STUDIES ON TRANSMISSIBLE LYMPHOID LEUCEMIA OF MICE*

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PLATES 23 AND 24

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One may expect studies on the transmissibility of leucemia to show whether it is an infectious, hyperplastic or neoplastic process and to determine the much debated etiological relation between leucemic and aleucemic lymphadenosis and lymphoma.

Frequent attempts to transmit leucemia in mammals, reviewed by Opie (1), have been made without success, until recently Snijders (2) transmitted lymphoid leucemia of guinea pigs. By intraperitoneal inoculations of blood or emulsions of organs, Snijders reproduced leucemia in 58 successive generations. The majority of the guinea pigs successfully inoculated developed leucemia, the rest, aleucemic lymphadenosis with or without tumor formation at the site of inoculation. He observed that transmissions were unsuccessful with filtrates free from cells. Snijders' investigations have been fully confirmed by Tio Tjwan Gie (3).

Richter and MacDowell (4) transmitted leucemia in a highly inbred strain of grey mice, of which almost every animal that lived longer than about 8 months developed leucemia spontaneously. Young mice of this strain, when inoculated intraperitoneally or subcutaneously with an emulsion of blood or lymph nodes of leucemic mice, developed leucemia within a few days; mice unrelated to them were found to resist similar inoculations. By crossing susceptible mice with mice resistant to transmissible leucemia MacDowell and Richter (5) reached the conclusion that susceptibility to transmission is inherited as a Mendelian dominant character.

Korteweg (6) inoculated mice intraperitoneally with an emulsion of a spontaneous lymphosarcoma of the mediastinum of a mouse. He observed the development of a lymphoid tumor at the site of inoculation in about 25 per cent of the inoculated animals and most of these showed a terminal leucemic blood picture. Attempts at transmission by cell-free material were unsuccessful.

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EXPERIMENTAL

The two transmissible strains described here originate from two spontaneous cases observed in a stock of albino mice designated A. This stock was purchased from a dealer and is being bred in our laboratory for the study of leucemia. Two other stocks of mice, called R and S, have been secured from different sources and are being bred under similar conditions in order to procure spontaneous cases of the disease. Failure to obtain a strain of mice in which leucemia is known to occur necessitated this laborious undertaking. Each mouse is examined for enlargement of lymph nodes and spleen at weekly intervals. The superficial lymph nodes and the spleen are easily palpable when

TABLE I

Incidence of Spontaneous Leucosis in the Stocks of Mice Used in Transmission Experiments

	Number of mice* examined		Number of cases of lymphoid leucosis	
	Living	Dead	Leucemic	Aleucemic
Stock A	350	220	2	2
Stock R	600	420	2	0
Stock S	250	470	1	4

* Above the age of 8 months.

they are distinctly enlarged. Blood smears are taken from all mice that have enlarged nymph nodes and from the inoculated mice at intervals of 1 or 2 weeks.

Spontaneous Leucosis in the Stocks of Mice Used for Transmission Experiments.*—Leucosis occurs in all three of our stocks, but it is very infrequent.† The incidence of spontaneous leucosis in our laboratory has not as yet been exactly determined; the accompanying approximate figures may be of value in interpreting the transmission experiments (Table I).

Four cases of leucemia were discovered during life, one in Stock S,

* Leucosis is used as a collective term for leucemia, lymphoma and related conditions.

† At the time a preliminary report (7) of these investigations was published, leucemia had been observed only in Stock A.

one in Stock R and two in Stock A. Only the last two cases were transmitted successfully.

Origin of the Transmissible Strain. Methods of Transfer

The donor of the first transmissible strain (A 8) was about 15 months old when a uniform enlargement of superficial lymph nodes so that each measured about 0.6 to 1 cm. across was noted. The microscopic picture of the lymph nodes showed the extensive lymphoid hyperplasia (Fig. 1) characteristic of lymphoid leucemia. The spleen was very much enlarged and extensive lymphoid infiltrations were found in the liver (Fig. 2) and in the kidney (Fig. 3). The white blood cells numbered 315,000; 68 per cent were lymphocytes and of these about 30 per cent showed signs of immaturity. The transmissions were made by injecting blood, to which heparin had been added to prevent clotting, or a fairly turbid suspension of lymph node tissue into the tail vein of normal mice. The material from lymph nodes was obtained by cutting them up with small scissors in the presence of Locke's solution and filtering through a small piece of cotton. In several parallel series the susceptibility of our three stocks to inoculations by the subcutaneous intraperitoneal and intravenous routes were compared.

The second transmissible strain was derived from a spontaneous case (A 984), which resembled the first case very closely. It was transmitted in a similar manner. The passages of the first strain are collected in Table II, those of the second strain in Table III.

The success of the inoculations varied with the individual passages. *E.g.*, both mice inoculated intravenously with leucemic blood of R 1742 and all three mice inoculated in a similar manner with leucemic blood of A 1396 developed leucemia. The blood in these cases was obtained from the heart of the animals and was injected in amounts of 0.05 to 0.15 cc. On the other hand, several other attempted transfers from other leucemic mice made in a similar manner were entirely negative. In one instance 4 of 10 mice inoculated intravenously with an emulsion of lymph nodes of Mouse R 1684 developed leucemia whereas a second similar transfer attempted 9 days later from the same donor to 13 mice was entirely unsuccessful.

