inc., Des Moines, Iowa.

The conditions of irradiation were: 220 PKV, 20 ma with 0.25 mm cu + 1.0 mm Al. added (with hvl of approximately 0.25 mm cu) at a dose rate of 6.4 R/min. A total dose of 800 R was given in 125 min. in air. The target distance was 31 inches square. Under these conditions the $LD_{50/35}$ was calculated to be 800 R. To place the chickens individually during irradiation, four cardboard boxes were constructed to hold 100 chickens, 25 chickens per box, one chicken per compartment. The four boxes were arranged in a square and rotated during irradiation in relation to each other as well as about their own axis to insure an even distribution of exposure to X-ray. In so doing, the calculated variation in the dose each chicken received was less than 2 percent (Mostosky, 1966).

In the experiments conducted to determine the $LD_{50/35}$ the conditions of irradiation were altered in the amount of total dose administered. The doses used were 650 R, 750 R, and 850 R. The above dosage was calculated from the results.

Differential Counts

Samples of blood were collected by puncture of the marginal wing vein. All samples were collected in the morning. Blood films were stained with Wright's stain.

At least three differential counts per sample per chicken were made (Lucas et al., 1961). All slides were scanned from right to left, left to right, and at several levels to diminish errors due to different distributions of

the cells on the slide because of size and/or density. The leukocytes were classified as lymphocytes, monocytes, heterophils, basophils, or eosinophils (Lucas et al., 1961). If the number of leukocytes on two slides was less than 300, as in the slides from irradiated chickens at 14 days post hatching, no differential counts could be recorded.

Total Leukocyte Counts*

Total leukocyte counts were made of the chickens at 14 and 38 days of age to determine the effect of irradiation on the total white blood cell counts. A free flowing drop of blood from the wing vein was drawn to the 0.5 mark on a standard white blood cell diluting pipette and diluted immediately to the 11 mark with Reese-Ecker stain as a diluent. Each pipette was shaken for a minimum of 30 seconds, after which a sample was transferred to a hemocytometer and total white blood cell counts made. The number of white blood cells per cubic mm was calculated.

Necropsy

After completion of all studies, the chickens were necropsied. The thymectomized chickens were examined grossly from the cranium to the thyroid gland for remnants of thymic tissue. Serial sections of the thyroid gland were examined microscopically for remnants of lymphoid tissue.

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*These determinations were made cooperatively with a fellow graduate student, Hugh T. Fauser.

When thymectomies were considered complete by gross examination, but remnants of lymphoid tissue were found on microscopic examination of the thyroid gland or fat deposits in the vicinity of the thymus gland locations, the results obtained from such chickens were included in the thymectomized group in the studies of the blood after a preliminary examination of the results indicated that somewhat less than complete removal was effective.

Statistical Analysis

All data was analyzed by the basic analysis of variance technique for cross classified data. Where supplementary testing was indicated orthogonal contrasts (Li, 1964) were used. Because the number of variables under study either exceeded 2, or analysis was further complicated by unequal sample sizes and sub-sampling (Ruble et al., 1968), the MSU computer was used.* Data was tested for variance homogeneity with Cochrans test (Kirk, 1968).

Infection

At 2½ months of age chickens from each of the four treatment groups (thymectomized, thymectomized irradiated, normal, and irradiated) were inoculated intradermally with 5 mg wet weight of viable M. avium. Twelve weeks after infection, blood samples were collected for differential

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*Use of the Michigan State University computing facilities was made possible through support, in part, from the National Science Foundation.

counts from the infected and control chickens. All chickens were then skin tested intradermally in the wattle with 0.1 cc of mammalian tuberculin (Panigrahi, 1969). Three days after skin testing, blood samples were taken from all chickens for differential counts.

RESULTS

The percent mortality due to 850 R, 750 R, and 650 R for 3 day old thymectomized and normal chickens is presented in Figure 1. The irradiation at 850 R produced a 79 percent mortality, and 750 R and 650 R caused a mortality rate below 50 percent.

Figure 2 represents the percent mortality and range of variation of the mortality rate for three separate groups of chickens subjected to 800 R, the $LD_{50/35}$.

The effect of thymectomy, sham thymectomy, and X-irradiation on the number of lymphocytes/100 leukocytes 14 and 38 days after hatching is shown in Tables 1 and 2. Table 3 indicates that the mean difference of the three combined scores within each group between 14 and 38 days after hatching was the same. Surgical manipulation did not affect the aging effect as reflected by the number of lymphocytes/300 leukocytes. The analysis of this data is presented in Table 4. For each of the three replicate scores, surgery produced a significant reduction of the number of lymphocytes/100 leukocytes. Supplementary testing indicated the reduction was found only in the thymectomized group. X-irradiation had no significant effect upon lymphocytes/100 leukocytes. No significant interaction between thymectomy and

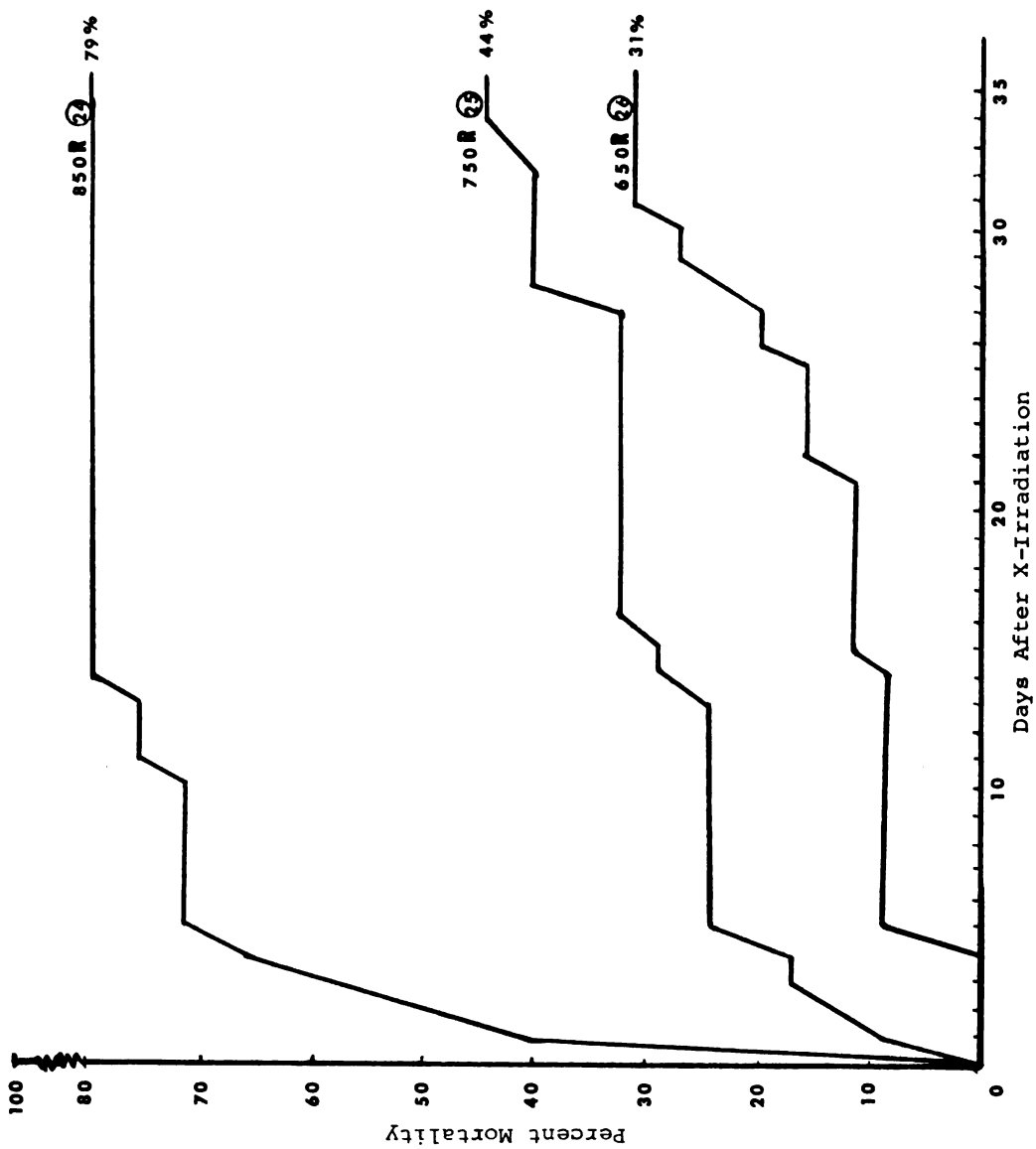

Figure 1. Percent mortality of 3 day old thymectomized and normal chickens following X-irradiation. The circled numbers indicate the number of chickens represented by each line at the dose indicated.