A comparison of the intravenous transmissions with blood and with lymph nodes in Stocks A and R gives the following figures:

	No. injected	Leucosis		Successful inoculations
		Leucemic	Aleucemic	
				per cent
Inoculated with lymph nodes	463	50	14	13.8
Inoculated with blood	133	16	6	16.5

TABLE II

Transmissions from Spontaneous Leucemia A 8

Transfer from mouse No.	Passage	Stock A, intravenous			Stock A, intraperitoneal			Stock A, subcutaneous			Stock R, intravenous			Stock S, intravenous		
			Leucosis			Leucosis			Leucosis			Leucosis			Leucosis	
		No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic
A 8	Orig.	22	2	0	15	0	0	15	0	0						
A 246	I	32	5	2**	18	1	1									
A 209	I	12	1	0	15	0	1									
A 40	II	18	3	0	9	0	0				8	1	0			
A 50	II	14	1	0	5	0	0									
A 96*	II	9	0	0				6	0	0						
A 931*	II	18	2	1	16	0	0									
A 181	III	14	3	0												
A 179	III	19	2	0	2	0	1									
R 135	III										26	6	2			
A 805*	III	25	1	1**				23	0	0						
A 804	III	6	0	0										12	0	0
A 1101	IV	8	1	0												
A 1239	IV	9	2	0							7	1	1			
A 1240	IV	12	0	0												
A 220	IV	25	0	0	10	0	0							15	0	0
R 771	IV										13	0	0			
R 774	IV										11	0	0			
R 1742	IV	2	2	0				8	0	0						
A 837	IV	9	0	0												
A 1383	V	25	1	1												
A 1297	V	24	6	1												
A 1398	V	17	1	1												
A 1519	VI	13	2	0												
A 1396	VI	3	3	0										3	2	0
A 1534*	VI	29	0	0										16	0	0
A 1707	VII	6	1	0										9	0	0
A 1633	VII	13	0	0												
A 1738	VIII	5	0	0										6	0	0
Total....		389	39	7	90	1	3	52	0	0	65	8	3	61	2	0

* These donors were aleucemic; all the others leucemic.

** One of these may be spontaneous for it developed several months after inoculation.

The factors governing the outcome of a transmission are not fully known. Some of them will be discussed later; others, such as the amount of inoculum necessary to produce leucemia, the influence of the medium used in its preparation, the physical condition of the recipient etc. have not as yet been sufficiently investigated. That hered-

TABLE III

Transmissions from Spontaneous Leucemia A 984

Transfer from mouse No.	Passage	Stock A, intravenous			Stock A and R, intraperitoneal			Stock R, intravenous			Stock S, intravenous		
			Leucosis			Leucosis			Leucosis			Leucosis	
		No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic	No. injected	Leucemic	Aleucemic
A 984	Orig.	14	1	0	(A) 10	0	0	18	1	1			
R 639	I							16*	1	1			
A 682	I	16	0	0	(R) 9	0	0						
					(A) 6	0	0	12	0	0			
R 1694	II							23	4	0			
R 1657	III							16	1	0			
R 1659	III							7	0	3	9	0	0
R 1663	III	6	1	0				23	4	3			
R 1660	III	16	6	0				9	1	0	7	0	0
A 1497	IV	15	2	0							20	2	1
R 1278	IV	17	0	2							13	1	0
A 1364	IV	21	0	0									
S 2457	V	10	0	0									
A 1612	V	6	0	1									
S 1678	V										9	0	0
S 2362	V	27	10	0							18	0	0
Total....		148	10	3	25	0	0	124	12	8	76	3	1

* 8 mice of Strain R were inoculated subcutaneously with this material without success.

itary susceptibility is an important factor has been demonstrated by MacDowell and Richter. It is noteworthy in this connection that in a passage (from R 1660) 8 of the 16 mice inoculated were grandchildren of A 8, an animal with spontaneous leucemia. Four of these 8 mice developed leucemia after inoculation, whereas only 2 of the inocu-

lations in the 8 mice not known to be closely related to a spontaneous case of leucemia were successful.

Notably unsuccessful were the attempts at transmission during the extremely hot summer of 1930, resulting in a loss of the transmissible strains described in this report. The negative inoculations made at that time during our absence, have been included in Tables II and III; they are responsible in part for the low mean percentage of successful inoculations.

The figures presented in Tables II and III show that Stocks A and R are equally susceptible to transmissible leucemia. It is noteworthy that Stock R was secured as a well observed normal stock in which the incidence of cancer was found to be low and leucemia was not known to occur. Stock S appears somewhat less susceptible although the data presented in Tables II and III are not sufficient for us to estimate its relative susceptibility. When transfers to Stock A were effective, mice of Stock S reacted well to simultaneous inoculations. Most of the transfers to Stock S were attempted during the summer of 1930 when some undetermined factor interfered with the success of inoculations. For this reason in the analysis that follows Stock S will not be considered.

Comparison of Intravenous, Intraperitoneal and Subcutaneous Inoculations. Etiological Relationship of Leucemic and Aleucemic Leucosis.—It is evident from Tables II and III that leucosis can be best transmitted by intravenous injections. The majority of the mice successfully inoculated by this route developed leucemia, namely 69 of the 726 mice inoculated or 9.5 per cent, and a smaller number, 21 (2.8 per cent), developed aleucemic lymphadenosis. Much less successful was attempted transmission by the intraperitoneal route; only 1 of 124 mice injected developed leucemia and in 3 there was a lymphoid tumor unaccompanied by a systemic enlargement of the lymph nodes. In 2 of these the tumor was situated at the site of inoculation (A 931 and A 1156) and in one (A 707) in the mediastinum. The latter case may have been spontaneous. Subcutaneous inoculations into 62 mice were entirely negative.

The figures presented in Tables II and III give sufficient evidence for the possibility of the development of aleucemic lymphadenosis by inoculations with organs of leucemic mice. The reverse possibility, namely the production of leucemia by intravenous inoculation of or-

gans from aleucemic cases, has been tested by inoculations from four cases (A 96, A 931, A 805 and A 1534, Table II), only twice with success.

One of these transfers was made from the above mentioned Case A 931, a mouse with a white blood count of 15,000 to 32,000 during a 2 weeks' period of observation. Two of 18 mice inoculated intravenously with an emulsion of the lymphomatous tumor of A 931 developed leucemia and one (A 805) aleucemic lymphadenosis. A 805 was under observation for 46 days following the discovery of enlarged lymph nodes measuring 0.6 to 1 cm. across. The white blood count varied from 12,500 to 27,000. The postmortem appearances were those of aleucemic lymphadenosis with pronounced lymphoid infiltration in various organs. Following intravenous inoculation of 25 mice with an emulsion of lymph nodes of this aleucemic mouse one of the inoculated mice developed leucemia.

These three cases of leucemia developed 14 to 19 days following intravenous inoculation of lymphoid tissues derived from aleucemic cases. The incubation period and the youth of these mice (4 to 6 months) almost certainly excludes the possibility that they were spontaneous.

These observations are in harmony with those described by the investigators mentioned above, indicating that leucemic and aleucemic lymphadenosis may occur with or without tumor formation and that they are varieties of the same condition. One factor that appears important in determining the type of leucosis is whether the leucotic cells have a free entrance into the circulation, for intravenous dissemination seems to favor leucemia. Another determinant would seem, according to the studies of Richter and MacDowell, to be the character of the cells of the donor (8). These investigators observed that the lesions produced by inoculation of lymph nodes deriving from several spontaneous cases show considerable differences depending solely on the cells of the donor.

Anatomical Changes in Transmissible Leucemia of Mice

It is not within the scope of this work to describe in detail the gross and microscopic appearance of the organs in leucemia and lymphoma produced by transmission. Leucemia as produced by intravenous inoculations resembles spontaneous leucemia very closely.

There is a systemic enlargement of the lymph nodes, the size of which frequently exceeds 1 cm. in diameter. The lymph follicles of the intestinal tract are very prominent and the size of the mesenteric lymph nodes usually exceeds those of the superficial lymph nodes. The retroperitoneal lymph nodes form frequently a confluent mass embedding the adrenal, parts of the kidneys and adjacent structures. Similarly the lymphoid mass extending from the mediastinal lymph nodes not infrequently covers most of the heart. The spleen measures about 3 to 4 cm. in its longest diameter. The microscopic picture of the lymph nodes and spleen is illustrated in Figs. 5 and 6. The lymphoid infiltration of various organs seen in spontaneous leucemia (Figs. 2 and 3) are equally characteristic for leucemia produced by transmission (Fig. 7).

The microscopic picture of the organs involved has the character of a highly invasive neoplastic growth composed of medium and large cells resembling lymphocytes, many of them in mitotic division.

The anatomical changes in aleucemic lymphadenosis were essentially the same, except that the circulating blood was not invaded.

In the cases with tumor formation at the site of inoculation (peritoneal cavity) several lymph nodes were considerably enlarged but the liver was not infiltrated. Fig. 8 is a view of the tumor A 931 described above.

Relation of Age to the Result of Inoculation.—Table IV records the results of the inoculations with transmissible leucosis in the mice grouped according to age.

TABLE IV

Relation of Age to the Results of Inoculation with Transmissible Leucosis

Age in mos.	Below 3	3 to 5	5 to 7	7 to 9	9 to 11	11 to 13	Over 13
No. of mice inoculated	612	218	124	70	86	32	20
No. of successful inoculations	48 (9)*	13 (1)	10 (3)	0	17 (5)	3 (2)	1
Percentage of successful inoculations	7.8	6	8	—	19.8	9	5

* The figures in parentheses indicate the number of cases of leucosis with aleucemic blood picture.

It would seem from Table IV that young as well as old mice may succumb to leucosis if leucotic material is introduced into the circulation. The age of mice with spontaneous leucemia observed in these stocks was from 12½ to 18 months; it appears from this table that mice

are most susceptible to inoculations during the period immediately preceding, when they are from 9 to 11 months old. However the number of mice in each group is too small to form a basis for a definite opinion as to the relation between age and susceptibility to leucemia.