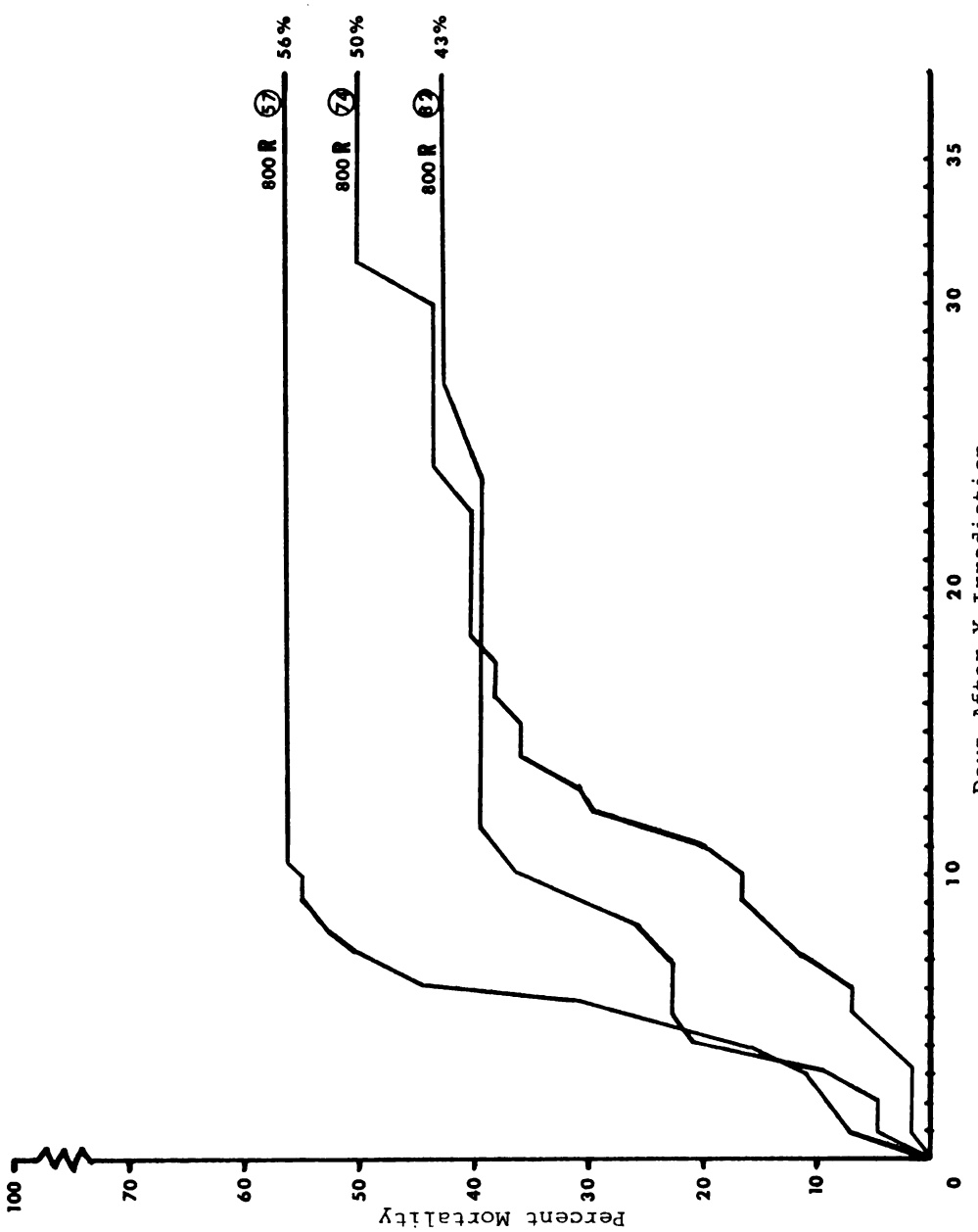

Figure 2. Percent mortality of 3 day old thymectomized and normal chickens following 800 R X-irradiation. The circled numbers indicate the number of chickens represented by each line.

Table 1. The number of lymphocytes/100 leukocytes 14 days after hatching, three counts/chicken

	Non-X-Irradiated	
	Chicken #	# Lymphocytes/100 Leukocytes
Normal	N-135	63,63,60
	N-134	63,50,64
	N-108	81,67,73
	N-123	62,65,74
	N-110	68,56,60
	N-107	44,55,47
	N-109	48,56,60
	N-115	44,58,59
	N-118	51,59,59
	N-113	66,66,62
	N-116	56,71,67
Sham Thymectomized	S-69	57,56,69
	S-64	63,60,50
	S-68	46,59,67
	S-61	59,70,73
	S-66	55,72,72
	S-75	79,63,59
	S-73	73,74,64
	S-60	51,52,58
	S-62	48,48,41
Thymectomized	Tx-37	35,40,38
	Tx-130	37,36,36
	Tx-39	30,42,39
	Tx-34	59,55,53
	Tx-41	46,52,55
	Tx-40	25,24,28
	Tx-42	40,39,43
	Tx-31	45,43,48
	Tx-36	30,38,31
	Tx-33	54,56,53
	Tx-131	28,39,39
	Tx-38	28,39,38
	Tx-43	48,36,32

Table 2. The number of lymphocytes/100 leukocytes 38 days after hatching, 3 counts/chicken

	Non-X-Irradiated		X-Irradiated	
	Chicken No.	No. Lymphocytes/100 Leukocytes	Chicken No.	No. Lymphocytes/100 Leukocytes
Normal	N-135	52,58,54	X-119	42,50,52
	N-134	21,29,16	X-86	52,51,56
	N-108	63,54,69	X-99	40,39,56
	N-123	27,33,32	X-85	61,56,55
	N-110	59,66,72	X-79	39,32,47
	N-107	32,32,36	X-100	52,55,48
	N-109	72,81,74	X-80	65,64,67
	N-115	58,62,78	X-94	43,49,39
	N-118	41,42,55	X-101	41,42,46
	N-113	51,56,60	X-84	55,56,49
	N-116	56,55,63	X-87	55,45,48
	N-105	28,29,29	X-102	41,33,33
			X-121	48,51,55
			X-93	32,43,37
			X-78	69,67,64
Sham Thymectomized	S-69	41,61,59	Sx-57	71,67,58
	S-64	23,34,42	Sx-59	55,56,59
	S-68	53,48,49	Sx-45	82,81,76
	S-61	46,46,41	Sx-54	58,63,60
	S-66	66,55,61	Sx-51	55,58,51
	S-75	56,67,62		
	S-73	61,61,61		
	S-60	49,42,45		
	S-62	31,41,47		
Thymectomized	Tx-37	19,24,23	Tx-X-18	22,40,36
	Tx-130	17,29,29	Tx-X-24	20,19,24
	Tx-39	14,08,05	Tx-X-13	27,20,24
	Tx-34	42,43,43	Tx-X-126	04,14,08
	Tx-41	25,23,28	Tx-X-128	29,42,45
	Tx-40	15,14,17	Tx-X-125	29,26,28
	Tx-42	33,31,31	Tx-X-5	23,22,23
	Tx-31	17,29,22	Tx-X-17	25,28,21
	Tx-36	19,31,35	Tx-X-3	23,30,32
	Tx-33	38,33,36	Tx-X-9	13,11,23
	Tx-131	12,12,17	Tx-X-129	39,29,30
	Tx-38	49,48,33	Tx-X-27	26,33,36
	Tx-43	22,38,38		