The Blood Picture in Transmissible Leucosis

The average count for normal mice of the breeds used was neutrophilic polymorphonuclears, 42 per cent, immature granulocytes (metamyelocytes, ring forms), 3.6 per cent, lymphocytes, 52 per cent, lymphoblasts, 0.4 per cent, "pro-lymphocytes,"* 1 per cent, monocytes, 1 per cent. Polychromatophilia is common in these apparently healthy stocks. Only one typical eosinophile was seen in a very large number of slides examined. The percentage of lymphocytes varied within very broad limits (from about 25 to 72 per cent) and under many conditions, such as age and external temperature. No less variable was the total leucocyte count, the figures ranging between 2,000 and 16,000, with an average of about 8,000. However the figures below about 5,000 were found in a single day when the temperature was unusually high. If these counts are disregarded the average is about 11,000.

The changes in the blood picture occur rather suddenly, the first definite leucemic blood change being a sharp relative increase in the number of immature lymphocytes or lymphoblasts preceding the leucocytosis (Tables VI and VII). These are rather large cells (18 to 24 μ) with dark blue cytoplasms, with no granules or a few fine azurophilic granules usually in the perinuclear zone, a large round or slightly oval nucleus with a very fine chromatin network and 2 to 5 nucleoli poorly differentiated in smears stained with Romanowsky stains. These lymphoblasts appear to differ somewhat from those occasionally observed in the normal circulating blood because of their larger size and the more pronounced basophilia of the cytoplasm. Biologically a distinct difference is shown by the capacity of the leucemic lymphocytes to produce leucemia. (*Cf.* Korteweg.) The "pro-lymphocytes" normally present in the blood (1 per cent) diminished at least relatively

* Very small lymphoid cells with narrow rim of intensely basophilic cytoplasm without granulation and with a very compact structureless nucleus (Ferrata).

in the leucemic blood. A few days after the appearance in the blood of lymphoblasts, the total number of leucocytes begins to increase (Tables VI, VII) with a continuous relative as well as absolute rise of the number of lymphoblasts. The peak, relatively speaking, of the lymphoblasts, is reached before the total leucocyte count is at its

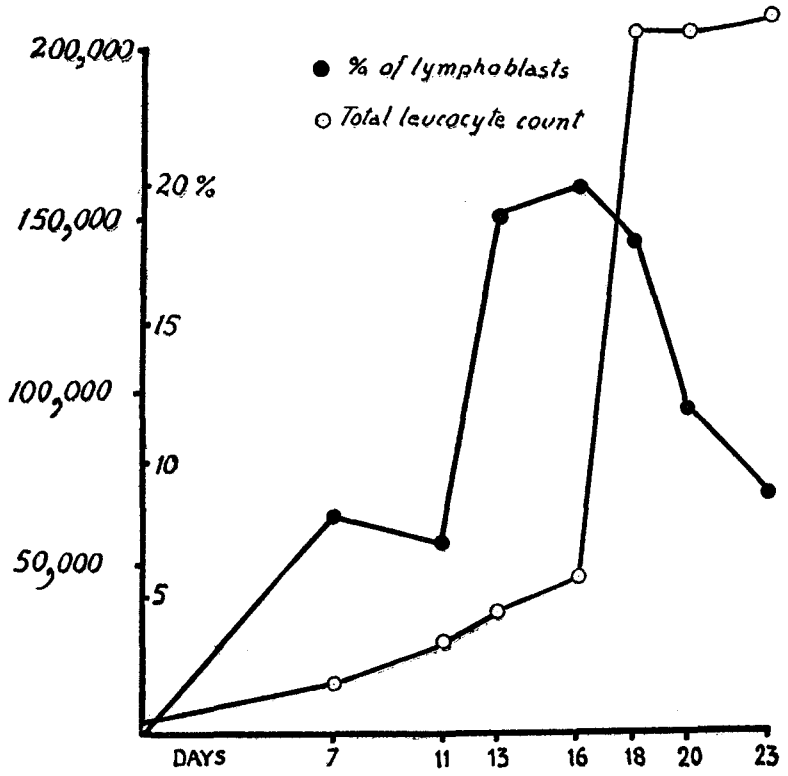


CHART 1 shows that in leucemia of the mouse produced by transmission (in Mouse A 1122) the immature lymphocytic cells (lymphoblasts) are greatly increased in the circulating blood before the total number of leucocytes reaches its maximum.

maximum, as is illustrated by the accompanying graph (Chart 1). The percentage of undifferentiated cells at the peak varied from 12 to 35 per cent.

The percentage of lymphocytes closely paralleled the total leucocyte increase. Polychromatophilia increased greatly with the development

TABLE V

Blood Counts in Aleucemic Lymphadenosis

No. of mouse	Blood counts at time of the discovery of enlarged lymph nodes				Subsequent blood counts												No. of days* when killed (k.) or died (d.)
	No. of days*	White blood count	Lymphocytes	Lymphoblasts	No. of days*	White blood count	Lymphocytes	Lymphoblasts	No. of days*	White blood count	Lymphocytes	Lymphoblasts	No. of days*	White blood count	Lymphocytes	Lymphoblasts	
			per cent	*per cent*			*per cent*	*per cent*			*per cent*	*per cent*			*per cent*	*per cent*	
A 1623	8	5,000	30	0	13	17,000	60	1	20	15,000	28	0	—	—	—	—	d. 22
R 673	27	20,500	65	4	—	—	—	—	—	—	—	—	—	—	—	—	d. 31
R 633	22	25,500	—	—	27	43,000	47	5	30	33,000	—	—	—	—	—	—	d. 32
R 1765	30	7,200	59	1	37	23,000	66	2	—	—	—	—	—	—	—	—	d. 44
R 1769	8	49,500	57	0	11	63,500	46	0	—	—	—	—	—	—	—	—	d. 16
R 1415	13	32,500	64	1	21	11,000	64	3	27	10,000	52	2	—	—	—	—	d. 32
R 1801	23	17,500	—	—	—	—	—	—	—	—	—	—	—	—	—	—	d. 30
A 805	20	15,000	—	—	25	14,500	42	0	49	12,500	—	—	62	27,000	50	1	k. 62
A 96	37	17,400	40	0	44	17,400	31	1	—	—	—	—	—	—	—	—	k. 44
A 941	44	13,000	58	1	—	—	—	—	—	—	—	—	—	—	—	—	d. 213
A 1425	22	15,000	58	1	28	23,500	62	0	33	30,000	64	1	35	37,500	51	0	d. 39
A 874	5	9,700	24	1	18	10,500	45	1	32	24,500	28	2	39	32,000	64	5	d. 116
A 1534	24	9,000	49	3	30	48,000	58	6	37	56,000	—	—	—	—	—	—	k. 37
A 931	36	15,000	—	—	43	16,000	60	8	50	32,000	71	5	—	—	—	—	k. 50
R 773	13	16,000	70	2	17	46,000	66	12	—	—	—	—	—	—	—	—	d. 23
R 784	27	23,500	59	3	35	46,000	50	2	42	45,000	63	3	49	56,500	49	4	d. 54
R 1059	21	18,500	28	0	28	24,500	33	0	—	—	—	—	—	—	—	—	d. 35

* After inoculation.

of the leucemic picture, as did the number of "shadow" cells (disintegrated nuclear bodies).

It is of great significance that almost all of the lymphocytes found in leucemic blood smears are more or less immature, although not immature enough to justify their classification as lymphoblasts. They are larger than normal lymphocytes, the cytoplasm is deeper in color and the nucleus has a finer structure with greater variations than occur in normal cells. The number of cells with azurophilic granules in the cytoplasm is also decidedly less.

In the cases of aleucemic lymphadenosis observed after the inoculation of leucemic blood the percentage of lymphoblasts was not so high as in the pre-leucemic stage of transmitted leucemia, as is shown in Table V.

Observations on the Pathogenesis of Leucemia

The first evidence of a successful inoculation was a uniform enlargement of all superficial lymph nodes observed from 7 to 25 days following the intravenous inoculation. The blood counts taken at this stage of the disease were normal; the differential counts showed a percentage of lymphocytes within the normal range, but the percentage of lymphocytes with signs of immaturity was as a rule above normal. Within a few days the number of the circulating leucocytes rose considerably and the aleucemic phase was soon followed by typical leucemia (Table VI). In a few instances the blood count was already leucemic when the enlargement of the lymph nodes was discovered (Table VI); this observation however was of little significance because the physical examinations were made only at 3 to 4 day intervals and the aleucemic stage could easily have been missed.

Thus it appears that when leucemic cells are injected into a vein of normal mice they leave the circulation and multiply in certain tissues favorable for their growth. The occasional high percentage of immature lymphocytes in the circulating blood during the aleucemic phase suggests that immature cells enter the circulation steadily but presumably leave it to "colonize" in some other location. This mechanism would seem adequate to explain the uniform enlargement of all lymphoid tissues.