Table 3. Aging effect: number of lymphocytes/300 leukocytes in normal, sham operated, and thymectomized chickens at 14 and 38 days post hatching

Normal (N)			Sham Operated (S)			Thymectomized (Tx)		
Chicken No.	14 Days	38 Days	Chicken No.	14 Days	38 Days	Chicken No.	14 Days	38 Days
N-118	169	138	S-73	211	183	Tx-33	163	107
N-113	194	167	S-69	182	161	Tx-34	167	128
N-107	146	100	S-62	137	119	Tx-31	136	68
N-115	161	198	S-66	199	182	Tx-37	113	66
N-123	201	92	S-64	173	99	Tx-40	77	46
N-135	186	164	S-60	161	136	Tx-42	122	95
N-109	164	227	S-68	172	150	Tx-130	109	75
N-110	184	197	S-75	201	185	Tx-41	153	76
N-134	177	66	S-61	202	133	Tx-43	116	98
N-116	194	174				Tx-39	111	27
N-108	221	186				Tx-36	99	85
N-105	131	86				Tx-131	106	41
						Tx-38	105	130

One way analysis of variance for unequal sample size:

$$H_0: u_{\text{difference}_N} = u_{\text{difference}_S} = u_{\text{difference}_{Tx}}$$

$$N_1: u_{d_N} = u_{d_S} = u_{d_{Tx}}$$

Source	d.f.	S.S.	MSS.	F
Among	2	428.619	214.310	0.177
Within	31	37461.499	1208.435	
Total	33	37890.118	At 0.05 level not sig.	

With addition transformation $F_{(2,31)} = 0.177$

Table 4. Two-way analyses of the variance of lymphocytes/100 leukocytes 38 days after hatching--three replicate scores

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
Score 1					
Surgical manipulation	11,178.45451	2	5,589.22725	36.5371	0.0005*
X-irradiation	507.85297	1	507.85297	3.3199	0.073
Interaction	734.19543	2	367.09772	2.3997	0.099
Error	9,178.44103	60	152.97402		
Total	20,687.75758	65			
Score 2					
Surgical manipulation	10,198.11540	2	5,099.05670	35.8718	0.0005*
X-irradiation	224.26496	1	224.26496	1.5777	0.214
Interaction	640.81247	2	320.40623	2.2541	0.114
Error	8,528.79530	60	142.14659		
Total	18,841.59091	65			
Score 3					
Surgical manipulation	10,292.88853	2	5,146.44426	34.4808	0.0005*
X-irradiation	56.33041	1	56.33041	0.3774	0.541
Interaction	311.93691	2	155.96846	1.0450	0.358
Error	8,955.31966	60	149.25533		
Total	19,274.48485				

X-irradiation occurred. The simple correlation between score 1 and 2, score 2 and 3, score 1 and 3 are: 0.91992, 0.92522, and 0.89022, respectively.

The number of leukocytes/mm^3 of blood for each of the four groups of chickens, thymectomized, thymectomized X-irradiated, X-irradiated and normal at 14 and 38 days after hatching is presented in Table 5. Table 6 presents the analyses of the effects of X-irradiation and thymectomy in the chicken on the leukocytes/mm^3 of blood.

The two way analysis of the leukocytes/mm^3 at 14 days after hatching indicated a significant reduction in the leukocyte/mm^3 due to X-irradiation but no significant effect due to thymectomy. At 38 days after hatching the affect of X-irradiation was no longer significant ($\alpha = 0.05$) and thymectomy produced no significant effect. The last analysis in Table 6 indicated that the difference in the effect due to X-irradiation at 14 and 35 days after hatching is statistically significant. No interaction between thymectomy and X-irradiation was detected in any of the analyses presented in Table 6.

Figure 3 shows the lack of separability of the thymus and the thyroid gland.

Data were collected at 5½ months after hatching to determine if thymectomy or X-irradiation caused a prolonged effect upon the number of lymphocytes/100 leukocytes. In addition, data to determine the effects of infection with M. avium and tuberculin testing were collected. The data

Table 5. Leukocytes/mm^3 in experimental chickens 14 and 38 days after hatching

Non-Irradiated			Irradiated		
Chicken #	14 Days	38 Days	Chicken #	14 Days	38 Days
Normal					
N-645	22,208	27,712	X-665	8,000	23,488
N-667	20,032	28,416	X-666	4,800	32,512
N-524	27,712	26,432	X-667	7,488	11,712
N-526	20,224	28,288	X-668	14,400	18,688
N-527	13,056	5,952	X-670	7,488	14,912
N-529	13,504	11,072	X-672	9,600	17,600
N-531	11,520	12,800	X-674	6,400	19,712
N-532	30,400	27,328	X-675	8,512	13,312
N-533	26,688	17,472	X-676	3,200	20,800
N-705	40,512	42,688	X-677	4,800	18,688
N-706	40,000	17,088	X-679	13,312	16,512
N-707	15,424	18,112	X-685	1,088	16,000
N-708	57,088	16,000	X-686	5,888	7,424
N-710	26,688	24,000	X-687	5,312	34,688
N-711	29,312	36,800	X-688	18,112	19,712
N-712	14,400	49,088	X-689	3,712	9,600
N-713	24,000	35,200	X-690	14,400	30,912
N-715	13,888	25,600	X-691	3,712	11,712
N-718	14,400	37,888	X-693	8,512	58,688
N-719	32,000	37,312	X-696	4,800	19,200
N-720	29,888	20,288	X-698	9,088	9,600
N-723	19,712	22,400	X-699	10,112	22,400
N-724	11,200	9,088	X-700	8,512	14,912
			X-701	7,488	18,688
			X-702	12,800	19,200
			X-703	11,712	21,888
Thymectomized					
Tx-640	5,888	27,712	Tx-X-601	8,000	25,600
Tx-641	26,688	9,088	Tx-X-602	20,800	10,848
Tx-648	40,000	41,600	Tx-X-603	12,800	26,112
Tx-649	30,400	16,512	Tx-X-604	16,000	14,400
			Tx-X-605	9,088	25,600
			Tx-X-606	11,200	13,824
			Tx-X-608	6,912	17,600
			Tx-X-609	17,088	12,288
			Tx-X-610	13,312	22,912
			Tx-X-612	5,312	24,512
			Tx-X-615	16,512	32,000
			Tx-X-618	8,000	18,688
			Tx-X-622	12,224	14,400
			Tx-X-623	18,688	19,712
			Tx-X-624	6,400	12,800
			Tx-X-625	14,400	29,888
			Tx-X-626	6,912	28,288
			Tx-X-627	9,600	13,824
			Tx-X-629	6,912	22,912
			Tx-X-630	4,288	6,400
			Tx-X-632	3,200	2,688
			Tx-X-635	9,600	16,512
			Tx-X-636	10,624	10,688
			Tx-X-639	10,112	14,400

Table 6. The analyses of leukocytes/mm^3 at 14 and 38 days after hatching and differences between 14 and 38 days

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
	Two-Way Analysis of Variance at 14 Days After Hatching of Leukocytes/mm^3				
X-irradiation	6,228,706,355.87500	1	6,228,706,355.87500	43.1619	0.0005*
Thymectomy	115,865,235.55469	1	115,865,235.55469	0.8029	0.373
Interaction	5,108,693.22009	1	5,108,693.22009	0.0354	0.851
Error	10,534,655,277.25000	73	144,310,346.26172		
Total	20,261,443,636.00000	76			
	Two-Way Analysis of Variance at 38 Days After Hatching of Leucocytes/mm^3				
X-irradiation	724,001,897.14063	1	724,001,897.14063	2.9585	0.090
Thymectomy	68,689,903.49609	1	68,689,903.49609	0.2807	0.598
Interaction	1,781,650.97873	1	1,781,650.97873	0.0073	0.932
Error	17,864,607,908.50000	73	244,720,656.28125		
Total	19,380,482,207.50000	76			
Two-Way Analysis of Variance Comparing the Differences of Leukocytes/mm^3 Between 14 and 38 Days After Hatching					
X-irradiation	2,705,546,731.93750	1	2,705,546,731.93750	7.9921	0.006*
Thymectomy	362,979,037.10156	1	362,979,037.10156	1.0722	0.304
Interaction	12,924,218.00439	1	12,924,218.00439	0.0382	0.846
Error	24,712,587,128.00000	73	338,528,590.79688		
Total	29,008,348,571.00000	76			

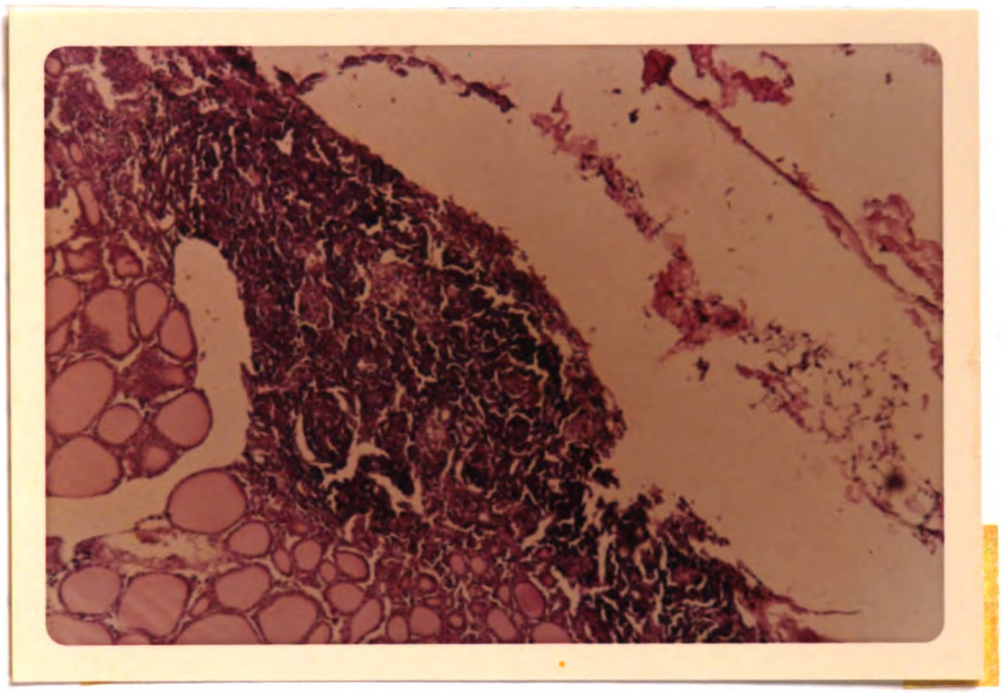

Figure 3. Continuity of thymus cells (darker staining area) with cells of the thyroid gland (Hematoxylin and Eosin stain).

is presented in Table 7. Table 8 shows the four way analysis of variance for unequal sample sizes performed on the data of Table 7. In the analysis for each of the three scores, there were significant interactions ($\alpha = 0.05$). The following interactions existed: (1) two three-way interactions: among thymectomy, X-irradiation and infection; and among irradiation, infection, and tuberculin testing. Two-way interactions between thymectomy and infection, thymectomy and X-irradiation, and infection and tuberculin testing were found. The existence of these interactions makes the tests of significance listed in Table 8 for the main effects, namely thymectomy, X-irradiation, infection, and tuberculin testing, invalid.

To facilitate the interpretation of the nature of interactions, the three scores from Table 8 were combined. The simple correlations between scores 1 and 2, scores 2 and 3, and scores 1 and 3 are: 0.91332, 0.88231, 0.83351, respectively. The four-way analysis of variance for unequal sample sizes with the average of the three scores taken per sample is presented in Table 9. The dominant interactions were ABC, CD, and AB: (1) thymectomy, with X-irradiation with infection, (2) infection with tuberculin testing, and (3) thymectomy with X-irradiation, respectively. The three-way interaction as graphed in Figure 4 was characterized by a different response in thymectomized and normal chickens, depending upon whether or not they had been X-irradiated; furthermore, this interaction between thymectomy and

Table 7. The number of lymphocytes/100 leukocytes in experimental chickens 5½ months of age

	Non-X-Irradiated		X-Irradiated	
	Chicken #	# Lymphocyte/100 Leukocytes	Chicken #	# Lymphocyte/100 Leukocytes
	Noninfected--Sampled Before Tuberculin Testing			
Normal	N-920	55,57,54	X-893	85,90,92
	N-924	52,52,42	X-912	76,80,62
	N-926	67,55,76	X-914	90,90,87
	N-929	80,66,84	X-915	86,85,86
	N-935	86,83,78		
Thymectomized	Tx-835	48,44,56	Tx-X-847	43,41,38
	Tx-836	68,69,63	Tx-X-848	81,73,58
	Tx-837	57,60,68	Tx-X-859	47,59,47
	Tx-842	36,27,31	Tx-X-864	29,38,36
	Noninfected--Sampled After Tuberculin Testing			
Normal	N-920	66,69,64	X-893	88,79,81
	N-924	55,47,50	X-912	80,75,82
	N-926	77,60,72	X-914	84,92,86
	N-929	62,55,51	X-915	83,81,86
	N-935	67,77,79		
Thymectomized	Tx-835	40,40,50	Tx-X-847	35,43,52
	Tx-836	59,70,67	Tx-X-848	49,56,55
	Tx-837	44,51,72	Tx-X-859	48,49,50
	Tx-842	45,41,45	Tx-X-864	36,31,25
	Infected--Sampled Before Tuberculin Testing			
Normal	N-922	91,94,94	X-892	60,63,65
	N-931	96,93,89	X-897	90,90,86
	N-934	73,84,88	X-898	91,93,88
	N-937	86,91,92	X-899	84,87,90
	N-938	94,88,86	X-902	83,76,88
			X-908	87,86,78
			X-909	91,80,79
Thymectomized	Tx-831	72,77,67	Tx-X-853	74,72,76
	Tx-833	75,81,87	Tx-X-855	82,80,85
	Tx-843	79,76,89	Tx-X-857	70,71,74
			Tx-X-861	74,65,56
			Tx-X-876	78,77,75
	Infected--Sampled After Tuberculin Testing			
Normal	N-922	46,67,79	X-892	87,81,76
	N-931	66,56,58	X-897	90,85,83
	N-934	66,73,63	X-898	85,88,94
	N-937	88,80,87	X-899	86,82,79
	N-938	84,69,80	X-902	82,84,76
			X-908	79,84,81
			X-909	83,87,91
Thymectomized	Tx-831	65,67,51	Tx-X-853	64,70,65
	Tx-833	40,52,58	Tx-X-855	73,72,75
	Tx-843	43,40,56	Tx-X-857	71,71,57
			Tx-X-861	58,56,55
			Tx-X-876	65,50,48

Table 8. The analysis of the number of lymphocytes/100 leukocytes in experimental chickens 5½ months of age (four-way analysis of variance, three scores)