The history of a small group of mice closely followed from the day

TABLE VI

Blood Counts in Leucemia Produced by Transmission

No. of mouse	At time of the discovery of enlarged lymph nodes					Subsequent blood counts												No. of days* when killed (k.) or died (d.)
	Size of lymph nodes	No. of days*	White blood count	Lymphocytes	Lymphoblasts	No. of days*	White blood count	Lymphocytes	Lymphoblasts	No. of days*	White blood count	Lymphocytes	Lymphoblasts	No. of days*	White blood count	Lymphocytes	Lymphoblasts	
	cm.			*per cent*	*per cent*			*per cent*	*per cent*			*per cent*	*per cent*			*per cent*	*per cent*	
A 1498	0.3–0.4	14	11,800	61	1	18	16,000	72	4	22	177,000	58	23	26	405,000	—	—	d. 30
R 1803	0.2	12	13,400	40	0	16	29,000	54	7	20	35,500	61	6	25	101,000	72	12	d. 27
A 1519	0.3	15	5,500	27	0	18	106,000	44	13	20	355,000	62	25	22	419,000	70	21	k. 22
A 813	0.5–1.0	16	19,200	31	2	20	36,000	58	3	25	135,000	50	24	28	295,000	47	18	
														35	19,000	40	6	d. 59
A 1101	0.3–0.4	18	11,500	45	3	20	32,500	39	23	24	174,000	67	16	28	222,000	69	19	
														32	407,000	88	5	k. 32
A 422	0.3–0.5	16	8,000	75	4	23	146,000	56	24	35	325,000	55	27	—	—	—	—	d. 66
R 639	0.2–0.4	22	13,200	68	12	27	32,000	78	12	30	125,000	76	14	42	187,000	—	—	d. 42
A 1054	0.5	22	10,000	41	4	26	60,000	37	17	32	153,000	52	17	—	—	—	—	d. 39
R 1663	0.2	14	11,000	66	4	22	35,500	61	3	29	130,000	60	17	41	284,000	55	23	k. 41
R 1775	0.2	16	11,500	71	5	21	20,000	71	6	24	25,000	63	16	28	66,000	59	22	
														35	212,000	74	15	d. 39
R 1802	0.2	12	5,800	30	2	16	46,000	66	2	25	103,000	42	11	—	—	—	—	d. 27
A 1240	0.2	17	22,500	41	14	20	48,000	60	19	27	226,000	70	20	29	516,000	68	23	k. 29
A 50	0.3	23	59,000	58	2	24	108,000	46	8	30	259,000	74	16	—	—	—	—	d. 40
A 926	0.3	16	44,000	56	21	23	174,000	80	16	28	258,000	75	18	43	252,000	72	10	d. 50
R 1735	0.4	15	32,000	58	4	23	196,000	73	17	27	220,000	75	15	—	—	—	—	d. 28
R 760	0.3–0.4	15	83,000	71	9	22	245,000	77	12	31	216,000	81	14	—	—	—	—	d. 34
R 771	0.4–0.5	15	285,000	68	13	23	280,000	73	13	24	485,000	—	—	—	—	—	—	k. 24

* After inoculation.

TABLE VII

The Development of Leucemia after Intravenous Inoculation

		Inoculated mice		Control mice (uninjected)		
No. of mouse		A 1122	A 1297	A 1002	A 1344	A 1115
Before inoculation	White blood count	2,600	2,200	3,800	1,500	3,600
	Per cent of lymphocytes	32	32	41	40	46
	Per cent of lymphoblasts	0	0	0	0	0
	Size of lymph nodes	Normal	Normal	Normal	Normal	Normal
7 days after inoculation	White blood count	15,000	18,500	6,000	9,000	14,500
	Per cent of lymphocytes	47	42	72	34	39
	Per cent of lymphoblasts	8	0	0	0	0
	Size of lymph nodes	Very slightly enlarged	Normal	Normal	Normal	Normal
11 days after inoculation	White blood count	27,600	27,500	7,800	12,000	20,500
	Per cent of lymphocytes	53	32	70	46	41
	Per cent of lymphoblasts	7	2	0	0	0
	Size of lymph nodes	3 to 4 mm.	Very slightly enlarged	Normal	Normal	Normal
13 days after inoculation	White blood count	36,000	28,000	10,000	14,000	16,000
	Per cent of lymphocytes	52	40	59	50	60
	Per cent of lymphoblasts	19	1	1	0	1
	Size of lymph nodes	4 to 5 mm.**	2 to 3 mm.	Normal	Normal	Normal

16 days after inoculation	White blood count	46,000	24,000	10,000	16,000	16,000
	Per cent of lymphocytes	62	58	68	48	77
	Per cent of lymphoblasts	20	1	0	0	0
	Size of lymph nodes	4 to 5 mm.	3 mm.**	Normal	Normal	Normal
18 days after inoculation	White blood count	205,000	42,000	8,000	6,500	14,000
	Per cent of lymphocytes	74	40	70	49	51
	Per cent of lymphoblasts	18	5	0	0	0
	Size of lymph nodes	4 to 5 mm.	3 to 6 mm.	Normal	Normal	Normal
20 days after inoculation	White blood count	205,000 (400,000)*	46,000			
	Per cent of lymphocytes	87	46			
	Per cent of lymphoblasts	12	4			
	Size of lymph nodes	5 mm.				
23 days after inoculation	White blood count	209,000 (500,000)*	158,000			
	Per cent of lymphocytes	89	—			
	Per cent of lymphoblasts	9	—			
	Size of lymph nodes	5 mm.				

** Spleen 2 cm. long.

* Estimated from smear.

of inoculation is fully given in Table VII. The leucocyte count of 3 control mice of the same age and breed, kept side by side with the inoculated mice, varied between 3,600 to 16,000, the percentage of lymphocytes from 41 to 77 per cent, the percentage of immature lymphocytes from 0 to 1 per cent. The first evidence of a successful inoculation was a very slight enlargement of all superficial lymph nodes observed 7 days after inoculation in one case and 11 days in another. At the same time there was a distinct increase in the percentage of immature lymphocytes (8 per cent in one and 2 per cent in the other). The absolute number of white blood cells as well as the percentage of mature lymphocytes appeared to be within the normal range.

SUMMARY AND CONCLUSIONS

Lymphoid leucemia of the mouse is readily transmitted by intravenous inoculations. The majority of the mice inoculated successfully develop leucemic, a smaller number of them, aleucemic lymphadenosis. The data presented favor the view that leucemic and aleucemic lymphadenosis are essentially the same condition.