Source[a]	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
Score 1					
A	7909.76558	1	7909.76558	69.8921	0.0005
B	821.28579	1	821.28579	7.2570	0.009
C	2743.54915	1	2743.54915	24.2425	0.0005
D	1299.48815	1	1299.48815	11.4825	0.001
AB	335.44329	1	355.44329	2.9640	0.090
AC	693.57627	1	693.57627	6.1286	0.016*
AD	220.94239	1	220.94239	1.9523	0.168
BC	0.00131	1	0.00131	0.0000	0.997
BD	331.71162	1	331.71162	2.9311	0.092
CD	358.64904	1	358.64904	3.1691	0.080
ABC	649.29101	1	649.29101	5.7372	0.020*
ABD	13.82974	1	13.82974	0.1222	0.728
BCD	356.95230	1	356.95230	3.1541	0.081
ACD	17.99532	1	17.99532	0.1590	0.692
ABCD	1.84069	1	1.84069	0.0163	0.899
Error	6563.92619	58	113.17114		
Total	22380.55405	73			
Score 2					
A	6323.35876	1	6323.35876	59.9295	0.0005
B	803.46046	1	803.46046	7.6148	0.008
C	2914.35746	1	2914.35746	27.6208	0.0005
D	1189.55090	1	1189.55090	11.2739	0.001
AB	645.93034	1	645.93034	6.1218	0.016*
AC	363.33582	1	363.33582	3.4435	0.069
AD	83.71257	1	83.71257	0.7934	0.377
BC	205.98249	1	205.98249	1.9522	0.168
BD	200.86511	1	200.86511	1.9037	0.173
CD	436.19497	1	436.19497	4.1340	0.047*
ABC	555.13712	1	555.13712	5.2613	0.025*
ABD	42.68259	1	42.68259	0.4045	0.527
BCD	714.53395	1	714.53395	6.7720	0.012*
ACD	49.79341	1	49.79341	0.4719	0.495
ABCD	1.69171	1	1.69171	0.0160	0.900
Error	6119.77143	58	105.51330		
Total	20963.09459	73			
Score 3					
A	6251.63939	1	6251.63939	49.6535	0.0005
B	36.88711	1	36.88711	0.2930	0.590
C	2656.80314	1	2656.80314	21.1016	0.0005
D	728.79115	1	728.79115	5.7884	0.019
AB	1076.60888	1	1076.60888	8.5509	0.005*
AC	305.30301	1	305.30301	2.4249	0.125
AD	82.01149	1	82.01149	0.6514	0.423
BC	52.84539	1	52.84539	0.4197	0.520
BD	287.54473	1	287.54473	2.2838	0.136
CD	917.64330	1	917.64330	7.2884	0.009*
ABC	792.26990	1	792.26990	6.2926	0.015*
ABD	48.48346	1	48.48346	0.3851	0.537
BCD	210.18776	1	210.18776	1.6694	0.201
ACD	246.53196	1	246.53196	1.9581	0.167
ABCD	5.28346	1	5.28346	0.0420	0.838
Error	7302.59714	58	125.90530		
Total	21105.36486	73			

[a]A = not thymectomized vs. thymectomized; B = not X-irradiated vs. irradiated; C = not infected vs. infected; and D = before tuberculin testing vs. after tuberculin testing.

Table 9. Four-way analysis of variance--three scores combined from Table 8

Source[a]	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
A	6807.55476	1	6807.55476	75.9501	0.0005
B	442.07779	1	442.07779	4.9321	0.030
C	2770.54412	1	2770.54412	30.9102	0.0005
D	1056.99574	1	1056.99574	11.7926	0.001
AB	650.96401	1	650.96401	7.2626	0.009*
AC	439.18367	1	439.18367	4.8999	0.031
AD	121.51100	1	121.51100	1.3557	0.249
BC	51.77015	1	51.77015	0.5776	0.450
BD	270.52338	1	270.52338	3.0182	0.088
CD	546.24946	1	546.24946	6.0944	0.017*
ABC	662.02993	1	662.02993	7.3861	0.009*
ABD	32.92860	1	32.92860	0.3674	0.547
BCD	401.62568	1	401.62568	4.4808	0.039
ACD	80.99922	1	80.99922	0.9037	0.346
ABCD	0.61604	1	0.61604	0.0069	0.934
Error	5198.64974	58	89.63189		
Total	19706.90240	73			

[a]A = not thymectomized vs. thymectomized; B = not X-irradiated vs. irradiated; C = not infected vs. infected; and D = before tuberculin testing vs. tuberculin testing.

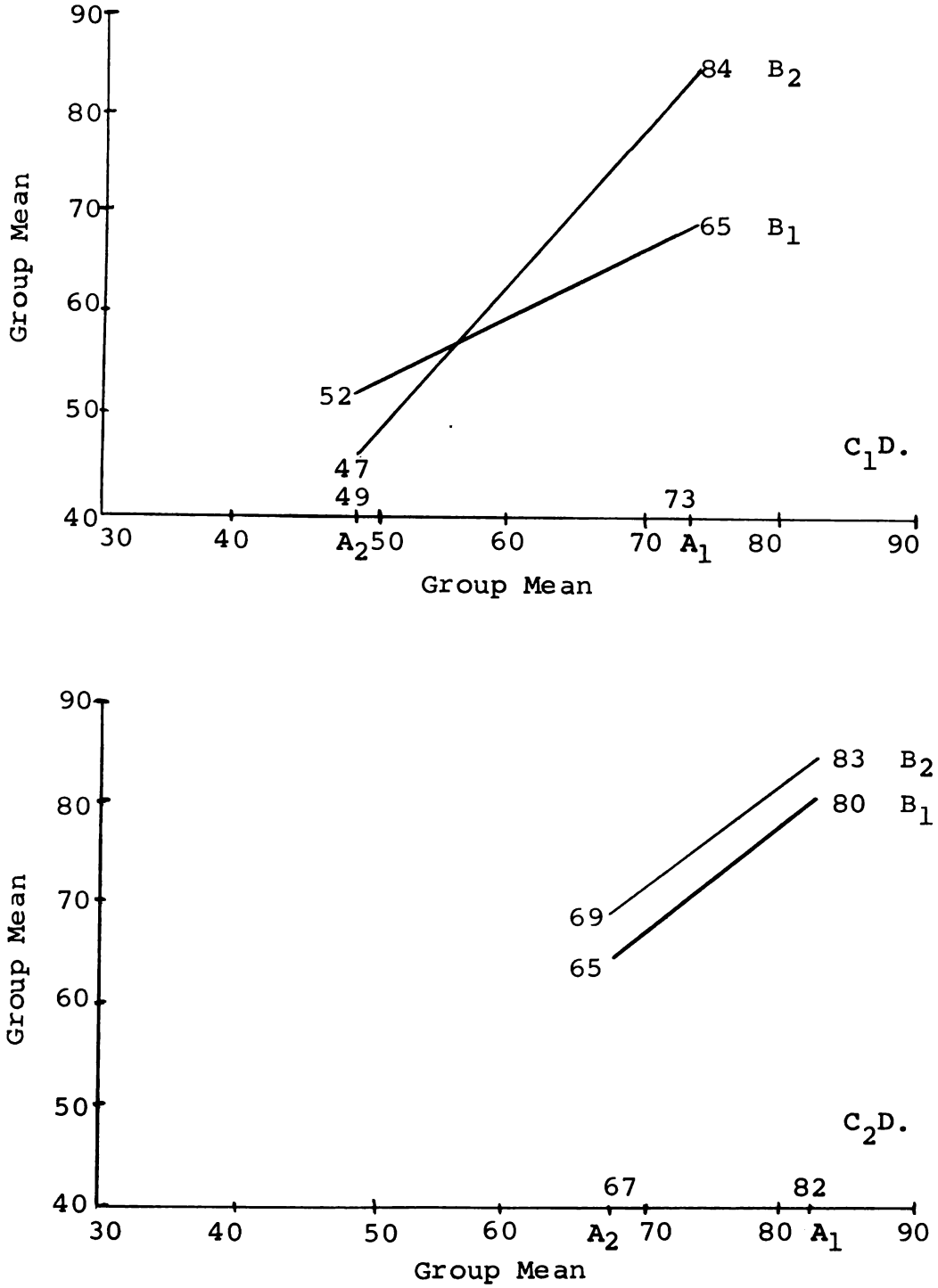

Figure 4. Representation of the three-way interaction between cross classified thymectomy, X-Irradiation, and infection. A_1 = Normal, A_2 = Thymectomized, B_1 = Not X-Irradiated, B_2 = X-Irradiated, C_1 = Not Infected, C_2 = Infected, D. = Summation Before and After Tuberculin Testing.