Leucemia produced by transmission is preceded by an aleucemic stage, in which the lymph nodes and the spleen are uniformly enlarged, and the white blood count and the percentage of lymphocytes are within the normal range but immature lymphocytes are numerous in the circulating blood.

Young as well as old mice may develop leucemia if leucotic material enters their circulation.

Studies of transmissible leucemia favor the view that leucemia of mammals is a neoplastic disease. The basic problem of leucemia would seem to be determination of the factors that bring about a malignant transformation of lymphoid cells.

REFERENCES

1. Opie, E. L., *Medicine*, 1928, **7**, 31.
2. Snijders, E. P., *Nederl. Tijdschr. Verlosk. Gynaec.*, 1926, **2**, 1256.
3. Tio Tjwan Gie, Over Leukaemie by Dieren, en over een overentbare Cavialeukose, Amsterdam, 1927.
4. Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **51**, 650.
5. MacDowell, E. C., and Richter, M. N., *J. Cancer Research*, 1930, **14**, 434.
6. Korteweg, R., *Z. Krebsforsch.*, 1929, **29**, 455.

7. Furth, J., and Strumia, M., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 834.
8. Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **52**, 823.

EXPLANATION OF PLATES

PLATE 23

FIG. 1. Lymph node in spontaneous lymphoid leucemia. The original architecture is obscured by a massive growth of lymphoid cells extending beyond the capsule of the lymph node (Mouse A 8). Hematoxylin, eosin and azure II. × 300.

FIG. 2. Spontaneous lymphoid leucemia. Infiltrations in the liver (Mouse A 8). Hematoxylin and eosin. × 200.

FIG. 3. Spontaneous lymphoid leucemia. Infiltrations in the kidney (Mouse A 8). Hematoxylin and eosin. × 200.

FIG. 4. The blood smear in lymphoid leucemia produced by transmission. White blood count 691,000. Wright and Giemsa's blood stain. × 1000.

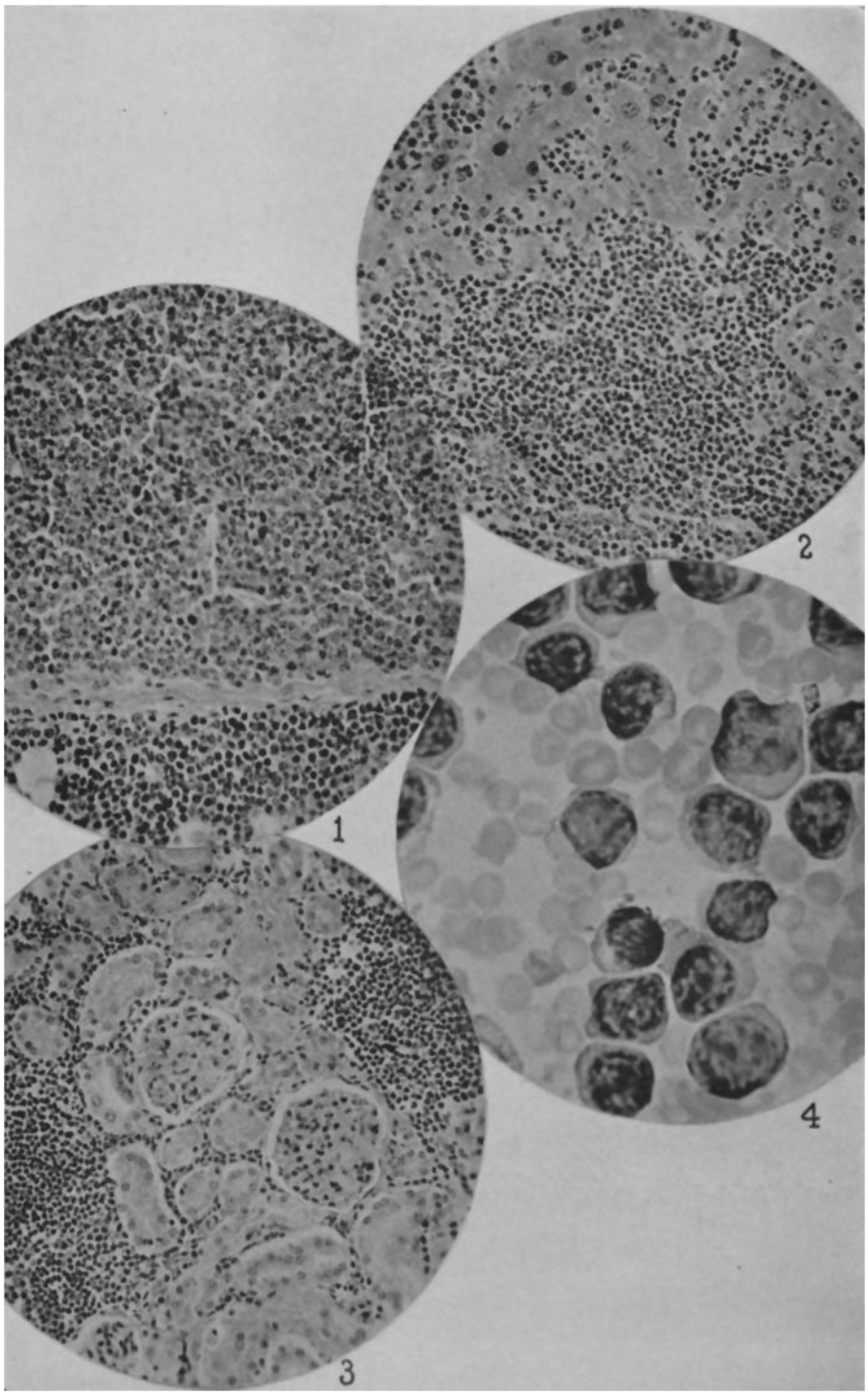
PLATE 24

FIG. 5. The lymph node in lymphoid leucemia produced by transmission. There is a massive infiltration of the fatty tissue surrounding the lymph node. Hematoxylin and eosin. × 200.