X-irradiation occurred only in the noninfected group. The two-way interaction between thymectomy and X-irradiation did not occur in the infected group of chickens. The interaction between thymectomy and X-irradiation as graphed in Figure 5 is such that X-irradiation resulted in an increase in the percent of lymphocytes in normal chickens but when coupled with thymectomy was followed by no detectable effect as compared with thymectomy. The manner in which infection and tuberculin testing interacted as shown in Figure 6 is the following: in infected chickens, tuberculin testing caused a lowering in the percent of lymphocytes, in noninfected chickens, tuberculin testing was not accompanied by such a response. In Table 9, as in Table 8, the significance of the four main treatments as given is invalid.

Because the significance of thymectomy, X-irradiation, infection, and tuberculin testing could not be taken directly from the four-way analyses of variance of either Table 8 or 9, orthogonal contrasts were made to test the effects of these four main treatments. The results obtained are in Table 10. The effects of thymectomy, X-irradiation, infection, and tuberculin testing were tested singly within different treatment combinations of the effects not being tested. For example, D/C_1 represents the following contrast comparison: the percent of lymphocytes prior to tuberculin testing of all chickens within the noninfected group, with the percent of lymphocytes after tuberculin testing within the noninfected group. There was no significant difference.

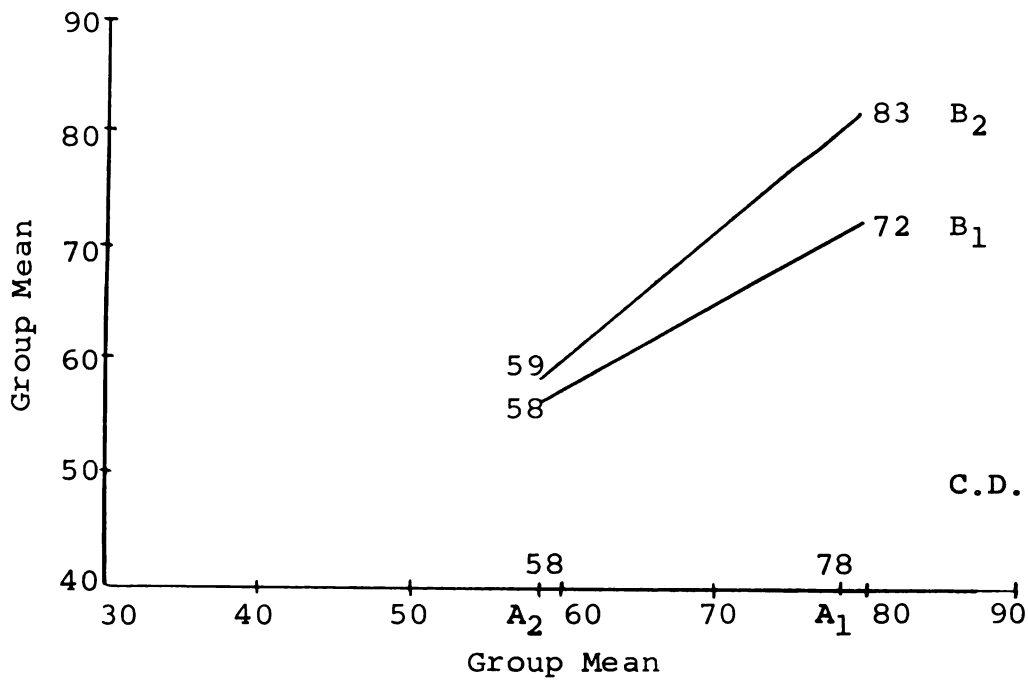

Figure 5. Representation of the two-way interaction between cross classified thymectomy and X-irradiation. A_1 = Normal, A_2 = Thymectomized, B_1 = Not X-Irradiated, B_2 = X-Irradiated. C.D. = Summation of Infected and Noninfected Chickens Before and After Tuberculin Testing.

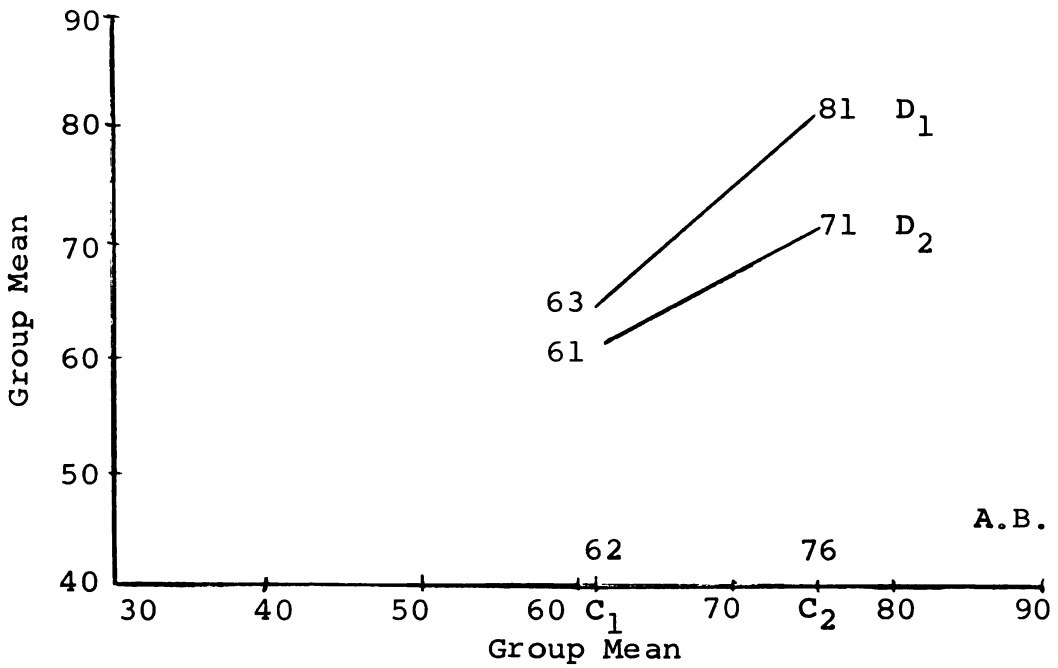

Figure 6. Representation of the two-way interaction between cross classified infection and tuberculin testing. C_1 = Noninfected Chickens, C_2 = Infected Chickens, D_1 = Before Tuberculin Testing, D_2 = After Tuberculin Testing. A.B. = Summation of Normal and Thymectomized Chickens Whether Irradiated or Not.

Table 10. The significance of treatments in the percentage of lymphocytes due to thymectomy, X-irradiation, infection, and tuberculin testing

Contrast[a]	Significance
D/C_1	n.s.
D/C_2	*
A/C_1	*
A/C_2	*
B/C_2	n.s.
A/B_1C_1	*
A/B_2C_1	*
B/A_1C_1	*
B/A_2C_1	*
$C/A_1B_1D_1$	*
$C/A_1B_1D_2$	*
$C/A_2B_1D_1$	*
$C/A_2B_1D_2$	n.s.
$C/A_2B_2D_1$	*
$C/A_2B_2D_2$	*

[a] A_1 = no thymectomy, A_2 = thymectomy; B_1 = no X-irradiation, B_2 = X-irradiation; C_1 = no infection, C_2 = infection; and D_1 = before tuberculin testing, D_2 = after tuberculin testing.

*Significant ($\alpha = 0.01$); n.s. = not significant.

The second contrast D/C_2 shows that tuberculin testing had a significant effect in decreasing the percent of lymphocytes in infected chickens. Tuberculin testing had a significant effect only in infected chickens.

The effect of thymectomy was tested within four levels of the other treatment combinations, within noninfected, within infected, within non-X-irradiated noninfected, and within X-irradiated noninfected. In all of these contrasts thymectomy produced a significant effect upon the percent of lymphocytes/100 leukocytes. The data shows the effect to be a decrease.

The significance of X-irradiation was tested within the infected chickens, within nonthymectomized noninfected chickens, and within thymectomized noninfected chickens. In chickens not infected with M. avium, there was no significant effect on the percentage of lymphocytes due to X-irradiation. In both nonthymectomized and thymectomized chickens infected with M. avium, X-irradiation had a significant detectable effect upon the percent of lymphocytes. The data shows the effect to be an increase in the percent of lymphocytes.

The significance of infection in affecting the percentage of lymphocytes also depends upon the combination of other treatments within which the effect of infection were being tested. Infection with M. avium had a significant effect on normal, nonirradiated chickens in increasing the percent of lymphocytes both before and after tuberculin testing. The thymectomized nonirradiated chickens had a

significant increase only before tuberculin testing but none after tuberculin testing. Infection had a significant effect in increasing the percent of lymphocytes in thymectomized X-irradiated chickens before and after tuberculin testing.

X-irradiation, infection, and tuberculin testing show significant effects upon the percent of lymphocytes depending upon the combination of other treatments. Thymectomy was associated with a significant decrease in the percent of lymphocytes in all of the meaningful contrasts tested.

The results obtained from the reactions to tuberculin are given in the Appendix.

DISCUSSION

The thymus is the first lymphoid organ to function in the chicken embryo and the thymus dependent white pulp of the spleen develops shortly after hatching; therefore, X-irradiation after thymectomy has been recommended to destroy any peripheral thymus dependent lymphocytes. The $LD_{50/35}$ was established for 3 day old chickens to determine whether the percent of lymphocytes in the peripheral blood was reduced more by X-irradiation and thymectomy than by thymectomy only; neonatal thymectomy in the chicken is followed by a depletion in the percent of lymphocytes in the peripheral blood.

The results of differential blood counts 14 days after hatching indicated that thymectomy has a statistically significant effect in lowering the percent of lymphocytes. No comparisons could be made with the X-irradiated chickens because of a marked anemia in this group at 14 days after hatching and there were not sufficient leukocytes on two blood smear slides for a differential count.

A comparison of the mean difference in the percent of lymphocytes between 14 and 38 days among thymectomized, sham-thymectomized and normal chickens showed all groups to

be equal. Therefore, the aging or maturation effect was not affected by thymectomy.

At 38 days after hatching, thymectomized chickens and thymectomized X-irradiated chickens had a decrease in the percent of lymphocytes. There was no difference between thymectomized and thymectomized X-irradiated chickens in the extent of the decrease. Thymectomy with X-irradiation did not cause any greater decrease of the lymphocytes in the peripheral blood than thymectomy only. The conclusion drawn is that X-irradiation is not required after thymectomy to reduce the percent of lymphocytes in the peripheral blood.

The X-irradiation was not without effect, but this effect was not detected as having long term infuence on the lymphocyte alone or as persistent as the effect of thymectomy. Fourteen days after hatching, the total leukocyte count from X-irradiated chickens was significantly lower than in nonirradiated chickens. This explains why there were insufficient leukocytes to perform differential blood counts 14 days after hatching. Significant to note is that there was no decrease in the total leukocyte count in the thymectomized group of chickens.

There was recovery with time of the lowered leukocyte count of the blood due to X-irradiation. By 38 days after hatching no effect on the total white blood cell count could be detected due to X-irradiation or to thymectomy. A comparison of the total leukocyte count made between 14 and 38 days after hatching indicated that a difference occurred

only in the X-irradiated group. In the X-irradiated group, recovery from the effects of X-irradiation was complete by 38 days after hatching or 35 days after X-irradiation. It was concluded that the thymus was not required for this recovery.

Thirty-eight days after hatching was chosen as the time to collect blood samples in these studies because the death rate due to X-irradiation reached its plateau by this time, and chickens in the group surviving X-irradiation were to be used in future studies. By 38 days after hatching the spleen which is a lymphogenic organ in the mature chicken has released the heterophils stored there during embryonation and is functioning in its adult capacity. If the effects of thymectomy in decreasing the percent of lymphocytes were to be counteracted by production of lymphocytes by the spleen, these should have been detected. No such counteracting effects were detected. At this time chickens are still considered immunologically incompetent (Wolfe et al., 1949). Differential blood counts showed a persistent decrease in lymphocytes in the thymectomized chickens at 38 days post hatching. The total leukocyte counts confirmed that X-irradiation decreased the numbers of all leukocytes and that recovery occurred by 35 days post X-irradiation. No such recovery occurred from the effects of thymectomy as detected by differential blood counts.

The results showed that thymectomy in chickens performed 24 hours post hatching was followed by a decrease in

the percent of lymphocytes in the peripheral blood. This decrease was persistent through 38 days after thymectomy. X-irradiation is followed by a significant decrease in all white blood cells as measured 11 days after irradiation but this decrease did not persist.

The data indicate that if thymectomy in the chicken was performed early, at least within 24 hours after hatching, X-irradiation was not necessary to cause a significant decrease in the percent of lymphocytes. So far as known, the chicken does not have lymphatics through which lymphocytes travel. Furthermore, leukocytes are not found in the peripheral circulation of the embryo. All leukocytes are released into the blood stream within 24 hours after hatching, and the lymphocytes are released after 24 hours after hatching (Lucas et al., 1961). This emphasizes the importance of performing thymectomy early enough to prevent the peripheralization of thymocytes into the circulation.

No attempts were made to classify the lymphocytes according to size. Szenberg and Shortman (1966) reported that lymphocytes represent a mixture of cells with different biological functions. Cell populations from the thymus and bursa differ in density and function, but this difference exists between cells of the same size and morphology. Also, the large bursa dependent lymphocytes are rarely found in the peripheral blood (Lucas et al., 1961).

Variability in determinations of total white blood cell counts depending upon the methods used has been reported

(Denington et al., 1955). The choice of Reese-Eckers stain as a diluent was made because it has been reported to have the lowest coefficient of variability. The use of a diluent with the lowest coefficient of variability increased the statistical power of the analysis.

The variability in the reported normal ranges of differential blood counts in the chicken is greater than in mammals (Lucas et al., 1961). This variability exists among chickens and within one chicken. A diurnal rhythm has also been reported (Glick et al., 1960; Khussar, 1966); therefore, all blood samples were collected in the morning. To determine how much variabiltiy occurred during the counting process, three separate blood counts were performed. The simple correlations between the three scores were considered to be high enough to indicate that subsequent differences detected between the experimental groups of chickens were indicative of a true difference and were not due to errors in counting.

Chickens which contained remnants of thymus tissue adhering to the thyroid gland at necropsy (incomplete-thymectomy) were included in the studies for two reasons. First, when the differential blood counts of complete-thymectomy chickens were compared with those of incomplete-thymectomy chickens, regardless of X-irradiation, no differences were detected. Therefore, all results should be reviewed in terms of the significance of the removal of substantial thymic tissue rather than the persistence of

small remnants. The second consideration for including incomplete-thymectomy chickens in the tabulation of results is that cells of the thymus invade the thyroid. In fowl, cells of the thymus lie in contact with the thyroid. In many cases, lymphoid area cells of the thyroid are directly continuous with the cells of the thymus, Figure 3. The membrane separating the two glands has disappeared (Payne et al., 1952). This strongly indicates that complete thymectomy, in the sense of complete removal of all thymic tissue can be obtained with certainty only if the thyroid gland is also removed.

Having shown that neonatal thymectomy in the chicken is followed by a significant decrease in the percent of lymphocytes of the peripheral blood at 38 days post hatching, a study was conducted to determine whether in 5½ month old thymectomized chickens the percent of lymphocytes was still lower than in normal chickens. In addition, the effect on the percent of lymphocytes of X-irradiation, infection with M. avium at 2½ months of age and tuberculin testing on 5½ month old chickens was determined. Interactions between various combinations of the four main conditions were found. Each of the conditions tested showed significant effects upon the percent of lymphocytes, but this significance was restricted to certain combinations within the other conditions. To facilitate the interpretation of the interactions, the three replicate scores of differential blood counts were

averaged. A three way interaction between thymectomy, X-irradiation and infection was found: (a) as illustrated in Figure 4 in the noninfected group there was an increase in percent of lymphocytes in the nonthymectomized group due to X-irradiation, (b) in the thymectomized group there was a decrease due to the effects of X-irradiation, (c) the effects of thymectomy upon the percent of lymphocytes was a decrease. In the infected group: (a) the effect of thymectomy upon the percent of lymphocytes was a decrease, (b) the effect of X-irradiation was measured as an increase in both normal and thymectomized groups, (c) all infected chickens showed a higher percent of lymphocytes than their uninfected controls. The prominent two-way interaction (lack of a parallel response) between thymectomy and X-irradiation as illustrated in Figure 5 was due to a greater increase in the percent of lymphocytes in normal irradiated chickens than in thymectomized irradiated chickens. The two-way interaction between infection and tuberculin testing as shown in Figure 6, indicates that tuberculin testing alters the percent of lymphocytes in the infected chickens only. The decrease in the percent of lymphocytes in infected chickens after tuberculin testing may be due to a total increase in some other leukocyte type; this could produce an apparent decrease in percent of lymphocytes, or it may be a real decrease in percent of lymphocytes.