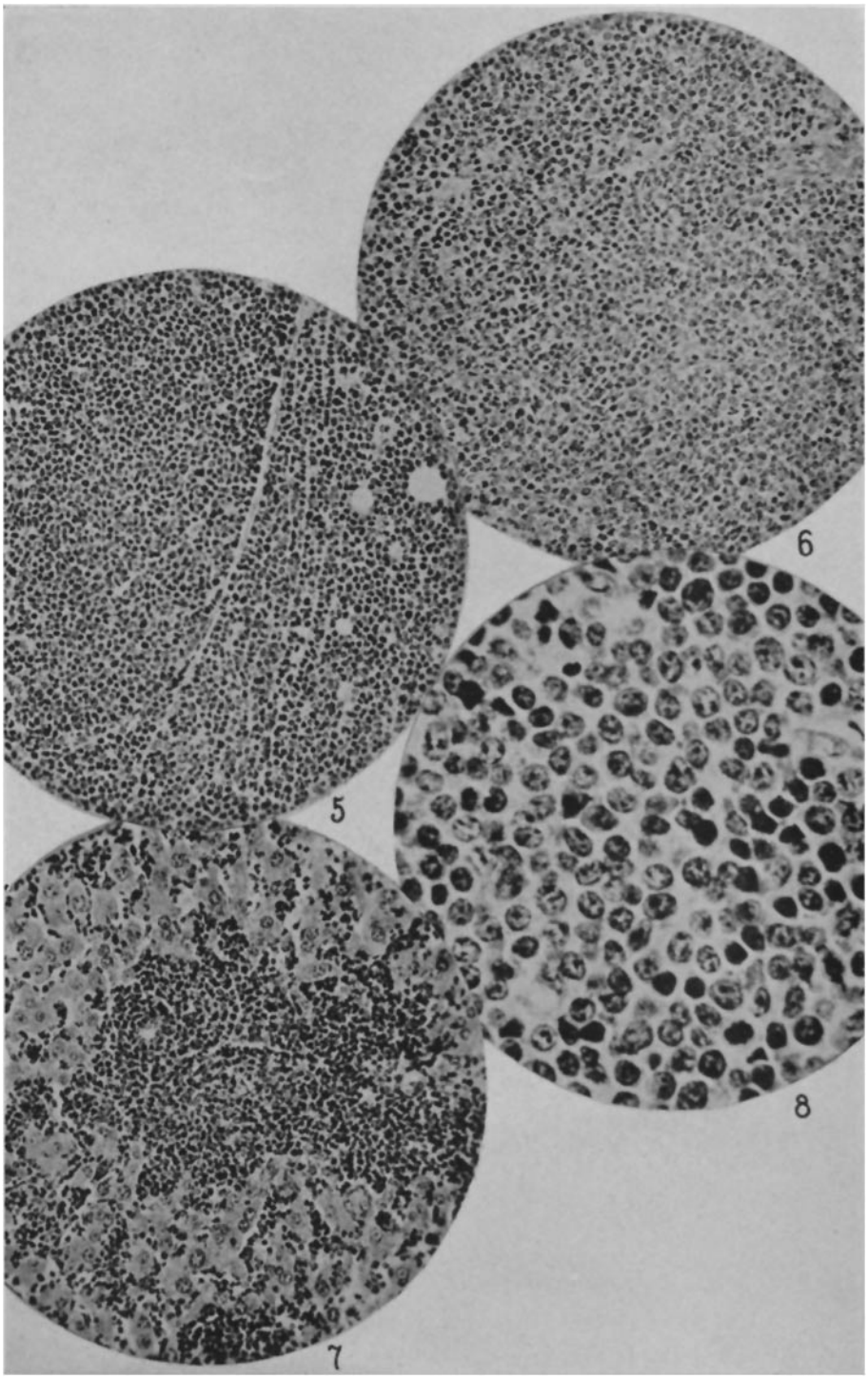
FIG. 6. The hyperplastic splenic follicle in transmitted leucemia of mice. Hematoxylin and eosin. × 200.

FIG. 7. Lymphoid infiltration in the liver of a mouse with leucemia produced by transmission. Hematoxylin and eosin. × 200.

FIG. 8. View of a lymphoid tumor produced by intraperitoneal inoculation in Mouse A 931. Hematoxylin and eosin. × 700.



(Furth and Strumia: Transmissible lymphoid leucemia of mice)



(Furth and Strumia: Transmissible lymphoid leucemia of mice)