Orthogonal contrasts were constructed, restricting each of the four main conditions within combinations of the other conditions to test what significance the conditions of thymectomy, X-irradiation, infection, and tuberculin testing had upon the percent of lymphocytes in the peripheral blood of chickens. Caution must be used in evaluating the significance of these analyses because the data was not orthogonal (independent). To take this into consideration, an alpha level of 0.01 was set, but the degree of confidence at which the hypothesis could be accepted or rejected is more closely representative of an alpha level of 0.05.

Having determined in Table 9 and illustrated in Figure 6 that an interaction exists between tuberculin testing and infection, the test for the significance of the effect of tuberculin testing was made separately for the noninfected and infected groups. Tuberculin testing in noninfected chickens regardless of thymectomy or X-irradiation caused no detectable change in the percent of lymphocytes. By contrast, tuberculin testing decreased the percent of lymphocytes in all chickens infected with M. avium.

Using this same procedure, it was shown that X-irradiation appeared to have no discernible effect upon the chickens subsequently infected. However, in noninfected X-irradiated chickens there was a significant effect due to X-irradiation in both normal and thymectomized groups. In normal chickens the effect the X-irradiation was an increase

in the percent of lymphocytes, but in thymectomized chickens, X-irradiation was followed by a decrease in the percent of lymphocytes.

This increase in the percent of lymphocytes in normal chickens following X-irradiation is a strong source of the interaction as well as an indication that the long termed effects of X-irradiation are not predicted by the initial sequence of events immediately following irradiation. After the destruction of leukocytes and the initial recovery to normal values, X-irradiated chickens appear to have been stimulated, presumably as a result of X-irradiation, to produce or maintain a higher percentage of lymphocytes than normal. To achieve a reduction in the percent of lymphocytes in thymectomized chickens, by coupling X-irradiation with thymectomy would not be justified, because X-irradiated chickens cannot function as proper controls.

Infection with M. avium increased the percent of lymphocytes in normal, thymectomized, X-irradiated and thymectomized X-irradiated chickens with one exception. The percent of lymphocytes in thymectomized chickens after tuberculin testing is the same in both infected and noninfected chickens. Prior to tuberculin testing, the infected thymectomized group did show a higher percent of lymphocytes than the noninfected thymectomized group.

Thymectomy was always followed by a decline in the percent of lymphocytes in both subsequently infected and noninfected chickens. Whether or not X-irradiation was

administered, there was a persistent decrease in the percent of lymphocytes in all noninfected chickens.

To achieve a reduction in the percent of lymphocytes of the peripheral circulation of the chicken, neonatal thymectomy is indicated. The decrease effected by thymectomy is persistent through 5½ months of age. The need of coupling X-irradiation with thymectomy is highly questionable and in fact contradictory because the longer term effect of X-irradiation was an increase in the percent of blood lymphocytes at 5½ months of age. Furthermore, X-irradiation following thymectomy did cause a greater decrease in the percent of lymphocytes at 38 days after hatching than did only thymectomy.

Because the lymphocyte has been credited as the cell type which mediates the delayed hypersensitivity reaction, the thymectomized chicken affords a unique biological model in which the controlling mechanism of delayed hypersensitivity can be studied *in vivo* as well as *in vitro*. The focus of the research reported here was to develop the technique of thymectomy and to determine its effects upon the percent of lymphocytes in the peripheral blood.

The kind of lymphocyte, and their function or biologic capacities has not been determined. Lischner *et al.* (1967) have reported in a human an analogous situation with the thymectomized chicken. They report that lymphocytes in congenital absence of the thymus fail to participate in a

host versus graft rejection and to develop tuberculin sensitivity. Moreover, the passive transfer for these capacities from immunologically competent cells was unsuccessful.

In the research reported here, what appears to be an increase or decrease in the percent of lymphocytes, may be the result of an unequal decrease or increase in leukocytes other than the lymphocyte; the increase or decrease reported here must be viewed as relative to their controls. Because the thrombocytes in the chicken are stained differently from the leukocytes so as to be differentiated from them when counting in a hemocytometer for total leukocyte counts, a simultaneous differential count is not possible. The morphology of the leukocytes is distinct from that of the thrombocytes but individual differences between them are lost. A technique in which total leukocyte counts and differential counts could be performed simultaneously would answer the question whether the increases and decreases in percent of lymphocytes are relative or absolute.

Of primary interest was the determination that neonatal thymectomy in the chicken is followed by a statistically significant decrease in the percent of lymphocytes up to 5½ months of age. X-irradiation is not necessary to effect a further decrease. The additional conditions of infection with M. avium and tuberculin testing were included as a pilot study to determine whether thymectomized and normal chickens responded in parallel fashion. There are strong indications that the effects of tuberculin testing

are dependent upon infection rather than upon thymectomy. On the other hand the influence of infection upon the percent of lymphocytes within thymectomized chickens only was the same after tuberculin testing as in noninfected thymectomized chickens. These are the first investigations into the possible functions of the lymphocyte during infection and tuberculin testing and must be further studied for a meaningful interpretation.

SUMMARY

The decrease in the percent of lymphocytes following thymectomy one day post hatching and X-irradiation three days post hatching was the same as the decrease in chickens thymectomized one day post hatching. These observations were made in chickens 38 days and 5½ months of age. The necessity of coupling X-irradiation with neonatal thymectomy to destroy thymocytes (thymic lymphocytes) which may have peripheralized prior to hatching was studied by comparing the percent of lymphocytes of X-irradiated normal and thymectomized chickens with non-X-irradiated normal and thymectomized chickens. It was found that complete thymectomized chickens could not be distinguished from incomplete-thymectomized chickens by percent of lymphocytes in differential counts regardless of X-irradiation.

The long term effects of X-irradiation and thymectomy were determined in 5½ month old chickens infected with M. avium at 2½ months before and after tuberculin testing at 5½ months of age. There were statistically significant interactions and only the most prominent were diagrammed. A three-way interaction between thymectomy, X-irradiation, and infection was found. Infection with M. avium increased the percent of lymphocytes in all chickens. In noninfected

chickens, X-irradiation caused a significant increase in the percent of lymphocytes, but when coupled with thymectomy caused a decrease in the percent of lymphocytes. In infected chickens, X-irradiation regardless of thymectomy was followed by increases in the percent of lymphocytes. Regardless of infection or tuberculin testing, X-irradiation increased the percent of lymphocytes in normal chickens more than in thymectomized chickens. There was a decrease in the percent of lymphocytes in infected chickens only after tuberculin testing.

Thymectomized chickens showed a persistent decrease in the percent of lymphocytes at 5½ months of age. The use of X-irradiation after thymectomy is not recommended because of the interactions with thymectomy at 5½ months of age and because no greater decrease in the percent of lymphocytes than that due to thymectomy only was found.

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APPENDIX

APPENDIX

REACTIONS ELICITED BY TUBERCULIN AT 48 HOURS OF *M. avium* INFECTED CHICKENS

	Positive (%)	Negative (%)	Questionable (%)
Normal	82	9	9
X-irradiated	86	0	14
Thymectomized	100	0	0
Thymectomized X-irradiated	82	0	18